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Abstract book

Oral communications

ABSTRACT N° ICBF22-482

USING PHYSIOLOGY TO IMPROVE CONSERVATION AQUACULTURE OF AN IMPERILED ESTUARINE FISH: LONGFIN SMELT (SPIRINCHUS THALEICHTHYS)

Nann Fangue* [1], Yuzo Yanagitsuru[1], Florian Mauduit[1], Dennis Cocherell[1], Tien-Chieh Hung[1], James Hobbs[2], Richard Connon[1]

[1]UC Davis, Davis,
[2]Ca Dept Fish and Wildlife, Sacramento, United States

Abstract:
The population of the once-abundant longfin smelt in the San Francisco Estuary has plummeted to <1% of historical abundances. To aid conservation efforts, an aquaculture program has been under development since 2009. However, low larval survival (<1%) has hindered the development of a captive culture for the species until 2019 (~13% survival) following changes to larviculture protocols informed by physiological studies. We present the key findings that have improved longfin smelt larviculture. We used a cardiac assay to determine a correlate of thermal optimum, Arrhenius breakpoint temperature (TAB), and two correlates of upper thermal limit, the temperature at which heart rate peaks (Tpeak), and when arrhythmia begins (TArr) for larvae. We found that larvae exhibited high variability in all three cardiac metrics, resulting in a large overlap between the temperatures where individuals are at their thermal optimum and others at their limit. By analyzing the proportion of individuals at TAB, Tpeak, and TArr, we identified that temperatures below 14 °C maximized the proportion of individuals at TAB (i.e. thermal optimum), the result of which was corroborated by improved larval growth performance at 9 or 12 °C. We additionally measured the growth performance of larvae at different salinities and discovered that larvae have improved survival and growth at moderately brackish salinities (5-10 ppt). Although further studies of longfin smelt are necessary to validate culture conditions that increase larval survival, the dramatically improved survival after 2019 is encouraging for the development of a longfin smelt conservation aquaculture program.

06-Developmental physiology of fishes

ABSTRACT N° ICBF22-260

LOCAL ADAPTATION SHAPES THERMAL TOLERANCE OF FUNDULUS HETEROCLITUS EMBRYOS

Tessa Blanchard* [1], Madison Earhart[1], Ariel Shatsky[1], Patricia Schulte[1]

[1]University of British Columbia, Vancouver, Canada

Abstract:

The early life stages of fish are particularly sensitive to thermal stressors as their thermal windows are often narrower than those of subsequent life stages. This sensitivity suggests that the thermal windows of larval life stages are likely to be shaped by natural selection. To test this hypothesis, we examined the thermal windows for development in populations of Fundulus heteroclitus, a species of topminnow that inhabit intertidal saltmarshes along the Atlantic coast of North America through a steep latitudinal thermal gradient. There is genetic differentiation with latitude such that northern and southern subspecies are recognized, and these subspecies differ in multiple aspects of thermal biology as adults, but less is known about variation at early life stages. Therefore, we raised fish from the northern and southern subspecies of F. heteroclitus and their reciprocal crosses at 15,18, 21, 24, 27, 30, and 33°C until hatching. We measured traits including developmental rate, yolk-sac volume, larval growth, heart rate, and survival in response to each temperature regime. We found that southern embryos had a right-shifted developmental thermal performance curve and higher thermal optimum compared to northern embryos. Larval length showed an inverse relationship with incubation temperature whereas yolk-sac volume displayed a positive relationship. Differences across larval traits and cross-type were also found, such that northern crosses hatched faster with larger yolk-sac volumes. Overall, these observed differences in embryonic and larval traits between southern and northern populations is suggestive of local adaptation via counter-gradient variation

06-Developmental physiology of fishes

ABSTRACT N° ICBF22-539

FUNCTIONALITY OF STRESS KINASES AND HEAT SHOCK PROTEINS DURING EARLY DEVELOPMENT OF REARED GREATER AMBERJACK (SERIOLA DUMERILI) (RISSO, 1810)

Nikolas Panteli*[1], Maria Demertzioglou[1], Konstantinos Feidantsis[1], Konstantinos Kormas[2], Elena Mente[2, 3], Efthimia Antonopoulou[1]

[1]School of Biology, Aristotle Unversity of Thessaloniki, Thessaloniki,
[2]School of Agricultural Sciences, University of Thessaly, Volos,
[3]School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

Abstract:
Adversities in reproduction performance of captive-reared greater amberjack (Seriola dumerili) are the main obstacle to its commercialization. Reproductive dysfunctions are speculated to arise due to captivity-induced stress or nutritional deficiencies in fishfeed. Throughout early developmental stages, activation of signal transduction pathways is crucial in embryos patterning since they are involved in proliferation and differentiation processes. The present study aims to clarify the potential role of mitogen-activated protein kinases (MAPKs) and their target heat shock proteins (HSPs) during greater amberjack development. Samples from five developmental stages (1-day prior to hatching fertilized eggs until 46 days post-hatching juveniles) were obtained from an aquaculture unit. HSPs displayed a higher induction at 3 days post-hatching and onwards compared to earlier stages. Additionally, the immediate significant increase of HSP90 induction at hatching stage, which was maintained at high levels throughout early development, indicates a potential prominent role in greater amberjack ontogeny. Regarding MAPKs, activation of both p38 and p44/42 MAPKs increased significantly at the hatching stage, suggesting an essential role of the MAPK signaling pathway in late stages of embryonic development and differentiation processes. However, an immense reduction occurred at the 3 days post-hatching stage, implying that initial activation of both MAPKs may simultaneously mediate embryonic responses to environmental stimuli. Subsequent increase in MAPKs phosphorylated levels in juvenile stages indicates further involvement in early development.

The project is funded by the Operational Program “Competitiveness, Entrepreneurship and Innovation 2014-2020 (EPAnEK)” (project code: 6-00068, MIS: 5045873, Acronym: MAGIATIKO), which is co-funded by Greece and the European Union.

06-Developmental physiology of fishes

ABSTRACT N° ICBF22-388

CRITICAL WINDOWS AND DEVELOPMENTAL PLASTICITY IN EARLY LIFE FISHES EXPOSED TO ENVIRONMENTAL STRESSORS

Gil Martinez Bautista*[1], Warren Burggren[1]

[1]University of North Texas, Denton, Texas, United States

Abstract:
Climate change alters individual's life history traits of fishes, especially during early development. The phenotype of developing fishes is most responsive to the environment during specific sensitive periods (critical windows), in which environmental stressors have the greatest effect on developmental trajectories. The degree of responsiveness depends on stressor type, stressor dose, and time of exposure. These alterations can lead to long-term effects, either adaptive or maladaptive, resulting in changes in individual developmental trajectories. These switches in the developmental trajectories are often described as permanent. However, numerous studies have shown that developing fish whose developmental trajectory has been altered by the action of a stressor can subsequently take an alternate developmental trajectory 'restoring' the changes when the stressor disappears, a process facilitated by early developmental plasticity. In this presentation we discuss developmental plasticity in early life stages, especially in the face of hypoxic, thermal or osmotic challenges. Examples to be discussed include early fish survival, growth, time to key developmental events and hypoxia- and hyperoxia-related gene expression as a function of stressor type, dose and time of exposure. Also discussed is hypoxia-induced branchial remodeling producing larger gills with reduced oxygen diffusion distances that persist in juvenile and/or adult fishes, and cardiovascular modifications supporting enhanced gas exchange and transport during environmental changes. Studying the dynamics of fish developmental trajectories will facilitate the understanding and predicting of the mechanisms that individuals, populations and species will employ to cope with predictable and unpredictable climate change.

ABSTRACT N° ICBF22-541

SEROTONERGIC SYSTEM DYNAMICS IN THE EMBRYONIC DEVELOPMENT OF GULF TOADFISH, OPSANUS BETA

Anastasiya Plotnikova* [1], Danielle McDonald[1]

[1]University of Miami, Miami, FL, United States

Abstract:
Serotonin (5-HT) is a highly conserved morphogen involved in vertebrate embryonic development. The serotonergic system, including the 5-HT transporter and 5-HT2A receptor, is present in developing mouse embryos, and the 8-day post-coitum mouse myocardium is capable of 5-HT uptake. In the developing zebrafish, disruption of 5-HT transporter dynamics via the selective serotonin reuptake inhibitor, fluoxetine, induces significant mortality, suggesting a role for 5-HT and the 5-HT transporter in developing fish. However, changes in mRNA expression of the 5-HT transporter and the 5-HT2A receptor within developing fish has never been investigated. The objective of our study is to determine how 5-HT transporter and 5-HT2A receptor mRNA expression changes to maintain adequate 5-HT levels throughout embryonic development of Gulf toadfish, Opsanus beta. It is hypothesized that 5-HT transporter and 5-HT2A receptor mRNA expression levels are related and will peak at organogenesis in developing Gulf toadfish. Toadfish will be sexed by ultrasound and placed into breeding pairs, within aquaria. Developing embryo (1-9 days post fertilization (dpf)) and larvae (10-19 dpf) 5-HT transporter and 5-HT2A receptor mRNA expression will be analyzed via quantitative real-time PCR (qPCR) and 5-HT immunoreactivity of paraffin-embedded embryos and larvae will be analyzed. Although mammalian mouse studies have established a role for the serotoninergic system in embryonic development and stable amounts of 5-HT in the zebrafish embryo are critical for proper development, few studies have addressed the role of 5-HT, the 5-HT transporter, and the 5-HT2A receptor in teleost organogenesis.

06-Developmental physiology of fishes

ABSTRACT N° ICBF22-186

COMPLEMENTARY CONTRIBUTIONS OF FLI1A AND THE HOX13 PARALOGOUS GROUP TO ZEBRAFISH PECTORAL FIN DEVELOPMENT

Mustafa Hamid[1], Robert L. Lalonde[2], Marie-Andrée Akimenko[* 1]

[1]Department of Biology, University of Ottawa, Ottawa, Canada,
[2]University of Colorado, Aurora, United States

Abstract:
The zebrafish embryonic pectoral fin is comprised of the endoskeletal disc (ED) and the pectoral fin fold (PFF). The ED made of chondrocytes gives rise to the endochondral skeleton, consisting of proximal and distal radials at the base of the adult fin, while the rays composing the fin exoskeleton are forming within the PFF. Pectoral fin development is dependent on the coordinated activity of various transcription factors including the Hox13 paralogous group (Hoxa13a, Hoxa13b, Hoxd13a) which are critical for appendicular patterning. The function of another transcription factor, Fli1a, in blood vessels is well characterized but its role in the ED remains elusive. Using a transgenic approach, we observed a complementary pattern of expression between fli1a and hox13 that persists as fin develops, suggesting a regulatory relationship between these genes that correlates with distinct cell behaviours. To further examine this regulation, fli1a was ectopically expressed in the hox13 domain. The mis-expression of fli1a was associated with an increase in hox13 expression and long-lasting aberrations in the migration of hox13-expressing cells, leading to extra bone nodules and ray defects in the adult fin. In support of the regulatory relationship between fli1a and hox13, studies in Ewing sarcoma, an aggressive bone and soft tissue cancer, report that FLI1 dysregulation is associated with an increase in HOXA13 and HOXD13 expression. Taken together, our results support a coordinated participation of fli1a and hox13 during pectoral fin development and provide insights into fin fold cell migration, distal radial formation, fin ray morphogenesis, and blood vessel remodeling.

ABSTRACT N° ICBF22-420

THE PHYSIOLOGICAL SIGNIFICANCE OF VENTILATORY RESPONSES TO HYPOXIA IN DEVELOPING ZEBRAFISH

Kathleen Gilmour* [1], Milica Mandic[1], Kevin Pan[1], Kaitlyn Flear[1], Steve Perry[1]

[1]University of Ottawa, Ottawa, Canada

Abstract:
The hypoxic ventilatory response (HVR) in fish is initiated during hypoxia to increase ventilation volume, which helps to maintain arterial PO2 as water PO2 declines. Aquatic surface respiration (ASR) is an alternative ventilatory response to hypoxia in which fish skim the better-oxygenated layer of water at the water-air interface. Zebrafish (Danio rerio) hyperventilate in hypoxia even during early development when the gills are undeveloped and gas exchange occurs primarily across the skin. Developing zebrafish engage in ASR from about 5 days post-fertilization (dpf). The physiological benefits of hyperventilation and ASR during hypoxia in larvae remain unclear. In a series of studies, we evaluated the relationship between the HVR and critical O2 tension (Pcrit) to probe the significance of the HVR in larvae between 4 and 15 dpf. We also assessed the use of ASR and whether use of ASR improved survival in larvae exposed to intermittent hypoxia. The relationship between peak HVR and Pcrit was dependent on developmental stage, with peak HVR occurring at a water PO2 higher than Pcrit in early stage larvae, suggesting that the energetic cost of sustaining the HVR may outweigh its benefit. Access to the water's surface significantly improved survival in larvae that were old enough to engage in ASR. However, larvae spent significantly less time in ASR during hypoxia than did adult fish, suggesting that ASR is energetically more costly in larvae than in adults. Collectively, however, our data point to a physiological benefit of HVR and ASR in developing zebrafish.

ABSTRACT N° ICBF22-308

EXPOSURE—MEDIATED ANTHROPOGENIC IMPACTS ON CARDIORESPIRATORY RESPONSES IN DEVELOPING RAINBOW TROUT (ONCORHYNCHUS MYKISS)

Erik Folkerts* [1, 2], Daniel Alessi[1], Greg Goss[1]

[1]University of Alberta, Edmonton, Canada,
[2]University of Miami, Miami, United States

Abstract:
The development of oil and gas resources is a continually growing aspect of many global energy sectors. However, environmental impacts related to increased hydraulic fracturing activities in the oil and gas sector remains relatively understudied. In an effort to better characterize cardiorespiratory toxicological implications associated with fracturing wastewater exposure (termed flowback and produced water; FPW), embryonic cardiorespiratory responses were investigated in developing rainbow trout (Oncorhynchus mykiss). Specifically, the implications of FPW dilution, length of exposure (acute versus chronic), and developmental state on embryonic and larval trout responses were examined. Concerning acute, 48 hr exposures, 5% FPW dilutions significantly increased rates of cardiac morphological deformities regardless of developmental state. However, exposures to 2.5% FPW dilutions at the earliest developmental state tested additionally induced cardiac deformities and altered embryonic respiration/metabolic rates. Generally, it was also observed that earlier developmental exposures produced larger changes in gene expression of key cardiac-related genes, while later developmental exposures produced more varied and less numerous alterations in gene expression profiles studied. Chronic (28-day) exposures to FPW at lower concentrations produced similar cardiorespiratory detriments to those observed in acutely exposed organisms at earlier developmental time points, although with often lower magnitude responses. Our results suggest that earlier periods within the cardiac developmental window are more sensitive and induce greater toxicological responses in trout when exposed to FPW. Furthermore, we confirm the cardiorespiratory system as a target for FPW toxicity. Such information may be used to help guide risk assessment protocols for FPW-related activities.

ABSTRACT N° ICBF22-200

RAPID EMBRYONIC DEVELOPMENT SUPPORTS THE EARLY ONSET OF GILL FUNCTIONS IN TWO CORAL REEF DAMSELFISHES

Jodie Rummer* [1, 2], Leteisha Prescott[1, 3], Amy Regish[4, 5], Shannon McMahon[1], Stephen McCormick[4, 5]

[1]James Cook University,
[2]ARC Centre of Excellence for Coral Reef Studies, Townsville, Australia,
[3]Cawthron Institute, Nelson, New Zealand,
[4]U.S. Geological Survey, Turner Falls,
[5]University of Massachusetts, Amherst, United States

Abstract:
The gill is one of the most important organs for growth and survival of fishes. Early life stages in coral reef fishes often exhibit extreme physiological and demographic characteristics that are linked to well-established respiratory and ionoregulatory processes. However, gill development and function in coral reef fishes is not well understood. Therefore, we investigated gill morphology, oxygen uptake and ionoregulatory systems throughout embryogenesis in two coral reef damselfishes, Acanthochromis polyacanthus and Amphiprion melanopus (Pomacentridae). In both species, we found key gill structures to develop rapidly early in the embryonic phase. Ionoregulatory cells appear on gill filaments 3–4 days post-fertilization and increase in density, whilst disappearing or shrinking in cutaneous locations. Primary respiratory tissue (lamellae) appears 5–7 days post fertilization, coinciding with a peak in oxygen uptake rates of the developing embryos. Oxygen uptake was unaffected by phenylhydrazine across all ages (pre-hatching), indicating that haemoglobin is not yet required for oxygen uptake. This suggests that gills have limited contribution to respiratory functions during embryonic development, at least until hatching. Rapid gill development in damselfishes, when compared with that in most previously investigated fishes, may reflect preparations for a high performance, challenging lifestyle on tropical reefs, but may also make reef fishes more vulnerable to anthropogenic stressors.

06-Developmental physiology of fishes

ABSTRACT N° ICBF22-210

DEVELOPMENT OF OXYGEN SENSING

Michael Jonz* [1]

[1]University of Ottawa, Ottawa, Canada

Abstract:
The ontogenesis of oxygen sensing in vertebrates is generally an unsolved mystery. Even in mammalian models, where much research has been focused for decades, the site of specialized oxygen chemoreceptors, which detect hypoxia and mount autonomic reflexes, changes multiple times during development. In fish, which undergo significant metamorphic changes, the story is just as complicated. Current models suggest that extrabranchial chemoreceptors detect hypoxia during embryonic development before the appearance of gills or a functional circulatory system. In zebrafish, embryos begin to sense hypoxia at 2 days postfertilization (dpf) and swim towards regions of higher oxygen availability as soon as the swim bladder inflates. Oxygen chemoreception transitions to the gills by 7 dpf as they continue to develop. While embryonic chemoreceptors disappear as development proceeds, this may not be the case for amphibious fish. The potential physiological role of specific cell types in oxygen sensing during development is an active area of investigation. Recent evidence is revealing an ever-increasing number of potential sites of chemoreception in fish during development. New advances, such as single-cell RNA sequencing and studies in regeneration, are leading the way to a deeper understanding of the transcriptomic profile of gill chemoreceptors and sensory neurons, and their neurogenesis.

ABSTRACT N° ICBF22-372

PHOTORECEPTION AND TRANSCRIPTOMIC RESPONSE TO LIGHT DURING EARLY DEVELOPMENT OF ATLANTIC SALMON (SALMO SALAR)

Mariann Eilertsen*[1], David Dolan[2], Charlotte Bolton[3], Rita Karlsen[1], Wayne Davies[4,5], Rolf Edvardsen[6], Thomasz Furmanek[6], Harald Sveier[7], Herve Migaud[3], Jon Vidar Helvik[1]

[1]Department of Biological Sciences, University of Bergen,
[2]Department of Informatics, University of Bergen, Bergen, Norway,
[3]Institute of Aquaculture, University of Stirling, Stirling, United Kingdom,
[4]Umeå Centre for Molecular Medicine, University, Umeå, Sweden,
[5]School of Life Sciences, College of Science, Health and Engineering, La Trobe University, Melbourne, Australia,
[6]Institute of Marine Research,
[7]Lerøy Seafood Group ASA, Bergen, Norway

Abstract:
Light cues vary along the axis of periodicity, intensity and spectrum and perception of light is dependent on the photoreceptive capacity encoded within the genome and the opsins expressed. A global approach has been taken to analyze the photoreceptive capacity and the effect of differing light conditions on a developing teleost prior to first feeding. The transcriptomes of embryos and alevins of Atlantic salmon exposed to different light conditions were analyzed, including a developmental series and a circadian profile. The results showed that genes mediating nonvisual photoreception are present prior to hatching at a stage when the retina is still poorly differentiated. These results are supported by a cellular localization of the nonvisual opsins by in situ hybridization. The clock genes were expressed early, but the circadian profile showed that only two clock genes were significantly cycling before first feeding. Not many genes were differentially expressed between day and night within a light condition, however, many genes were significantly different between light conditions, indicating that light environment has an impact on the transcriptome during early development. Comparing the transcriptome data from constant conditions to periodicity of white light or different colors revealed overrepresentation of genes related to photoreception, eye development, muscle contraction, degradation of metabolites and cell cycle among others, and in constant light, several clock genes were upregulated. The study implies a direct influence of light conditions on the transcriptome profile at early developmental stages, by an advanced photoreceptive system where few clock genes are cycling.

06-Developmental physiology of fishes

ABSTRACT N° ICBF22-499

ULTRASTRUCTURAL CHARACTERIZATION OF CHORION MALFORMATIONS IN ATLANTIC SALMON EMBRYOS.

Iván Valdebenito* [1], Elías Figueroa[1], Jorge Farías[2], Jennie Risopatron[2]

[1]Universidad Católica de Temuco,
[2]Universidad de la Frontera, Temuco, Chile

Abstract:
The chorion is the protein and acellular structure that protects the embryo during development in fish. Chorion malformations are one of the most significant problems in salmon farming and studies of their causes are scarce. The objective of this research was to characterize by electron microscopy (SEM and TEM) the chorion malformations recorded in Chilean salmon farming during the 2021 spawning season. Chorion malformations occurred in 12% of the embryos and corresponded to soft chorion (6%), perforated chorion (3.5%) and chorion with discs (2.5%). The last two malformations are associated with the accumulation of sediment on the outer surface of the chorion and infection with bacteria and fungi that penetrate the different protein layers that make up this structure. These microorganisms finally perforate the chorion, producing perforations of different sizes that make it difficult to transport embryos from one fish farm to another.
The discs observed are located on the inner face of the chorion and finally alter the arrangement of the protein fibers that form the chorion, producing perforation of the chorion, regularly generating embryonic abortions as a result of the strangulation of the yolk sac and causing the death of the embryo during handling. or transportation. The causes of these malformations are the low quality of the water (soft and perforated chorion) and the crystallization of minerals present in the calcium-rich waters in which the Atlantic salmon incubation process takes place.
Research financed by FONDECYT project 1211246

ABSTRACT N° ICBF22-104

EVOLUTIONARY NEUROSCIENCE OF SOCIAL BEHAVIOR: CICHLIDS AND BEYOND

Hans Hofmann* [1]

[1]The University of Texas at Austin, Austin, United States

Abstract:
Social behavior varies tremendously across species. Nevertheless, we have a fundamental understanding of how social systems evolved. And, we have some insight into how the brain processes and stores socially salient information, how it generates context-appropriate behavior, and how behavior and its neural substrates develop during ontogeny. We can now begin to integrate these seemingly disparate approaches to unravel the causes and consequences of variation in brain and behavior in diverse species; and reconstruct the evolution of the neuromolecular mechanisms that regulate and generate complex behavior. These studies demonstrate remarkably conserved roles of hormonal and neuromodulatory systems in the regulation of social behavior, even in cases of social systems that evolved convergently in distantly related taxa. Recent genome-scale studies provide support for the intriguing hypothesis that similar gene sets underlie independent evolutionary transitions to similar social phenotypes. In addition, neural circuits such as the vertebrate Social Decision- Making Network are highly conserved, suggesting that much of the behavioral diversity in nature reflects variations of an evolutionarily ancient theme. This may not be surprising, as the most recent common ancestor of all animals already had to meet challenges imposed by fluctuating internal states and external environments (finding mates, defending resources, avoiding predators, etc.). The mechanisms used by these ancestral organisms to maintain homeostasis likely served as the building blocks for the evolution of more derived behavior. I will introduce a conceptual framework and present results from experimental and comparative studies that together provide insight into the origins and evolution of complex behavioral and neuromolecular phenotypes.

ABSTRACT N° ICBF22-282

INVESTIGATING THE IMPACT OF ANTHROPOGENIC CHANGES BY NEUROENDOCRINE INDICTORS OF ALLOSTATIC LOAD IN FISH

Erik Höglund* [1]

[1]Norwegian Institute for Water Research, Oslo, Norway

Abstract:
The brain's serotonergic system a play a key role in the integration of behavioral and physiological stress responses in vertebrates and as such is a central mediator of allostatic processes. Specifically, the serotonergic system modulates the release of cortisol, the major stress coping hormone in fish, by interacting with the hypothalamic–pituitary–interrenal (HPI) axis. Accordingly, changes in central serotonergic neurochemistry and the responsiveness of the HPI axis have been associated with prolonged/chronic stress and allostatic load. Still, central allostatic neural regulation is seldom included in studies investigating the effects of environmental contaminants in fish. In this talk, results from a series of lab studies where serotonergic neurochemistry and HPI reactivity was used to investigate the impact gas supersaturation and acidification, two main human induced environmental changes which potentially threatens a landlocked population of Atlantic salmon (Salmo salar), is presented and discussed.

ABSTRACT N° ICBF22-522

PAIN IS IN THE BRAIN: WHAT WE KNOW ABOUT CENTRAL PROCESSING OF PAIN IN FISHES

Lynne Sneddon* [1]

[1]University of Gothenburg, Gothenburg, Sweden

Abstract:
Central nervous system processing of pain is comprehensively studied in mammals yet we know very little with regards fishes. Many areas of the mammalian brain are involved in processing pain but what about fish? By studying fishes we can learn not only about their neurobiology, but also about the evolution and comparative aspects of pain processing as well as decide whether they could be a valid model for biomedical studies. Using a variety of approaches from molecular biology, functional magnetic resonance imaging (fMRI) and in vivo confocal imaging this question of how the fish brain responds to potentially painful stimuli will be discussed. Techniques in molecular biology can yield insights into the underlying mechanism and a number of genes were found to respond to painful treatment in common carp and rainbow trout that are similar to those found in mammalian studies. Further a novel candidate, Vangl2, was explored using in situ hybridisation. In vivo imaging tools are powerful ways in which to understand how the fish brain is responding in real time. Using fMRI in common carp, a number of brain areas in the forebrain and midbrain responses to painful treatment. Real time imaging was also conducted for transgenic larval CaMPARI zebrafish to assess which areas of the brain responded to noxious heat. These approaches can help us understand central pain processing and fill the gaps in our knowledge.

09-Brain and behavior in fish: From evolutionary ecology to biomedicine

ABSTRACT N° ICBF22-170

NEUROMOLECULAR EFFECTS OF ENVIRONMENTAL CHANGE

Celia Schunter* [1]

[1]Swire Institute of Marine Science, The University of Hong Kong, Hong Kong, Hong Kong, China

Abstract:
Anthropogenic activities are causing the global climate to change at an unprecedented rate. A plethora of studies has now shown that such rapid changes in the environment, including thermal anomalies and acidification of aquatic ecosystems, can have severe consequences on the ecology as well as crucial behaviours of aquatic organisms. Over the past years, I have been investigating the impacts of environmental change on marine fishes in particular on the brain, through experiments, unique field collections, molecular work, and computational analyses to understand the neuromolecular responses. Through transgenerational exposure to elevated CO2, we deciphered short-term, developmental effects as well as the influence of parental effects on the brain. Furthermore, collections in the wild at CO2 seeps allowed us to understand the common and variable responses among species and revealed different levels of plasticity and adaptive potentials owing to evolutionary rates. Lastly, we find that climate change-stressors also alter crucial cooperative behaviors in fish, such as cleaning interactions, and we exhibit the changes to underlying molecular mechanisms in different brain regions revealing a major influence on mutualism maintenance with potential large-scale effects on the coral reef ecosystems.

09-Brain and behavior in fish: From evolutionary ecology to biomedicine

ABSTRACT N° ICBF22-202

DOPAMINE AND REWARD BEHAVIOR IN FISH

Marco Vindas*[1], Petronella Kettunen[2], Lars Ebbesson[3]

[1]Norwegian University of Life Sciences, Ås, Norway,
[2]University of Gothenburg, Gothenburg, Sweden,
[3]NORCE Norwegian Research Center , Bergen, Norway

Abstract:
In mammals, reward conditioning and processing has been linked to activation of dopamine (DA) neurons in the mesocorticolimbic system in the mid brain, particularly the ventral tegmental area. In fish, however, the mesocorticolimbic system is yet to be elucidated. Notably, DA neurons located in other brain areas may have similar functions. To explore this possibility, we conducted a series of experiments in order to identify the activity of DA neurons during reward learning. Zebrafish were sequentially sampled during reward conditioning (a light was used to signal a food reward) after 1, 3, 6 and 9 trials to investigate DA neuronal activity in the fore and midbrain by means of cfos and tyrosine hydroxylase co-expression. The main DA associated neuronal populations activated during reward were found in the forebrain Vd and the Vv, and the midbrain TPp areas. RNA sequencing analysis showed upregulation of neuronal plasticity genes and downregulation of inhibitory genes preconditioning, and upregulation of energy balance genes postconditioning, in the Vv. In addition, fish learned quicker after being treated with a DA 1 receptor antagonist which increased overall DA concentrations in the forebrain. Further analysis of signaling molecule gene expression pre and postconditioning in the Vd, Vv and TPp is still pending. Our result so far, provide evidence of forebrain subpallial areas (particularly the Vv) being important in fish during reward conditioning.

ABSTRACT N° ICBF22-162

TOPOLOGY OF THE CENTRAL MELANOCORTIN NEUROPEPTIDES IN THE ATLANTIC SALMON PARR BRAIN

Sissel Norland* [1], Ivar Rønnestad[1], Mariann Eilertsen[1], Jon Vidar Helvik[1], Ana S. Gomes[1]

[1]Department of Biological Sciences, University of Bergen, Bergen, Norway

Abstract:
The melanocortin system is a key regulator of appetite and food intake in vertebrates. This network includes the central melanocortin neuropeptides neuropeptide y (npy), agouti-related peptide (agrp), cocaine- and amphetamine-related transcript (cart), and pro-opiomelanocortin (pomc). In mammals the hypothalamic arcuate nucleus is considered an important center for appetite control with neurons coexpressing npy/agrp or cart/pomc. Compared with mammals, salmonids typically express multiple paralogs of these genes due to whole-genome duplication events. In this study, we have mapped three npy, two agrp, ten cart, and three pomc paralogues using in situ hybridization and double fluorescent in situ hybridization in the Atlantic salmon (Salmo salar) parr brain. We have found that the mRNA distribution of the melanocortin neuropeptides was to a large extent located in sensory and neuroendocrine brain regions. In the hypothalamic lateral tuberal nucleus, the putative homolog to the mammalian hypothalamic arcuate nucleus, we observed that npya, agrp1, cart2b, and pomca were predominantly localized in distinct neurons, although a few neurons coexpressing npya/agrp1 or cart2b/pomca were observed. This is the first study that shows colocalization of npya/agrp1 and cart2b/pomca in the teleost's hypothalamus, thus, establishing an important advancement in understanding the appetite control system in fish. In addition, our results may indicate that sensory brain regions play a role in the modulation of signals affecting appetite. .

09-Brain and behavior in fish: From evolutionary ecology to biomedicine

ABSTRACT N° ICBF22-181

A COMPARATIVE ANALYSIS OF LOCOMOTION AND SUPRASPINAL MOTOR CIRCUITS IN FISH LARVAE

Michael Orger*[1], Pedro Silva[1], Adrien Jouary[1], Aaron Ostrovsky[1], Ruth Diez del Corral[1], Sabine Renninger[1], Edite Figueiras[1], Alexandre Laborde[1], João Marques[1], Adriana Correia[2]

[1]Champalimaud Foundation,
[2]Instituto Superior Técnico, Lisbon, Portugal

Abstract:
A comparative approach in behavioral neuroscience can help us to distinguish general organizing principles in the brain from specialized adaptations to a particular ecological niche, and to understand how changes in neural circuits shape behavior on an evolutionary timescale. With this in mind, we have studied the locomotor behaviour of the larvae of three closely related species of Danioninae: Danio rerio, Danionella cerebrum and Devario aequipinnatus. Although these species share similar anatomy at the larval stage, they exhibit different locomotor styles in terms of tail oscillation frequency, duration of movements, and interbout intervals. We measured the locomotion of a cohort of larvae from each species in a set of automated assays including phototaxis, optomotor response, visually and acoustically triggered escape, social behaviour and prey capture. Compared to zebrafish, the Devario larvae show more rapid development of behavioral responses, in particular showing clear social attraction in the first week of life. They also show increased maneuverability and performance in the optomotor response and prey capture. We have used dextran dye back-fills of reticulospinal neurons to compare the anatomy of the descending motor system across the three species, which shows a high degree of conservation, although with some small differences in dendritic projection patterns. Using transgenic lines expressing the calcium indicator GCaMP panneurally in all three species, we are investigating differences in functional circuit organization that underlie the observed behavioral differences.

ABSTRACT N° ICBF22-245

FOREBRAIN NEUROCHEMICAL AND NEUROGENESIS EVENTS UNDERLIE SOCIAL DEFICITS IN ADULT ZEBRAFISH

Catherine R Dermon* [1], Panagiotis Perdikaris[1]

[1]University of Patras, Dept of Biology, Patras, Greece

Abstract:
Social deficits are core clinical symptoms of many neuropsychiatric disorders including Autism Spectrum Disorders. Elevated anxiety is a common comorbid psychopathology of individuals with aberrant social behavior, but the neurobiological mechanisms remain unknown. The present study aimed to determine the underlying forebrain functional and structural alterations in a social withdrawal model of zebrafish (Danio rerio). For this, zebrafish were treated sub-chronically with MK-801, a non-competitive antagonist of glutamate N-methyl-D-aspartate receptor.

Following treatment, the social phenotype, anxiety levels and the presence of behavioral stereotypies were evaluated in MK-801 and control zebrafish. The underlying brain neurochemical changes were determined in GABAergic, glutamatergic, and noradrenergic transmission, using western blot, immunohistochemistry (IHC) and immunofluorescence. In addition, structural changes in forebrain cell proliferation zones were studied, by means of bromo-deoxy-uridine (BrdU) IHC.

Behavioral data established that MK-801-treated zebrafish exhibit a social withdrawal phenotype. Neurobiological data revealed the presence of excitation/inhibition imbalance and forebrain neurogenesis defects. Importantly, social deficits were accompanied by increased anxiety levels, possibly associated to the altered beta2-ARs' expression across the Social Decision-Making network nodes. Moreover, co-localization of beta2-ARs with elements of GABAergic and glutamatergic systems, as well as with GAP-43, a protein characterizing a high plasticity potential, suggests a key-role of noradrenergic neurotransmission in the pathophysiological mechanisms linking anxiety with social deficits. Taken all together, our findings support that behavioral and neurobiological features underlying the social withdrawal phenotype in zebrafish are evolutionary conserved.

Partly funded by the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (project code: T1EDK-04290) co-financed by the European Union and Greek National Funds

ABSTRACT N° ICBF22-346

OPTIMIZING ZEBRAFISH WELFARE - EFFECTS OF ENVIRONMENTAL ENRICHMENT ON AGONISTIC BEHAVIOR AND STRESS RESPONSE IN GROUPS OF ZEBRAFISH KEPT IN DIFFERENT DENSITIES.

Oly Sen Sarma* [1], Svante Winberg[1], Erika Roman[2]

[1]Uppsala University,
[2]Swedish University of Agricultural Sciences, Uppsala, Sweden

Abstract:
Despite its popularity in research, there is very little scientifically validated knowledge about the best practices on zebrafish (Danio rerio) husbandry. This has led to several facilities having their own husbandry protocols.
Zebrafish were reared in tanks with or without environmental enrichment at different densities. The welfare was assessed by three main methods. Firstly, the agonistic behavior of the fish was observed manually directly in the housing tanks. Secondly, cortisol(stress hormone) secretion in response to stress was analyzed for each group. Thirdly, cortisol secretion in response to acute stress in individual fish was assessed. This study was performed to expand knowledge on the effects of enrichment and fish density on the welfare of zebrafish, with hopes of providing a scientific basis for future legislation and recommendations. The obtained results showed a significant effect of fish density on the agonistic behavior and stress responses of zebrafish. Low density has a negative effect on zebrafish welfare, causing increased aggression and higher cortisol release. Enrichment had no observable effect on the well-being of the fish.

ABSTRACT N° ICBF22-451

ZEBRAFISH AND MAJOR NEUROBEHAVIORAL DOMAINS: TRANSLATIONAL INSIGHTS FOR CNS DISEASE MODELING

Allan Kalueff* [1, 2], Konstantin Demin[2, 3], Konstantin Zabegalov[1], Murilo de Abreu[4, 5], Fabiano Costa[1, 6], Tatyana Kolesnikova[1]

[1]Sirius University of Science and Technology, Sochi,
[2]St. Petersburg State University,
[3]Almazov Medical Research Center, St. Petersburg,
[4]Moscow Institute of Physics and Technology, Moscow, Russia,
[5]University of Passo Fundo, Passo Fundo,
[6]University of Santa Maria, Santa Maria, Brazil

Abstract:

The zebrafish (Danio rerio) is currently widely used in translational neuroscience and biological psychiatry research. Here, we provide a general outline of zebrafish neurobiology and its major neurobehavioral domains, to emphasize the rapidly expanding applications of zebrafish to model a wide spectrum of brain disorders, including anxiety, post-traumatic stress, schizophrenia, autism and drug abuse. We will further discuss how adult zebrafish-based models of aberrant locomotor activity, emotionality, cognition and sociality can be relevant to a wide range of human psychiatric conditions. In addition to well-recognized zebrafish phenotypes, novel own data will be presented on novel zebrafish models of despair, highly relevant to modeling depression-like phenotypes in this species. Complementing rich behavioral data, zebrafish models also employ a wide range of biological markers of CNS pathology, including stress- or drug-related alterations in neuroplasticity, neuroendocrine (e.g., cortisol) responses, as well as robust changes in gene brain expression of selected brain neurotrophins and cytokines. The involvement of neuroglia, assessed by altered expression of various microglial and astrocytal biomarkers, in zebrafish CNS syndromes will also be discussed. Finally, we will also evaluate the unique role of zebrafish screens in innovative CNS drug discovery, including their applications to testing traditional medicines, as well as high-throughput drug and mutation screening. Recent pilot data from our group on artificial intelligence-driven neurophenotyping of zebrafish will also be discussed in terms of the value of this approach for unbiased dissection of zebrafish behaviors, characterizing novel zebrafish mutant strains, as well as the search for novel CNS drugs.

09-Brain and behavior in fish: From evolutionary ecology to biomedicine

ABSTRACT N° ICBF22-221

AUTS2A PARTICIPATES IN INTERGENERATIONAL REGULATION OF BEHAVIOR IN MEDAKA (ORYZIAS LATIPES)

Antoine CLEMENT* [1], Constance MERDRIGNAC[1], Thaovi NGUYEN[1], Amaury HERPIN[1], Violaine COLSON[1], Julien BOBE[1]

[1]INRAE LPGP, Rennes, France

Abstract:
Maternal stress is suspected to have intergenerational effects on behavior, especially at early stages. In rainbow trout (Oncorhynchus mykiss), a maternal stress led to behavioral changes in offspring and induced a dysregulation of several neurodevelopment genes in unfertilized eggs. Among them, the auts2a gene (activator of transcription and developmental regulator) is involved in several pathologies in humans (AUTS2) including autism spectrum disorder and mental retardation. The aim of the study was to investigate the importance of maternally-contributed (MC) auts2a in behavior and to decipher underlying molecular mechanisms. We generated a mutant auts2a medaka line using CRISPR/Cas9 technology targeting the N-terminal region of the Auts2a protein. To assess maternal auts2a contribution, we used fish with different genotypes. In addition to wild type and homozygous auts2a mutants, we generated heterozygous +/- mutants that received, or not, maternal auts2a contribution.

We observed significant differences in the macroscopic developmental phenotypes, depending on auts2a maternal contribution. Heterozygous fish without MC auts2a exhibited a lower survival rate and smaller head size compared to fish exhibiting the same genotype but had not received MC auts2a. New environment test and habituation tests were used to assess emotional responses. Together, our observations show that MC auts2a triggers differences in fish behavior. The behavioral phenotype of fish lacking MC auts2a suggests a lower anxiety level and lower learning abilities in comparison to fish with MC auts2a. In addition, we have obtained preliminary evidence suggesting that the temporal expression profile of key neurodevelopment genes is impaired in fish lacking MC auts2a.

ABSTRACT N° ICBF22-214

ARGININE VASOPRESSIN MODULATES ION BALANCE AND SOCIAL BEHAVIOR DURING HYPO-OSMO ADAPTATION IN ZEBRAFISH

Chih-Wei Fu* [1], Sok-Keng Tong[1], Ming-Yi Chou[1]

[1]National Taiwan University, Taipei, Taiwan

Abstract:
Arginine vasopressin (AVP) is a conserved and osmo-regulatory hormone across vertebrates. Besides, AVP is a neurotransmitter that released into many regains of brain and regulates social and aggressive behavior. However, the effects of AVP signaling on osmo-regulation and central nervous system during osmo-adaption remain largely unclear. In this study, we used zebrafish as animal model to investigate the mechanism of AVP induced ion-regulation and behavior change after hypo-osmo stimulus. We found de-ionic water treatment increased AVP mRNA expression in zebrafish larvae. Whole body Cl- content and H+ secretion was decreased in AVP knockdown embryos. AVP MO treatment also decreased the number of foxi3a and p63-expressing cells, and decreased NCC and HR type ionocyte number. Furthermore, AVP MO downregulated calcitonin gene-related peptide (cgrp) and calcitonin receptor-like 1 (crlr1) mRNA expression. To better evaluate the effects on central nervous system, adult zebrafish were conduct series behavior test after de-ionic water treatment. Zebrafish exhibited higher level social preference after de-ionic water incubation. The biting number was decreased in mirror biting test. Our results suggested AVP might modulate ionocyte differentiation and proliferation, and affect Cgrp and Crlr1 for Cl- homeostasis. Moreover, AVP transmission after osmo-stimulation might modulate aggressive behavior and social behavior. We reveal the central and peripheral effects of AVP, providing new and comprehensive insight to the response after osmo-stress.

ABSTRACT N° ICBF22-151

PHARMACOLOGICAL REDUCTION IN SEROTONERGIC SIGNALLING MIMICS PARASITE-INDUCED BEHAVIOURAL MANIPULATION OF CALIFORNIA KILLIFISH

Ida B Johansen* [1], Lauren E. Nadler[2], Marco A. Vindas[1], Andrew Turner[3], Rebecca Hernandez[3], Ryan Hechinger[3], Øyvind Øverli[1]

[1]Norwegian University of Life Sciences, Ås, Norway,
[2]Nova Southeastern University, Fort Lauderdale,
[3]University of California, San Diego, San Diego, United States

Abstract:
Some parasites change their host's behaviour to aid transmission to the next host in their life cycle. However, mechanisms driving host modification in parasite-host systems remains poorly understood. Using the California killifish (Fundulus parvipinnis) and its brain-infecting trematode parasite Euhaplorchis californiensis (Euha) as a model system, we investigated the role of serotonin (5-HT) in parasite manipulation of vertebrate hosts. Euha-infected killifish exhibit up to four times greater conspicuous behaviours and are 10 times more likely to be eaten than uninfected conspecifics. The central monoamine neurotransmitter 5-HT is known for its role in regulating vertebrate behaviour and 5-HT activity was previously found to be reduced in an infection intensity-dependent manner in Euha-infected killifish. Here, we hypothesized that behavioural manipulation caused by E. californiensis is mediated through decreased serotonergic activity. To test this hypothesis, we compared routine and exploratory behaviour in groups of uninfected and experimentally infected killifish with pharmacologically manipulated serotonergic activity (sea water sham, 5-HT receptor agonist, 5-HT receptor antagonist). The results showed that parasite infection increases the frequency of conspicuous behaviours, but reduces activity and exploration. Intriguingly, the data indicates that pharmacologically reducing 5-HT activity mimics the effects of infection in uninfected killifish. These data point to disruption of serotonergic signalling as a proximate mechanism driving E. californiensis behavioural manipulation of killifish.

ABSTRACT N° ICBF22-271

GREAT LAKES BURBOT LIFE HISTORY VARIATION

Jill Leonard* [1], Thornton Ritz[2], Michael Woodworth[1], Andrew Shapiro[1]

[1]Northern Michigan University, Marquette, MI,
[2]State University of New York ESF, Clayton, NY, United States

Abstract:
Burbot (Lota lota) are native to all the Laurentian Great Lakes and are still extant in these systems. They typically occupy high trophic levels and are associated with deep water. In Lake Superior, they are recreationally fished during the winter, typically at night, but there is little management of the harvest. They have also been the traditional target of river netting, which suggested life history variation in the winter spawning species. Our recent work has focused on characterizing burbot spawning and early life stages in the southern Lake Superior region. Adults show at least two major reproductive life history strategies: lacustrine and adfluvial spawning, with fluvial residency also likely. These strategies not only differ in selection of spawning areas, but also in reproductive timing. We have shown that larval morphology varies between Great Lakes populations and also differs from western U.S. populations. We also show substantial hatching asynchrony within families that is likely ecologically relevant to the planktonic embryos and larvae. While many questions remain, our initial results suggest that burbot display substantial life history variation that has ecological relevance and may be important for future management of the increasingly sought-after fish.

ABSTRACT N° ICBF22-115

JUVENILE BURBOT (LOTA LOTA) IN THE "SPOTLIGHTS"

Johan Auwerx* [1], Inne Vught[1], Daniel De Charleroy[1]

[1]RESEARCH INSTITUTE NATURE AND FOREST, Brussels, Belgium

Abstract:
In 2005, a reintroduction programme for burbot was launched in Flanders (Belgium) to establish new populations. For several years, reared juveniles were released in selected waters and the populations were subsequently monitored.
Reintroductions can only be considered fully successful if a sustainable population is created, with successful reproduction and a sufficient number of offspring growing into sexually mature animals. In order to evaluate this reintroduction, new insights and methods have been tested in recent years to search for evidence of natural reproduction.
Since burbot has a hidden lifestyle and is nocturnal, it is difficult to catch by traditional fishing gear e.g. electrofishing. The variability in growth, which can be observed in the first summer, also complicates the interpretation of age. We therefore decided to search for larvae as a clear indication of a successful reintroduction.
Visual inspection of the riparian zone to find newly hatched larvae can be carried out, provided the necessary experience is available. In order to be independent of weather conditions, light traps can be used on stagnant, clear water bodies such as gravel pits, depressions in flood plains, lakes, etc. These light traps are very attractive to young burbot because they are positively phototactic and swim in the direction of a light source. In Western Europe, the traps can be used from the beginning of March till the end of April and significantly increase the chance of observations in locations with a low density of burbot larvae.

ABSTRACT N° ICBF22-251

THE ROAD TO COMMERCIAL BURBOT AQUACULTURE

Joachim Claeye* [1], Wouter Meeus[1], Thomas Abeel[1], Jurgen Adriaen[1], Stef Aerts[1]

[1]Aqua-ERF, Odisee University of Applied Sciences, Sint-Niklaas, Belgium

Abstract:
The European inland aquaculture production has been stagnating and even declining for some species. One of the
possible strategies to enhance inland production is species diversification. The idea is to aim for high-value species,
which allow for bigger investments needed for commercially profitable production in Recirculating Aquaculture Systems
(RAS). We will present the different steps to be taken to establish a new species in aquaculture, using as a case study
our over a decade-long work on burbot.

Because of its potential fast growth rate and high market value, the European burbot (Lota lota lota) was selected in 2009
by AQUA-ERF (Odisee University of Applied Sciences) to investigate its aquaculture potential as a commercial food fish.
Several tasting panels confirmed the high consumer acceptance and willingness to purchase.

At that point, little to no knowledge was available on the culture of this species, and nothing at all on its culture in RAS.
Since 2009 our scientists investigated in several projects many aspects of the culture of burbot. These aspects ranged
from grow-out trials to determine the growth potential, optimal temperature for growth and stocking densities, benchmark
commercial feeds. Subsequently, larval culture techniques were developed with as result the first-ever milestone of
weaning being reported. Other milestones are the artificial reproduction of RAS born broodstock and out-of-season
reproduction. Having solved all these practical bottlenecks, large-scale commercial production in RAS both for human
consumption as restocking and stock enhancement is now within reach!

Since 2015 commercial grow-out of the species in Belgium has been slowly undertaken both in flow-through and RAS,
followed by the start-up of a commercial hatchery operating in RAS which has been founded from our lab and started
operations in 2017.

ABSTRACT N° ICBF22-135

HISTORY OF BURBOT AQUACULTURE IN THE US: MOVING FROM A CONSERVATION FOCUS TO A POTENTIAL HIGH VALUE COMMERCIAL SPECIES!

Kenneth Cain* [1]

[1]University of Idaho, Moscow, United States

Abstract:
Burbot, the only freshwater members of the cod family, inhabit northern latitudes across the globe. Burbot aquaculture was initiated by the University of Idaho (UI) as part of a recovery program for the Kootenai river, where populations were critically low. Through partnerships with the Kootenai Tribe of Idaho (KTOI), and numerous state, federal, and international agency partners, the UI developed aquaculture methodology that led to successful stocking of juvenile burbot into the Kootenai River from 2009 to 2014. The KTOI now leads this recovery program, which has resulted in dramatic increases in the wild burbot population in this river. In fact, success of this program prompted the Idaho Department of Fish and Game (IDFG) to open a burbot sport fishery for the first time in 27 years. The UI has now shifted focus from the conservation program to feasibility testing to assess burbot as a new commercial (foodfish) species for aquaculture operations in the US. All aspects of burbot aquaculture are being optimized at the UI and have included broodstock management, egg incubation, larval rearing/weaning, juvenile and sub-adult growth and diet preference, and potential marketability. Burbot represent an appealing new coldwater aquaculture species and research highlighting the recent history of conservation and commercial culture efforts will be presented.

ABSTRACT N° ICBF22-159

THE EFFECTS OF ACUTE AND CHRONIC EXPOSURE OF AMMONIA ON JUVENILE BURBOT GROWTH AND SURVIVAL

Christopher Myrick* [1], Benjamin Vaage[1]

[1]Colorado State University, Fort Collins, United States

Abstract:
Burbot, Lota lota are a candidate species for commercial aquaculture because of their palatability and optimal growth at temperatures similar to those used in freshwater trout aquaculture. However, data on burbot environmental tolerances and requirements are sparse, especially with reference to water quality parameters relevant to aquaculture, such as un-ionized ammonia (UIA) concentration. We used a two-phased approach to evaluate the effects of un-ionized ammonia on the growth and survival of burbot. First, we measured the acute toxicity of ammonia to juvenile burbot (mean SL: 144 ± 6 mm; mean wet weight: 27.3 ± 3.4 g) and calculated a 96-hr LC50 of 0.58 mg/L UIA. We then measured the 60-d growth, food consumption rate, and performance of burbot (mean initial SL: 190 ± 6.9 mm; mean initial weight: 67.0 ± 4.5 g) reared in 0.00, 0.03, 0.06, 0.12, or 0.19 mg/L UIA using a 20-tank flow-through system under optimal temperature (14.7°C) and dissolved oxygen (DO > 80% saturation) conditions. Elevated ammonia concentration significantly reduced daily food consumption and subsequent growth. Fish exposed to 0.03 and 0.06 mg/L UIA showed temporal acclimation to UIA, achieving food consumption and growth rates on par with control fish after 30 days of exposure. The estimated effective UIA concentrations for 10 and 20 percent reductions in growth based on our data are: EC10 = 0.03 ± 0.006 mg/L and EC20 = 0.050 ± 0.004 mg/L. The authors recommend rearing burbot at densities that maintain UIA levels ≤ 0.03 mg/L.

ABSTRACT N° ICBF22-509

THE STRESS AXIS AND RESPONSE TO ACUTE STRESSORS IN NOTOTHENIA ROSSII ACCLIMATED AT DIFFERENT TEMPERATURES

Pedro M. Guerreiro* [1], Sandra Silva[1], Bruno Louro[1], Alexandra Alves[1], Elsa Couto[1], Adelino Canario[1]

[1]CCMAR- Centre of Marine Sciences, University of Algarve, Faro, Portugal

Abstract:
Antarctic fish evolved in a stenothermal environment, subject to very small fluctuations in temperature throughout their life (–1°C - 2°C). Their ability to respond to increased temperature is uncertain. We aimed at evaluating the mechanism and capabilities of the HPI axis in Antarctic fish in three sets of experiments.

Four groups were placed at 2C. Upon a standard stress test (SST: chasing+netting +1min air exposure) fish were returned to tank and sampled after 1,4,24h. Six groups were acclimated to 2,5,8C for 10-days. At this point the control group of each temperature was sacrificed. The other group received SST and sacrificed 90-min after. Plasma and tissue samples were collected for cortisol and stress-related genes and the interrenal used in-vitro to determine sensitivity to ACTH. Eight groups at 2C were injected with drugs involved in blockage or stimulation of cortisol release/action (saline, cortisol, dexamethasone, metyrapone, spironolactone, mifepristone) and then kept at control or transferred to 6C and sampled after 36 hours.

After SST cortisol peaks between 1-4 hours and reduces to basal between 24-48 hours. Temperature influenced the cortisol response to SST. At higher temperatures cortisol levels in non-stressed group are as high as in fish subjected to SST. Interrenal sensitivity at high temperature showed little response to ACTH, suggesting low sensitivity and/or exhaustion. Manipulation of the HPI-axis showed these fish to respond in a way similar to what has been reported in other fish families in temperate or tropical environments.

Supported by FCT through Propolar and grants PTDC/BIAANM/3484/2014 and UIDB/04326/2020.

ABSTRACT N° ICBF22-439

HYPOXIA AND THERMAL EFFECT ON OSMOREGULATION OF ANTARCTIC FISH HARPAGIFER ANTARCTICUS

Luis Vargas-Chacoff*[1, 2], Kurt Paschke[2, 3], Juan P. Pontigo[4], Ricardo Oyarzún-Salazar[1, 2], Pedro M. Guerreiro[5], Jorge M. Navarro[1, 6]

[1]Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile,
[2]Fondap Center for High Latitude Research (IDEAL), and Millennium Institute Biodiversity of Antarctic and Subantarctic Ecosystems, BASE, University Austral of Chile, Valdivia,
[3]Instituto de Acuicultura, Universidad Austral de Chile,
[4]Laboratorio de Biotecnología Aplicada, Facultad de Medicina Veterianaria, Universidad San Sebastián, Puerto Montt, Chile,
[5]Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Faro, Portugal,
[6]Fondap Center for High Latitude Research (IDEAL), University Austral of Chile, Valdivia, Chile

Abstract:
Climate change is modifying Antarctic waters and teleosts therein are very sensitive to temperature. The increase in temperature changes the oxygen concentration, enhancing the probability of fish facing hypoxia events. In addition to energy yielding processes, such changes are affecting another important physiological process for teleost fish, since "osmoregulation" is greatly affected. The objective of this study was to evaluate the changes in the osmotic response in relation to hypoxia and temperature in the Antarctic Notothenioid Harpagifer antarcticus. Adults of H. antarcticus (n = 60) were captured in the lowest intertidal zone of King George Island, Antarctica. We performed an experiment decreasing the concentration of oxygen (100 normoxia, 60, 40, 30, 20 and 10% oxygen) at 2 temperatures (control of 2 ° C and 5 ° C). In normoxia (100%), the weight-specific oxygen consumption rate (QO2) increased 1.3 times with the temperature rise from 2 to 5 ° C. A 40% decrease in oxygen causes a 42% reduction in QO2; while at 2 ° C the same decrease in oxygen causes only a 9% decrease in QO2. At 40 and 30% O2 saturation, QO2 decreases in a similar way at both temperatures. Between 20 and 10% oxygen, all the fish died, at both temperatures. The osmotic parameters were modified by temperature and hypoxia. This study provides relevant information to understand how climate change is affecting the osmotic response and energy metabolism of this Antarctic fish. This work was funded by Fondap-Ideal Grant Nº15150003, ANID-Millennium Science Initiative Program-Center code “ICN2021_002”.

07-Biology and Ecology of Antarctic Fishes

ABSTRACT N° ICBF22-447

AN INTEGRATIVE FRAMEWORK FOR UNDERSTANDING THE RESILIENCE OF ANTARCTIC FISHES TO CLIMATE CHANGE

Anne Todgham*[1], Amanda Frazier[1], Andrew Naslund[1], Kenneth Zillig[1], Milica Mandic[1]

[1]University of California Davis, Davis, United States

Abstract:
Although the Southern Ocean is experiencing some of the fastest rates of ocean change, few studies have explored how Antarctic fishes may be affected by co-occurring changes in environmental factors, such as warming (OW) and acidification (OA). Organisms within these oceans may be some of the most vulnerable to environmental change, having evolved under stable conditions for millions of years. Early life stages are of particular concern as they are thought to be more sensitive to changes in climate-related variables than adults; however, there is limited research to date on the sensitivity of early life stages of Antarctic fishes to environmental change. Research in our lab group is aimed at understanding the metabolic capacity of embryos, larvae and juvenile Antarctic fishes to acclimate to environmental change projected over the next 80 years. Taking an integrative and comparative approach across ontogeny, our research is focused on characterizing the physiological plasticity of early life stages of Antarctic fishes to increases in temperature as well as understanding how co-occurring stressors can interact synergistically to impact performance during early development. Our results provide evidence of stressor-induced energetic trade-offs in physiology and behaviour, with insights on mechanisms from cellular energy metabolism, which may identify physiological weak links leading to vulnerability of Antarctic fishes to future ocean change.

ABSTRACT N° ICBF22-275

EVOLUTION OF MOLECULAR CHAPERONE REGULATION IN ANTARCTIC FISHES

Sean Place* [1]

[1]Sonoma State University, Rohnert Park, United States

Abstract:
Antarctic fishes of the notothenioidei suborder have lost their ability to rapidly upregulate molecular chaperones in response to stress. Rather, these proteins are constitutively expressed even under normal environmental conditions. We endeavored to identify alterations in the genomes of notothenioid fishes that may underlie this loss of regulatory control. To this end, we performed a genome-wide scan to screen for HSF1-binding sites, a master trans-acting regulator of chaperone expression, to look for modifications in promoter regions that could have impacted inducibility of these genes. Furthermore, we determined copy number of the major HSP gene families in two notothenioid fishes to probe for evidence of the potential for neofunctionalization of inducible HSP genes via duplication events. Lastly, we performed homology and structural-based comparisons of the transcription factor HSF1 and the transcriptional inhibitor, NELF-E, across notothens varying in their induction of the HSR. Broadly, our results reveal a dynamic evolution of cis- and trans-regulatory elements throughout notothens' adaptation to the Southern Ocean. In particular we found evidence of significant turnover in molecular chaperone promoter regions of Notothenioidei compared to other perciform fishes, along with evidence of relaxed purifying selection on ancestrally conserved cis-regulatory elements, which may have been supported by gene duplication events in the genome of at least some Antarctic notothens. Lastly, we also identified putative functional modifications in regulatory elements that offer strong candidates for future in vivo functional analyses. These findings demonstrate that strong selective forces have acted upon regulatory elements of chaperone genes among Antarctic notothens.

ABSTRACT N° ICBF22-422

THERMAL SENSITIVITY AND ACCLIMATION CAPACITY OF NOTOTHENIOID MITOCHONDRIAL METABOLISM

Felix Mark* [1]

[1]Alfred-Wegener-Institute Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany

Abstract:
Antarctic fish of the suborder Notothenioidei display remarkable metabolic adaptations to life in the Southern Ocean. These comprise very low, energy saving metabolic rates, higher mitochondrial densities, cold adapted enzymes, anti-freeze proteins and last but not least, the loss of hemoglobin in the icefishes (family Channichthyidae). Yet, those adaptations come at a premium and render Antarctic notothenioids especially vulnerable to a warming ocean. As the central players in aerobic energy metabolism, mitochondria and their acclimatory plasticity play an important role in buffering the effects of climate change and much depends on their thermal stability and acclimatory capacity.
I will present an overview of my studies on notothenioid mitochondria under acute and chronic thermal exposure and explore the differences in thermal reaction norms, leak rates and thermal stability of the individual respiratory complexes in Antarctic nototheniids (N. rossii, N. coriiceps), trematomids (T. eulepidotus, T. loennbergii) and channichthyids (C. hamatus, C. wilsoni) and contrast them to Austral nototheniids (N. angustata) as well as Arctic and temperate gadoids.

ABSTRACT N° ICBF22-161

ACUTE WARMING INDUCED CHANGES OF PROTEIN SYNTHESIS RATES IN BOREAL AND ANTARCTIC FISH

Nina Krebs* [1], Jan Tebben[1], Felix Mark[1], Magnus Lucassen[1], Gisela Lannig[1], Christian Bock[1], Hans-Otto Pörtner[1]

[1]Alfred-Wegener Institute, Bremerhaven, Germany

Abstract:
Temperature-dependent mechanisms behind growth and respiration are important indicators for understanding the thermal tolerance of marine ectotherms. We investigated the temperature-dependent rate of protein synthesis of the stenothermal Antarctic eelpout Pachycara brachycephalum and compared it to the eurythermal Common eelpout Zoarces viviparus.

We determined the protein synthesis rates (Ks) in vivo in P. brachycephalum and Z. viviparus within their species-specific thermal window. Both species were exposed to acute warming: 0°C acclimated P. brachycephalum to 2°C day-1 until 10°C, 4°C acclimated Z. viviparus to 3°C day-1 until 22°C. Samples of gills and muscle tissue were taken at different time points after injection of labeled phenylalanine (1.5h and 3h) and temperature steps.

Thermal response of protein synthesis rate was species- and tissue-specific. With warming, both species showed an exponential rise in gill protein synthesis rates (P. brachycephalum: Ks = 1.1 – 4.5 % day-1 vs. Z. viviparus: Ks =2.4 – 15.4 % day-1) with similar values at equal temperatures (4°C Ks = 2.5 vs. 2.6; 10°C Ks= 4.5 vs 4.3) reflecting the high oxygen uptake/metabolic rate of gill tissue during acute warming. In contrast, Ks was much lower in white muscle. While Ks was not altered by acute temperature change in P. brachycephalum (0.4 – 0.6 % day-1) it showed a temperature-dependent maximum at 16°C in Z. viviparus (4°C: Ks= 0.1; 16°C Ks= 0.4 % day-1; 22°C Ks= 0.3% day-1), which seems to correspond to maximal whole-animal growth performance of Z. viviparus at 12-14°C. The methodological and ecophysiological implications of these findings will be discussed.

ABSTRACT N° ICBF22-353

THERMAL PLASTICITY OF GROWTH, METABOLIC RATE AND STRESS RESPONSES IN THE COLD WATER, BENTHIC ATLANTIC WOLFFISH (ANARHICHAS LUPUS)

James Hinchcliffe* [1], Jonathan Roques [1], Andreas Ekström[1], Ida Heden[1], Kristina Sundell[1], Henrik Sundh[1], Erik Sandblom[1], Björn Thrandur Björnsson [1], Elisabeth Jönsson [1]

[1]University of Gothenburg , Gothenburg, Sweden

Abstract:

The Atlantic wolffish (Anarhichas lupus), is a stenothermal cold-water teleost that has been identified as a candidate for cold-water aquaculture. Previous studies have shown that growth is compromised when exposed to elevations of temperature above its optimal temperature range. However, the mechanism(s) underlying the impaired growth response remains unknown. Moreover, little is known about its stress response and how this may have implications for future farming practices. The present study addresses whether the reduction in growth can be linked to chronically elevated stress levels, impairment of aerobic scope and/or gut barrier function. To this end, we evaluated the effects of both an acute (24 hours) and chronic (50 days) exposure to 10°C (control) and 15°C on the aerobic metabolic capacity in juvenile wolffish, by assessing standard (SMR) and maximum metabolic (MMR) rates in concordance with growth measurements. We also assessed plasma stress indicators (cortisol, glucose, lactate), plasma hydromineral balance and blood oxygen carrying capacity. For the chronically exposed groups, we compared these parameters with gut barrier function and growth rates. Overall, no difference in plasma cortisol was found, individuals exposed to 15°C had both higher SMR and higher MMR than fish at 10°C, even after a 50-day period. Fish exposed to 15°C showed reduced growth rates and an altered gut barrier function. The results suggest that the increased energy consumption at 15°C is a mechanistic explanation for the decreased growth. The increased metabolic costs at elevated temperatures may leave the species vulnerable with current warming oceans due to climate change.

ABSTRACT N° ICBF22-148

PHYLOGEOGRAPHY, POPULATION GENETICS AND ADAPTATION IN HARPAGIFER (NOTOTHENIIDAE) ALONG THE SOUTHERN OCEAN

Elie Poulin* [1, 2], Nicolás Segovia[1, 3], Claudio Gonzalez-Wevar[1, 4], Valentina Bernal[1, 2], Paul Brickle[5], Javier Naretto[2], Thomas Saucede[6]

[1]Millennium Institute of Antarctic and Subantarctic Biodiversity (BASE),
[2]Universidad de Chile,
[3]Instituto de Ecologia y Biodiversidad, Santiago,
[4]Universidad Australe de Chile, Vladivia, Chile,
[5]South Atlantic Environmental Research Institute, Stanley, Falkland Islands (Islas Malvinas),
[6]Université Bourgogne Franche-Comté, Dijon, France

Abstract:
Within the nototheniid, the dominant group of Antarctic fishes both in biomass and in abundance, the genus Harpagifer represents an interesting biogeographical model. According to its current taxonomy, this genus includes a single Antarctic species, Harpagifer antarcticus, restricted to the west Antarctic Peninsula, and several shallow subantarctic species, found in southern South America, Falkland/Malvinas, South Georgia, Marion, Crozet, Kerguelen and Macquarie islands. Molecular evidence suggests Pleistocene divergence between South America and Antarctica (0.8-1.7 ma), being much more recent than those recorded in benthic marine invertebrates, macroalgae, and other nototheniid fishes, suggesting recent diversification of the genus. In this work, 12000 GBS-SNPs and COI sequences obtained from 300 individuals of seven Harpagifer species in the Southern Ocean were analyzed. Our results suggest the presence of three genetic groups: Antarctica (Antarctica, South Georgia, Signy Island), South America (Patagonia, Falkland/Malvinas Islands), and Subantarctic Islands (Kerguelen, Crozet, Marion). Data associated with partial COI sequences show phylogeographic structuring between biogeographical provinces but low levels of genetic distance between these 3 groups. Using landscape genomics tools, our results support the existence of 3 main genetic units but suggest that patterns of genetic structure are more associated with microevolutionary processes, particularly thermal gradients (i.e. summer temperatures) across the Southern Ocean than with historical divergences. Given the Antarctic origin of Harpagifer, our results suggest that long-distance dispersal and subsequent local adaptation played an important role in the recent Quaternary biogeography of Harpagifer from Antarctic to sub-Antarctic.
ANID postdoc 3190482, MI-BASE ICN2021_002, FONDAP IDEAL 15150003, PROTEKER 1044

ABSTRACT N° ICBF22-281

COLD FISH DON'T MISS A BEAT: INTERNAL HEART RATE BIO–LOGGING OF POLAR COD (BOREOGADUS SAIDA)

Luis Kuchenmueller[1, 2], Sarah Kempf[1], Kurt Gamperl[3], Felix Mark* [1]

[1]Alfred-Wegener-Institut, Helmholtz-Centre for Polar- and Marine Research (AWI), Bremerhaven, Germany,
[2]Deakin University, Geelong, Australia,
[3]Memorial University of Newfoundland, St. John's, Canada

Abstract:

In this study, we adapted Star-Oddi micro-HRT (G2) bio-loggers for improved function in the cold-adapted Arctic key species Polar cod (B. saida) that generally displays a very low heart rate (ʃH) of 8bpm. To integrate ʃH data with oxygen consumption rates (MO2), we conducted critical swim speed (Ucrit) tests in a swim-tunnel respirometer within the ecologically relevant temperature range of 0–8°C.

A significant correlation ($p < 0.01$) of observed cardiorespiratory parameters indicated primary dependency of ƒH and MO2 during acute warming, suggesting a species-specific potential of ƒH as a proxy for energy expenditure. Despite present Ucrit (2.3 ± 0.3 BL/s) being 20% lower than in untagged conspecifics at similar temperature, maximum metabolic rates were 35% higher for bio-logger-bearing individuals. Apparent excess potential to increase MO2 suggests that polar cod's performance limitations are not dictated by the absolute capacity of oxygen supply. Hence, alternative explanations determining Ucrit, such as behavioral termination of swimming trials to save energy, or potential limitations in ATP supply to the muscle, are discussed.

Heart rate was significantly impacted by both temperature and swimming velocity ($p < 0.0001$, respectively). Past the optimal temperature range of polar cod (2.8–4.4°C), heart rate ceased to increase, with incremental Q10 values levelling off from 2.63±0.43 at 0–2°C, to 1.73±0.74 at 6–8°C. Consequently, potential impacts of insufficient heart rate scaling with acute temperature rise are discussed in the light of projected Arctic warming.

ABSTRACT N° ICBF22-219

ATLANTIC COD FACING CLIMATE-DRIVEN WARMING: REPRODUCTIVE PHYSIOLOGY UNDER CHALLENGING ENVIRONMENTAL CONDITIONS

Maud Alix* [1], Lene Kleppe[1], Eva Andersson[1], Birgitta Norberg[2], Ørjan Karlsen[1], Paul Whatmore[1], Jorge MO Fernandes[3], Artem Nedoluzhko[3], Kaja H Skjærven[1], Jon Egil Skjæraasen[1], Olav Sigurd Kjesbu[1]

[1]Institute of Marine Research, Bergen,
[2]Institute of Marine Research - Austevoll Research Station, Storebø,
[3]Faculty of Biosciences and Aquaculture - Nord University, Bodø, Norway

Abstract:
Ambient temperature is a prime regulator of teleost reproductive physiology and is consequently the most studied among climate change-related stressors. The spawning phase seems particularly sensitive to warming due to the narrow thermal tolerance window. The aim of this study was to explore how temperature may impact the reproductive physiology of Atlantic cod, Gadus morhua – a species of great ecological and commercial interest in the North Atlantic – and the subsequent consequences on developmental success in the context of climate-change scenarios.

To address this series of related, fundamental topics, 200 wild-caught, Norwegian coastal cod of different sizes were held in triplicate tanks at optimal (3, 6 °C) and higher-than-optimal (9, 12 °C) temperatures in a recirculating, advanced aquaculture system. From the onset of the reproductive cycle to the end of the spawning season, PIT-tagged fish were tracked monthly to assess body condition, plasma steroid concentrations and oocyte development. During the spawning season, egg batches were collected daily to determine volume, embryonic stage, and egg quality.

Higher-than-optimal temperature accelerated oocyte growth and negatively affected the estradiol-17 plasma concentration. The prevalence of atresia during oogenesis became more noticeable at higher temperatures, whereas there was no sign of increased atresia intensity as such between treatments. Even though individuals were able to spawn at all temperatures, the developmental success was negatively impacted by rising temperatures. Temperature-induced adjustments in the level of mRNAs of maternal origin in cleavage-stage embryos were observed, which may be one of the first physiological signals that control embryo development and provide an adaptation for the next generation to a warmer environment.

ABSTRACT N° ICBF22-284

PARENTAL HYPOTHERMIC EXPERIENCE MODULATES TRANSGENERATIONAL METABOLIC ACCLIMATION IN TROPICAL TILAPIA

Min-Chen Wang* [1], Mao-Ting Hsu[2], Fumiya Furukawa[3], Tzu-Hao Lin[4], Yung-Che Tseng[1]

[1]1. Institute of Cellular and Organism Biology, Academia Sinica, Taipei, Taiwan,
[2]2. Neurobiology Research Unit, Okinawa Institute of Science and Technology Graduate University, Okinawa,
[3]3. School of Marine Biosciences, Kitasato University, Tokyo, Japan,
[4]4. Biodiversity Research Center, Academia Sinica,, Taipei City, Taiwan

Abstract:
Global climate change has resulted in reduced daily temperature variations in the winter of the tropical/subtropical transition zone. So far, related studies on the energy trade-off significance in tropical fish are less discussed. The climatic variability hypothesis proposes that organisms that experienced wider thermal fluctuation might behave with greater physiological thermal-tolerant capacity and vice versa. Since thermal tolerance is energy-dependent, the present study aims to transgenerationally investigate the effect of thermal experiences on the energy allocation in tropical tilapia. According to serious studies toward the growth characterization, behavior paradigms, metabolomic features, and transcriptomic profiling, we found that, tropical tilapia (Oreochromis mossambicus), that were transgenerationally acclimated in regular temperature (cold- naïve, CN) had different energy provision traits from the fish that ever been suffered the cold stress during the juvenile stage in each generation (cold-experienced strain, CE). Accordingly, the tropical fish could adjust respective energy provision traits to cope with ambient thermal variance. Our study also provides a piece of feasible nutrient supply information that benefits to aquaculture improvement under cold stress in the tropical/subtropical area.

ABSTRACT N° ICBF22-417

ESCAPE AND PHYSIOLOGICAL PERFORMANCE IN A TROPICAL NEWBORN SHARK: TEMPERATURE EFFECTS

José Trujillo* [1], Ian Bouyoucos[2, 3], Willian Rayment[1], Paolo Domenici[4], Serge Palnes[5], Jodie Rummer[2, 6], Bridie Allan[1]

[1]Department of Marine Science, University of Otago, Dunedin, New Zealand,
[2]Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Australia,
[3]3PSL Research University, EPHE-UPVD-CNRS, USR 3278 CRIOBE, Universite de Perpignan, Perpignan, France,
[4]CNR-IBF Istituto di Biofisica, Pisa, Italy,
[5]Laboratoire d'Excellence "CORAIL", EPHE, PSL Research University, UPVD, CNRS, USR 3278 CRIOBE, Moorea, French Polynesia,
[6]Marine Biology, College of Science and Engineering, James Cook University, Townsville, Australia

Abstract:
Escape responses are crucial manoeuvres that fish employ to avoid predation. Escape responses are anaerobically-powered accelerations that require a phase to physiologically recover after the response. A successful escape is crucial for survival; however, the recovery phase may constrain other vital processes (e.g., growth, foraging). Thus, establishing this trade-off between escape and physiological performance is elemental to understand survival in fish, especially during early life stages. This is of interest for sharks, in which only adults of one species have been studied (Squalus suckleyi). Because sharks are ectotherms, temperature is likely to modulate this trade-off. We used newborn blacktip reef sharks (Carcharhinus melanopterus) as a model species. Escape performance, after mechano-acoustic stimulation, was determined at four temperatures (25, 27, 29 and 31ºC). We used intermittent-flow respirometry to quantify rates of oxygen uptake ($\dot{M}O_2$) and recovery times at three temperatures (27, 29 and 31ºC). All individuals performed a characteristic C-start double-bend response and showed higher turning rates than expected, although at comparable speeds and accelerations. In addition, small turning radii (3-11% of body length), puts C. melanopterus among turning specialists. We also measured reaction times (escape latency) and found shorter latencies (4.17-8.33 ms) contrary to previous work on sharks, suggesting activation of Mauthner cells, as these neurons are known for being associated with fast responses in teleosts. We found that escape traits are sensitive to short-term increases in temperature. In contrast, $\dot{M}O_2$ was not affected by experimental temperatures. Nonetheless, recovery data suggests that newborn sharks may not be able to cope with exhaustive exercise at higher temperature. Our results are paramount for the study of survival in newborn sharks.

ABSTRACT N° ICBF22-130

WOUND HEALING AND IMMUNE FUNCTION IN A NEWBORN TROPICAL REEF SHARK EXPOSED TO SIMULATED HEATWAVE AND COLD SNAP CONDITIONS

Shamil F. Debaere* [1], Elena M. C. Milanesi[2], José E. Trujillo[3], Gudrun De Boeck[1], Serge Planes[4, 5], Jodie L. Rummer[6, 7]

[1]Department of Biology, Systemic Physiological and Ecotoxicological Research (SPHERE), University of Antwerp, Antwerp, Belgium,
[2]Department of Earth and Environmental Sciences (DISAT), University of Milano-Bicocca, Milan, Italy,
[3]Department of Marine Science, University of Otago, Dunedin, New Zealand,
[4]PSL Research University, EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan, Perpignan, France,
[5]Laboratoire d'Excellence CORAIL, EPHE, PSL Research University, UPVD, USR 3278 CRIOBE, Papetoai, Moorea, French Polynesia,
[6]College of Science and Engineering, James Cook University,
[7]Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Australia

Abstract:
Sharks can incur a range of external injuries throughout their lives that originate from various sources. The most prominent wounds observed in viviparous shark neonates are the natal umbilical wounds. The high recovery potential generally reported in sharks may be particularly important during these early life stages, since open wounds are potential sources of infection that may cause complications in neonates whilst immune systems are still developing. However, the energy allocated to recovery may considerably reduce the energy investment in metabolically demanding activities such as neonate growth; although, the impact of energy allocation to wound healing remains largely unexplored. Furthermore, exposure to environmental stressors, such as those associated with anthropogenic climate change, may profoundly affect wound healing and early development of neonatal sharks. In order to maintain steady growth rates the neonates may therefore downregulate other physiological functions (e.g., immune function).
We tested whether wound healing rates are affected by simulated heatwave and cold snap conditions by evaluating the healing rates of umbilical wounds of neonatal blacktip reef sharks (Carcharhinus melanopterus). Preliminary results suggest that increased ambient water temperatures accelerate healing (29°C vs 25°C: + 69.4-79.0%), but they can also be expected to increase infection risk. We consequently assessed whether infection risk increases with temperature and how this may influence wound recovery, and we evaluated the potential downregulation of immune function by investigating the physiological response to bacterial infection between temperatures. Our results reveal an intriguing interplay between wound healing, immune function and growth in a neonatal shark in future ocean conditions, and advance our understanding of the tolerance of wound healing in sharks.

ABSTRACT N° ICBF22-490

INVESTIGATING THE THERMAL PHYSIOLOGY OF WILD GILTHEAD SEABREAM SPARUS AURATA, TO BETTER INTERPRET THEIR PATTERNS OF SEASONAL MIGRATIONS

Alexandre Mignucci* [1], David McKenzie[2], Théo Navarro[3], Fabien Forget[4], Hossein Allal[5], Gilbert Dutto[6], Eric Gasset[6], Jérôme Bourjea[1]

[1]IFREMER, Sète,
[2]CNRS, Montpellier,
[3]CNRS, Palavas-les-Flots,
[4]IRD, Sète,
[5]CHU de Montpellier, Montpellier,
[6]IFREMER, Palavas-les-Flots, France

Abstract:
The gilthead seabream Sparus aurata is a highly prized species that uses coastal lagoons as summer feeding grounds in the Gulf of Lions (NW Mediterranean Sea). It migrates offshore for reproduction at sea before winter, returning in spring, but may also perform short excursions out to sea in summer. The environmental drivers of these migrations, and by extension the potential impact of climate change on the seabream lifecycle, are unknown. We monitored the movements of nearly 400 seabream in a lagoon over 4 years by acoustic telemetry, along with various environmental parameters in the lagoon and at sea. Dominance analysis indicated that temperature was a predominant driver for both seasonal migrations and summer excursions. We captured 30 seabream from local lagoons in summer, to investigate mechanisms of thermal physiology underlying these migrations. Fish were implanted under anaesthesia with intraperitoneal archival tags (Star-Oddi®) that log heart rate, then exposed them to stepwise progressive warming or cooling while swimming spontaneously in a tank or swimming steadily in a swim tunnel. We found a response threshold based upon the inability of the heart to compensate for temperatures above 29°C. This corresponded to the temperature associated with seaward excursions from the lagoon during summer heat waves, which indicates that seabream were looking for a thermal refuge at sea. Heat waves are increasing in frequency and duration due to global change, which could significantly impact seabream fitness over the long term.

ABSTRACT N° ICBF22-194

IMPACTS OF TEMPERATURE ACCLIMATION ON THE PHYSIOLOGY OF PACIFIC SARDINE (SARDINOPS SAGAX)

Joshua Lonthair* [1, 2], Lisa Komoroske[1], Nick Wegner[2], Nann Fangue[3]

[1]University of Massachusetts Amherst, Amherst,
[2]NOAA - SWFSC, San Diego,
[3]University of California Davis, Davis, United States

Abstract:
The impacts of temperature acclimation on marine teleosts have been well-documented, with alterations to respiratory constraints and enzymatic activity potentially affecting population and ecological dynamics. Recent studies have shown alterations in fish body size across species and ecosystems associated with ocean warming. However, studies on the impacts of ocean warming on Pacific sardine (Sardinops sagax), a key forage fish in California food webs and fisheries, have been less well-documented. Although we know that the Pacific sardine is physiologically sensitive to temperature, the impacts of ocean warming on metabolic rate, and other physiological parameters have not been examined. Here we sought to explore this knowledge gap and observe the physiological alterations during the changes associated with both acute and long-term temperature acclimation. Key metrics analyzed were metabolic rate, which includes maximum metabolic rate and routine metabolic rate, critical thermal maximum (CTmax), and critical oxygen threshold (Pcrit). Overall, this data allows us to begin to elucidate the physiological responses associated with different temperature exposure in a critical California coastal pelagic species, which has significant implications for future fisheries yield projections, stock assessments, and ecosystem stability.

ABSTRACT N° ICBF22-527

RNA-SEQ ANALYSIS OF THERMAL STRESS RESPONSE IN BLACK CUSK-EEL LARVAE (GENYPTERUS MACULATUS).

Phillip Dettleff* [1], Rodrigo Zuloaga[2], Camila Godoy[2], Marcia Fuentes[2], Alfredo Molina[2], Juan Antonio Valdes[2]

[1]Pontificia Universidad Católica de Chile,
[2]Universidad Andres Bello, Santiago, Chile

Abstract:
The Genypterus genus contains native species of economic relevance with high farming potential, including the black cusk-eel (Genypterus maculatus). This species is distributed on the south Pacific coast of Chile. Environmental factors such as temperature can generate stress in fish, however the knowledge on thermal stress is limited for black cusk-eel. The objective of this work was to study the effect of heat stress in black-cusk-eel larvae.

Black cusk-eel larvae (9 days post hatch) were collected from CIMARQ and separated in control and stress groups. The stress group was subjected to high-temperature stress (19°C) while the control group was maintained at 14°C, sampling at 24 (T1) and 48 hrs (T2) for cortisol, DNA oxidative damage and RNA-seq analysis.

High temperature produces a significant increase in cortisol levels at T1 in the stress group, with no DNA damage observed. For the RNA-seq analysis, the first de novo assembly of larvae was generated with more than 56,000 transcripts identified. Thermal stress generates a differential expression at T1 and T2 with several process enriched related to nucleosome, DNA packing, protein folding, heat response and oxidative damage. This study showed that thermal stress affect early stages of black cusck-eel modulating the expression of relevant processes, information that should be considered in a climate change world scenario. Funding: CONICYT FONDECYT Postdoctorado 3180283.

15-The future of fishes: how will they cope physiologically with a changing planet?

ABSTRACT N° ICBF22-384

SUB-LETHAL THRESHOLDS IN CELLULAR RESPONSES TO ACUTE TEMPERATURE STRESS IN A SALMONID

Ken Jeffries* [1], Travis Durhack[2], Theresa Mackey[1], Matthew Thorstensen[1], Michael Lawrence[1], Eva Enders[2]

[1]University of Manitoba,
[2]Fisheries and Oceans Canada, Winnipeg, Canada

Abstract:
There are numerous methods for estimating the thermal tolerance limits in fishes. However, from a conservation perspective for wild fish populations, it is important to identify sub-lethal temperature thresholds where temperature begins to adversely impact the animal. Several lines of evidence suggest there are sub-lethal thresholds at multiple levels of biological organization in the response of fishes to temperature increases. At the cellular level, there are sub-lethal thresholds that include activation of an inducible heat shock response at moderate temperature increases and activation of apoptosis at more extreme levels of cellular stress. We aimed to determine if thresholds in the cellular response occurred at temperatures that match with common endpoints in temperature stress studies in fishes. We first estimated the temperature preference (15.1±1.1°C), Topt for aerobic scope (~15°C), agitation temperature (22.0±1.4°C) and the CTmax (28.2±0.4°C) for 1 yr old Brook Trout (Salvelinus fontinalis). We then acutely exposed a different subset of fish from the same cohort to temperatures that match the temperatures of the common endpoints. Fish were sampled for gill, liver, and blood plasma when they reached the target temperature or after 60 min of recovery at the acclimation temperature (10°C). We used qPCR to estimate mRNA transcript levels of genes associated with heat shock proteins, oxidative stress, apoptosis, and inducible transcription factors. We found that a major shift in the transcriptome response occurred near the agitation temperature, which we interpret as a link between the cellular stress response and a behavioural avoidance response in the fish.

ABSTRACT N° ICBF22-465

ROLE OF METABOLISM IN STRUCTURING TROPHIC INTERACTIONS ACROSS TEMPERATURES

Kenneth Zillig* [1], Alexandra McInturf[2, 3], Scott Burman[4], Dennis Cocherell[1], Nann Fangue[1]

[1]Wildlife, Fish, and Conservation Department, University of California - Davis, Davis,
[2]Wildlife, Fish, and Conservation Department, University of California - Davis, Davis, CA,
[3]Coastal Oregon Marine Experiment Station, Oregon State University, Newport, OR,
[4]Land, Air and Water Resources Department, University of California - Davis, Davis, CA, United States

Abstract:
Salmonids are broadly considered a coldwater fish due to poor performance and recruitment of wild populations during warm-water periods. However, recent laboratory research has demonstrated that Chinook salmon (Oncorhynchus tshawytscha) juveniles possess impressive swim and metabolic performance at temperatures exceeding 23°C. This implies that temperature-dependent ecological processes (e.g. predation, disease, competition) may be underlying reducing salmonid performance at warm temperatures. Chinook salmon from California's Central Valley have to contend with a large assemblage of aquatic predators. We hypothesized that warm water temperatures may advantage these predators against juvenile salmon, and explain the poor performance of juvenile Chinook salmon in the wild. We tested metabolic and burst swim performance (velocity and fatigue rate) at five temperatures (11 - 25°C) for juvenile Chinook salmon and two predators; non-native largemouth bass (Micropterus salmoides) and native rainbow trout (O. mykiss). We also observed trophic interactions between juvenile Chinook salmon and these two predator species. We found that the effect of temperature varied between physiological metrics and among the predator species and prey populations. Largemouth bass were more likely to consume salmon at warm temperatures, while rainbow trout predation was unaffected. Likewise, largemouth bass exhibited increased burst performance at warm temperatures (>20°C), while rainbow trout showed little influence of temperature on burst capacity. Absolute burst ability and the relative burst performance between predator and prey were stronger indicators of trophic dynamics than aerobic scope. Our results are consistent with our hypothesis of thermal advantage and offer a mechanism to explain field observations indicating that temperatures in excess of 20°C lead to reduced salmon abundance.

ABSTRACT N° ICBF22-179

FUTURE OF FARMED FISH IN WARMING WORLD: AEROBIC EXERCISE TRAINING IMPROVES CARDIAC PERFORMANCE AND CARDIAC THERMAL TOLERANCE IN SALMONIDS.

Luca Pettinau* [1], Eila Seppänen[2], Katja Anttila[1]

[1]University of Turku, Turku,
[2]Natural Resources Institute Finland (Luke), Enonkoski, Finland

Abstract:
Domestication and selection for fast growth have impaired the cardiorespiratory system of salmonids and compromised their capacity to face environmental stressors, like heatwaves. In the future climate change scenario, heatwaves are predicted to increase in magnitude and frequency. Thereby, farmed salmonids might be especially vulnerable to heatwaves. Exercise swimming training has been proposed as useful tool to enhance cardiac function and robustness against diseases in farmed fish and therefore improve aquaculture production. However, whether exercise training could improve cardiac robustness against heatwaves remains unknown. Consequently, we trained juvenile rainbow trout with different swimming velocities: 0.7 (control), 1.3 and 2.1 body lengths per second (bl*s-1) with interval protocols of 6h per day for 5 weeks. We repeated the training protocol with adult reproductive brown trout to see if different species and life stages respond differently. The adult fish were trained with 0.3 (control) and 0.7 bl*s-1. Measuring maximal heart rate (fHmax) during acute warming and cardiac thermal tolerance variables [i.e. Breakpoint temperature (TABP), arrhythmia temperature (TARR)], we demonstrated that training at 1.3 bl*s-1 for juveniles and at 0.7 bl*s-1 for adults increased significantly the upper thermal tolerance of the cardiovascular function. In particular, the TARR increased up to 3.6°C in juveniles and 3.4°C in adults as compared to the control fish. These findings present valid training programmes to increase the cardiac thermal tolerance of salmonids and therefore possibly their capacity to face heatwaves with direct applications in the aquaculture facilities to enhance fish production by possibly reducing the mortality during heatwaves.

ABSTRACT N° ICBF22-302

THERMAL TOLERANCE OF WILD AND CULTURED ARCTIC CHARR FROM ICELAND WHILE SWIMMING: ARE WE BREEDING OUT CLIMATE RESILIENCY?

Jay Nelson* [1], Helgi Thorarensen[2]

[1]Towson University, Towson, United States,
[2]Holar University, Holar, Iceland

Abstract:
Climate projections predict changes in magnitude and stochasticity of both temperature and flow for many freshwaters. Predicting the fate of fishes that inhabit these waters will require knowing how they respond to variations in both temperature and flow. Some species, such as the Arctic charr in Iceland, have already experienced these changes. Glacial retreat over the past 10,000 years, and geothermal activity have created a mosaic of freshwater habitats inhabited by char that vary substantially in both temperature and flow. We raised cultured char from hatching under a 2 X 2 matrix of flow and temperature conditions in the laboratory for a single generation and compared them to wild char captured from variant flow and temperature environments. Thermal tolerance (critical thermal maximum (CTmax)) while swimming in a flow selection chamber and the current speed selected by the fish, acclimated to two different temperatures was measured. Wild fish were more thermally tolerant than cultured fish at both acclimation temperatures and were more thermally plastic. Flow selection during the CTmax test was more variable at the high acclimation temperature and was more variable in wild fish.

ABSTRACT N° ICBF22-418

MECHANISMS OF ION AND ACID-BASE REGULATION IN EARLY LIFE STAGE RED DRUM (SCIAENOPS OCELLATUS).

Andrew Esbaugh* [1], Joshua Lonthair[2]

[1]University of Texas at Austin, Austin,
[2]University of Massachusetts Amherst, Amherst, United States

Abstract:
Ocean acidification (OA) caused by elevated CO2 is an imminent environmental stress to marine organisms, and is hypothesized to have a suite a detrimental effects. In fish, elevated CO2 causes a respiratory acidosis that is compensated through ion transport pathways at the gills. This disturbance is thought to be the underlying cause of many of the effects of OA, including reduced larval survival. Importantly, little is known about the development and function of acid-base pathways in fishes with an obligate marine life history. We therefore sought to explore the development of acid-base pathways in a representative marine fish, the red drum, and assess the role of phenotypic plasticity in early life resilience to a CO2 induced acidosis. We first explored the dose response effects of CO2, which resulted in significant reductions in larval survival at OA relevant partial pressures. However, a significant proportion of tested individuals also exhibited surprising resilience to CO2 with approximately 50% survival when exposed to partial pressures over 10x those relevant to OA. Gene expression and confocal microscopy were used to assess acid-base pathways, which provided evidence for functional pathways and CO2-induced plasticity as early as 36 hours post-fertilization. A scanning ion-selective electrode technique was used to verify the function of these pathways, which was evident from a dose-dependent increase of proton flux across the larval epithelium. Interestingly, proton flux was both bafilomycin-sensitive and EIPA-sensitive suggesting the presence of multiple acid excretion mechanisms, which may contribute to the observed resilience of red drum to CO2 stress.

06-Developmental physiology of fishes

ABSTRACT N° ICBF22-175

DEVELOPMENT OF ION REGULATION IN KILLIFISHES

Patricia Wright* [1], Jonathan Wilson[2], Louise Tunnah[1]

[1]University of Guelph, Guelph,
[2]Wilfrid Laurier University, Waterloo, Canada

Abstract:
For most fishes the gills are the primary site of exchange with the environment. However, reliance on gill-exchange varies over both developmental time and amongst life-history strategies. For example, many larval fishes hatch with poorly developed gills and rely instead on the skin for exchange in early life. Some fishes with amphibious life-histories experience diminished gill function during terrestrial sojourns and also rely on the skin for exchange, similar to larval fishes. Given these similarities between larval and amphibious fishes we tested the hypothesis that larval skin traits (i.e. cutaneous ionocytes and neuroepithelial cells) are retained in adult amphibious killifishes to enable the skin to be a major site of exchange on land. We used 6 closely related species spanning a range of amphibiousness to test this hypothesis. We found skin barrier properties (i.e. thickness and complexity) increased over development in all species. Surprisingly, all species, whether amphibious or not, retained a larval skin trait - cutaneous ionocytes – from hatch to maturity, albeit at varying densities. Ionocyte density tended to increase across development in amphibious species, while in exclusively water-breathing species ionocytes were retained at consistently low-levels. Overall, our results show that amphibious killifishes continuously express larval ionoregulatory traits until adulthood and at much higher densities than water-breathing species, highlighting distinct differences in skin function between these two groups.

06-Developmental physiology of fishes

ABSTRACT N° ICBF22-322

EFFECTS OF TRIIODOTHRYONINE ON THE STRESS AXIS IN LARVAL STURGEON

Ian Bouyoucos*[1], Alyssa Weinrauch[1], Gary Anderson[1]

[1]University of Manitoba, Winnipeg, Canada

Abstract:
Stress experienced during early ontogeny impacts development in fish larvae. The capacity of larvae to respond to stress is regulated by endocrine systems; notably, the hypothalamus-pituitary-interrenal (HPI) axis regulates energy and mineral homeostasis via the corticosteroid, cortisol. Triiodothyronine (T3), plays an essential role in fishes by regulating metamorphosis. Shared function of the HPI and hypothalamus-pituitary-thyroid (HPT) axes is mediated through neuropeptides, corticotropin-releasing factor (CRF) and thyrotropin-releasing hormone (TRH), that activate the HPT axis in fishes. Because cortisol and CRF are causally linked via negative feedback on the HPI axis, and CRF and TRH play a dual role in stimulating the HPT axis, we hypothesized that exposure to exogenous T3 should affect development and function of the HPI axis, as has been documented in various physiological systems. To that end, we exposed gametes of lake sturgeon (Acipenser fulvescens) – a freshwater, chondrostean fish – to exogenous T3 during the process of fertilization. To further test the effect of exogenous T3 on HPI axis, sturgeon embryos were reared using two methods, including one that has been previously documented to delay development of the HPI axis in A. fulvescens. Upon hatch, larvae were sampled over the first month of life (i.e., ~30 days post-fertilization). Whole-body homogenates were processed for cortisol concentration and the abundance of mRNAs encoding genes involved in the HPI axis (e.g., CRF, proopiomelanocortin, steroidogenic acute regulatory protein, glucocorticoid receptors). Together, this study suggests a possible role for T3 in HPI axis development in sturgeon.

ABSTRACT N° ICBF22-354

EXPLORING EUTHANASIC PROTOCOLS IN EARLY STAGES OF ZEBRAFISH

Juan Ramos* [1], Lluis Tort[1], Ali Khansari[1]

[1]Universitat Autonoma de Barcelona., barcelona, Spain

Abstract:
The use of zebrafish Danio rerio has been growing in an exponential way since the early 2000´s. Therefore, a number of protocols are needed to breed and maintain zebrafish welfare of this model species. Among them, the influence of the environmental conditions and their impact on husbandry protocols, is more evident in the early stages. Until day 5 post fertilization, no legal welfare considerations are applied to the early stages of fish, but some considerations could be adopted to better select an appropriated protocol, not just for the zebrafish species, but also for the specific developmental stage.
Although aversion or scape reactions could serve as welfare markers during the early stages of zebrafish, other functional indicators may not be mature enough to be adequately monitored, as most of current protocols are just an adaptation from the adult stages. For instance, larvae respiration is performed through the skin, so oxygen can be spread to tissues without significant heart work, which compromises the use of heart beat as an indicator in the anesthesia and euthanasia processes. Thus, regarding euthanasic agents, Tricaine and thermal shock have been traditionally used as euthanasic protocols but our results show that they are no effective, as heartbeat is recovered even after the exposition to these agents. We also observed that Lidocaine and Clove Oil are more effective in these early stages.

ABSTRACT N° ICBF22-163

THE COMPOSITION OF IONOCYTES IS BALANCED BY THE SPATIOTEMPORAL DYNAMICS OF FOXI3A/B IN ZEBRAFISH

Zi-Hua Huang* [1], Bo-Kai Liao[1]

[1]National Taiwan Ocean University, Keelung, Taiwan

Abstract:
Ionocytes are specialized cells scattered on epithelia and fish gills for maintaining ionic homeostasis, and five sub-types were identified in zebrafish by their physiological properties and ion channel/transporter markers. Delta-Notch signaling pathway determines ionocyte progenitors, and two downstream transcription factors, foxi3a and foxi3b, control the fates of sub-types via their positive regulatory loop. However, the determination mechanisms from common progenitors to such various types of ionocytes are not clear. To reveal the differentiation trajectories of skin ionocytes and the temporal-spatial dynamics, we generated a foxi3b::foxi3b-venus/yfp transgenic zebrafish line, named Boötis, by bacterial artificial chromosome recombineering and meganuclease transgenesis. Boötis recapitulated expression patterns of the endogenous foxi3b except more foxi3b-venus+ cells in yolk extension regions during 24~36 hpf resulted from the imbalanced regulatory loop by more transgene copies. Surprisingly, we discovered that Boötis lost all NaRCs (Na+-K+-ATPase-rich cells) and NCCs (Na+-Cl- cotransporter cells) but showed significantly more HRCs (H+ pump-rich cells) detoured from the NaRC lineage. To further explore the regulatory dynamics, mathematical modeling on 2D cell grids was conducted. We found that distinct cell types could be generated simultaneously, given that both the expression and degradation rates were higher from one foxi3 to the other. Moreover, the cell types became more uniform by matching the expression rates of both foxi3, as expected in Boötis. In summary, the ionocyte determinations in zebrafish are sensitive to the temporal dynamics and the balance of the regulatory loop between foxi3a and foxi3b, which further influences the composition of ionocyte sub-types.

ABSTRACT N° ICBF22-414

USING GENE EDITING TO TEST CURRENT ASSUMPTIONS OF IONOREGULATORY MODELS IN LARVAL ZEBRAFISH

Alex Zimmer* [1]

[1]University of Alberta, Edmonton, Canada

Abstract:
The regulation of Na and Cl absorption in zebrafish larvae is coordinated by several different ionocyte subtypes expressing various ion uptake pathways. Genetic knockout approaches have recently allowed for testing of current models and for determining the plasticity of these physiological systems. This talk will review three separate case studies using CRISPR/Cas9-generated knockout mutant lines that together demonstrate the genetic and physiological plasticity of ionoregulatory systems and suggest an incomplete picture of ionoregulatory mechanisms in larval zebrafish. First, in hypothesizing that nhe3b mutants lacking Na/H-exchanger 3b would have a reduced capacity for Na uptake under low Na conditions, we found that this transporter appears to be dispensable for Na uptake and that there may exist additional, yet unknown pathways for Na uptake in zebrafish. Second, in a similar result, rhcgb mutants lacking the ammonia-transporting Rhesus glycoprotein Rhcgb displayed an increased ammonia excretion relative to wild-type fish. In these mutants, we found evidence for transcriptional regulation of other Rh genes, highlighting the plasticity of this system. Third, knockout of ca17a, the cytosolic carbonic anhydrase expressed in H-ATPase-rich ionocytes resulted in an increase in Na uptake. This increase in Na uptake rate presumably occurred via Na-Cl-cotransporter because this is the only pathway described to date whose function does not rely on H+ generated by Ca17a. However, Cl uptake was significantly reduced in ca17a mutants, casting doubt on the completeness of current ionoregulatory models in larval zebrafish and further suggesting a yet unknown pathway for Na uptake in this species.

ABSTRACT N° ICBF22-461

GR AND MR PLAY A ROLE IN THE CORTISOL-MEDIATED FEEDING SUPPRESSION IN ZEBRAFISH

Niepukolie Nipu[1], Femilarani Antomagesh[1], Erin Faught[1], Mathilakath M. Vijayan[*1]

[1]University of Calgary, Calgary, Canada

Abstract:
Chronic stress in fish suppresses feeding, but the molecular mechanism(s) by which glucocorticoids (GCs) play a role in the appetite regulation is unclear. We hypothesized that cortisol-driven changes in brain glucose uptake would alter the energy status leading to feeding inhibition. We first established the effects of glucose on feeding by exposing zebrafish (Danio rerio) to glucose, which resulted in hyperglycemia, reduced phosphorylation of the nutrient sensor AMP-activated kinase (AMPK) in the brain, and suppression of food intake. Similarly, chronic cortisol exposure also resulted in hyperglycemia and suppressed feeding, but glucose uptake in the brain remained unchanged and there was no difference in the phosphorylation status of AMPK. This suggests that cortisol-mediated hyperglycemia may not be driving the reduction in feeding. We next tested whether cortisol acted through the corticosteroid receptors to alter feeding by utilizing a ubiquitous knockout of the glucocorticoid receptor (GR) or the mineralocorticoid receptor (MR) in zebrafish. Interestingly, the feeding inhibition seen with cortisol was also evident in fish lacking GR or MR suggesting that either receptor may be sufficient to modulate the feeding behavior. When we examined fish lacking GR, they had a higher capacity for glucose uptake in the brain, but the phosphorylation of AMPK remained unchanged. In sum, cortisol-driven feeding suppression is not just due to hyperglycemia but dependent on the nutrient status, which may be dictated by the energy substrate repartitioning mediated by GR and MR activation.

Acknowledgement: This study was funded by the Natural Sciences and Engineering Research Council of Canada Discovery Grant to MMV.

ABSTRACT N° ICBF22-232

SEROTONIN, A CENTRAL PLAYER IN THE HOMEOSTATIC REGULATION OF FOOD INTAKE.

Mauro Chivite[*1], Sara Comesaña[1], Maria Alborja-Valados[1], Jessica Calo[1], Jose Luís Soengas[1], Marcos Antonio López-Patiño[1], Jesús M Míguez[1]

[1]Centro de Investigación Mariña, Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía, Universidade de Vigo, Spain, Vigo, Spain

Abstract:
Serotonin has been postulated as one of the main neurotransmitters involved in the integration of satiety-related information in mammals. Raphe serotonin neurons are activated in response to food-related signals and this neurotransmitter, specifically through serotonin receptors 5HT2C and 5HT1B, modulates the activity of hypothalamic neuropeptides involved in the homeostatic control of food intake. In fish, several pharmacological studies have indicated that serotonin inhibits food intake through specific receptors. However, the mechanism involved in the anorexigenic action of serotonin and the putative regulation of hypothalamic neuropeptides by these receptors are unclear. In this study, we performed several pharmacological experiments in the rainbow trout to evaluate the possible role of serotonin 5HT2C, 5HT1B and 5HT1A receptors in different conditions both in fed and unfed fish, and with short- and long-term treatments with serotonergic drugs. The results obtained indicate that serotonin promotes an anorexigenic response in rainbow trout that is mainly mediated by 5HT2C receptors. In addition, 5HT1B and 5HT1A receptors also play a role in the regulation of feeding behaviour acting at presynaptic level to down-regulate serotonergic activity, but also at post-synaptic locations. Neuropeptidergic neurons in the hypothalamus are the target of serotonin, so that activation of 5HT2C receptors up-regulates the anorexigenic pathways, while 5HT1A and 5HT1B mediates feeding-dependent responses that generally down-regulate the expression of orexigenic neuropeptides.
Acknowledgments: Supported by Spanish AEI and European Fund of Regional Development (PID2019-103969RB-C31 and FEDER).

ABSTRACT N° ICBF22-468

CART AS AN ANOREXIGENIC NEUROPEPTIDE AND A POTENTIAL PARTICIPANT IN THE REGULATION OF STRESS IN NILE TILAPIA

Nikko Alvin Cabillon*[1], Adi Segev-Hadar[2], Jakob Biran[2]

[1]Department of Animal Sciences, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot,
[2]Department of Poultry and Aquaculture, Institute of Animal Science, Agricultural Research Organization, Rishon LeTsiyon, Israel

Abstract:
Cocaine- and amphetamine-regulated transcript (CART) is a pleiotropic neuropeptide involved in the central regulation of stress, anxiety, depression, reproduction, and circadian functions. However, it is mainly known for its regulation of body weight and appetite. While mammals possess a single CART gene, bird genomes contain two carts, and fish may possess up to seven cart genes. Nile tilapia (Oreochromis niloticus) is the third most cultured species in global aquaculture. Hence, understanding the central regulation of tilapia feeding may help improve aquaculture. In the present study, we identified the expression of seven cart genes in the brain of Nile tilapia. Tissue distribution analysis demonstrated that while the brain is the main expression site for some carts, other cart genes are highly expressed in peripheral organs. Acute starvation of adult tilapia significantly decreased the hypothalamic expression of four cart genes, and two of them displayed suppressed hypothalamic expression in response to chronic starvation. Interestingly, chronic starvation also elicited the expression of two cart genes and plasma cortisol, suggesting that some cart genes may be involved in the central regulation of stress. Lastly, we utilized the CRISPR/Cas9 method to generate germline tilapia carrying loss of function mutations in the primary appetite-regulating cart gene.

ABSTRACT N° ICBF22-441

NEW INSIGHTS IN GROWTH, METABOLIC AND HORMONAL MILIEU AFTER CURCUMIN ADMINISTRATION IN OREOCHROMIS MOSSAMBICUS

Divya Lekha* [1, 2], M.V. Sruthi[1]

[1]Dept of Zoology, Central University of Kerala, Kasaragod, Kerala,
[2]Dept of Zoology, University of Calicut, Kerala, India

Abstract:
Phytochemicals function as growth promoters by acting on hormones of the growth hormone- insulin like growth factor (GH-IGF) axis. Curcumin have been shown to have wide spectrum of biological activities. The present study was designed to investigate the effects of long term dietary supplementation of curcumin on growth and the potential roles that curcumin may play in the regulation of appetite regulating genes in tilapia (Oreochromis mossambicus). Curcumin in 0.5 and 1% doses were given as feed additive to tilapia for 100 days. Supplementation of dietary curcumin improved the growth performances in tilapia. The feed intake (FI) was significantly reduced by curcumin. Curcumin enhanced digestive enzyme activities and improved the enzymatic antioxidant capacities, reduced lipid peroxidation product (malondialdehyde) and cortisol level. Curcumin influenced energy metabolism by downregulating NKA pump activity in gills. The study also proves that curcumin supplementation improved nutritional value and general health in tilapia as indicated by the increase in omega-3 and omega-6 polyunsaturated fatty acids (n-3 and n-6 PUFAs), index of thrombogenecity (TI) value, polyene index (PI) value and improved amino acid levels. Different doses of curcumin significantly upregulated the mRNA expressions of the components of GH-IGF axis and appetite regulating peptides such as neuropeptide Y (NPY), pro- opiomelanocortin (POMC), agouti-related peptide (AgRP), ghrelin, ghrelin receptor (GHSR), leptin and leptin receptor in tilapia. In silico docking studies carried out validated the vivo results.
This study proves that curcumin can be supplemented as a functionally and environmentally oriented aquafeed to improve the quality and quantity of aquaculture.

ABSTRACT N° ICBF22-342

COPING STYLES AND STRESS-INDUCED ANOREXIA IN SALMONIDS

Marco Vindas*[1], Ida Beitnes Johansen[1], Maren Høyland[1], Ole Folkedal[2], Tore S Kristiansen[2], Erik Höglund[3], Øyvind Øverli[1]

[1]Norwegian University of Life Sciences, Ås,
[2]Institute of Marine Research, Bergen,
[3]NIVA, Oslo, Norway

Abstract:
In response to stress, salmonids typically stop eating, a phenomenon that may last from a few hours to several days. While this stress-induced anorexia is related to the magnitude and duration of the stressor, it is also associated with individual stress coping style (i.e. personality). Reactive fish are characterized by pronounced behavioral inhibition in concert with high cortisol and brain serotonergic activity levels whereas the opposite is true for proactive individuals. While both proactive and reactive fish suffer from stress-induced anorexia (ignore or are not able to ingest food), the phenomenon is more pronounced in reactive individuals where it typically lasts ten times longer than for proactive fish. Notably, pharmacologically elevating both cortisol and serotonin levels (i.e. up to levels observed post- stress) also reduces feed-intake, suggesting that cortisol and serotonin are important regulators of stress-induced anorexia. In aquaculture, reactive copers generally grow slower than proactive individuals. However, if fish are exposed to an early life stress regime, such coping style-related differences in growth rate are abolished, presumably due to a reduction in serotonergic stress-reactivity in both reactive and proactive fish. In conclusion, although stress normally leads to anorexia and reduced growth rates, habituating fish early in life to stressful environments appears to attenuate physiological stress responses (i.e. serotonergic activity) regardless of stress coping style, resulting in more homogenous growth.

ABSTRACT N° ICBF22-484

SOCIAL STRESS-DEPENDENT REGULATION OF THE SOMATOTROPIC AXIS IN JUVENILE RAINBOW TROUT

Kathleen Gilmour* [1], Carol Best[1], Brett Culbert[2], Julianne Magnan[1], Katherine Jennings[1], Kenan Touma[1], Nick Bernier[2], Jan Mennigen[1]

[1]University of Ottawa, Ottawa,
[2]University of Guelph, Guelph, Canada

Abstract:
Juvenile rainbow trout (Oncorhynchus mykiss) confined in pairs form social hierarchies in which subordinate fish experience chronic stress as evidenced by prolonged elevation of the stress hormone cortisol. A combination of elevated cortisol and low food intake induces a catabolic state in subordinate fish, including mobilization of lipid reserves and decreased rates of muscle protein synthesis. By contrast, dominant fish monopolize and defend food resources, entering an anabolic state with increases in de novo lipogenesis. These differences provide a useful system in which to study the somatotropic axis and in particular, context-dependent actions of growth hormone (GH) in promoting lipolysis versus protein accretion. Synteny analysis supported the existence of four GH receptor paralogues in trout, ghra1 (formerly ghr1), ghra2, ghrb1 (formerly ghr2a) and ghrb2 (formerly ghr2b). After 4 d of social interaction, subordinate trout exhibited elevated transcript abundances of ghrb1 and ghrb2 in red and white muscle together with elevated hsl1 (hormone-sensitive lipase) transcript abundance. Transcript abundances of ghra1 and ghra2 did not differ from control values in white muscle of subordinates, but were reduced in red muscle, and correspondingly, transcript abundances of igf1 and igf2 (insulin-like growth factor) also were lowered in red muscle of subordinates. These results are consistent with a role for ghra in stimulating somatic growth via igf1 versus a role for ghrb in promoting lipolysis via hsl.

ABSTRACT N° ICBF22-263

PERSONALITY, STRESS AND GROWTH IN FARMED FISH. THE IMPORTANCE OF THE SOCIAL CONTEXT

Manuel Gesto* [1], Peter Vilhelm Skov[1]

[1]DTU Aqua, Hirtshals, Denmark

Abstract:
Inter-individual differences in the growth rates of farmed fish are believed to be determined by genotypic traits and by phenotypic plasticity upon facing the social and environmental factors associated to the farming conditions. Repeated acute stress or chronic mild stress are known potent inducers of growth suppression in fish. Fish personality, particularly in fish showing social hierarchies such as salmonids, is also believed to affect the individual capacity to cope with the farming environment; It is often assumed that farmed subordinate (reactive) individuals live in a state of chronic mild stress, and therefore they might be in disadvantage, from the point of view of growth performance, when compared to dominant individuals. We will review some recent data about the relationship between fish personality, stress sensitivity and growth, highlighting the role of the domestication level, specific farming conditions and particularly the social context in shaping fish performance under captivity.

ABSTRACT N° ICBF22-127

PHYSIOLOGICAL RELEVANCE OF STRESS-INDUCED CHAPERONE-MEDIATED AUTOPHAGY IN MAJOR METABOLIC PATHWAYS IN THE RAINBOW TROUT

Simon Schnebert* [1], Emilio J Vélez[1], Maxime Goguet[1], Karine Dias[1], Vincent Véron[1], Karine Pinel[1], Florian Beaumatin[1], Amaury Herpin[2], Beth Cleveland[3], Iban Seiliez[1]

[1]Université de Pau et des Pays de l'Adour, E2S UPPA, INRAE, UMR1419 Nutrition Métabolisme et Aquaculture, Saint-Pée-sur-Nivelle,
[2]INRAE, UR1037 Laboratory of Fish Physiology and Genomics, Rennes, France,
[3]National Center for Cool and Cold Water Aquaculture, ARS/USDA, Kearneysville, United States

Abstract:
Chaperone-mediated Autophagy (CMA) is established as a major lysosomal-dependent protein degradation pathway that maintains cellular proteostasis in response to multiple stressors such as starvation, oxidative stress or hypoxia. While it is well described in mammals, CMA was only recently evidenced in medaka fish. This discovery provides new exciting perspectives towards its role in fish homeostasis and metabolism. In the present study, we first show that CMA-related factors are ubiquitously expressed during early development as well as in several adult tissues of rainbow trout (Oncorhynchus mykiss), a major aquaculture species. In order to firmly establish the existence of functional CMA activity in this species, we then performed an in vitro CMA activity assay using isolated lysosomes. Our results show that rainbow trout undoubtedly exhibits CMA activity, which increases 21-fold during fasting ($p<0.05$). Finally, to describe the physiological role of CMA in trout, we generated a knock-out (KO) fish line lacking a key CMA rate-limiting gene, the lysosomal-associated membrane protein 2a (Lamp2A), by using the genome-editing tool CRISPR-Cas9. Interestingly, preliminary results suggest that Lamp2A-KO fish show faster growth than their wild type counterparts. Future analyses will characterize the significance of CMA as a crucial mechanism regulating numerous physiological and stress-related processes in fish.

ABSTRACT N° ICBF22-423

CONSERVED METABOLIC AND STRESS REGULATORY FEATURES OF TENEURIN C-TERMINAL ASSOCIATED PEPTIDES (TCAP) IN FISH.

Peggy Biga* [1], Ross Reid[1], Andrea Reid[1], David Lovejoy[2]

[1]UAB, Birmingham, United States,
[2]University of Toronto, Toronto, Canada

Abstract:
Teneurins have well established roles in the function and maintenance of the central nervous system of vertebrates. A bioactive peptide found on the c-terminal portion of teneurins, named teneurin c-terminal associated peptide (TCAP), has been shown to regulate glucose metabolism and can be independently transcribed. The current depth of literature on TCAPs in fish is limited, but here we show conserved regulation of glucose metabolism and stress-related physiology. TCAP peptides are well conserved across taxa, and zebrafish has maintained two TCAP-2 paralogs (TCAP-2a and TCAP-2b), and single TCAP-1 and TCAP-4 orthologs. Previous work has shown that TCAP shares 22% sequence similarity with the CRF superfamily and thus initial studies focused on targeting the roles of TCAP in relation to the stress axis. Studies in rodents established TCAP-1 as a potent anxiolytic consistent with decreased anxiety, while also exhibiting metabolic effects as demonstrated by 40% decrease in blood glucose in healthy and type II diabetic insulin-insensitive pathological rats. Furthermore, TCAP-1 decreased insulin and increased serum glucagon levels, suggesting systemic metabolic action. Evolutionary analysis of TCAP peptides reveals that it predates insulin, suggesting the metabolic functions are highly conserved. We tested the effects of TCAP-3 on metabolic function in zebrafish, and show a dose-dependent increase in metabolic activity in larval and adult zebrafish. Additionally, TCAP-3 protects zebrafish from cold temperature stress in relation to metabolic activity, while also enhancing heart rate and growth rate. TCAPs appear to exhibit highly conserved functions in regulating metabolic function and stress physiology across taxa.

ABSTRACT N° ICBF22-206

ENDOCANNABINOID RECEPTORS ARE INVOLVED IN INTAKE RESPONSE TO PALATABLE FEED IN RAINBOW TROUT

Mauro Chivite[1], Sara Comesana[1], Jessica Calo[1], Ayelen M Blanco[1], Marta Conde-Sieira[1], José L Soengas[*1]

[1]Universidade de Vigo, Vigo, Spain

Abstract:
The regulation of feed intake in fish is framed into homeostatic (feed intake necessary to maintain energy balance and metabolic function) and hedonic (feed intake driven by sensory perception and/or pleasure). The mechanisms involved in hedonic systems are scarcely known. One of the putative systems involved is the endocannabinoid system (ECs). This is composed mainly by the ligands N-arachidonylethanolamine (Anandamide; AEA) and 2-arachidonoylglycerol (2-AG), the enzymes involved in EC synthesis and degradation, specific EC receptors 1 (CNR1) and 2 (CNR2), but other receptors could be involved like G-protein coupled receptor 55 (GPR55) and transient receptor potential cation channel-1 (TRPV1). We recently demonstrated in rainbow trout the presence of a rewarding response mediated by ECs in hypothalamus and telencephalon when fish fed a lipid-enriched diet, and that central administration of AEA or 2-AG exert a bimodal effect on feed intake in fish with low doses inducing an increase that disappeared with the high dose of both endocannabinoids. To assess the precise involvement of the different receptors of the ECs (CNR1, GPR55, and TRPV1) in this response we carried out two studies. In a first study, we injected ICV AEA or 2-AG in the absence/presence of specific receptor antagonists. In a second study, we feed fish with a palatable feed (HFD diet) in the absence/presence of the same receptor antagonists. The results are discussed in the context of hedonic regulation of food intake in fish.

Acknowledgments: Supported by Spanish AEI and European Fund of Regional Development (PID2019-103969RB-C31 and FEDER)

ABSTRACT N° ICBF22-183

IMPACT OF TWO DIETS WITH DIFFERENT VEGETABLE PROTEIN CONTENT ON GASTROINTESTINAL AMINO ACID SENSING AND FEED INTAKE REGULATION IN RAINBOW TROUT

Jessica Calo Rodríguez* [1], Sara Comesaña[1], Mauro Chivite[1], María Alborja[1], Marta Conde[1], Ayelén M. Blanco[1], Sofía Morais[2], José Luis Soengas[1]

[1]Universidad de Vigo, Vigo,
[2]LUCTA S. A., Madrid, Spain

Abstract:
The regulation of feed intake in fish is dependent upon different neuroendocrine and metabolic mechanisms including amino acid sensing occurring in the gastrointestinal tract. In previous studies, we demonstrated the impact of specific amino acids alone on such mechanisms and their impact on feeding regulation. However, there is no information regarding the impact of diets with different protein levels or sources on those mechanisms. Therefore, in this study, we evaluated the response of amino acid sensing systems along the gastrointestinal tract (GIT) of rainbow trout (Oncorhynchus mykiss) fed with two different diets differing in the amount of fishmeal and vegetable protein: control diet with 20% fishmeal and 10% soy protein concentrate (NF/LV), and the other with 10% fishmeal and 20% soy protein concentrate (LF/HV). Fish were fed for 4 weeks and feed intake was registered daily with no significant differences found. At the end of the feed experimental trial, we collected samples of different areas of the GIT (stomach, anterior intestine and posterior intestine) and hypothalamus at different times: time 0, after 48h fasted; and times 1, 2 and 3, at 1h, 4h and 24h respectively after refeed. Gastrointestinal levels of mRNAs encoding amino acids receptors and transporters as well as mRNA and protein levels of key gut hormones (GHRL, CCK, PYY, GLP-1) were measured by RT-qPCR and Western Blot, respectively. Moreover, we assessed neuropeptide expression (NPY, AgRP, CART and POMC) in the hypothalamus and enzyme activity of pepsin in the stomach and trypsin and quimiotrypsin in the anterior intestine.

Acknowledgments: Supported by Spanish AEI and European Fund of Regional Development (PID2019-103969RB-C31 and FEDER).

ABSTRACT N° ICBF22-316

GASTROINTESTINAL GLUCOSE ACQUISITION IN LAKE STURGEON (ACIPENSER FULVESCENS) PROVIDING FURTHER INSIGHT INTO A UNIQUE INTESTINAL FEATURE: THE SPIRAL VALVE

Jenna Drummond* [1], Alyssa Weinrauch[1], W. Gary Anderson[1]

[1]University of Manitoba, Winnipeg, Canada

Abstract:
Unique to the gastrointestinal tract (GIT) of non-teleost Actinopterygii (e.g. lake sturgeon, Acipenser fulvescens) and Chondrichthyes is an organ with scroll-like folds termed the spiral valve. Due to its structure, the spiral valve presumably slows down the passage of chyme and increases the gastrointestinal epithelial surface area to maximize nutrient absorption. While morphological data suggests the spiral valve as the primary GIT region of nutrient uptake, there is currently minimal functional data to support this claim. Here we hypothesize that glucose digestion and transport will show regional separation in the GIT of lake sturgeon with elevated levels in the spiral valve. To test this, we assessed glucose digestibility and transport capacity in fed and fasted fish by measuring the activity of the digestive enzyme maltase, relative mRNA abundance of sodium-glucose linked transport protein-1 (SGLT1), and glucose transport rates. Measurements were obtained along the length of the GIT from the stomach, pyloric caeca, anterior intestine, and spiral valve. Maltase activity was highest in the pyloric caeca, suggesting this region as particularly important in glucose digestion. SGLT1 mRNA expression was elevated in the anterior intestine of fed fish, identifying this region as important for glucose absorption. These results indicate that carbohydrates may be primarily digested in the pyloric caeca, therefore liberating glucose for acquisition in the anterior intestine. Thus, the spiral valve may not be the predominant area of dietary glucose absorption as predicted, highlighting the importance of comparing functional and morphological results to determine the roles of GIT regions in digestion.

ABSTRACT N° ICBF22-380

KELP FLY (COELOPA FRIGIDA) LARVAE AS PROTEIN SOURCE IN DIETS FOR RAINBOW TROUT (ONCORHYNCHUS MYKISS)

Niklas Warwas* [1, 2], Xintian Xie[1], Emma Berdan[2, 3], Jonathan Roques[1, 2], Markus Langeland[1, 4], Henrik Sundh[1, 2], Henrik Pavia[1, 2], James Hinchcliffe[1, 2], Elisabeth Jönsson [1, 2], Kristina Sundell[1, 2]

[1]University of Gothenburg ,
[2]Swedish Mariculture Research Center (SWEMARC), Gothenburg ,
[3]University of Stockholm , Stockholm ,
[4]Research Institutes of Sweden (RISE), Gothenburg , Sweden

Abstract:
Insects are among the most promising alternative feed ingredients for salmonid aquaculture but current research is predominantly focused on a few terrestrial species that are generally poor in essential amino- and fatty acids compared to fishmeal. The aim of the present study was to cultivate a marine insect, the kelp-fly (Coelopa frigida, CF), on side streams of the kelp industry (Saccharina latissima) to be tested as a feed ingredient for rainbow trout (Oncorhynchus mykiss). The CF larvae contained 60% protein (3.5% lysine, 1.2% methionine) and 17% fat (of which 5.3% were EPA). A 10-week feeding trial was carried out where 40% of the fishmeal in a control diet was substituted with either CF or commercial black soldier fly meal (Hermetia illucens, HI). A commercial BioMar diet was used as reference. Specific growth rate (SGR) was similar among the CF, control and reference diet while feed intake (FI) was higher in CF and reference diet compared to the control. Compared to HI, CF inclusion increased both SGR and FI. The feed conversion ratio (FCR) of both insect diets increased compared control and reference diets, indicating reduced bioavailability of the insect protein compared to fishmeal. Additionally, net ion transfer in the small intestine increased for fish fed HI compared to CF, possibly due to a higher salt content. The results indicate that C. frigida is a promising feed ingredient. Compared to the black soldier fly meal, C. frigida contained higher levels of methionine, lysine and omega-3 fatty acids, and appeared more palatable.

ABSTRACT N° ICBF22-436

GENOTYPE-INULIN INTERACTION AFFECTS HOST METABOLISM IN RAINBOW TROUT FED A SUSTAINABLE ALL PLANT-BASED DIET

Lokesh Jep[1], Mickael Le Bechec[2], Thierry Pigot[2], Mathilde Dupont Nivet[3], Laurent Labbé[4], Lionel Goardon[4], Thierry Kerneis[4], Stéphane Panserat[1], Karine Ricaud* [1]

[1]Université de Pau et des Pays de l'Adour, INRAE E2S UPPA NUMEA, Saint Pée Sur Nivelle,
[2]Universite de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IMT Mines Ales, IPREM, Pau,
[3]INRAE, Jouy-en-Josas,
[4]INRAE PEIMA, Sizun, France

Abstract:
Inulin affects nutrition, metabolism, and immune status in many animals. Although inulin is widely used in the diet of teleosts, not much is known about its mechanism of action. In this study, we investigated the effect of inulin (2%) on the intestinal microbiome and metabolism in different organs of rainbow trout (Oncorhynchus mykiss; mean weight: 128.6g±8.4) selected for efficient utilization of a 100% plant-based diet (Suave) compared to a control line (Temoin). Metabolic responses to the two factors in liver, intestine, muscle, and adipose tissue were tissue specific, with line and interaction between the two factors influencing overall expression in liver. In the intestine, inulin and lineage and in muscle, lineage influenced the expression of metabolic genes. The microbial signature between the mucus and contents was significantly different, with genera from the Proteobacteria group being more abundant in the mucus, whereas genera from the Firmicutes and Planctomycetes groups were more abundant in the contents. An effect of inulin and the interaction between the lineage and inulin on the microbiome was evident in the contents. The significant features between the control diet and the 2% inulin diet differed greatly, and Streptococcus and Weissella were significantly abundant in the inulin-fed group. In addition, an OTU belonging to the Ruminococcaceae was significantly abundant in suave. The tissue- specific correlations between OTUs and gene expression may indicate the link between the microbiome and metabolism. Taken together, these results suggest a significant effect of inulin on rainbow trout metabolism through the action of specific members of the microbiota and their metabolites.

ABSTRACT N° ICBF22-144

ACCLIMATION TO ELEVATED TEMPERATURES INDUCES TRANSCRIPTOME WIDE PATTERNS OF POPULATION-SPECIFIC RESPONSES IN DEVELOPING LAKE STURGEON (ACIPENSER FULVESCENS)

William Bugg[*,1], Matt Thorstensen[1], Katie Marshall[2], Gary Anderson[1], Ken Jeffries[1]

[1]University of Manitoba, Winnipeg,
[2]University of British Columbia, Vancouver, Canada

Abstract:
Rising mean and variance in temperatures elevate threats to endangered freshwater species like the lake sturgeon, Acipenser fulvescens. Higher temperatures in early development result in physiological consequences for latitudinally distributed lake sturgeon populations throughout Manitoba, Canada, with alteration of metabolic rate, thermal tolerance, transcriptional responses, growth, and mortality. Thus, we acclimated lake sturgeon from northern and southern populations within Manitoba to current and future projected environmental temperatures of 16, 20, and 24°C for 30 days, and measured gill transcriptional responses using RNAseq. Transcriptional processes demonstrated unique population-specific and acclimation-specific responses to the thermal treatments, as well as conserved molecular responses between northern and southern sturgeon populations. Gene expression profiles revealed a gradient in transcriptional responses consistent with acclimation temperature, and a higher magnitude of transcriptional change observed in the southern lake sturgeon population as temperatures increase. Overall lake sturgeon populations responded to thermal acclimation by upregulating the expression of genes involved in oxygen sensing/transport, pathogen responses, and DNA damage as well as pre and post transcriptional modifications (i.e., methylation, alternative splicing). Further, both populations upregulated gene expression related to damage signaling and repair responses as temperatures increased to 20°C, but the northern population also responded with increases in damage signaling and recruitment of mitochondrial processes involved in energy production. Ultimately, these transcriptional responses highlight molecular consequences of increasing temperatures for divergent lake sturgeon populations during vulnerable early development periods.

02-Physiology of primitive fishes and their relatives

ABSTRACT N° ICBF22-411

ALLOMETRY IN GROWTH AND METABOLISM OF LARVAL GREEN STURGEON AT SUBOPTIMAL REARING TEMPERATURE (ACIPENSER MEDIROSTRIS)

Vanessa Lo* [1], Dennis Cocherell[1], Anne Todgham[1], Nann Fangue[1]

[1]University of California, Davis, Davis, United States

Abstract:
The southern Distinct Population Segment (sDPS) of green sturgeon (Acipenser medirostris) has declined precipitously due to anthropogenic changes to the Sacramento River basin (SRB) in California. Inadequate recruitment is thought to be a major contributor to population decline, but little is known about the mechanisms that regulate recruitment. Temperatures experienced during early life stages can shift developmental trajectories. As river management in the spawning reaches of the SRB are historically managed for cold water salmonids, we assessed how cold temperatures affect embryonic and larval stages for green sturgeon by measuring metabolic rate through development for green sturgeon incubated and reared at suboptimal and normal temperatures: 11℃ and 15℃. We found three distinct developmental periods when assessing the length-weight relationship, but the variability in slope appeared to converge in the third period when the fish completed metamorphosis into their juvenile form. The inflection points characterizing these changes in development occurred at similar lengths and weights (11℃: inflection 1 at 23mm and 0.06g, inflection 2 at 36mm and 0.34g; 15℃: inflection 1 at 23mm and 0.06g, inflection 2 at 37 mm and 0.36g), but 11℃ fish were severely stunted, requiring 118 days post-hatch to achieve the same size of 15℃ fish at 42 days post-hatch. These results suggest that suboptimal temperatures for green sturgeon can be a major detriment for rapid growth needed in larval and juvenile fish to avoid predation and may contribute to their population decline.

ABSTRACT N° ICBF22-315

DIFFERENCES IN WATER CHEMISTRY BETWEEN HATCHERY AND RIVER CONDITIONS EFFECT NUTRIENT ABSORPTION BY LARVAL AND YOUNG-OF-YEAR LAKE STURGEON

Janet Genz* [1], Rachael N. Hicks[1], Trevor Hindman[1], Chazz Edwards[1]

[1]University of West Georgia, Carrollton, GA, United States

Abstract:
Stocking is commonly used for conservation of depleted populations of lake sturgeon (Acipenser fulvescens). The differences in environmental factors between the rearing and stocking environments are predicted to influence growth and survival of stocked juveniles. Furthermore, naturally recruited fish may differ physiologically from stocked fish due to differences in their developmental environments. In this study, growth and nutritional status were compared between lake sturgeon exposed to either hatchery or riverine conditions during the larval and juvenile stages.

Larval lake sturgeon (1-40 dph) were sampled weekly, and measured for growth and whole-body ion accumulation. Ion concentrations mirrored environmental availability, with increased [Ca2+] in hatchery-reared fish, and higher [Zn+] and [Mg2+] in larval sturgeon exposed to river water. Both overall growth and rate of development of protective bony scutes were reduced in the river-water group, clearly indicating that environmental water chemistry is an important driver of ionic regulation in larval lake sturgeon, and influences early growth and development.

Hatchery-reared juveniles from the same cohort were held for 4 months in standard laboratory water, then exposed to river water for 3 weeks. No differences between treatments were observed in growth, condition, or HSI over this exposure duration, but results did suggest that environmental ion availability influences intestinal function. Differences were observed for FCR, intestinal anatomy, and the proximate composition of digesta 6 h post-feeding. Changes to nutritional absorption and allocation in young-of-year lake sturgeon at the time of stocking may have impacts on survival and thus the effectiveness of stocking programs for population recovery.

ABSTRACT N° ICBF22-259

PHYSIOLOGICAL CONSEQUENCES OF ELEVATED TEMPERATURES ON WHITE STURGEON EMBRYOS AND LARVAE

Madison Earhart* [1], Tessa Blanchard[1], Nicholas Strowbridge[1], Phillip Morrison[1], Dan Baker[2], Colin Brauner[1], Patricia Schulte[1]

[1]UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER,
[2]Vancouver Island University, Nanaimo, Canada

Abstract:
White sturgeon (Acipenser transmontanus) are threatened or endangered throughout their range in British Columbia. While rapid declines in larvae and juveniles in these populations is likely caused by many factors, without doubt irregular water discharge from dams, resultant sedimentation changes, and increasing temperatures are playing a role. Thus, it is crucial to understand how current and future river temperatures affect sturgeon thermal phenotypes and associated early life stage survival. To assess the effects of various river temperatures on larval phenotypes and survival we reared Nechako river white sturgeon embryos at three different temperatures from shortly following fertilization to expulsion of their yolk-plugs. As the Nechako River has some inherent temperature control available due to modifying dam discharge, we chose rearing temperatures that embryos and larvae encounter in this system: 14°C, 18°C, or 21°C. Throughout embryogenesis and following hatch, fish were sampled throughout development to assess morphology, metabolism, thermal tolerance, and gene expression. Embryo metabolic rate, measured one day prior to hatch, increased between 14°C and 18°C but not 18°C and 21°C ($P<0.001$). Further, we measured the thermal tolerance of larvae using CTMax. Thermal tolerance, measured as CTMax at the time of yolk-sac plug ejection, increased with acclimation temperature ($P<0.001$). Gene expression across a panel of thermal stress, metabolic, and hypoxia related genes differed among acclimation temperature groups in control fish ($P<0.01$) and less so following exposure to CTMax. Lastly, survival was reduced at both 18°C and 21°C, suggesting reduced likelihood of sturgeon surviving these conditions in the river.

ABSTRACT N° ICBF22-184

DNA DAMAGE RESPONSE IN STURGEON (ACIPENSER RUTHENUS) EMBRYO

Ievgeniia Gazo* [1]

[1]University of South Bohemia in eské Budjovice, Faculty of Fisheries and Protection of Waters, Vodnany, Czech Republic

Abstract:
The early stages of embryo development are characterized by rapid cell proliferation, growth and DNA replication. In externally fertilizing organisms the developing embryo is exposed to a variety of genotoxic stressors throughout development, which include environmental pollution and radiation. Embryos are able to minimize adverse effects of external factors and/or overcome the consequences by using stress response pathways including DNA damage repair. Sturgeons (Acipenseridae) are important in aquaculture, mainly due to production of black caviar and boneless meat. Most of sturgeon species have been classified as endangered. However, very little is known about pathways of DNA damage repair in sturgeon embryos.

In the current study we have analyzed DNA damage response in embryos of small sturgeon (Acipenser ruthenus) exposed to camptothecin (CPT) and olaparib. We have evaluated the level of induced DNA damage by comet assay and western blotting with anti-phospho-H2AX antibody. DNA damage response (DDR) in embryos was evaluated by RNA sequencing and proteomics.

The results of this study indicate that DDR in sturgeon embryos is stage-dependent. Further, we observed a correlation between phenotype formation and changes in transcriptomic and proteomic profiles. CPT and olaparib downregulated oxidative phosphorylation and metabolic pathways, and upregulated pathways involved in nucleotide excision repair, base excision repair, and homologous recombination. The analysis of gene expression revealed several markers of DDR and adaptive stress-response, which could be applied in toxicological studies on fish embryo.

ABSTRACT N° ICBF22-394

FILLING THE GAPS IN THE RESPIRATORY PHYSIOLOGY OF PRIMITIVE FISHES

Charlotte Nelson[1], Peter Allen[2], Colin Brauner[*1]

[1]University of British Columbia, Vancouver, Canada,
[2]Mississippi State University, Starkville, United States

Abstract:
Dogfish (Squalus suckleyi) and rainbow trout (Oncorhynchus mykiss) have very different respiratory physiologies. While rainbow trout possess a large Root effect (reduced O2 affinity and carrying capacity under acidic conditions), red blood cell (RBC) intracellular pH (pHi) protection, a high activity RBC carbonic anhydrase (CA) isoform, and inhibitors of CA in the plasma, they lack plasma accessible CA (paCA) in the gills. Dogfish show a very different pattern. They have no Root effect, no RBC pHi protection, a low activity RBC CA isoform, they lack plasma inhibitors of CA and possess paCA isoforms in the gills. Much work has focussed on characterising these two systems, however whether they represent two end points of a mechanistic spectrum remains unknown. Here we investigated the respiratory physiology of several basal fishes which lie at the phylogenetic intersection of the elasmobranchs and teleosts. From previous work we know that many basal fishes, including sturgeons (Acipenseriformes) and gars (Lepisosteiformes), lack a physiologically relevant Root effect and corresponding RBC pHi protection. We characterised the missing pieces of this puzzle by establishing whether these basal representatives have high or low activity RBC CA, whether they possess gill paCA activity and circulating plasma isoforms, and whether they have plasma inhibitors of CA. We find that the compliment of respiratory characteristics expressed by these basal representatives is far from simple, and suggest that dogfish and rainbow trout, which have previously been held as model species for the elasmobranchs and teleosts respectively, should instead be viewed as opposing ends of a highly variable spectrum.

ABSTRACT N° ICBF22-446

GROWTH, ACTIVITY, AND DEVELOPMENT OF DIPLOID AND TRIPLOID WHITE STURGEON

Anne Todgham* [1], Michaiah Leal[1], Joel Van Eenennaam[1], Andrea Schreier[1]

[1]University of California Davis, Davis, United States

Abstract:
Previous studies in our lab have provided evidence of a reduced aerobic metabolic capacity, both at the cellular (metabolic enzyme activity) and the whole organism (aerobic scope) level. However, the downstream costs of this reduced metabolic capacity are still unclear, yet a lower aerobic scope suggests triploid white sturgeon have less energy to allocate to biological process like growth and development. We conducted a 15-week growth trial to assess energy allocation to somatic growth in diploid and triploid white sturgeon. Swimming activity, hepatosomatic index, condition factor, and deformities were measured throughout the growth trial to also examine energy allocation to activity and development. In general, our results indicate that triploid white sturgeon may have not have less energy available for processes beyond maintenance as evidenced by lower weights, more deformities, and less swimming activity when compared with their diploid counterparts. Given this lower performance seen in triploid white sturgeon, aquaculture farms should take special precaution and monitor and manage ploidy of sturgeon.

ABSTRACT N° ICBF22-269

THIRTEEN TISSUE-SPECIFIC TRANSCRIPTOMES FOR THE LAKE STURGEON (ACIPENSER FULVESCENS) REVEAL MECHANISMS OF DIGESTION AND MICROBIOME REGULATION

Matt Thorstensen* [1], Alyssa Weinrauch[1], William Bugg[1], Ian Bouyoucos[1], Gwangseok Yoon[1], Hossein Haghighi[1], Ken Jeffries[1], W. Gary Anderson[1]

[1]University of Manitoba, Winnipeg, Canada

Abstract:

Lake sturgeon (Acipenser fulvescens) are octoploid, long-lived fish that face conservation challenges across their range. The development of tissue-specific transcriptomes enables molecular analyses of physiological responses to ecological and anthropogenic influences. A tissue-specific perspective to gene expression allows analyses of shared and divergent regional functions. Here, we present 13 tissue-specific transcriptomes for lake sturgeon: brain, gill, head kidney, white muscle, liver, heart, esophagus, glandular stomach, muscular stomach, pyloric caecum, anterior intestine, spiral valve, and rectum. The transcriptomes for each tissue were sequenced and assembled individually from a mean of 57.9 million (±6.0 million std. dev.) reads each. Gene set enrichment analyses were performed on transcriptome annotations, with tissue-specific implications for lake sturgeon physiology. By focusing on annotated genes, we found patterns of immune regulation and microbial diversity along gut tissues. In the pyloric caecum, genes related to photoperiodism and entrainment of circadian clocks were observed, which indicated the potential for periodicity in the physiology of lake sturgeon digestion. In addition, shared functions across all tissues were identified, such as those related to cell division, mitochondrial activity, and chromatin organization. The transcriptomes were used as a resource for research on lake sturgeon molecular physiology, used in various applications including thermal tolerance, thermal acclimation, diet, and hypoxia. These transcriptomes represent a publicly available resource, with applications to both conservation programs and fundamental research.

02-Physiology of primitive fishes and their relatives

ABSTRACT N° ICBF22-542

ADAPTATIONS FOR ENVIRONMENTAL CHANGE IN A PRIMITIVE GENERALIST SPECIES: RESPONSES OF ALLIGATOR GAR TO SALINITY

Peter Allen* [1], Tibor Pechan[1], Jonathan Wilson[2], Olga Pechanova[1], Claudio Alvarez[3], Brandon Sorrell[1], Orion Rivers[1]

[1]Mississippi State University, Mississippi State, United States,
[2]Wilfrid Laurier University, Waterloo, Canada,
[3]Centro de Estudios Avanzados en Zonas Aridas, La Serena, Chile

Abstract:
The seven extant members of the Order Lepisosteiformes or gars possess many primitive characteristics and span a wide range of habitats, including freshwater rivers, estuaries, and saline coastal marshes. Euryhalinity is important to many populations, but relatively little is known about adaptations to compensate for salinity differences. This information is important for their conservation, culture and restoration. Therefore, several experiments were conducted to better understand physiological changes associated with acclimation to a range of salinities in alligator gar (Atractosteus spatula). To guide an understanding of changes in mucosal composition which may be important for general health and immune defenses, mucus peptides were compared between gar acclimated to freshwater (0 ppt) or saline water (20 ppt). Scanning and transmission electron microscopy were used to examine changes in gill ultrastructure and compared with gill enzyme activities and localization of ion transporters via immunohistochemistry. In addition, to understand the ability to rapidly compensate for a change in salinity, gill and kidney proteomic responses were evaluated during a time-course of salinity acclimation, alongside blood osmoregulatory measurements. Collectively, results of this study will be discussed in the context of providing a baseline of information important for guiding conservation practices for gar.

ABSTRACT N° ICBF22-292

THE ROLE OF SALINITY IN RECOVERY OF WHITE STURGEON (ACIPENSER TRANSMONTANUS) FROM EXHAUSTIVE EXERCISE

Ryan Shartau[1], Jacelyn Shu[2], Daniel Baker* [3]

[1]University of Texas at Tyler, Tyler, United States,
[2]University of British Columbia, Vancouver,
[3]Vancouver Island University, Nanaimo, Canada

Abstract:
The consequence of salinity levels on recovery from exercise is still poorly characterized, despite theoretical implications of its importance. Here we used the anadromous white sturgeon Acipenser transmontanus as a model organism to investigate the effects of differing salinity (0 ppt, 10 ppt, and 20 ppt) on recovery from exhaustive exercise induced through manual chasing. This challenge elicited the traditional physiological responses such as ion homeostasis disturbance, increases in secondary stress indicators and metabolic acidosis; however, environmental salinity altered both the magnitude of change and the timing of recovery in some of the parameters measured. In particular, the magnitude of the intracellular pH disturbance in both heart and red blood cell appeared to be mediated in freshwater, while the recovery of plasma chloride and bicarbonate ions to baseline was slightly more rapid in higher salinity. In general, responses were similar but not identical, leading us to conclude that the role of salinity on recovery from exercise is complex but not insignificant, and may play a role in behaviors exhibited by white sturgeon in their respective saline environments. This work has implications both for the iono-regulatory mechanisms associated with anadromy in white sturgeon, but also for the current catch-and-release industry, which may be angling white sturgeon in freshwater areas with an undetected halocline, thus potentially putting this endangered species at further risk.

ABSTRACT N° ICBF22-515

USING PHYSIOLOGY TO HELP RESOLVE THE CONFLICTING GOALS OF LAKE STURGEON (ACIPENSER FULVESCENS) CONSERVATION AND INVASIVE SEA LAMPREY (PETROMYZON MARINUS) CONTROL IN THE GREAT LAKES.

Michael P Wilkie* [1], Oana Birceanu[2], Scott LJ Hepditch[3], Jonathan M Wilson[1], Lisa O'Connor[4]

[1]Dept. Biology, Wilfrid Laurier University, Waterloo,
[2]University of Western Ontario, London,
[3]Institut National de la Recherche Scientifique, Centre Eau Terre Environment , Québec,
[4]Great Lakes Laboratory for Fisheries and Aquatic Sciences ,Fisheries and Oceans Canada, Sault Ste. Marie, Canada

Abstract:
There is concern that lake sturgeon (LS) population recovery initiatives in the Laurentian Great Lakes could be compromised by efforts to control invasive sea lampreys using the pesticide 3-trifluoromethyl-4-nitrophenol (TFM). TFM is applied to larval lamprey nursery streams, which may overlap with the habitat of juvenile LS, which are particularly vulnerable to TFM in their early life stages and in waters of high alkalinity. Our goals were to determine how TFM sensitivity and accumulation by LS was influenced by (i) water alkalinity and (ii) life stage, and (iii) to refine TFM application protocols to minimize non-target mortality in LS. Survival studies revealed that LS tolerance to TFM increased at higher (350 mgL-1 CaCO3) compared to lower water alkalinity (50 mgL-1 CaCO3) due to reductions in TFM bioavailability. However, alkalinity limited TFM toxicity more in sea lamprey than in LS, making LS more vulnerable to typical TFM treatment doses. TFM uptake rates, measured using 14C-TFM, were significantly higher in smaller, age 0+ LS compared to larger, 1+ LS, explaining why younger LS were more susceptible to TFM. When TFM was applied for longer periods (24h vs 12h), but at lower concentrations than typical field doses, toxicity was reduced in LS, but not in sea lamprey which had lower capacity to detoxify TFM. We conclude that delaying lampricide treatments to late summer/fall when LS are larger and using a “long and low” protocol to apply TFM in waters containing vulnerable LS, can protect them from TFM toxicity without compromising sea lamprey control.

ABSTRACT N° ICBF22-248

PHENOTYPIC FLEXIBILITY OF LONG-LIVED WHITE STURGEON

Angelina Dichiera* [1], Madison Earhart[1], William Bugg[2], Rachael Penman[1], Colin Brauner[1], Patricia Schulte[1]

[1]The University of British Columbia, Vancouver,
[2]The University of Manitoba, Winnipeg, Canada

Abstract:
The ability of a single genotype to display phenotypic variation in response to environmental change is thought to be especially beneficial in dynamic environments that fluctuate on a fine temporal or spatial scale. However, little research has focused on how slow-growing, long-lived species may also display similar phenotypic flexibility regardless of whether their environment is dynamic or static, as they encounter environmental change over large temporal scales. White sturgeon (Acipenser transmontanus) represent one of the largest and longest-lived freshwater fish in North America, with lifespans that extend decades. Unfortunately, in addition to historical overfishing and current range restrictions, white sturgeon of the Fraser River (British Columbia) must increasingly withstand elevated water temperatures and decreased oxygen availability. Thus, thermal and hypoxia sensitivity are growing concerns for species conservation. Previous data from early life stages demonstrate that the long-lived species is more tolerant to thermal stress than other short-lived species of the same environment. We expect this intrinsic tolerance is extended to subadult and adult life stages, and that these individuals also demonstrate tolerance across stressors (e.g., high critical thermal maxima and low critical oxygen thresholds). However, we also expect absolute tolerance is reduced in these older life stages, which are more capable of avoiding minor disturbances in suboptimal environmental conditions than early life stages. Importantly, this understanding of phenotypic flexibility across life history stages will allow us to more holistically assess if long-lived white sturgeon can adapt to environmental change.

ABSTRACT N° ICBF22-300

ALLELE SPECIFIC EXPRESSION REVEALED IN RNA-SEQ DATA IS ASSOCIATED WITH TILAPIA RESPONSE TO SALINITY CHALLENGE

Aurora Campo[1], Avner Cnaani* [1]

[1]Agricultural Research Organization, Rishon LeZion, Israel

Abstract:
Allelic imbalance (AI) is a phenomenon that depicts cis- and trans-effects on gene expression regulation, can illustrate imprinting events, and emphasise the importance of genetic variation in the level of the individual organism. It is an arduous effort to retrieve and characterize AI due to the multiple sources of bias present in a pipeline for RNA-seq and variant calling tasks. While tools for variant calling and AI detection have successfully used to score individual variability, very few of them spread outside of the medical research to be applied into the aquaculture field. In the present study, we introduce a pipeline to detect AI reducing the bias in critical steps such as genome mapping. The identification of significant AI is resolved by binomial test after removal of monoallelic gene expression. We employed the pipeline on transcriptome data of tilapia grown in fresh and salty water. We then validated the pipeline SNP calling from the transcriptome with whole genome sequencing of the same individuals. Our results indicate that specific imbalanced expression of alleles is a further factor in the fish transcriptomic response to a changing environment. Calculation of allele frequencies confirmed differences in these AI between tissues, revealing further differences in allele expression related to water salinity. Overall, AI analysis add an additional layer of information that can be obtained from transcriptome sequences, and enable wider view of genetic basis underlying phenotypic variation.

ABSTRACT N° ICBF22-136

METABOLIC DEPRESSION IN NILE TILAPIA AS A MECHANISM TO COPE WITH SEVERE HYPOXIA

Julie Hansen Bergstedt* [1], Tilo Pfalzgraff[1], Peter Vilhelm Skov[1]

[1]Technical University of Denmark, Hirtshals, Denmark

Abstract:
Nile tilapia (Oreochromis niloticus) is well known for its tolerance to hypoxic conditions, a trait that has contributed to the success in aquaculture. However, the coping mechanisms and metabolic response to the increasing duration and severity of hypoxia have not been fully accounted for. In the present study, the effects of hypoxia on metabolic rate and recovery were examined through measured oxygen uptake (MO2). Nile tilapia were exposed to periods of prolonged severe hypoxia at 2.1 kPa, lasting between 2 and 24 H. To assess tolerance to severe hypoxia, the fish were subjected to decreasing pO2 to determine the critical oxygen saturation (Pcrit), in addition to the lower pO2 threshold where loss of equilibrium (LOE) occurred. When exposed to hypoxia Nile tilapia show a considerable capacity for metabolic rate depression for up to 24 h, with little excess post-hypoxic oxygen debt upon restoration of normoxia. A severe decrease in pO2 further demonstrated the tolerance to severe hypoxia, as LOE did not occur before <0.4. Measurement of blood metabolites and pH showed a significant acidosis and elevation in glucose and lactate during oxygen levels that caused LOE. Conclusively, the study shows that metabolic depression is employed as a strategy to cope with severe hypoxia and that Nile tilapia do not switch to anaerobic metabolism due to hypoxia until pO2 <2.1 kPa.

ABSTRACT N° ICBF22-469

HOMOLOGOUS EXPRESSION SYSTEM FOR IN VITRO CHARACTERIZATION OF ENVIRONMENTAL EFFECTS ON TILAPIA PEPTIDE TRANSPORTERS.

Pazit Con*[1,2], Jens Hamar[3], Dietmar kueltz[3], Avner Cnaani[2]

[1]The Hebrew University of Jerusalem, Rehovot,
[2]Agricultural Research Organization - The Volcani Institute, Beit Dagan, Israel,
[3] University of California Davis, Davis, United States

Abstract:
The peptide transporter (PepT) systems are well-known for their importance to protein absorption in all animal species. These symporters use proton gradient at the cell apical membrane to mediate the absorption of small peptides. In fish, there are three peptide transporters variants, all found to express in the intestinal epithelia (enterocytes). The fish intestine is a multifunctional organ, involved in osmoregulation, acid-base regulation, and nutrient absorption. With the continues flow of water into the intestine through eating and drinking, the enterocytes are constantly exposed to changes in environmental factors, which can affect the function of various pumps, transporters and channels mediating transmembrane movement of different substances between the enterocytes and the intestinal lumen environment. Tilapia is an important aquacultured fish as well as an emerging model for environmental physiology research. In order to study the functionality of PepTs under different environmental conditions related to fish physiology, we have established homologous expression system, based on tilapia cell line, to study PepT systems activity. The Tmb cell line is an endothelial cell line originated from Mozambique tilapia, that had been shown as useful tool for studying the response to osmotic stress. In this study, we have established three modified Tmb cell lines, each of them expressing only one of the PepT variants. The expression and functionality of the three transporters was verified using PCR and fluorescence imaging of the dipeptide β-Ala-Lys(AMCA) accumulation in the cells. The absorption of β-Ala-Lys(AMCA) dipeptide was tracked under different environmental conditions.

ABSTRACT N° ICBF22-166

EFFECTS OF TEMPERATURE ON PROLACTIN RELEASE FROM THE TILAPIA PITUITARY

Andre P. P. Seale*[1], Daniel W. Woo[1], G.H.T. Malintha[1], Fritzie Celino-Brady[1], Yoko Yamaguchi[2], Jason P. Breves[3]

[1]University of Hawai'i at Mnoa, Honolulu, United States,
[2]Shimane University, Matsue, Japan,
[3]Skidmore College, Saratoga Springs, United States

Abstract:
Prolactin (PRL) cells of the Mozambique tilapia, Oreochromis mossambicus, have historically enabled investigations into how osmoreception is linked with adaptive patterns of hormone secretion. PRL cells exist as a nearly homogenous population in the rostral pars distalis (RPD) and rapidly respond to a hyposmotic stimulus by releasing PRL188 and PRL177, the two isoforms of PRL produced in this species. In addition to its wide salinity tolerance, O. mossambicus is known to survive temperatures ranging from 14 to 38 ºC. While the responses of tilapia PRL cells to hyposmotic stimuli are well documented, the effects of temperature on the operation of these cells have not been investigated. In the current study, we described the effects of incubation temperature (20, 25, and 30 ºC) on hormone release and mRNA expression of prl188 and prl177 from both RPDs and dispersed cells. Release of both PRLs increased by 6 and 24 h after a rise in temperature; gene expression of prl188, however, was inversely related to temperature, while prl177 was unaffected. Gene expression of the stretch-gated transient potential vanilloid 4 (TRPV4) channel was induced in cells incubated at 25 and 30 ºC compared with cells incubated at 20 ºC. In parallel, PRL cell volume increased with a rise in temperature. Our findings provide the first evidence that PRL cells of tilapia are sensitive to temperature and may thus mediate adaptive hormonal responses to fluctuations in environmental temperature. Supported by NSF (IOS-1755016 and -1755131), NIH (1R21DK111775-01), NOAA/UH Sea Grant (#NA18OAR4170347) and NIFA Hatch (HAW02051-H).

ABSTRACT N° ICBF22-332

METABOLOMICS MEETS MACHINE LEARNING: DYNAMIC VISUALIZATION OF ENERGY TRADE-OFF FEATURES UNDER TEMPERATURE VARIATIONS IN TILAPIA

Min-Chen Wang[1], Hui-Wen Peng[1], Yung-Che Tseng*[1]

[1]Marine Research Station, Institute of Cellular and Organism Biology, Academia Sinica, I-Lan, Taiwan

Abstract:
Plenty of studies proved that ectothermic fish showed adaptive capacities to ambient temperature changes through phenotypic and molecular adjustments. Those studies also investigated the mechanisms by which ectothermic fish respond to and survive in temperatures beyond thermal tolerance limits. These cellular protections against stress require the orchestration of many energy-dependent processes, and animals must allocate limited energy resources to maintain homeostasis and stress resistance. Therefore, delicately utilizing efficiency in different metabolic pathways and energy fuels is essential for energy maintenance under temperature changes. However, previous research endpoints can only reflect the primary metabolic responses by conventional approaches.

The machine learning (ML) technique has been successfully applied to assist biological/medical professionals and researchers in improving clinical prediction and drug screening in the past decade. ML applications in pre-processing massive datasets from the conventional metabolic performances estimation and metabolomics profiling could also benefit the identification and visualization of regime projections. In this study, we utilized the applicability of ML to metabolic performance assay and metabolomics while representing some examples in tilapia, from juveniles to adults. We emphasized the application of ML in projecting a high-dimensional dataset onto a low-dimensional space to visualize the heterogenety of trackling energy trade-off features in metabolite identification, metabolic pathways and marker discovery. Finally, we discuss how ML could reveal potential mechanisms of energy trade-off regulations and will be an essential research topic on dynamic physiological balances in fish under the future environmental system.

03-Environmental Physiology of Cichlids

ABSTRACT N° ICBF22-407

THE HOMEOSTATIC RESPONSE TO COLD STRESS OF NILE TILAPIA IS CENTRALLY REGULATED BY OXYTOCIN

Jakob Biran* [1], Adi Segev-Hadar [1], Anouk M. Olthof[2], Rahul Kanadia[2], Avner Cnaani[1]

[1]Agricultural Research Organization, Rishon LeTsiyon, Israel,
[2]University of Connecticut, Storrs, United States

Abstract:
As poikilotherms, cultured fish exposed to environmental extremes of cold temperatures experience a stressful metabolic challenge, which elicits physiological responses required to maintain cellular homeostasis. These responses include modified glucose or lipid metabolism, altered gene expression and alternative splicing, and endocrine and immune system activity. Additionally, heat seeking may not resolve the homeostatic needs of tropical poikilotherms under unpredictable extreme cold events, which occur frequently due to global climate change and aquaculture conditions. In spite of the physiological responses that occur in poikilothermic vertebrates, the prevailing notion is that these reactions are passive. Here, we explored molecular hypothalamic and physiological responses to cold stress in the tropical cichlid, Nile tilapia (Oreochromis niloticus). When exposed to cold, tilapia exhibited complex homeostatic responses, including plasma glucose and cortisol concomitant with reduced plasma lactate and metabolic rate. Hypothalamic transcriptome analysis revealed increased oxytocin expression. Blockage of oxytocin signaling using zebrafish knockout lines for oxytocin or its receptors, and pharmacological antagonist in tilapia, further affected temperature-dependent metabolic rate in two cold-exposed fish species. This indicates that oxytocin, a known thermoregulator in homeotherms, actively regulates temperature-related homeostasis in fish. Overall, our findings show that the fish brain actively responds to cold temperature by regulating metabolic physiology. Moreover, we identify oxytocin signaling as an adaptive and evolutionarily conserved metabolic regulator of temperature-related homeostasis.

ABSTRACT N° ICBF22-524

TEMPERATURE-INDUCED MASCULINISATION OF NILE TILAPIA: EFFECTS SEEN IN THE ADULT GONAD

Helena D'cotta* [1], Rokyatou Sissao[2], Laureana Rebordinos[3], Christoph Grunau[4], Khalid Belkhir[5], Cristian Chaparro[4], Aboubacar Toguyeni[2], Jean-François Baroiller[1]

[1]CIRAD-ISEM, Montpellier, France,
[2]UR ABAQ, Université of N Boni, Bobo-Dioulasso, Burkina Faso,
[3]Área de Genética, Fac. de Ciencias del Mar y Ambientales, Universidad de Cadiz, Cadiz, Spain,
[4]UMR-IHPE, Université de Perpignan, Perpignan,
[5]UMR-ISEM, Montpellier, France

Abstract:
Sex of Nile tilapia is under a complex control. It is determined by sex chromosomes following an XX/XY system, but it is also influenced by parental factors and by temperature. Elevated temperatures >32°C applied during early sex-differentiation can induce a female to male sex reversal producing functional testis and a male reproductive behaviour. The male proportion induced by temperature (the thermosensitivity) is an inherited trait, and varies between families, strains and populations. We now know that one of the mechanisms by which temperature exerts its masculinization effects is through the methylation of the DNA. Here we analyzed the effects of high temperature induced-masculinization in adult gonads between two families showing respectively a high and a low thermosensitivity. After fry exposure to 36°C, Family K14 had 90% male proportion whereas, Family K13 only showed 59.8% males. We compared the methylation marks persistent in testis and ovaries of the 27°C and 36°C exposed groups from both families and found differences in DNA methylation. Likewise, differences in RNA expressions were also seen. Correlation between DNA methylated levels and gene expressions will be discussed.

ABSTRACT N° ICBF22-521

EVIDENCE FOR SEX REVERSAL IN NILE TILAPIA IN LAKE KOU (BURKINA FASO): SHALLOW WATER TEMPERATURES PLAY A KEY ROLE

Rokyatou Sissao[1, 2], Helena D'Cotta* [3, 4], Jean-François Baroiller[3, 4], Aboubacar Toguyeni[2]

[1]Institut de l'environnement et de recherches agricoles, Centre national de la recherche scientifique et technologique,
[2]Laboratoire d'etudes et de recherche sur les ressources naturelles et sciences de l'environnement, Université Nazi BONI, Bobo-Dioulasso, Burkina Faso,
[3]UMR-ISEM, CNRS, Montpellier University, IRD, EPHE,
[4]UMR-ISEM, CIRAD, Campus International de Baillarguet, Montpellier, France

Abstract:
Temperature-induced sex reversal has been widely reported in Nile tilapia. While most studies under controlled conditions show that high temperatures can masculinize XX individuals, rare studies suggest a feminizing effect of such treatments in YY genotypes. The effects of low temperature treatments have rarely been explored. In the wild, this species can be exposed to various thermal regimes/ranges that may influence sexual differentiation of undifferentiated fry. Temperature monitoring over a year in the shallow areas where undifferentiated Nile tilapia fry live revealed periods of high (32-42°C) and low (12-20°C) temperatures in Lake Kou. Simulation of "hot periods" (27-36°C) under controlled conditions induces a high proportion of males compared to the control group (27°C). Sexual genotyping using amh markers, specific for X and Y chromosomes, confirmed the masculinizing effects of thermal fluctuations and allowed the identification of an XX male in Lake Kou. Progeny tests confirmed this sexual genotype. These results suggest the probable masculinization of thermosensitive XX fry induced by high temperature fluctuations in the shallow areas of the lake. Regarding cold temperatures, although constant treatment at 20°C leads to a balanced sex ratio, some XY females were nevertheless identified in the treated batch. However, no XY females were identified among the wild-caugth individuals in the lake, questioning the physiological reality of a possible feminizing effect of low temperatures.

13-Intraspecific and inter-individual variation in fishes facing environmental challenges and stressors

ABSTRACT N° ICBF22-273

INTRASPECIFIC VARIATION IN TOLERANCE TO ENVIRONMENTAL STRESSORS

Patricia Schulte* [1]

[1]University of British Columbia, Vancouver, Canada

Abstract:
Rapid adaptation from standing genetic variation has the potential to buffer populations against environmental change, but the extent of variation among individuals in genes encoding climate-change relevant traits remains poorly understood. Here I present data from our studies in a variety of fish species, exploring the extent of phenotypic variation in upper thermal tolerance and hypoxia tolerance and its genetic basis. There is substantial variation in both upper thermal tolerance and hypoxia tolerance within and between populations of all of the species examined, but the extent of correlation between these traits differs among species, populations, and even among brood years of a single species, suggesting that these two traits can evolve independently and that there is no evidence of a functional trade-off between them. Using genotyping-by-sequencing and other approaches for high-throughput genotyping at a whole-genome level, we have explored the genetic basis of variation in these traits. These data suggest that both thermal and hypoxia tolerance have a complex underlying genetic basis in which variation at multiple genes, each with relatively small effect, is involved in determining the phenotype, and that the genes involved do not overlap between the traits. Taken together, these results suggest that there is potential for fish to undergo at least some adaptive evolution in response to climate change.

ABSTRACT N° ICBF22-301

THE EFFECT OF TEMPERATURE AND GROUP COMPOSITION OF METABOLIC PHENOTYPES ON LEADERSHIP AND COLLECTIVE BEHAVIOURS IN FISH

Lucy Cotgrove* [1], Shaun Killen[1]

[1]University of Glasgow, Glasgow, United Kingdom

Abstract:

Cotgrove, L., Persson, A. S. M., Pettinau, L., Crespel, A., Jolles, J., Marras, S., Norin, T., Domenici, P., Killen, S.

Group living is ubiquitous among taxa and comes with costs and benefits associated with predator avoidance, foraging and reproduction. There is increasing evidence that consistent behavioural differences or personalities can drive collective behaviour but so far the physiological underpinnings in such processes are rarely considered. For ectotherms, collective group behaviour seems to be an emergent property of the phenotypes present within the group, particularly with regard to traits related to individual activity rate and energy use. We know very little about how environmental temperature affects group behaviours, despite known effects of temperature on individual activity level and metabolic rate. Using common minnows (Phoxinus phoxinus), we measured the standard metabolic rate (SMR) of fish and examined the routine shoaling behaviour of free-swimming groups in an open field (9 fish per group) using three different group compositions: (1) homogenous low SMR;(2) homogenous high SMR; and (3) mixed SMR (equal distribution of high, medium and low SMR). Each group was tested at two different temperatures (15 and 18oC) and each fish tracked individually. Novel results will be presented showing how metabolic composition of groups affect their cohesion, structure and coordination. We also examined how group leadership of was related to metabolic phenotype, and how leadership was affected by shifting temperatures. Our results provide insight into the mechanistic underpinnings of group functioning, and in a wider perspective, how changing environmental temperatures may affect the functioning of fish social groups.

13-Intraspecific and inter-individual variation in fishes facing environmental challenges and stressors

ABSTRACT N° ICBF22-242

INTER-INDIVIDUAL VARIATION IN MITOCHONDRIAL PHOSPHORYLATION EFFICIENCY PREDICTS TIME IN SHELTER FOR JUVENILE SALMON (SALMO SALAR)

Neal Dawson* [1], Darryl Mclennan[1], Agnieszka Magierecka[1], Neil B. Metcalfe[1]

[1]University of Glasgow, Glasgow, United Kingdom

Abstract:

At cold temperatures juvenile salmonids typically spend much of their daytime sheltering from predators, preferring to forage at night. The proportion of time in refuges is thus negatively related to their need for food, suggesting a link to metabolism. There is increasing evidence that individual variation in metabolic rate is linked to variation in mitochondrial function. Previous work on salmonids has shown that inter-individual variation in mitochondrial efficiency can explain variation in food intake and growth rates. Therefore, our aim for this study was to examine if inter-individual variation in mitochondrial efficiency can help predict foraging patterns or time spent sheltering for overwintering juvenile salmon (Salmo salar). PIT-tagged salmon of wild origin were housed individually under winter conditions and their use of a shelter recorded automatically. In line with previous research, fish showed a broad preference for hiding in the shelter during the day and emerging to feed at night, but exhibited marked among-individual variation in use of shelter. This was unrelated to body size but was predicted by mitochondrial function: there was a positive relationship between muscle mitochondrial phosphorylation efficiency and proportion of time spent in the shelter during the night, but not during the day. This is to say, the individuals with the most efficient mitochondria were able to spend more time safe from predation risk. This suggests that individual heterogeneity in cellular function may drive variation in the foraging or sheltering patterns of aquatic animals, which has implications for selection pressures acting on wild populations.

13-Intraspecific and inter-individual variation in fishes facing environmental challenges and stressors

ABSTRACT N° ICBF22-339

MORPHOLOGICAL VARIATION OF FISH ALONG ENVIRONMENTAL GRADIENTS IN MOROCCAN DESERT RIVERS (CASE OF LUCIOBARBUS BARBELS)

Abdelghani Guechchani* [1], Miguel Clavero[2], Mohamed Naimi[3], Mohammed Znari[1], Elisabeth Berger [4]

[1]Water, Biodiversity and Climate Change laboratory [WBCC] - Cadi Ayyad University, Marrakech, Morocco,
[2]Doñana Biological Station - CSIC, Seville, Spain,
[3]Polyvalent Laboratory for Research & Development,Sultan Moulay Slimane University, Beni Mellal, Morocco,
[4]University Koblenz Landau - iESLandau Institute for Environmental Sciences, Landau, Germany

Abstract:
Luciobarbus barbels (Fam. Cyprinidae) are the most important components of the ichthyofauna of Moroccan Desert Rivers. In spring 2013 we sampled fish communities at 88 sites in the Draa, Ziz-Gheriss and Ghir basins, the largest Moroccan river systems, finding barbels (L. lepineyi and L. pallaryi) in 64 of them. Both barbel presence and abundance had unimodal responses to the main environmental gradient in the area (i.e. upstream-downstream), peaking at intermediate elevations. We analyzed morphological variation by recording 17 morphometric variables in field photographs of 1182 anaesthetized individuals from 56 sites. Gradients of morphological variability were summarized by means of a principal component analysis. Surprisingly, average morphological characteristics were not related to species identity or to the basin of origin of the populations analyzed, but to the environmental conditions experienced by those populations, particularly to those related to water current. These results highlight the flexibility of morphological features of Luciobarbus barbels and the adaptability of these taxa to highly contrasting environments.

ABSTRACT N° ICBF22-143

THE EFFECT OF TEMPERATURE AND PH ON THE METABOLIC RATES AND HYPOXIA TOLERANCE OF REDFISH (SEBASTES SPP.)

Joelle Guitard* [1], David Deslauriers[1], Dominique Robert[1], Denis Chabot[2]

[1]Université du Québec à Rimouski, Rimouski,
[2]Department of Fisheries and Oceans Canada, Mont-Joli, Canada

Abstract:
Understanding the effects of global change, including changes in water temperature, oxygen availability, and pH, on commercially important marine species is crucial to improve current stock assessment practices. After more than 30 years under fishing moratorium, a massive recruitment of redfish (Sebastes spp.) was observed in 2011–2013. Still, little is known about their metabolic and thermal physiology and so it is difficult to predict how they will respond to rapidly changing conditions, including the alarming decreases in dissolved oxygen currently being observed in the deep channels of the GSL. To address this issue, we quantified the effect of four acclimatation temperatures (2.5, 5.0, 7.5 and 10.0 ℃) combined with two pH levels (7.35 and 7.75) on standard and maximum metabolic rates, aerobic scope, and hypoxia tolerance (Pcrit) in redfish (n = 64). We observed that higher acclimation temperatures, within the range of temperatures in our study, translated into higher standard and maximum metabolic rates and aerobic scope. We also found that hypoxia tolerance was higher for fish acclimated to lower temperatures (2.5 and 5.0℃). This suggests that redfish will have increasing difficulty coping with their changing habitats under the rapid rise of both hypoxia and temperature currently occurring in the GSL. These results indicate that while GSL redfish could still live comfortably at temperatures up to 10 ℃, there may be important effects to consider on their long-term energy requirements due to increased maintenance costs.

ABSTRACT N° ICBF22-153

ROLE OF INTER-INDIVIDUAL VARIATION IN FRESHWATER TOP PREDATOR INTERACTIONS AND ADAPTABILITY

Silviya Ivanova* [1], Timothy Johnson[2], Aaron Fisk[1]

[1]University of Windsor, Windsor,
[2]NDMNRF, Picton, Canada

Abstract:
Animal movements are influenced by the environment and their interactions with other species and some species are more sensitive to environmental change and competitors. Understanding individual variation in response to those gradients can improve our predictions of how such species would cope with climate change challenges. Here, we used acoustic telemetry over two-year period to infer a freshwater top predator's variation in behaviour related to habitat preference and quantify the interactions with a competitor species. Lake trout (Salvelinus namaycush), a native top predator in the Laurentian Great Lakes, is considered an indicator species and thus used as model species. Lake trout competes for a common food resource with Chinook salmon (Oncorhynchus tshawytscha), an introduced top predator. Three-dimensional (latitude, longitude and depth) utilization distribution was used to identify habitat overlap in Lake Ontario, and joint potential path area for extracting spatio-temporal co-occurrences. Inter-individual variation was observed for lake trout home ranges, and for post-spawning migration. Overall low mean overlap was observed for lake trout and Chinook in the eastern basin occurring July through October, when most lake trout occupy offshore waters. Lake trout inter-individual variation in habitat preference is a strong determinant for the amount of interactions with Chinook. With predictions for climate change associated increase in water temperatures, the interactions between these species are likely to increase as thermoregulation demands drive more lake trout into offshore areas. However, the existence of inter-individual variation in the populations of sensitive species may be key to their adaptability to new environmental challenges.

13-Intraspecific and inter-individual variation in fishes facing environmental challenges and stressors

ABSTRACT N° ICBF22-399

TEMPERATURE INFLUENCES INTRA-SPECIFIC SCALING OF METABOLIC AND HEART PERFORMANCE IN AN INDICATOR BEACH ZONE SPECIES, BARRED SURFPERCH

Krista Kraskura* [1], Emily Hardison[1], Erika Eliason[1]

[1]University of California Santa Barbara, Santa Barbara, United States

Abstract:
Body size and temperature are two of the most prominent factors shaping physiological performance in ectotherms. Various studies demonstrate that as temperature increases body size decreases, which suggests that larger individuals may be more vulnerable to climate change. The mixed evidence for these trends across taxa begs for a mechanistic physiological framework to better finetune these predictions. To address this, we acclimated live-bearing barred surfperch (Amphistichus argenteus) across a body size range of 5-700 g to 16ºC; a temperature that they commonly see in the wild. We experimentally measured i) metabolic performances (maximum and resting metabolic rates, and aerobic scope; MMR, RMR, AS) after acute (2ºC/h rate) temperature change from 16ºC to ecologically relevant 12ºC, 20ºC, and a predicted warm 22ºC, and ii) heart thermal performance in the same individuals. We found temperature dependent and performance metric-specific scaling relationships. The scaling of AS, MMR, and RMR was strongly hypoallometric suggesting a decrease in mass specific metabolic performance with body mass. Also, maximum heart rates decreased with body size but increased with temperature. We also found a clear correlation between cardiac thermal capacity and metabolic thermal limits, which suggests that ~22ºC is a functional thermal limit for barred surfperch. Our work provides a case study demonstrating that different physiological constraints may operate with growing size at species-specific levels. Scaling up, this work provides evidence that complex physiological mechanisms may be responsible for changing size-structures in communities, and ultimately ecosystem stability and function.

ABSTRACT N° ICBF22-141

ECOTOXICOLOGICAL VIEW ON THE EVOLUTION OF CANCER DEFENCES: INTER- AND INTRASPECIFIC ADAPTATIONS IN FISHES

Randel Kreitsberg* [1], Ciara Baines[1], Richard Meitern[1], Tuul Sepp[1]

[1]University of Tartu, Tartu, Estonia

Abstract:
Current knowledge of natural cancer defence mechanisms is limited, often using model organisms living in controlled environments. In natural habitats, anthropogenic contamination has resulted in increases of oncogenic substances. Studies have shown that wild populations can adapt to highly contaminated environments. We suggest that this process can be used to study the evolution of cancer defences, and polluted seas provide the perfect ‘wild laboratories’ for this.

Moreover, gene databases allow us to gain novel information about the evolution and function of these genes in different species, but also to understand cancer as a driving force in biological systems and species life histories. As fish are evolutionarily old and a genetically diverse group, comparative studies with cancer-related genes in different species could yet be a largely unexplored treasure trove for understanding the evolution and ecology of cancer.

Here, we provide an overview of two studies on adaptations to cancer defences:

First, intraspecific research on different populations of the flounder (Platichthys spp.) and dab (Limanda limanda) in the North and Baltic Seas. Flatfish populations inhabit the whole gradient of contamination. We have found different prevalences of liver tumours and cancer related gene expressions along that gradient.

Secondly, we present a comparative study of cancer-related gene copy number variation (CNV) in different fish species. Our study demonstrates a relationship with cancer-related CNV and maximum lifespan in fish species, suggesting that higher tumour suppressor gene CNV lengthens and oncogene CNV shortens lifespan. In addition, other potential defence mechanisms, including antioxidant defences and biotransformation, are discussed.

ABSTRACT N° ICBF22-479

RESPONSE TO TRANSPORTATION CHALLENGE OF RAINBOW TROUT ISOGENIC LINES

Isabelle Leguen* [1], Sandrine Peron[2], Thierry Kerneis[3], Lionel Goardon[3], Edwige Quillet[4], Patrick Prunet[2]

[1]INRAE-MARBEC, Montpellier,
[2]INRAE, Rennes,
[3]INRAE-PEIMA, Sizun,
[4]INRAE-GABI, Jouy-en-Josas, France

Abstract:
Successive or cumulative exposure to stressors may compromise adaptive capacity of fish and lead to allostatic overload and poor welfare. Among other breeding practises, transportation of fish is well known for involving a multitude of stressful practises including handling for capture and transport itself with confinement, noise, vibrations and exposure to deteriorated water quality which overall induce severe physiological stress responses. A previous study suggests intraspecific and individual differences for the trait 'coping with transportation stress". The present study aimed to contribute to our understanding of the genetic basis of this trait using isogenic trout lines. The effect of transportation stress was measured on 6 heterozygous rainbow trout isogenic lines. This stress transportation was a confinement stress (200 kg/m3) for 3 hours in bags inflate with oxygen at 10-12°C. Parameters analyzed in water and gill tissue are in relation with gill physiology (ionic and acid-base balances, nitrogen secretion, physical and chemical protections and organ regulation-gill remodeling). O2, CO2, N-NH4, Cl-, Na+, Ca2+ and pH were measured in water. Gene expression and Na/K-ATPase activity were performed in the gills tissue. In conclusion, use of isogenic trout lines appears a very fruitful strategy to study genetic basis of the trait 'coping with transportation stress'. It showed that genetic variability exists for this complex trait leading to high or low responsive lines. Results suggest an important role of gill physiology to support this trait variability

ABSTRACT N° ICBF22-503

MULTIGENERATIONAL EXPOSITION TO WARMING REDUCES MITOCHONDRIAL OXYGEN FLUXES IN THE MEDAKA FISH (ORIZIAS LATIPES)

Julie MORLA* [1], Remy LASSUS[1], Karine SALIN[2], Arnaud SENTIS[1], Martin DAUFRESNE[1]

[1]INRAE, Aix en provence ,
[2]ifremer, Plouzané, France

Abstract:
Thermal conditions experienced by previous generations can lead to modifications of metabolic efficiency in offspring, which is determining for their response to global warming. Mitochondria are crucial as they generate adenosine triphosphate (ATP) energy through oxidative phosphorylation but also produce harmful Reactive Oxygen Species (ROS). Warming can increase cellular oxygen demand (higher energy production) and ROS production (higher cellular damages).
To determine whether thermal acclimation can modulate thermal effects we investigated the impact of a multigenerational exposure to high or low (30°C or 20°C) temperature on the mitochondria response in the medaka fish at two acute temperature (20 or 30°C). We hypothesized that a long-term exposure to 30°C can lead to either (1) higher oxygen fluxes compared to fishes maintained at 20°C indicating a live fast die young strategy (higher reproduction and growth but shorter lifespan). (2) Lower oxygen fluxes and respiration required to compensate proton leakage (RCR) potentially indicating modifications in mitochondrial density and activity or a warm-multigenerational acclimation by uncoupling.
Our results indicate a decrease in mitochondrial oxygen fluxes for warm exposed fish but no RCR differences. These results are consistent with a warm-acclimation strategy to compensate consequences of high temperature indicating that acclimation can reduce respiratory efficiency and ROS production induced by warming. In contrast, acute warming led to higher mitochondrial fluxes. Our study highlights the opposite effects of short and long temperature exposure and indicates that taken into account both types of response is important to understand how organisms can cope with increased temperatures.

ABSTRACT N° ICBF22-114

INDIVIDUAL VARIATION IN METABOLIC RATE AND ITS PLASTICITY: LINKS WITH BEHAVIOUR AND TOLERANCE TO RAPID ENVIRONMENTAL CHANGE

Tommy Norin* [1]

[1]DTU Aqua | National Institute of Aquatic Resources, Technical University of Denmark, Kgs. Lyngby, Denmark

Abstract:
Conspecifics of the same age and size differ consistently in the pace with which they expend energy. This individual variation in metabolic rate has been proposed to influence behavioural variation, since differences in energy requirements should motivate differences in behaviours that facilitate energy acquisition, such as being bold and aggressive to gain preferential access to food, or being more active in foraging for food. While there is evidence for links between metabolic rate and behaviour in a constant environment, we know relatively little about if and how metabolic and behavioural plasticity co-vary or trade off when the environment changes. Here, I will present work on the links between metabolic rate and behaviour of fishes across environmental gradients, and how such trait (co)variation affects growth and tolerance to rapid changes in ambient conditions. Our results show that individual fish usually differ predictably in the plasticity of their metabolic rates, from resting to maximal levels, but that environmentally-induced modulations of metabolic rates do not necessarily induce parallel changes in behaviour. Variation in either metabolic rate or behaviour, including their plasticity, is also unrelated to variation in tolerance to rapid environmental changes among individual fish. These individual-level mismatches between physiological and behavioural plasticity and performance reveal how key organismal traits such as metabolic rate and activity can change independently of one another when the environment changes, suggesting that physiology and behaviour are not interdependent in fishes facing environmental challenges and stressors.

13-Intraspecific and inter-individual variation in fishes facing environmental challenges and stressors

ABSTRACT N° ICBF22-487

CAN CARDIAC MITOCHONDRIAL FUNCTION EXPLAIN FAMILY-BASED DIFFERENCES IN ATLANTIC SALMON (SALMO SALAR) UPPER TEMPERATURE TOLERANCE?

Julie Nati* [1], Kathy Clow[1], Eric Ignatz[1], Kurt Gamperl[1]

[1]Memorial University, St John's, Canada

Abstract:
Given the impacts of climate change, it is important to understand what factors determine fish thermal tolerance, and to be able to select for these important indices within populations used in aquaculture. Thus, we challenged twenty families of domesticated Atlantic salmon to an incremental (0.2°C day-1) thermal maximum (ITMax) test. ITmax varied significantly between the 20 families (range 25.0 to 23.3°C). Fish from the four most and least tolerant families that were being held at 10°C were then given a critical thermal maximum (CTMax) test (n=8). In addition, fish from the two most (F19 and F4) and least (F1 and F6) tolerant families were warmed to 18°C, acclimated at this temperature for > 2 weeks, and had their cardiac mitochondrial function assessed at test temperatures of 20, 24, 26 and 28°C using Oroboros fluororespirometry (n=7-9). CTMax was not different between the 8 families (range 27.8 to 28.2°C). With regard to mitochondrial function, there was no clear separation between the temperature tolerant and intolerant families. However, a number parameters of mitochondrial function were significantly different between family F1 and the other families. For example, values for respiration (OXPHOS I, OXOHOS I+II, CIV and LEAK) and RCR, and absolute reactive oxygen species (ROS) production, were consistently or often higher for Family F1 at the various test temperatures. These data suggest that ROS production, not respiratory capacity, limits a fish's ability to tolerate long-term exposure to high temperatures. However, more comprehensive experiments need to be performed to directly examine this hypothesis.

ABSTRACT N° ICBF22-495

INTER-INDIVIDUAL VARIATION IN AEROBIC METABOLISM VARIES WITH GILL MORPHOLOGY AND HEART MITOCHONDRIAL METABOLISM

Bernard Rees*[1], Jessica Reemeyer[2], Brian Irving[3]

[1]University of New Orleans, New Orleans, United States,
[2]McGill University, Montreal, Canada,
[3]Louisiana State University, Baton Rouge, United States

Abstract:
This study asked whether inter-individual variation in maximum and standard aerobic metabolic rates of the Gulf killifish, Fundulus grandis, held under common garden conditions correlate with gill morphology and cardiac mitochondrial bioenergetics, traits reflecting critical steps in the oxygen-supply cascade from the environment to the tissues. Maximum metabolic rate (MMR) was positively related to body mass, total gill filament length, and myocardial oxygen consumption during maximum oxidative phosphorylation (multiple R2 = 0.82). Standard metabolic rate (SMR) was positively related to body mass, total gill filament length, and myocardial oxygen consumption during maximum electron transport system activity (multiple R2 = 0.69). After controlling for body mass, individuals with longer gill filaments, summed over all filaments, or greater cardiac respiratory capacity had higher whole-animal metabolic rates. The overall model fit and the explanatory power of individual predictor variables were better for MMR than for SMR, suggesting that gill morphology and myocardial bioenergetics are more important in determining maximum rather than resting metabolism. Heart ventricle mass was not related to mass-independent variation in MMR or SMR, indicating that the quality of the heart (i.e., the capacity for mitochondrial metabolism) was more influential than heart size. Finally, myocardial oxygen consumption required to offset the dissipation of the transmembrane proton gradient in the absence of ATP synthesis was not correlated with either MMR or SMR. The results support the idea that inter-individual variation in aerobic metabolism, particularly maximum metabolic rate, is explained, in part, by variation in specific steps in the oxygen-supply cascade.

ABSTRACT N° ICBF22-450

MITOCHONDRIAL METABOLISM IN RED BLOOD CELLS: METHODOLOGY, RELEVANCE AND LIMITATION.

Karine Salin* [1], Léna Jégo[2], Jean-Baptiste Quéméneur[1], Sophie Collet[1], Antoine Stier[3], Damien Roussel[3], Anthony Hickey[4]

[1]Ifremer, Laboratoire des sciences de l’environnement marin, UMR 6530, Plouzané,
[2]Ecosystèmes, BIOdiversité, Evolution, UMR 6553, Rennes,
[3]Laboratoire d’écologie des hydrosystèmes naturels et anthropisés, UMR 5023, Villeurbanne, France,
[4]University of Auckland, Auckland, New Zealand

Abstract:
The physiological causes of intraspecific differences in animal performance are currently a source of debate. It has been suggested that individual variations in mitochondrial energy metabolism may drive variation in performance. Attempts to validate this relationship are restricted to single time point correlations, thus failing to distinguish within and between individual relationships. Mitochondrial properties in ectotherm are primarily inferred from lethal samplings, which prevents to track intra and inter- individual variation in mitochondrial metabolism.
In this study we tested the temporal repeatability of individual mitochondrial function in red blood cells (RBC) in fish, as well as relationship between mitochondrial phenotype and both past and future rates of growth, using juvenile European seabass (Dicentrarchus labrax). RBC mitochondrial function were estimated from measurement of rates of LEAK and OXPHOS respiration and repeatability was evaluated three times across a 6-week period. Duplicated assays of LEAK and OXPHOS respiration were significant reliable whereas individual LEAK and OXPHOS respiration showed a lack of repeatability over time. Interestingly, we found a negative relationship between past growth rate and OXPHOS respiration rate: Individuals with the fastest growth had lower OXPHOS respiration rates compared to fish that grew slowly. We are currently running further statistical analyses to test for effects in mitochondrial number and body mass on temporal changes in mitochondrial properties.

13-Intraspecific and inter-individual variation in fishes facing environmental challenges and stressors

ABSTRACT N° ICBF22-477

THE INFLUENCE OF PARASITE INFECTION ON AEROBIC METABOLISM AND TRAPPING VULNERABILITY OF PUMPKINSEED SUNFISH (LEPOMIS GIBBOSUS)

Davide Thambithurai* [1], Shaun Killen[1], Isabel Lanthier[2], Sandra Binning[2]

[1]University of Glasgow, Glasgow, United Kingdom,
[2]University of Montreal, Montreal, Canada

Abstract:
Recreational and commercial fishing can be a potent selective agent across fish populations in freshwater and marine environments. The evolutionary impact resulting from fishing is referred to as fisheries-induced evolution (FIE). Two key aspects of FIE that remain poorly understood are: (i) how do individuals within a population vary in their vulnerability to fishing given their metabolic phenotype?, and (ii) how do additional stressors, such as parasite infection, influence an individual fish's proclivity to capture? Using pumpkinseed sunfish as a model species, we examined the effects of Uvulifer sp. – a parasitic trematode worm, on individual metabolic physiology and vulnerability to capture in simulated trap fishing. We firstly used respirometry to assess inter-individual differences in metabolic phenotype given parasite load; we then tested groups of fish, changing the number of infected individuals between trials to see how variation in group composition would affect individual capture. This work provides insight into how a common stressor, parasite infection, influences individual metabolic physiology, and how this has the potential to affect fishery selection and downstream evolution in fish populations.

ABSTRACT N° ICBF22-483

IS INTRASPECIFIC VARIATION IN METABOLISM AND HYPOXIA TOLERANCE RELATED TO INDIVIDUAL MITOCHONDRIAL FUNCTION IN FISH?

Elisa Thoral[*1], Julie J H Nati[2], Jean-Baptiste Quéméneur[3], Léann Lozac'h[3], Germain Salou[4], Alain Vergnet[5], Mathieu Besson[4], François Allal[4], David J McKenzie[4], Karine Salin[3], Loïc Teulier[1]

[1]Université Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR 5023 LEHNA, F-69622, Villeurbanne, France,
[2]Department of Ocean Sciences, Memorial University of Newfoundland and Labrador, A1C 5S7, St. John's, NL, Canada,
[3]Université de Brest, Ifremer, CNRS, IRD, Laboratory of Environmental Marine Sciences, Plouzané, 29280,
[4]MARBEC, Université Montpellier, CNRS, IFREMER, IRD,, Montpellier,
[5]Ifremer, Chemin de Maguelone, F-34250, Palavas-Les-Flots, France

Abstract:

Individuals within fish species can vary in their baseline metabolism (standard metabolic rate, SMR) and physiological tolerance of environmental stressors. Understanding the subcellular processes underlying this variation is important to determine specific tolerance phenotypes and to predict future environmental resilience of fish populations. Therefore, we measured whole animal SMR and hypoxia tolerance, and then mitochondrial respiration rates in liver and heart, of n = 96 European sea bass (Dicentrarchus labrax) at 21°C. Relationships among individual variation in SMR, critical saturation for regulation of SMR in hypoxia (Scrit) and mitochondrial function will be investigated to test the following predictions: (1) that a high whole-animal SMR will be linked to a high mitochondrial basal oxygen consumption (LEAK respiration); (2) that a low whole-animal tolerance of hypoxia (high Scrit) will be linked to a low mitochondrial efficiency in energy production as estimated from the respiratory control ratio (RCR), and finally (3) that a low SMR will be linked to a low Scrit.

ABSTRACT N° ICBF22-258

LOCAL ADAPTATION IN THERMAL PERFORMANCE OF CHINOOK SALMON, ONCORHYNCHUS TSHAWYTSCHA, FROM EIGHT HATCHERY POPULATIONS

Kenneth Zillig[*1], Alyssa FitzGerald[2, 3], Robert Lusardi[1, 4], Dennis Cocherell[1], Nann Fangue[1]

[1]Wildlife, Fish, and Conservation Department, University of California - Davis, Davis,
[2]Institute of Marine Sciences, University of California Santa Cruz,,
[3]Fisheries Ecology Division, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Santa Cruz,
[4]Center for Watershed Sciences, University of California - Davis, Davis, United States

Abstract:
Fish populations, and salmonids specifically, are known to adjust their physiology to the local thermal environment. This physiological plasticity can be a result of acclimatization and/or local adaptation. Chinook salmon, fish that require cold water to survive, exist across a large latitudinal range, with individual populations experiencing a variety of temperature regimes. Chinook salmon living at the southern range boundary in California appear to be quite thermally tolerant; however, without direct comparisons with other populations and iterated across acclimation regimes, our understanding of the thermal capacity of Chinook salmon remains limited. Over the course of three years, we reared juvenile Chinook salmon from eight hatchery populations spanning the states of California, Oregon and Washington. Each population was reared at three acclimation temperatures (11, 16 and 20°C) to explore interpopulation variation in acclimation capacity and thermal physiology. We measured temperature dependent growth rate, acute thermal tolerance and aerobic metabolic scope in order to assess whether Chinook salmon populations exhibit interspecific variation in thermal capacity and potentially local adaptation. We found several patterns indicative of variation among populations, and consistent with local adaptation. For example, the critically endangered Winter-run population, historically native to cold, high elevation rivers in California, exhibited a thermal phenotype suited for cooler waters. Additionally, we found that local water-temperature was associated with greater thermal performance, and longer migration distance with increased metabolic capacity. Overall, we found that Chinook salmon across the sampled range exhibited impressive thermal capacity, challenging the moniker of a 'cold-water fish' for specific populations.

ABSTRACT N° ICBF22-488

A COMPARISON OF METHODS USED TO EVALUATE THE ACUTE THERMAL AND HYPOXIA TOLERANCE OF FISHES

Rebeccah Sandrelli* [1], Anthony Gamperl[1]

[1]Memorial University, St. John's, Canada

Abstract:
Accelerated climate change's effects on global ocean temperatures, on the frequency and severity of heat waves, and on the expansion of hypoxic areas/zones are of great concern with regard to the survival, management and conservation of aquatic organisms. Thus, there has been significant focus recently on determining the temperature and hypoxia tolerance of fishes. However, methods to determine acute temperature and hypoxia tolerance vary greatly, and it is not clear how lab-based measures of these parameters relate to those in free-living fishes. To obtain heart rate (fH) measurements from Atlantic salmon (Salmo salar), fish acclimated at 10oC were: 1) fitted with Doppler flow probes around their ventral aorta, placed in a respirometer to recover for 24 hours, and exposed to a step-wise decrease in water oxygen levels (from 100 to 30 percent air saturation) and a CTMax test (at 2oC h-1) with 2 days recovery between tests; 2) anesthetized, implanted with Star-Oddi data loggers that measure fH, ECGs and temperature, and exposed to a 10oC h-1 increase in temperature (i.e., a 'ramp protocol') or the above decrease in water O2 levels; and 3) implanted with Star-Oddi data loggers, allowed a month to recover, and given the hypoxic challenge and a CTMax (at 2oC h-1) test when free-swimming in a tank with conspecifics. In this talk we will compare important indices of hypoxia and thermal tolerance (e.g., Arrhenius breakpoint temperature, temperature at maximum fH and arrhythmias, critical thermal maximum, and the oxygen tension at which bradycardia occurs) between these methods.

ABSTRACT N° ICBF22-149

MOTHER KNOWS BEST: ADULT ZEBRAFISH EXPOSURE TO DIEL CYCLING HYPOXIA AND ELEVATED TEMPERATURE INCREASES HEAT TOLERANCE OF LARVAL OFFSPRING

Michael Lim* [1], Nicholas Bernier[1]

[1]University of Guelph, Guelph, Canada

Abstract:
The maternal-match hypothesis predicts that parental exposure to stressors, such as hypoxia and elevated temperatures, can be conveyed to offspring and help them cope with the same stressors in later life. Although there is some support for this hypothesis, the signals involved in non-genetic inheritance are unclear. In this study we tested how adult zebrafish exposure to combined diel cycling hypoxia (30-85% dissolved oxygen) and thermal stress (27-36°C) for two weeks affects the embryonic (1 hr post-fertilization) and larval (5 days post-fertilization) levels of key effectors of the cellular stress response, heat shock proteins (HSPs) 47, 70, and 90. While embryos derived from exposed parents had higher HSP70 and HSP90 mRNA and protein levels, and lower HSP47 mRNA levels, larvae had higher HSP70 and HSP47 mRNA levels, and lower HSP90 mRNA and HSP47 protein levels. Relative to control larvae, those derived from exposed parents and subjected to a combined hypoxia and thermal stress (30% dissolved oxygen, 36°C) had higher HSP47 protein levels and a blunted increase in cortisol. While the 2-week exposure increased both thermal and hypoxia tolerance in parents, their larvae had higher heat tolerance but no change in hypoxia tolerance. Our results demonstrate that in response to environmental stressors, parental investment of HSPs can have lasting effects on offspring basal and inducible HSP levels. Moreover, in support of the maternal-match hypothesis, we show that parental exposure to combined diel cycling hypoxia and thermal stress can increase larval heat tolerance and offspring stress coping abilities.

ABSTRACT N° ICBF22-476

IMPAIRING CARDIAC OXYGEN SUPPLY IN SWIMMING COHO SALMON COMPROMISES THEIR HEART FUNCTION AND TOLERANCE TO ACUTE WARMING

Andreas Ekström*[1], Jacey van Wert[2], Brian Hendricks[3], Matthew Gilbert[3, 4], Steven Cooke[5], Anthony Farrell[3], Scott Hinch[3], Erika Eliason[2]

[1]University of Gothenburg, Gothenburg, Sweden,
[2]UC Santa Barbara, Santa Barbara, United States,
[3]University of British Columbia, Vancouver,
[4]University of New Brunswick, New Brunswick,
[5]Carleton University, Ottawa, Canada

Abstract:
The essential, once-in-a-lifetime spawning migration of Pacific salmon comprises a physically strenuous challenge that is exacerbated with climate change. Indeed, recent climatic warming has led to an elevated mortality in many salmonid populations during their migrations, but the mechanisms underlying the increased mortality is not fully understood. One hypothesis posits that an oxygen limitation of the heart´s capacity to provide an adequate oxygen supply to the body tissues is central to swimming performance and hence for migratory success in salmon, especially in warm water. Here, we examined whether experimentally removing the coronary oxygen supply to the heart, which may be naturally impaired by coronary arteriosclerosis found in almost all mature Pacific salmon, impairs cardiac and swimming performance of coho salmon (Oncorhynchus kisutch) when exposed to acute warming. Fish with either an intact or blocked coronary artery (by surgical ligation) were instrumented with a blood flow probe facilitating assessments of cardiac capacity in vivo in fish swimming close to their maximum swim speed in a swim tunnel. We show that a restriction of the coronary oxygen supply caused drastic cardiac deterioration during swimming even at benign temperatures, and caused a substantial reduction in cardiac output which ultimately constrained metabolic rate when swimming at progressively higher temperatures. Removing the coronary supply to the heart also reduced the upper thermal tolerance limit while swimming by almost 5°C. Thus, our findings show that the coronary circulation is vital for the migratory capacity in salmon facing a warming environment with climate change.

ABSTRACT N° ICBF22-334

BODY MASS AND GENOME SIZE SHAPE THE TOLERANCE OF FISHES TO LOW OXYGEN IN A TEMPERATURE-DEPENDENT MANNER

Wilco Verberk* [1], Jeroen Sandker[1], Iris van de Pol[1], Mauricio Urbina[2], Rod Wilson[3], David McKenzie[4], Félix Leiva[1]

[1]Radboud University, Nijmegen, Netherlands,
[2]Universidad de Concepción, Concepción, Chile,
[3]University of Exeter, Exeter, United Kingdom,
[4]Centre for Marine Biodiversity Exploitation and Conservation, Montpellier, France

Abstract:
Aerobic metabolism generates 15-20 times more energy than anaerobic metabolism and thus is crucial in maintaining energy budgets in animals, fueling basal metabolism, activity, growth and reproduction. For ectothermic water-breathers such as fishes, low dissolved oxygen may limit oxygen uptake and hence aerobic metabolism. Here we explore abiotic and biotic drivers within a phylogenetic context to explain the variation in hypoxia tolerance observed in fishes. We compiled a database with 600 records on critical oxygen pressures (Pcrit) as a proxy for hypoxia tolerance from 171 fish species. Overall we found that hypoxia tolerance has a clear phylogenetic signal and it is further modulated by temperature, mass, genome size, salinity and metabolic rate. Marine fishes were more susceptible to hypoxia than freshwater fishes, a pattern that is consistent with greater fluctuations in oxygen and temperature in freshwater habitats. Fishes with relatively high oxygen requirements were also more susceptible to hypoxia. Intriguingly, we found that the influence of body mass and genome size on hypoxia tolerance varied with temperature. Specifically, fishes with larger body masses or larger genome sizes are more susceptible to hypoxia in warmer waters, but less susceptible in colder water. These thermal dependencies likely reflect constraints in oxygen uptake related to cellular surface-area to volume ratios and effects of viscosity on the thickness of the boundary layers enveloping the gills. Previous studies have found a wide diversity in the direction and strength of relationships between Pcrit and body mass. By including the thermal dependency, our study resolves the size dependency of hypoxia tolerance in fish.

ABSTRACT N° ICBF22-519

METABOLIC REPONSES AND RESILIENCE TO ENVIRONMENTAL CHALLENGES IN THE SEDENTARY BATRACHOID HALOBATRACHUS DIDACTYLUS (BLOCH & SCHNEIDER, 1801).

Juan M. Molina[1, 2], Andreas Kunzmann[2], João Reis[3], Pedro M. Guerreiro[* 3]

[1]Universidad Nacional del Sur, Bahia Blanca, Argentina,
[2]Leibniz-Zentrum für Marine Tropenforschung (ZMT), Bremen, Germany,
[3]CCMAR - Centre of Marine Sciences - Universidade do Algarve, Faro, Portugal

Abstract:
In a context of climate change, warming of the seas and expansion of hypoxic zones are challenges that most species of fish are, or will be subjected to. Understanding how different species cope with these changes in their environments at individual level can shed light on the how populations and ecosystems will be affected. We provide first-time estimates on metabolic rates, thermal and oxygen-related limits for Halobatrachus didactylus, a coastal sedentary fish that lives in intertidal environments of north-east Atlantic.
Using respirometry in different experimental designs we found that this species is highly resistant to acute thermal stress (CTmax: 34.82 +/- 0.66 ºC) and acute hypoxia (Pcrit: 0.404 +/- 0.034 mg O2L-1). We found size-specific differences in this stress response, with smaller individuals being more sensitive. We quantified its routine aerobic scope and daily activity patterns, finding this fish to show one of the lowest standard metabolic rates found in temperate fish (SMR: 16.93 mg O2 kg–1h–1). H. didactylus activity increases at night, when its metabolic rate increases twofold (RMR: 33.93 mg O2 kg–1h–1). The resilience of this species combined with its changing environment could force it to move northward and also eastward further into the Mediterranean. Anecdotal evidence exists of some individuals found as far as Greece.
Further, studies combining respirometry with biomarkers such as oxidative stress enzymes, damaged DNA and haematological parameters would provide a more mechanistically complete panorama of how H. didactylus and other batrachoids cope with the unpredictable and variable world they thrive in.

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ABSTRACT N° ICBF22-277

LOW PCRIT BUT NO HYPOXIA TOLERANCE? HYPOXIA COMPENSATION IN THE ARCTIC KEYSTONE SPECIES BOREOGADUS SAIDA

Sarah Kempf* [1, 2], Carolin Julie Neven [1], Guy Claireaux[3, 4], Felix Christopher Mark[1]

[1]Alfred Wegener Institute for Polar and Marine Research, Bremerhaven,
[2]University of Bremen, Bremen, Germany,
[3]Université de Bretagne Occidentale,
[4]Centre Ifremer de Bretagne, Brest, France

Abstract:
Global warming has already caused various environmental changes, including a loss of almost 50% Arctic sea-ice coverage since the 1980s. Sea-ice loss strengthens summer stratification and, consequently, hypoxic zones in the deep-water layers may form. The deep fjord systems of the Svalbard archipelago are particularly at risk from this long-lasting stratification. Thus, the present study aims to investigate the hypoxia tolerance of the Arctic keystone species Polar cod, Boreogadus saida. We measured the respiratory capacity (standard, routine and maximum metabolic rates, SMR, RMR, MMR) and swimming performance under progressive hypoxia (100% to 5% air saturation) at cold habitat temperatures (2.5 °C) and after warm-acclimation to close to its thermal limit (10 °C) via flow-through and swim-tunnel respirometry. The observed metabolic patterns were consistent at both acclimation temperatures: Over the whole range of its SMR and in part also for MMR (above 40% and 70% air saturation, respectively), Polar cod displayed oxyregulating behaviour under progressive hypoxia, with SMR never below aerobic baseline metabolism. Despite the common paradigm that polar organisms are not hypoxia tolerant, our study revealed that Polar cod can handle very low oxygen saturations down to a Pcrit of 5.9 % air saturation at typical habitat temperatures. Closer to critical temperatures (10°C), and Pcrit rose to 21.6% air saturation. However, we did not observe any metabolic downregulation and no anaerobic component of the hypoxia response in Polar cod, usually mentioned in the definition of hypoxia tolerance.

Therefore, we describe the observed response rather as metabolic hypoxia compensation than hypoxia tolerance as the mechanisms involved here actively seek to improve oxygen supply instead of (anaerobically) tolerating hypoxia through metabolic depression.

ABSTRACT N° ICBF22-410

WARMING-INDUCED “PLASTIC FLOORS” IMPROVE HYPOXIA VULNERABILITY, NOT AEROBIC SCOPE, IN RED DRUM

Adam Zambie* [1], Benjamin Negrete, Jr[1], Kerri Lynn Ackerly[1], Andrew Esbaugh[1]

[1]The University of Texas at Austin Marine Science Institute, Port Aransas, TX, United States

Abstract:
Ocean warming is a prevailing threat to marine ectotherms, as it has been shown to increase standard metabolic rate (SMR) and constrain aerobic scope (AS). Recent work has put forth the “plastic floors, concrete ceilings” hypothesis, which suggested that fish can acclimate to warming temperatures by reducing SMR while keeping maximum metabolic rate (MMR) stable, therefore recovering AS. Here we tested this hypothesis on an estuarine-dependent species native to the Gulf of Mexico, the red drum (Sciaenops ocellatus). Fish were subjected to a 12-week temperature acclimation to water temperatures of either 20 (control) or 28 (warm) degrees C. Intermittent-flow respirometry was performed at week 0, 4, 8, and 12 of the acclimation to obtain measurements of SMR, MMR, and critical oxygen threshold (Pcrit; a measure of hypoxia vulnerability). As expected, warm-acclimated fish had a significantly higher SMR, MMR, and Pcrit than control fish at time 0. Fish that were warm-acclimated conformed to the “plastic floors” hypothesis, as SMR declined by 35.3% over the 12-week acclimation. No change in SMR was observed in the control treatment. Contrary to expectations, the reduction in SMR did not improve AS relative to time 0 owing to a progressive decline in MMR over the course of 12-weeks. Interestingly, Pcrit decreased by 27% in the warm acclimated fishes, which resulted in temperature treatments having statistically similar values by 12-weeks. Our results suggest that the warming-induced reductions in SMR for red drum may be intended to reduce hypoxia vulnerability, and not to improve AS.

ABSTRACT N° ICBF22-351

HYPOXIA ACCLIMATION SELECTIVELY INDUCES MITOCHONDRIAL PLASTICITY IN AEROBIC TISSUES OF RED DRUM (SCIAENOPS OCELLATUS)

Kerri Lynn Ackerly* [1], Benjamin Negrete Jr. [1], Angelina Dichiera[2], Andrew Esbaugh[1]

[1]The University of Texas at Austin, Port Aransas, United States,
[2]The University of British Columbia , Vancouver, Canada

Abstract:
Hypoxia (low dissolved oxygen), a pervasive stressor in aquatic environments, is a significant threat facing fishes. As animals require oxygen to produce ATP, hypoxia can significantly limit aerobic capacity in fishes. However, some fishes show respiratory flexibility that rescues aerobic performance, including plasticity in mitochondrial performance. This plasticity may result in increased mitochondrial efficiency (e.g., less proton leak), increased oxygen storage capacity, and higher citrate synthase activity under hypoxia. To test this, we acclimated red drum (Sciaenops ocellatus) to 8-days of chronic hypoxia to induce a hypoxic phenotype. Fish were terminally sampled for cardiac and red muscle tissue, which was immediately dissected, gently homogenized, and placed in an Oxygraph 2K high resolution mitochondrial respirometer. A standard multiple-substrate-uncoupler-inhibitor titration protocol was used to quantify oxidative phosphorylation, proton leak, and maximum respiration in both hypoxia-acclimated and control fish. Tissue was also collected to assess mitochondrial plasticity through citrate synthase activity and gene expression for oxygen storage. We found that mitochondrial function and efficiency was maintained in cardiac tissue, though citrate synthase activity was higher under hypoxia. Interestingly, mitochondrial performance in red muscle significantly improved under hypoxia. Hypoxia-acclimated fish had significantly higher oxidative phosphorylation than control, but were performing at maximum capacity with no change to leak respiration. There was no significant change to citrate synthase activity in red muscle, suggesting that mitochondria more efficiently utilize oxygen without increasing uptake capacity. Overall, our results show the dynamic ability of fish to acclimate to hypoxia to improve aerobic performance.

ABSTRACT N° ICBF22-250

RED DRUM (SCIAENOPS OCELLATUS) DO NOT INCREASE TISSUE OXYGEN EXTRACTION MECHANISMS UNDER HYPOXIA

Angelina Dichiera* [1], Benjamin Negrete, Jr.[2], Kerri Lynn Ackerly[2], Andrew Esbaugh[2]

[1]The University of British Columbia, Vancouver, Canada,
[2]The University of Texas at Austin, Port Aransas, United States

Abstract:
With the growing prevalence of hypoxia (oxygen levels < 2 mg/L) in aquatic and marine ecosystems, we are increasingly interested in the adaptive mechanisms fish may employ to better their performance in poor environments. We investigated the contribution of a proposed strategy for enhancing tissue oxygen extraction – plasma-accessible carbonic anhydrase (CA-IV) – under hypoxia in a species of estuarine fish (red drum, Sciaenops ocellatus) that thrives in fluctuating habitats. We proposed that hypoxia-acclimated fish would increase the prevalence of CA-IV in aerobically-demanding tissues to confer more efficient oxygen delivery. Furthermore, we proposed the predicted phenotypic changes to tissue oxygen extraction under hypoxia may improve respiratory and swim performance under 100% oxygen conditions (i.e., normoxia) when compared with fish that have not been acclimated to hypoxia. Interestingly, there were no significant differences in relative CA-IV mRNA expression, protein quantity, nor enzyme activity between the two treatments, suggesting CA-IV function is simply maintained under hypoxia. Likewise, upon reoxygenation after 8 days of hypoxia, red drum respiratory performance was similar to that of control fish. Critical swim speed (Ucrit) was significantly higher in hypoxia-acclimated fish ($P<0.03$; one-tailed t-test), but this was most likely achieved due to an increased reliance on anaerobic metabolism during their swim trials. While the maintenance of CA-IV may still be an important contributor for hypoxia tolerance, our evidence suggests hypoxia-acclimated red drum are using other means to cope in a poor environment.

15-The future of fishes: how will they cope physiologically with a changing planet?

ABSTRACT N° ICBF22-139

HORMONAL RESPONSES TO HYPOXIA AND TURBIDITY AND THEIR BEHAVIORAL CONSEQUENCES IN AN AFRICAN CICHLID

Bethany Williams* [1], Lauren Pintor[1], Suzanne Gray[1]

[1]The Ohio State University, Columbus, OH, United States

Abstract:
Although hormones are vital to an organism's ability to respond to environmental stressors, they can be directly altered by the environment and impact reproductive behavior and fitness. For example, hypoxia inhibits the aromatase enzyme that converts testosterone (T) to estradiol (E) which is important for regulating aggressive and reproductive behaviors. The goal of this study was to examine the effects of oxygen and turbidity on hormones and reproductive behavior in male Pseudocrenilabrus multicolor cichlids from two populations that experience extremes of these two stressors. Specifically, we tested for the effect of rearing under normoxic/hypoxic x clear/turbid conditions on T and E levels in male cichlids. Next, we compared male-male competition and male courtship behavior between control fish and those treated with an aromatase inhibitor from two populations reared under normoxic/clear conditions. We found that rearing under normoxic and/or clear conditions decreased the ratio of T to E (Oxygen: $t= -3.36$, $p= 0.001$; Turbidity: $t= -3.01$, $p= 0.002$) while population had no effect. Secondly, we found that the rate of competitive behaviors was unaffected by treatment, population, or individual hormone level. However, courtship behavior increased with increasing ratios of T to E ($t= 2.64$, $p= 0.017$), but was negatively affected by the aromatase inhibition treatment ($t= -2.46$, $p= 0.026$). Overall, this indicates that changes in hormone levels due to the environment are likely to have behavioral consequences, though they may differ depending on the context. Additionally, the mechanism of hormonal change (e.g. aromatase inhibition) may also influence behavioral responses.

ABSTRACT N° ICBF22-526

GILL NEUROEPITHELIAL CELLS OF GULF TOADFISH (OPSANUS BETA) EXPOSED TO DIFFERENT ENVIRONMENTAL STRESSORS

Orianna A. Duh* [1], Meghan N. Roberts[1], M. Danielle McDonald[1]

[1]Rosenstiel School of Marine and Atmospheric Science - University of Miami , Miami, FL, United States

Abstract:

Neuroepithelial cells (NECs) within the fish gill are full of the monoamine neurochemical serotonin (5-HT) and are believed to initiate the cardiovascular and respiratory responses to hypoxia, potentially by releasing 5-HT. The distribution of the NECs within the gill is known for some fish species but not for the Gulf toadfish, Opsanus beta. Furthermore, whether the NEC size or number changes after chronic exposure to different stressors, such as hypoxia or pharmaceuticals, has never been tested. To analyze NECs in the gill, fish (N=24) were exposed to either normoxia (160.4 ± 0.1 torr), mild hypoxia (76.4 ± 2.1 torr), or severe hypoxia (22.9 ± 1.8 torr) for one week. NEC surface area did not change across the different oxygen regimes, however, fish exposed to mild hypoxia had significantly less NECs (34.9 ± 2.90) than both the control (51.8 ± 6.17) and severe hypoxia exposed fish (62.8 ± 3.69) ($p > 0.05$). Currently, we are running a similar experiment to determine if any changes occur in the NECs when exposed to 35-d of fluoxetine (FLX), a selective serotonin reuptake inhibitor that targets the serotonin transporter (SERT) which moves 5-HT into cells, and bupropion (BUP), a norepinephrine-dopamine reuptake inhibitor that targets the dopamine and norepinephrine transporters that also move 5-HT into cells. Fish were exposed to control and nominal concentrations of 0.1 micrograms per liter FLX and 0.5 BUP micrograms per liter (10-fold higher than environmentally realistic) and 10 micrograms per liter FLX and 50 BUP micrograms per liter (100-fold higher than environmentally realistic). Future work will investigate the impact of hypoxia and pharmaceutical exposure on the time to lose equilibrium, as well as tissue SERT mRNA expression.

ABSTRACT N° ICBF22-220

WHY SARDINES SHRINK IN THE NW MEDITERRANEAN SEA?

Quentin Queiros* [1, 2], David McKenzie[2], Shaun Killen[3], Gilbert Dutto[2], Claire Saraux[4], Quentin Schull[2]

[1]DECOD (Ecosystem Dynamics and Sustainability), INRAE, Institut Agro, IFREMER, Rennes,
[2]MARBEC, Univ Montpellier, CNRS, IFREMER, IRD, Sète, France,
[3]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Graham Kerr Building, Glasgow, United Kingdom,
[4]Université de Strasbourg, CNRS, IPHC UMR 7178, Strasbourg, France

Abstract:
Small pelagic fish used to be a major component of commercial fisheries in NW Mediterranean Sea but landings have fallen drastically over the last decade due to a severe decline in fish size and condition. This situation seems to result from bottom-up control constraints due to changes in plankton size and composition. Using an experimental framework, we investigated synergic effects of temperature as a direct consequence of climate change and an indirect effect through food modulation (size and quantity) on the energy expenditures of a captive population of sardines. Interestingly, energy costs of filtering mode on small particles were twice greater than energy costs of direct capture on large particles. Despite lower energy costs of digestion, daily energy expenditures of sardines fed on small particles were greater than those fed on large items and the gap widened when temperature increased. This shows how a change in prey size modified sardines' feeding behavior, affecting their metabolism, likely altering energy allocation towards life-history traits such as growth or survival and explaining the current population situation. The extra cost associated with filter feeding strategies, especially so in a warming environment, bode not well for the future of small pelagic fishes if they don’t adapt in regards to the last IPCC forecasts.

ABSTRACT N° ICBF22-368

EFFECTS OF CLIMATE CHANGE AND AMMONIA ON THE PHYSIOLOGY AND ZOOTECHNICAL PERFORMANCE OF THE AIR-BREATHING ARAPAIMA GIGAS

José RAMIREZ[1], Renan SILVA[1], Adalberto VAL*[1]

[1]INPA - Brazilian National Institute for Research of the Amazon , Manaus, AM, Brazil

Abstract:
Carbon dioxide is driving global climate change and so global warming, posing food security at risk, including fish farming. Increased water temperature adversely affects fish biology. In addition, it causes an increase in the toxic fraction of ammonia, requiring further biological adjustments. In the present study, we analyzed the combined effects of ammonia and the extreme environmental scenario on the Amazonian, carnivorous, air-breathing fish Arapaima gigas (pirarucu). For 30 days, juvenile pirarucu were subjected to a sublethal concentration of ammonia in both current and extreme scenarios, real-time simulated in environmental rooms, as predicted by the IPCC for the year 2100. Zootechnical parameters were affected by ammonia in the current and in the extreme scenario. The [K+] increased in animals exposed to the extreme scenario, and glucose and triglycerides increased under combined effects of ammonia and extreme scenario. Acetylcholinesterase (Ache) activity increased in the presence of ammonia in the current scenario and in both conditions (presence/absence of ammonia) in the extreme scenario, in relation to the control without ammonia. The opposite occurred with H+-ATPase, i.e., there was a decrease in its activity in the presence of ammonia in the control scenario and in both conditions in the extreme scenario, compared to control. This study shows that a combination of the extreme climate change scenario and ammonia potentiates physiological and zootechnical imbalances to pirarucu. (INCT ADAPTA: CNPq, FAPEAM, CAPES)

ABSTRACT N° ICBF22-486

CARDIORESPIRATORY FUNCTION AND SWIMMING CAPACITY OF ATLANTIC SALMON (SALMO SALAR) AT COLD TEMPERATURES

Emma Porter* [1], Anthony Gamperl[1]

[1]Memorial University of Newfoundland, St. John's, Canada

Abstract:

We investigated how acclimation to 8, 4 and 1oC, and acute cooling (8-1oC overnight), affected the aerobic metabolism and cardiac function of salmon during a critical swim speed (Ucrit) test. This study revealed several interesting temperature-dependent effects. First, while differences in resting heart rate (fH) between the groups were predictable based on previous research (range ~ 65 to 28 min-1, with that of 8-1oC fish slightly less than 1oC-acclimated conspecifics), the former group had an ~2-fold greater resting stroke volume (VS) as compared to the other groups, and the cardiac output (Q) of 1oC-acclimated fish was much lower and compensated for by enhanced tissue oxygen extraction (MO2/Q). Second, increases in fH (1.2 to 1.4-fold) contributed little to the higher Q when swum, and the contributions of Q (VS) vs. oxygen extraction to aerobic scope (AS) were very different in the two groups tested at 1oC and reflected the available scope for these parameters. Finally, Ucrit was 2.08 and 1.69 body lengths s-1 in the 8 and 4oC-acclimated groups, but only 1.27 and 1.44 in the 1oC-acclimated and 8-1oC fish, respectively; this lower value in 1oC vs. 8-1oC fish despite higher values for maximum metabolic rate and AS. These data: support recent studies which suggest that maximum fH is constrained at low temperatures; show that cardiorespiratory function at cold temperatures, and its response to increased demands, display considerable plasticity; and suggest that factors independent of oxygen delivery limit swimming capacity in salmon when chronically exposed to temperatures approaching their lower limit.

ABSTRACT N° ICBF22-216

FISH PHYSIOLOGY AS A USEFUL TOOL TO MANAGE ARTISANAL FISHERIES IN EUROPE

Blanca Partida[1], Jorge Saez[2], Ignacio Ruiz-Jarabo* [1]

[1]University Complutense of Madrid, Madrid,
[2]SolDeCocos, Seville, Spain

Abstract:
European fisheries have been recently forced to ensure their ecological sustainability. The European Common Fisheries Policy (Article 15, European Regulation 1380/2013) states that all fish caught below commercial size must be landed unless there is scientific evidence of their survival and recovery. In this study, in collaboration with artisanal fisheries, physiological responses (primary and secondary) to fishing stress in several teleost species are described. The objective is to minimize the adverse effects of fishing to improve the survival of discards, guaranteeing a correct physiological recovery. Fish were introduced in sea cages after capture, and blood and dermal mucus were collected during the first 48 hours to analyse stress biomarkers. The results show that fish caught by longline fishing in southern Europe have high survival rates and rapid physiological recovery (in less than 24 hours). This study provides tools that have proven to be useful for fisheries management.

ABSTRACT N° ICBF22-419

THE EFFECTS OF INDIVIDUAL PHYSIOLOGY AND MAZE COMPLEXITY DESIGN ON FISH SPATIAL LEARNING

Daphne Cortese* [1], Amelia Munson[1], Nick Jones[2], Shaun Killen[1]

[1]University of Glasgow, Glasgow,
[2]University of St Andrews, St Andrews, United Kingdom

Abstract:
The ability to locate food and other essential resources within the environment is an important cognitive challenge faced by many species. Individuals within a species often vary in their ability to locate and remember the location of food resources. Variation in physiological traits, such as metabolic phenotype, can result in different behavioral capacity and expression leading to variation in performance across environments. For example, individuals with higher metabolic rate are generally bolder and more active, which may lead to the ability to learn faster about the location of food compared to individuals with lower metabolic rate. However, the link between individual physiology and spatial learning has often been overlooked. In addition, it is poorly understood how spatial learning varies across environments of different complexity. To test this, using 3D printed mazes with two different levels of complexity (2-doors vs 4-doors), we trained European minnows (Phoxinus phoxinus) for 20 days to find the door associated with a food reward. We then measured fish metabolic phenotype (i.e. standard and maximum metabolic rate and aerobic scope). Our results will provide insights into the role of individual metabolism on spatial learning, measured as a change in the time to find the reward over training, and explore the effects of maze complexity on spatial learning performance. We hope this can give important ecological information on how learning varies across variable environments and will help improve the design of mazes in cognitive performance tests.

ABSTRACT N° ICBF22-391

VISUALIZATION OF BLOOD FLOW IN CARTILAGINOUS FISH USING CONTRAST-ENHANCED ULTRASOUND IMAGING

Marianna Horn*[1], Markus Hecker[1], Steven Machtaler[1]

[1]University of Saskatchewan, Saskatoon, Canada

Abstract:
Environmental monitoring of animal health is essential to conservation measures for species at risk. Data collection is typically limited to catch and release, as terminal monitoring would be unethical. However, the parameters collected through this type of monitoring are often limited (length, weight, body index) and do not provide comprehensive assessments of health. We propose the use of a minimally invasive imaging tool, contrast-enhanced ultrasound imaging, to quantitatively measure blood flow in organs and tissues. We present a proof-of-concept project in which we show that perfusion in different organs in an endangered cartilaginous fish, white sturgeon, can be both visualized and measured using ultrasound imaging. Microbubbles, an ultrasound contrast agent, were synthesized and injected intravenously into white sturgeon immediately after euthanasia (anaesthetic overdose with MS-222 and subsequent pithing in accordance with federal regulations). Vascular perfusion using non-linear contrast mode was detected using a high-resolution ultrasound system. By taking advantage of spontaneous contractions of the heart which occur post mortem to circulate the contrast agent via the blood stream, we observed a contrast-specific acoustic signal in organs including liver, upper and lower gastrointestinal systems, heart, gills, and barbels. We recognize that this does not represent proper in vivo circulation, but present it as a proof-of-concept for the potential use of contrast-enhanced ultrasound imaging as a minimally invasive tool to quantitatively measure perfusion in organs of endangered fish. Improved understanding of health in wild populations will allow better targeted conservation efforts and selection of relevant metrics for ecological monitoring.

ABSTRACT N° ICBF22-189

CHROMOSOMAL INVERSIONS MAY FACILITATE LOCAL ADAPTATION IN FRESHWATER FISHES INHABITING ENVIRONMENTS OF VARYING CONNECTIVITY

Matt Thorstensen* [1], Peter Euclide[2], Jennifer Jeffrey[1], Yue Shi[2, 3], Jason Treberg[1], Douglas Watkinson [4], Eva Enders[4], Wesley Larson[2, 5], Yasuhiro Kobayashi[6, 7], Ken Jeffries[1]

[1]University of Manitoba, Winnipeg, Canada,
[2]University of Wisconsin-Stevens Point, Stevens Point,
[3]University of Alaska Fairbanks, Fairbanks, United States,
[4]Fisheries and Oceans Canada, Winnipeg, Canada,
[5]National Oceanographic and Atmospheric Administration, Juneau,
[6]Fort Hays State University, Hays,
[7]The College of St. Scholastica, Duluth, United States

Abstract:
Freshwater fishes often inhabit waterbodies of varying connectivity and fluctuating opportunities for gene flow over time. Because gene flow tends to work in contrast to local adaptation, even limited or periodic gene flow may decrease the ability of populations to adapt to local conditions. Chromosomal inversions, where a chromosome segment is reversed in one group relative to another, may support local adaptation by preventing gene flow between two populations. Here, reduced representation sequencing was used to study n = 345 walleye (Sander vitreus) from Cedar Bluff Reservoir (Kansas, USA), Lake Manitoba (Manitoba, Canada), and Lake Winnipeg (Manitoba, Canada) in North America. A putative chromosomal inversion was revealed by haplotype and outlier-based tests. This putative inversion contained three expressed genes (measured via mRNA transcript abundance), indicating the potential for its functional significance. In addition, the putative inversion was nearly fixed for alternate genotypes in each Canadian lake. These patterns exist despite several opportunities for gene flow between these proximate Canadian lakes, suggesting that the inversion may facilitate adaptive divergence between the two lakes. Our results provide additional evidence that chromosomal inversions may facilitate local adaptation in freshwater fishes that inhabit connected, heterogenous environments.

ABSTRACT N° ICBF22-274

EFFECTS OF REPLACEMENT OF DIETARY FISHMEAL WITH PLANT SOURCE ON SEVERAL PHYSIOLOGICAL PARAMETERS IN GIANT FRESHWATER SHRIMP: A MODEL FOR CARNIVOROUS FISH

Khanakorn Phonsiri* [1], Surintorn Boonanuntanasarn[1]

[1]Suranaree University of Technology, Nakhon Ratchasima, Thailand

Abstract:
This study aimed to investigate the effects of replacement of dietary fishmeal with soybean meal in giant freshwater shrimp (Macrobrachium rosenbergii)which could be used as a model of carnivorous fish. Two isonitrogenous (360 g kg-1 crude protein) dietary treatments included fishmeal-based diet (FM) and soybean meal-based diet (SBM) were formulated and fed to shrimp for 3 months. There were no significant differences (P>0.05) in growth performances in shrimp fed experimental diets. The muscle composition of experimental shrimp appeared to be similar (P>0.05). Shrimp fed with SBM had higher glucose level, total protein and alanine amino transferase in hemolymph (P<0.05). In contrast, significant lower triglyceride and cholesterol were observed in shrimp fed with SBM (P>0.05). Among digestive enzyme activities, amylase enzyme activity was increased in shrimp fed with SBM (P<0.05). Expression of myhc, chi, hc, lgbp, bgbp, per, cru, clec, sod, sod2, cMnsod, hsp was higher in shrimp fed SBM (P<0.05). However, up-regulation in gpx was observed in shrimp fed FM (P<0.05). Taken together, replacement of fishmeal with SBM modulated several metabolic and physiologic parameters in shrimp.

ABSTRACT N° ICBF22-205

AN INTEGRATIVE PERSPECTIVE ON FISH HEALTH: ENVIRONMENTAL AND ANTHROPOGENIC PATHWAYS AFFECTING FISH STRESS

Quentin Schull[* 1], Vincent A. Viblanc[2], Luisa Metral[1], Lina Leclerc[1], Diego Romero[3], Fabrice Pernet[4], Claudie Quéré[4], Valérie Derolez[1], Dominique Munaron[1], Christopher W. McKindsey[5], Claire Saraux[1, 2], Bourjea Jérome[1]

[1]MARBEC, Univ Montpellier, Ifremer, CNRS, IRD, Sète,
[2]Université de Strasbourg, CNRS, IPHC, UMR 7178, Strasbourg, France,
[3]Área de Toxicología, Facultad de Veterinaria, Campus Regional de Excelencia Internacional Campus Mare Nostrum, Universidad de Murcia, Espinardo, Murcia, Spain,
[4]Ifremer/LEMAR UMR 6539, Technopole de Brest-Iroise, Plouzané, France,
[5]Fisheries and Oceans Canada, Maurice Lamontagne Institute, Mont-Joli, Québec, Canada

Abstract:

Session 16: FISH IN A TOXIC WORLD: BIOMARKERS AND IMPACTS OF EXPOSURE

Multifactorial studies that aim at assessing the cumulative effects of natural and anthropogenic stressors on individual health are crucial to understand how organisms cope with their environment, and to infer how populations may react to changes in their ecosystem. Here we investigate direct and indirect causal pathways through which environmental pressures affect the stress and health of wild Gilt-head seabreams (Sparus aurata) in Mediterranean costal lagoons using an integrative partial least squares path modelling approach. We monitored the bio-chemical features of 10 contrasted Mediterranean lagoons ecosystems and assessed their consequences on fish physiology. We integrated 38 environmental and physiological variables gathered into 7 latent variables reflecting lagoons features and fish health including individual reserves, structure, inorganic pollutant load, and individual trophic and stress levels. This approach allowed to explain 30 % of the total variance measured in our 10 different lagoon ecosystems. More importantly 54% of fish stress was explained by the dependent lagoon features, fish age, fish diet, fish reserve, fish structure and fish pollutant load latent variables included in our model. We identified direct negative consequences of lagoons eutrophication on fish health and were also able to highlight indirect antagonistic effects mostly passing through a reduction of inorganic pollutant loads. We discuss the importance of integrative studies in shedding light on how individuals deal with contrasting environments and multiple ecological pressures. A similar framework for studying complex multifactorial interactions is likely to prove useful in a number of species and ecosystems.

ABSTRACT N° ICBF22-336

CONTAMINANT CONSEQUENCES ON FISH HEALTH, INVESTIGATING COCKTAIL EFFECT IN THE WILD

Anaïs Beauvieux* [1], Fabrice Bertile[2], Jérôme Bourjea[1], Claire Saraux[2], Tarek Hattab[1], Luisa Metral[1], Diego Romero[3], Jean-Marc Fromentin[1], Quentin Schull[1]

[1]MARBEC, Univ Montpellier, IFREMER, CNRS, IRD, Sète,
[2]IPHC-CNRS & Université De Strasbourg, 67200 Strasbourg, France,
[3]Área de Toxicología, Facultad de Veterinaria, Campus de Espinardo, Universidad de Murcia, ,
30100 Murcia, Spain

Abstract:
One of today's greatest challenges in environmental toxicology is to understand outcomes of mixture toxicity, commonly referred to as cocktail effects, in humans and in wildlife. Fish contaminant loads are routinely measured to assess exposure of anthropogenic chemicals in the aquatic environment. However, little is known on how cocktail effects may affect fish health. In recent years, proteomics has emerged as a powerful tool to investigate mechanistic consequences of environmental stress in wildlife, and hence new and potentially more accurate exposure and effect biomarkers. The main goal of this study was to evaluate trace metals and organic contaminants accumulation and their possible impacts on the health of juveniles of two highly prized fish in the Mediterranean Sea: European sardines (Sardina pilchardus) and Gilthead seabreams (Sparus aurata). We assessed 32 trace metals and 50 organic contaminants bioaccumulated in wild sardines (n = 106) and gilthead seabreams (n = 135) muscle samples collected in the Golf of Lions. Both species displayed contrasted contamination signature. Moreover we examined the usefulness of non-targeted protein expression for biomarker discovery in these 2 species by investigating differences in the response to contamination. Comparison of the global protein profiles of S.pilchardus and S.aurata revealed numerous proteins which were differentially regulated between the different contamination clusters. Overall, this study identified promising biomarker candidates associated to cocktail of contaminants in wild individuals of 2 species that may be used in future monitoring allowing for a temporal follow-up of these organisms health.

ABSTRACT N° ICBF22-233

CO-EXPOSURE TO CRUDE OIL AND ULTRAVIOLET (UV) RADIATION INDUCES CATARACT FORMATION IN FISHES: A NOVEL ENDPOINT OF PHOTO-INDUCED CRUDE OIL TOXICITY

Rachel Leads* [1], Aaron Roberts[1]

[1]University of North Texas, Denton, United States

Abstract:
Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment due to both natural and anthropogenic activity. Exposure to ultraviolet (UV) radiation can significantly increase the toxicity of PAHs to aquatic organisms through photo-induced toxicity. While increased mortality is a well-documented effect of photo-induced toxicity, few studies have characterized potential sublethal effects. Impaired visual function is one sublethal effect that may greatly impact fitness and ecological performance. In fishes, the eyes are particularly vulnerable to contaminant exposure which can induce cataract formation and impair vision. The present study developed a novel method to quantify cataract formation in fish lenses following PAH exposure by measuring changes in lens absorbance and optical density. In addition, fixed wavelength fluorescence was used to assess adsorption of PAHs to lenses. Lenses were dissected from field-collected spotted gar (Lepisosteus oculatus) and were exposed to PAHs in crude oil water accommodated fractions in the presence or absence of UV (12 h/d) for 24 h. Absorbance and fluorescence were measured using a BioTek Synergy 2 multi-mode microplate reader at 48, 72, 96, and 120 h. Optical density of lenses significantly increased following co-exposure to PAHs and UV at 96 and 120 h, indicating an effect of photo-induced toxicity. Increased fluorescence was also observed in lenses following crude oil exposure, indicating adsorption of PAHs to tissue. These results provide a novel endpoint of crude oil photo-induced toxicity in fishes and are important for understanding the effects of oil on fishery resources.

ABSTRACT N° ICBF22-295

EXPOSURE TO HYPERSALINITY AND PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) CAUSE DEVELOPMENTAL EFFECTS IN RED DRUM

Kathleen Roark* [1], Kerri Lynn Ackerly[1], Kristin Nielsen[1]

[1]University of Texas at Austin Marine Science Institute , Port Aransas , United States

Abstract:
Estuaries are important sites for many biological and ecological processes, including acting as a nursery habitat for several fish species. Freshwater inflow from terrestrial sources and tidal inflow from the saline ocean cause natural salinity fluctuations and gradients that estuarine biota must tolerate. Changes in flow regimes can result from anthropogenic activity in coastal watersheds, as well as from rising temperatures associated with climate change. Salinities outside of tolerated ranges are known to significantly impact development in fishes, but experimental data is limited in regard to certain species. Estuaries are also vulnerable to contamination via per- and polyfluoroalkyl substances (PFAS), a large class of manmade organofluorine compounds that are used in a variety of industrial and commercial applications. These compounds are highly mobile, persistent, and ubiquitously detected in sediment, soil, surface waters, wildlife, and humans. Estuarine habitats along the Gulf of Mexico are particularly vulnerable to PFAS contamination from a variety of sources, and many commonly detected PFAS remain chronically understudied, with little to no ecotoxicological data available for evaluating ecological risk in marine species or impacted estuaries. Early life stage red drum (Sciaenops ocellatus) were selected as an estuarine model for the present study due to their commercial and ecological importance, as well as their abundance in the Gulf of Mexico, a system which experiences both fluctuations in salinity and contamination by PFAS. Results of the present study address important gaps in our knowledge regarding the effects of PFAS contamination and hypersalinity, separately and in combination with one another.

ABSTRACT N° ICBF22-287

INFLUENCES OF ANTHROPOGENIC STRESS ON THE SOCIALITY OF THREE-SPINED STICKLEBACK (GASTEROSTEUS ACULEATUS)

Sienna Overduin* [1], Isabel Arago[1], Lucy Cotgrove[2], Shaun Killen[2], Kelly Rozanitis[1], Daniel Alessi[1], Tamzin Blewett[1]

[1]University of Alberta, Edmonton, Canada,
[2]University of Glasgow, Glasgow, United Kingdom

Abstract:
Social context influences all aspects of life, including how organisms interact with their surroundings, perceive stressors, and respond to toxicants in their environment. With the condition of aquatic environments declining worldwide, furthering our understanding of the factors that affect the success of species will be essential for their survivability. As such, we examined how copper (Cu) contamination affects behaviour and physiology of three-spined stickleback (Gasterosteus aculeatus) as a function of their social environment. We hypothesized that exposing sticklebacks to Cu, while experiencing the stress of isolation, will lead to greater uptake of Cu and subsequent behavioural responses, as opposed to exposing sticklebacks to Cu in a group setting. Wild-caught fish were exposed to environmentally relevant Cu concentrations of 50μg/L and 150μg/L for 96 hours then assessed for activity level, social cohesion, and foraging. Furthermore, tissues (gill, liver, and intestine) were collected to measure organ-specific bioaccumulation of ions and enzyme activity (Na+/K+/ATPase, H+-ATPase). The interplay between toxicology and social behaviour is not well understood, and as such is not considered in risk assessment standard practice. This potentially leaves a significant gap in our understanding of organismal response to toxicants. Observing response to copper after isolation and group exposures may highlight the need to account for the social environment to truly represent the vulnerability of populations.

ABSTRACT N° ICBF22-231

THE PHENOTYPIC RESPONSES OF FISH TO A COMPLEX ENVIRONMENTAL SCENARIO

Giovanna Mottola* [1], Elli Keituri[1], Alexander Pape[1], Katja Anttila[1]

[1]Department of Biology, University of Turku, Turku, Finland

Abstract:
Climate change is one of the greatest anthropogenic threats for fish. Fish can, however, respond to it with certain extent through phenotypic plasticity. While it has been shown that long exposure to high temperatures could result in an acclimation response, it is still unclear whether and how fish could respond phenotypically to short and extreme exposures, like heat waves. Moreover, since environmental stressors are unlike to occur singularly, it is important to consider whether the thermal phenotypic response is affected by other stressors, like presence of pollutants. Among those, copper has been widely discharged in aquatic environments. Copper has been shown to cause mortality only in high concentrations. However, the potential effect that temperature could cause on copper toxicity, and its effect on the thermal tolerance, has not been uncovered yet. The present study assesses the individual phenotypic plasticity of thermal tolerance in zebrafish exposed for one week to a heat wave, copper run-off and the combination of both. The thermal tolerance was initially assessed in each fish using the Critical Thermal Maximum (CTmax). Afterwards, fish were divided to four different groups (27 ºC - 27 ºC + Cu – 33 ºC - 33 ºC + Cu) after which the CTmax was measured again to evaluate its individual plasticity. Furthermore, the expression of Heat Shock Protein and Hypoxia-Inducible factor was evaluated to reveal if these molecular pathways would be behind the physiological responses to heat wave and copper.

ABSTRACT N° ICBF22-448

BEHAVIORAL DEFECTS IN ZEBRAFISH LARVAE FOLLOWING EXPOSURE TO A SET OF 18 CHEMICALS INTERFERING WITH ESTROGENIC SIGNALING: NO SUSPECTED KEY-ROLE FOR BRAIN AROMATASE

Mélanie Blanc* [1], Sacha Sire[1], Harmony Lautrette[1], Armelle Christophe[2], François Brion[2], Marie-Laure Bégout[1], Xavier Cousin[1]

[1]MARBEC, Palavas,
[2]INERIS ESMI, Verneuil-en-Halatte, France

Abstract:
Exposure to some endocrine disrupter chemicals (EDC) produces behavioural disruptions. There are evidences that early exposure may induce persistent effects such as a decrease in cognitive abilities or an increased risk of neurodegenerative syndromes. Identification of underlying mechanisms is missing in most cases and several mechanisms seem to be at play. This lack of mechanistic understanding is one of the reasons for not integrating behavioural disruptions in evaluation of EDC neurotoxicity. As it is playing a central role in oestrogens synthesis in the brain, brain aromatase (aroB) was a candidate in the mediation of such effects.

We have selected a set of 18 chemicals modifying the expression of aroB to various extents. Then we have conducted zebrafish embryo-larval exposures and tested for behavioural disruptions in 5 dpf larvae. No effects were observed after exposure to 10 of these chemicals while several behavioural traits were disrupted for 8 of them. Behavioural disruptions e.g. distance travelled, path sinuosity, response to light change were observed but no clear pattern could be identified. In addition there was no correlation between behavioural and aroB disruptions. This suggests that aroB does not play a key role in triggering behavioural disruptions resulting from exposure to EDCs. To evaluate the long-term effects of early exposure to these chemicals, we investigated the behaviour of adults exposed during embryonic stage only to a subset of 5 compounds. Seven different tests were used to encompass various behavioural traits relevant for evaluation of individual abilities. Results still under investigation will be presented in an ecological perspective.

This work was supported by the French National Research Agency, FEATS project (ANR-19-CE34-0005-05)

ABSTRACT N° ICBF22-171

ESTRADIOL AND BISPHENOL A AFFECT EARLY SKELETOGENESIS IN THE EUROPEAN SEA BASS D. LABRAX

Emilie Farcy* [1], Camille Martinand-Mari[2], Nicolas Leurs[3], Eric Gasset[4], Gilbert Dutto[4], Melanie Debiais [3]

[1]Montpellier University / MARBEC,
[2]CNRS / I-SEM,
[3]Montpellier University / I-SEM, Montpellier,
[4]Ifremer / MARBEC, Palavas Les Flots, France

Abstract:
Natural or synthetic estrogens are pollutants found in aquatic ecosystems at low concentrations reaching ng.L-1 to µg.L-1. At these concentrations, (xeno-)estrogens are able to interfere with fish endocrine system. If water-borne exposure occurs at early life stages, when blood estrogens concentrations are low, this may have significant consequences for estrogen-sensitive functions such as skeletal development. To better understand how (xeno-)estrogens may affect skeletogenesis, 12 days post-hatch (dph) and 16 dph larvae of the European sea bass Dicentrarchus labrax were experimentally exposed to the natural estrogen estradiol (E2, 0.4 and 40 ng/L) and to the xenoestrogen bisphenol A (BPA, 1.6 and 160 µg/L). Morphological characteristics of the larvae (growth, developmental abnormalities) were recorded together with bone mineralization levels using alizarine red staining. RNA expression levels of several genes playing key roles in skeletogenesis and estrogen signaling pathways were also quantified. When exposure to E2 and BPA started before initiation of bone mineralization, several osteoblast, chondrocyte and osteoclast marker genes were transcriptionally overexpressed. Interestingly, this was correlated to an increase in bone mineralization in larvae head exposed to 0.4 and 40 ng/L E2 or to 1.6 µg/L BPA. In constrast, after the initiation of bone mineralization, exposure to E2 0.4 ng/L had a negative effect on head, vertebrae and tail fin bone mineralization and few genes were differentially transcriptionally regulated. This study brings new insights into the regulatory mechanisms of skeletogenesis by estradiol and into the effects of waterborne exposure to (xeno-)estrogens on the early skeletal development of teleost fishes.

ABSTRACT N° ICBF22-412

PROBIOTIC ADMINISTRATION COUNTERACTS BISPHENOL A REPRODUCTIVE TOXICITY IN ZEBRAFISH

Christian Giommi* [1], Hamid Habibi[2], Francesca Maradonna[1], Oliana Carnevali[1]

[1]Università Politecnica delle Marche, Ancona , Italy,
[2]University of Calgary, Calgary, Canada

Abstract:
Despite Bisphenol A (BPA) use has been limited in several countries, it is widely present in the environment and previous studies in zebrafish revealed that at environmentally relevant concentrations it is able to impair fertility. On the contrary, several studies demonstrated the ability of different probiotic strains to improve organism reproduction, immune system and metabolism. To investigate the ability of SLAb51 probiotic mixture to counteract the adverse BPA effects on reproduction, a 28-day trial was set up with four groups: BPA (10 µg/L BPA); P (10^9 CFU/g SLAb51 trough diet); BPA+P (10 µg/L BPA and 10^9 CFU/g SLAb51 trough diet); C (control group). Fertility results did not show significant differences among experimental groups compared to C fish. PCA analysis of RT-qPCR results (fshr, lhcgr, ar, esr1, esr2a, esr2b, pgrmc1 and pgrmc2) demonstrated that male BPA+P fish result in a phenotype closer to C or P levels, than to BPA. Similarly, in females, gene expression profile (fshr, lhcgr, pgrmc1 and pgrmc2) in BPA+P class III follicles were closer to C ones, and in class IV follicles C and BPA+P showed good similarity based on PC2, supporting the hypothesis that SLAb51 can antagonize BPA toxicity. Histological analysis showed that SLAb51 positively interact with spermatogenesis, increasing the number of spermatogonia and spermatozoa respect to C and increasing the number of spermatogonia in BPA+P respect to BPA, while regarding oocyte growth and maturation processes further investigation are in progress. Altogether the results would help in building up a comprehensive scenario regarding SLAb51 effects in zebrafish, to encourage further research on probiotics use as tool to mitigate the effect of the many toxicants ubiquitously present worldwide.

ABSTRACT N° ICBF22-298

COMPARATIVE EFFECTS OF DEVELOPMENTAL METFORMIN EXPOSURE ON WILD AND CULTURED EMBRYOLARVAL FISHES

Kristin Nielsen* [1], Lily DeCamp[1], Mona Birgisson[1], Mark McMaster[2], Joanne Parrott[2], Vince Palace[3], Karen Kidd[4], Erin Ussery[2]

[1]University of Texas, Port Aransas, United States,
[2]Environment and Climate Change Canada, Burlington,
[3]IISD-ELA, Kenosha,
[4]McMaster University, Hamilton, Canada

Abstract:
The increasing environmental occurrence of widely prescribed pharmaceuticals, like metformin (the first line treatment for Type 2 diabetes), is of considerable concern from an ecological risk perspective. Laboratory exposure studies have shown that metformin adversely impacts multiple species of non-target aquatic biota; however, the degree of exposure required to elicit effects varies according to species and ontogeny. Moreover, it is unknown whether laboratory-derived toxicity values are representative of those for wild fishes, as some studies have suggested that metformin will undergo environmental processes that will prevent adverse effects on biota in natural systems. Therefore, we conducted exposures with environmentally relevant (0, 5, 50- µg/L) metformin concentrations in duplicate, using laboratory-reared and wild-spawned fathead minnow (Pimephales promelas) embryos. Exposure solutions for the lab-spawned cohort were prepared by dosing reconstituted freshwater with metformin just prior to study commencement, while the wild-spawned cohort was exposed via previously spiked lake water retrieved from 5,000-L limnocorrals deployed in a naturally occurring boreal lake system. Limnocorrals were spiked a week before study commencement to account for environmental processes that may alter the bioavailability of metformin. Metformin exposure altered developmental endpoints in both cohorts that are consistent with energy dyshomeostasis; however, the magnitude and direction of change varied considerably between groups. When evaluated through the lens of fish fitness, impacts to wild-spawned larvae were disproportionately severe. These results also indicate that metformin (and/or its metabolically active metabolite, guanylurea) remains bioavailable in naturally occurring systems, and that current exposure scenarios may be sufficient to adversely impact wild fishes.

ABSTRACT N° ICBF22-365

MESOCOSM INVESTIGATIONS OF THE ECOTOXICOLOGICAL EFFECTS OF METFORMIN ON FISH HEALTH

Oana Birceanu* [1], Mark McMaster[2], Kristin Nielsen[3], Vince Palace[4], Joanne Parrott[2], Karen Kidd[5], Nicholas Blandford[6], Joanna Wilson[5], Jessie Cunningham[2], Abby Wynia[2], Thomas Clark[2], Erin Ussery [2]

[1]University of Western Ontario, London,
[2]Environment and Climate Change Canada, Burlington, Canada,
[3]The University of Texas at Austin, Austin, United States,
[4]International Institute for Sustainable Development-Experimental Lakes Area, Winnipeg,
[5]McMaster University, Hamilton,
[6]University of Manitoba, Winnipeg, Canada

Abstract:
The occurrence and fate of pharmaceuticals in the aquatic environment is an emerging issue in aquatic toxicology. Currently, one of the most prevalent contaminants is the type-2 diabetic drug, metformin, which is released in freshwaters via wastewater effluent. Levels of metformin in surface waters range from 0.4-30 µg/L. Although this chemical is continuously released in the environment, little is known about its fate and effects in freshwater organisms. In the laboratory, metformin reduces larval growth and alters whole-body metabolome, but little is known about its impact in wild fish. To this end, we conducted an 8 week in-lake mesocosm study at the IISD-Experimental Lakes Area, to investigate the fate and effects of metformin in adult, wild-caught fathead minnows. Four replicate mesocosm were assigned to one of three treatments: 0, 4, or 40 µg/L metformin. At the end of the 8-week exposure, fish condition factor, hepatosomatic index, gonadosomatic index and carcass water remained unaffected. In addition, carcass lipid levels were lower in the metformin exposed fish, with transient effects on liver metabolic capacity. To date, our study shows that exposure of wild fish to environmentally relevant levels of metformin leads to minimal disturbances in energy allocation, liver metabolism and gonadal investment. Our work will explore the liver metabolic capacity and energy breakdown in more detail, to determine the range of effects that metformin can have on wild fish populations.

ABSTRACT N° ICBF22-224

PHYSIOLOGICAL CONSEQUENCES OF CHRONIC EXPOSURE OF FISH TO VIRGIN OR CONTAMINATED MICROPLASTICS

Xavier Cousin*[1], Bettie Cormier[2], Florane Le Bihanic[3], Steffen Keiter[4], Jérôme Cachot[5], Marie-Laure Bégout[1]

[1]MARBEC, Palavas, France,
[2]NTNU, Gløshaugen, Norway,
[3]OFB, Bordeaux, France,
[4]Orebro Uni, Orebro, Sweden,
[5]Uni Bordeaux, Bordeaux, France

Abstract:
The occurrence of microplastics (MPs) in the digestive tract of fish varies by species, but can reach 75% of individuals. The toxicity of MPs remains poorly known and may be of physical or chemical origin. We have carried out trophic exposures of marine medakas (O. melastigma) and zebrafish (D. rerio) to industrial MPs (PE 11-13 µm, PVC >250 µm) either virgin or doped with environmental concentrations of organic pollutants. Another exposure was carried out using MPs obtained after grinding (at 50 µm) plastics collected on two beaches in Guadeloupe, Marie-Galante (lightly polluted beach) and Petit-Bourg (highly polluted beach). MPs were introduced into the food at a rate of 1% by weight and exposure began at different ages depending on the size of the MPs, from the first food intake (PE), at 1 month (Guadeloupe) and 2 months (PVC). Molecular markers (genotoxicity, oxidative stress, endocrine status) and individual variables (survival, growth and reproduction, behaviour) were monitored throughout exposure.

For both species, we have demonstrated:

- The absence of acute toxicity, genotoxicity or oxidative stress
- A decrease in growth after several months of exposure for all MPs and a reduction in reproduction for some of them
- The disruption of some offspring traits (length and behavior)

These results indicate that MPs can be toxic through different mechanisms depending on the biological defects considered. These results suggest that long-term exposures to MPs induce physiological alterations that may have consequences on recruitment and populations.

Work performed within JPI-Oceans project EPHEMARE (FORMAS, 2015-01865; ANR-15-JOCE-0002-01)

ABSTRACT N° ICBF22-234

BIOACCUMULATION AND DEVELOPMENTAL TOXICITY OF POLYCHLORINATED BIPHENYLS ON EARLY LIFE STAGE ZEBRAFISH (DANIO RERIO)

Corey Green* [1], Jeffery Morris[2], Rachel Leads[1], Claire Lay[2], Michel Gielazyn[3], Lisa Rosman[4], Aaron Roberts[1]

[1]University of North Texas, Denton,
[2]Abt Associates, Denver,
[3]NOAA, St.Petersperg,
[4]NOAA, New York, United States

Abstract:
Polychlorinated biphenyls (PCBs) are long-lived synthetic compounds that were widely used until 1979. We investigated the cardiac, neurologic, growth, reproductive, and behavioral effects of exposure to Aroclor 1254 in zebrafish. Embryos were exposed at 6 hpf via aqueous solution for 96 hr with and without renewal. Nominal concentrations of Aroclor 1254 ranged from 8.7% to 870% of measured concentrations. We measured PCB concentrations in both bioassay and tissue exposure solutions and tissue samples. Tissue for RNA-Seq was collected and heart rate, neurological endpoint (eye tremors), and cardiac edema were measured at 102 hpf and 174 hpf. Cardiac edema was not present in Aroclor 1254-treated zebrafish but was observed in those exposed to PCB-126 as a positive control. However, dose-dependent bradycardia was observed in zebrafish exposed to Aroclor 1254 and PCB-126 at both 102 and 174 hpf. Similarly, a dose dependent increase in eye tremor behavior, duration, and intensity was observed in embryos exposed to Aroclor 1254 at both 102 and 174 hpf, while severely deformed PCB-126 exposed larvae exhibited no eye tremors or response to external stimuli and negative control fish exhibited no eye tremors but a positive response to external stimuli. Eye tremor behavior appears similar to that of other dopaminergic-related neurodegeneration associated with PCB exposure. Bioinformatic analyses found that the top biological processes affected included visual function pathways supporting the eye tremor phenotypes.

ABSTRACT N° ICBF22-116

TRANSFERABLE APPROACHES TO EVALUATE THE HEALTH OF EARLY LIFE STAGES OF A THREATENED ANADROMOUS FISH SPECIES, THE LONGFIN SMELT.

Florian Mauduit* [1], Amélie Segarra[1], Yuzo Yanagitsuru[1], Tien-Chieh Hung[1], Nann Fangue[1], Richard Connon[1]

[1]University of California, Davis, United States

Abstract:
Transferable approaches to evaluate the health of early life stages of a threatened anadromous fish species, the Longfin Smelt.

Mauduit F., Segarra A., Yanagitsuru Y., Hung T.C., Fangue N.A., Connon R.E.

Conservation efforts are sometimes constrained by lack of knowledge about the species' biology and the impact of environmental stressors upon them. Longfin Smelt (Spirinchus thaleichthys) is a threatened anadromous fish species found in estuaries and lakes along the northern Pacific coast of North America. To evaluate potential contributions of anthropogenic activities to their decline, we aimed to 1) transfer tools and approaches commonly used on model species and/or later life stages to assess the health of Longfin Smelt larvae and 2) apply these tools to determine effects of an exposure to a frequently detected pyrethroid insecticide; Bifenthrin. For this, we tested the relevance of the light/dark stimuli induced behavior test and of the thermal susceptibility test for the Longfin Smelt larvae. Movement tracking of 1 to 4 dph larvae during alternating light-dark periods revealed a pattern of increased larvae's locomotion in the light followed by resting state in the dark, allowing the establishment of a behavioral model for this species. In addition, we tested larvae's thermal tolerance by monitoring the heart rate of anesthetized individuals submitted to a stepwise temperature increase. We observed that larvae heart rate gradually increased with temperature until becoming arrhythmic. Combined, these two tests allow for the assessment of Longfin Smelt larvae health and provide sensitive endpoints to evaluate the impact of environmental stressors upon this species. Exposure to environmentally relevant concentrations of bifenthrin for 96h induced hyperactivity but did not affect cardiac thermal tolerance of the 1-4 dph Longfin Smelt larvae.

ABSTRACT N° ICBF22-283

THE OLFACTORY SYSTEM OF CHONDRICHTHYES: NUMBERS AND FEATURES OF A PUZZLING SENSORY STRUCTURE

Sara Ferrando* [1]

[1]University of Genoa, Genoa, Italy

Abstract:
The olfactory system of Chondrichthyes resembles that of all vertebrates, as it is constituted by sensory organs, olfactory nerves, and olfactory bulbs which are connected to the rest of the brain by olfactory tracts; it also resembles that of most bony fishes, as it is not subdivided between a properly olfactory part and a vomeronasal one. Deeper analyses, ranging from gross morphology to genomic level, showed in the olfactory system of Chondrichthyes a strong reduction of the main olfactory system components compared to other fishes. The ciliated olfactory neurons have never been described, while the genomes show a very low number of genes belonging to the family of the Olfactory Receptors (ORs). The largest family of chemical receptors in Chondrichthyes, the Vomeronasal type-2 Receptors (V2Rs), is a relatively small family, thus the overall number of chemical receptors (ORs, V1Rs, V2Rs, and TAARs) in these fishes is quite low, compared to other vertebrates (an average of 38 in the 4 investigated species). On the other hand, the sensory organ and the bulb are relatively large, and the shape of the sensory organ is complex and very diversified among species. Thus, chondrichthyan olfaction seems to rely on the largest and morphologically most complex vomeronasal system in the whole vertebrate subphylum, and on a small set of receptors. Beside evolutionary considerations, it is noteworthy that any knowledge about the effects of environmental changes on the olfaction of bony fishes cannot be used as it is to predict effects on Chondrichthyes.

11-Sensing the Environment: Molecules to Populations (Sensing the Anthropocene)

ABSTRACT N° ICBF22-155

THE AGE-RELATED CHANGES IN OLFACTORY-MEDIATED BEHAVIOURAL RESPONSES OF ZEBRAFISH MAY NOT BE EQUAL FOR ALL ODOURANTS

Arash Shahriari* [1], Keith Tierney[1], Connor Stewart[1], Gurvinder Dhaliwal[1], Nojan Mannani[1]

[1]University of Alberta, Edmonton, Canada

Abstract:
Animals are enveloped in a chemosensory world. Olfaction, in particular, drives important behaviours such as finding food and avoiding predators. The olfactory system is also considered to be one of the first sensory systems to decrease in functionality as animals age. This hyposmia is due to a natural decline in olfactory sensory neurons (OSNs), which detect olfactory cues known as odourants. In the popular vertebrate model, the zebrafish, there are five OSN classes, in which each class detects its own set of odourants. It is therefore possible that aging-associated hyposmia in zebrafish will depend on the odourant being detected as the population of various OSN classes may be affected differently. We demonstrate how aging affects the population size of ciliated and microvillus OSNs, which are the two classes most abundant in zebrafish. Because ciliated and microvillous OSNs detect bile acids and amino acids, respectively, the effects aging has on zebrafish behavioural response towards a mixture of amino acids or of bile acids are observed. This research will demonstrate that the degree of aging-associated hyposmia may not be equal for all odourants and therefore, aging animals may better respond to certain chemical components of their environment over others.

ABSTRACT N° ICBF22-188

MOLECULAR AND PHENOTYPIC EVIDENCE THAT ZEBRAFISH EMBRYOS CHEMICALLY PROPAGATE HEAT STRESS TO EACH OTHER

Lauric Feugere*[1], Adam Bates[1], Joerg Hardege[1], Pedro Beltran-Alvarez[2], Katharina Wollenberg Valero[1]

[1]Department of Biological and Marine Sciences, University of Hull,
[2]Department of Biomedical Sciences, University of Hull, HULL, United Kingdom

Abstract:
Fish face rising temperatures and increasingly recurrent thermal stress that accompany climate change. Such stressors alter fish biology with effects from molecular to ecological levels. We have recently shown that aquatic animals can propagate stress from stressed donors to naive receivers in response to abiotic stressors. Our recent research found that (i) zebrafish embryos alter their development, behaviour, and gene expression when incubated in the medium of a heat-stressed embryo, and (ii) that marine invertebrates change their behaviour when exposed to the medium of pH-stressed animals. We further hypothesised that the propagation of stress between conspecifics is mediated by released “stress metabolites”. We aimed to investigate further the molecular aspects of stress communication using transcriptomics and metabolomics. Zebrafish embryos were exposed to repeated thermal stress and to the “stress medium” in which another embryo was stressed beforehand. Embryos exposed in the control medium in which a control embryo was incubated beforehand served as negative controls. The metabolome of the stress and control media showed distinct chemical profiles, with the stress medium containing several candidate stress metabolites. Furthermore, zebrafish embryos incubated in the stress medium showed behavioural and developmental alterations. The transcriptomic signature of embryos was regulated by stress metabolites. Altogether, our results indicated that the effects of thermal stress can be propagated between a stressed donor and a naive receiver zebrafish embryo through means of stress metabolites. Further work is warranted to refine the composition of the stress medium and the molecular pathways they activate in receivers.

ABSTRACT N° ICBF22-375

WHEN THE NOSE FAILS: A TALE OF ANTHROPOGENIC POLLUTION.

Mar Huertas* [1]

[1]Texas State University, San Marcos, United States

Abstract:
The sense of smell is involved in the control of several physiological processes in fish (e.g. reproduction and feeding). Consequently, a loss of this sense can have deleterious consequences for the survival of fish species. Nitrogen is a dangerous agricultural pollutant because it undergoes nitrification, producing toxic nitrogenous products, such as ammonia, nitrate and nitrite. Moreover, these compounds often accumulate in intensive aquaculture systems as a result of nitrogenous excretion from fish. Thus, fish in natural environments and aquaculture set-ups can be continuously exposed to sub-lethal concentrations of environmental nitrite. Although the effects of nitrite at lethal concentrations has been studied for decades, the effects of long-term exposure to sub-lethal nitrite is less explored, and its impact on fish senses is unknown. We anticipate that continuous sub-lethal exposure to nitrite can have subtle effects in the sensory nose, which projects the sensitive cilia of its neurons to the external aquatic media. Our research in goldfish and catfish models showed that nitrite accumulates first in the nose and brain, and later in the gill, and gut. Surprisingly, we also found that nitrite has a “double-edged sword effect.” Thus, at very low concentrations, nitrite improves olfaction and feeding behavior. However, at increasing concentrations the nasal microbiome composition changes, damage appears in the olfactory epithelia and feeding behavior declines. These changes can result in higher susceptibly of fish to pathogen nasal infections and starvation. In the long term, chronic exposure to nitrite can have negative implications for fish culture and fish wellbeing.

ABSTRACT N° ICBF22-192

EFFECTS OF L-ALANINE EXPOSURE DURING EARLY LIFE STAGE ON OLFACTORY DEVELOPMENT AND OVERALL SURVIVAL IN AGE-0 LAKE STURGEON (ACIPENSER FULVESCENS)

Tyler Edwards* [1], Ian Bouyoucos[1], Caleb Hasler[2], Gary Anderson[1]

[1]University of Manitoba,
[2]University of Winnipeg, Winnipeg, Canada

Abstract:
Rearing environment plays an important role in phenotypic development of fishes, however, little research has examined the influence of environment on the development of olfaction. The olfactory epithelium in fishes is composed of three well-described olfactory sensory neurons (OSNs) the microvillous, ciliated, and crypt cells. The goal of this study was to determine if olfactory development in lake sturgeon (Acipenser fulvescens) and overall survival could be enhanced by treatment of the tank water with the addition of L-Alanine. L-alanine was added daily from days 17 through 20 post fertilization. We chose L-alanine as it is a standard stimulus of microvillous OSNs related to food cues and thus may assist in recognition of food as the fish transition from endogenous yolk to exogenous feeding. Body length, mass, mortality, and relative mRNA abundance of microvillous OSN receptor genes (V2R 26, V2R 1) and ciliated OSN receptor genes (OR 1, TAAR 1) were assessed throughout development. Behavioural assays were conducted to assess if there were differences between foraging behaviour in L-alanine-exposed larvae and control. Individuals were sampled prior to treatment then throughout development at days 17, 21, 26, 31, 50, 65 and 80 post-fertilization. There were no significant differences observed in body measurements or mortality. However, significant differences were observed between control and L-alanine treatments in expression of all-genes at points throughout development. The data indicates an environment phenotype interaction in the development of the olfactory system in early development, that may manifest as a change in behaviour to the presence of food.

ABSTRACT N° ICBF22-131

THE RELATIONSHIP BETWEEN LIFE HISTORY AND PHYSIOLOGICAL SENSITIVITY TO ELEVATED MARINE CO2 IN DIFFERENT NORTH PACIFIC OCEAN FISH SPECIES

Chase Williams* [1], Evan Gallagher[2], Paul McElhany[1], Shallin Busch[1], Andrew Dittman[1]

[1]NOAA NWFSC,
[2]University of Washington, Seattle, United States

Abstract:
The effects of predicted levels of elevated CO2 in marine waters on invertebrates and fish species is an increasing concern. Numerous studies have found significant physiological and behavioral effects in fish induced by elevated CO2 exposures, including our earlier study that demonstrated olfactory injury from elevated CO2 in coho salmon (Oncorhynchus kisutch). However, the effects of elevated CO2 may be species-specific, and there have been few studies addressing the effects of elevated CO2 on benthic fish. In the current study, we investigated the effects of elevated CO2 exposures on the deep-water benthic dwelling species, sablefish (Anoplopoma fimbria). Sablefish were exposed to three different levels of CO2 for two weeks, followed by behavioral, neurophysiological and gene expression analysis of the central olfactory system. Analysis of mRNAs in sablefish encoding genes that maintain GABA-mediated olfactory bulb signal processing showed olfactory bulb genes modulated by CO2 in our previous salmon study were generally unaffected by high CO2. Analysis of food odorant-driven behaviors did not differ from fish maintained under control CO2. The sablefish olfactory behavior results were consistent with electro-olfactogram (EOG) recordings of odorant signaling that did not differ among treatment and controls, suggesting that the transient effects of CO2 observed on gene expression did not lead to alterations in sablefish olfactory neurobehavior. The results of our study contrast other studies demonstrating adverse effects of elevated CO2 in pelagic fish, but support differences among fish species to susceptibility to elevated CO2, potentially associated with life history traits.

ABSTRACT N° ICBF22-303

TESTING THE ROLE OF TURBIDITY ON THE VISUAL SENSITIVITY OF AN AFRICAN CICHLID (PSEUDOCRENILABRUS MULTICOLOR VICTORIAE)

Jai Tiarks* [1], Suzanne Gray[1]

[1]Ohio State University, Columbus, United States

Abstract:
Turbidity is increasing in freshwaters globally due to human activities and is known to affect visually-mediated behaviors dependent on visual cues in fish. As anthropogenic impacts continue to degrade aquatic environments, it is critical to determine how sensory systems are affected and what this might mean for population persistence. Our aim is to determine the effect of turbidity on behavioral and visual traits in an African cichlid fish (Pseudocrenilabrus multicolor victoriae) that experiences extremes of turbidity across its East African range. We used a behavioral test of visual sensitivity (optomotor response) with P. multicolor collected from two natural populations in the Lake Nabugabo system, Uganda. The two sampling locations differ in environmental characteristics: Ndyabusole (high turbidity, high dissolved oxygen), Lwamunda (low turbidity, low dissolved oxygen). Each fish (n=40) was acclimated for 24 hours before optomotor response trials were conducted. During the trial, the optomotor screen was rotated and a turbidity solution was added to the tank every 2 minutes until an optomotor response was no longer observed (i.e. the fish reached a detection threshold and stopped following the screen). Preliminary results show higher visual detection thresholds in the Ndyabusole (turbid) population compared to the Lwamunda (clear) population ($F(3, 35) = 6.676$, $p < 0.001$).This research improves our understanding of the effect of elevated turbidity on African cichlid visual sensitivity and contributes to growing knowledge of how animals respond to environmental change.

ABSTRACT N° ICBF22-459

ION REGULATION AT THE GILLS PRECEDES GAS EXCHANGE AND THE ORIGIN OF VERTEBRATES

Michael Sackville[1], Christopher Cameron[2], J. Andrew Gillis[3], Colin Brauner* [4]

[1]Marine Biological Laboratory, Woods Hole, United States,
[2]Université de Montréal, Montréal, Canada,
[3]University of Cambridge , Cambridge, United Kingdom,
[4]University of British Columbia, Vancouver, Canada

Abstract:
Gas exchange and ion regulation at the gills play key roles in vertebrate evolution. Gills are hypothesized to first acquire these important homeostatic functions from the skin in stem vertebrates, facilitating the evolution of larger, more active modes of life. However, this hypothesis lacks functional support in relevant taxa. Here, we characterize gill and skin function in a hemichordate (acorn worm, Saccoglossus kowalevskii), cephalochordate (amphioxus, Branchiostoma floridae) and vertebrate (lamprey ammocoete, Entosphenus tridentatus) with the burrowing, filter-feeding traits of vertebrate ancestors. We provide functional support for a vertebrate origin of gas exchange at the gills with increasing body size and activity, as direct measurements in vivo find only ammocoete gills as the dominant site of gas exchange, and only with increasing body size or challenges to oxygen supply and demand. Conversely, gills of all three taxa are implicated in ion regulation. Direct measurements in vivo find ammocoete gills responsible for all ion flux at all body sizes, while molecular markers for ion regulation are higher in amphioxus and acorn worm gills than skin. This suggests an earlier origin for ion regulation at the gills unrelated to vertebrate size and activity, perhaps at the very inception of pharyngeal pores in stem deuterostomes.

05-Ion and acid-base regulation in fishes

ABSTRACT N° ICBF22-247

HOLDING WATER: AN EVOLUTIONARY AND PHYSIOLOGICAL EXAMINATION OF MARINE-FRESHWATER TRANSITIONS IN FISHES

Andrew Whitehead* [1], Lauren Sallan[2], Stephen McCormick[3]

[1]University of California Davis, Davis,
[2]University of Pennsylvania, Philadelphia,
[3]University of Massachusetts Amherst, Amherst, United States

Abstract:
Marine ecosystems provide a niche volume that is vastly larger than for freshwater, yet the number of fish species occupying these distinct ecosystems is approximately equal. Evolutionary transitions between marine and freshwater are therefore important for explaining the extraordinary phyletic diversity of fishes. Yet much mystery and controversy is associated with explanations for 1) the large apparent asymmetry in transitions between marine and freshwater, and 2) the nature of osmotic habitats (marine, estuarine, or freshwater) that were ancestral to major clades of fishes. We integrate research findings from paleontology, evolutionary genetics, and comparative osmoregulatory physiology, to provide deeper understanding of fish evolution; we provide mechanistic insight into the evolutionary patterns and processes that produced the broad diversity of osmoregulatory strategies evident among contemporary fishes. We synthesize evidence to assert that 1) intertidal/subtidal, possibly estuarine, nearshore habitats are the most likely ancestral osmotic environment for vertebrates, 2) asymmetry in the impact of mass extinctions between marine and freshwater taxa, and evidence for large-scale habitat transitions thereafter, had major implications for ancestral osmotic habitats of fishes, 3) rare bursts of speciation and complex patterns of transitions confined to a few clades account for the high freshwater species diversity, and 4) the preponderance of marine-to-freshwater directionality helps to explain the greater diversity of physiological strategies that have evolved for osmoregulation in freshwater compared to seawater. We conclude by providing guidance for future research that should further illuminate the evolutionary history and mechanisms that underpin osmoregulatory diversity displayed by these highly-speciose vertebrate groups.

ABSTRACT N° ICBF22-458

EVOLUTION OF THE HORMONAL CONTROL OF OSMOREGULATION: INSIGHTS FROM STUDIES IN SEA LAMPREY

Ningping Gong[1], Diogo Ferreira-Martins[2], Jessica Norstog[3], Stephen McCormick[2, 3], Mark Sheridan*[1]

[1]Texas Tech University, Lubbock,
[2]U.S. Geological Survey, Turners Falls,
[3]University of Massachusetts, Amherst, United States

Abstract:
We previously isolated and characterized a prolactin-like cytokine from the pituitary of sea lamprey (lPRL-L), a representative of one of the oldest lineages of vertebrates (jawless fish). Juvenile sea lamprey reared in fresh water (FW) were exposed to ion-poor water (IPW) or seawater (SW). Pituitary lPRL-l mRNA abundance increased in IPW relative to levels observed in FW. Immunoreactive-lPRL-l in the pituitary gland was significantly greater in juveniles exposed to IPW compared to FW controls. Exposure of juveniles to SW also affected branchial mRNA expression of several key ion transporters critical for osmoregulation, including several isomers of Na+/K+ ATPase (ATP) and of the solute carrier family 12 (SLC12). Notably, branchial expression of ATP1a1.1, SLC12a3.3, and SLC12a3.2 mRNAs decreased after SW exposure, whereas branchial expression of ATP1b3 and SLC12a2.2 increased after SW exposure. Treatment with recombinant lPRL-l (rlPRL-l) blocked the SW-associated decreases in ATP1a1.1 and SLC12a3.3 mRNA expression as well as elevated their expression above levels seen in SW. These data suggest that ATP1a1.1, SLC12a3.2, and SLC12a3.3 are involved in FW adaptation of lamprey and that ATP1b3 and SLC12a2.2 are involved in SW adaptation. In addition, lPRL-l modulates FW adaptation in lamprey, a role which is conserved for PRL hormones in jawed vertebrates. (Supported by NSF grant 1558037 to MAS and SDM.)

ABSTRACT N° ICBF22-359

SEAWATER TOLERANCE AND FEEDING IN LANDLOCKED AND ANADROMOUS POPULATIONS OF SEA LAMPREY

Jessica Norstog* [1], Stephen McCormick[1, 2]

[1]University of Massachusetts Amherst, Amherst,
[2]USGS EESC Conte Anadromous Fish Research Center, Turners Falls, United States

Abstract:
The life histories of anadromous and landlocked Sea Lamprey are similar, though landlocked populations lack exposure to seawater and thus experience relaxed selection on traits associated with survival in seawater, including salinity tolerance and associated physiological traits. This study investigated differences in one anadromous and three landlocked populations of Sea Lamprey in the capacity of metamorphosed juveniles for ion regulation in seawater. Landlocked lamprey had lower survival in 35 ppt seawater compared to anadromous lamprey. Plasma ion concentrations after exposure to 30 ppt seawater were elevated in two upper Great Lakes populations compared to the anadromous population. Freshwater ion transporter mRNA levels were significantly greater in landlocked populations in FW and remained higher compared to the anadromous population for the duration of the SW-exposure experiment. All populations showed strongly elevated gill NKA activity compared to larvae, which increased over time after exposure to 30 ppt seawater. These results suggest that there are population-based differences in salinity performance that are consistent with relaxed selection on traits for seawater entry in landlocked populations.

ABSTRACT N° ICBF22-297

ROLE OF GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR IN METAMORPHOSIS AND OSMOREGULATION OF SEA LAMPREY (PETROMYZON MARINUS)

Diogo Ferreira-Martins* [1], Emily Walton[1], Mark Sheridan[2], Stephen McCormick[1, 3]

[1]Department of Biology, University of Massachusetts UMASS, Amherst, MA,
[2]Department of Biological Sciences, Texas Tech University, Lubbock, TX,
[3]U.S. Geological Survey, Eastern Ecological Science Center, Conte Anadromous Fish Research Laboratory, USGS, Turners Falls, MA, United States

Abstract:
Growth hormone (GH) and Insulin-like growth factor 1 (IGF1) promote seawater tolerance in advanced teleost fishes, but the endocrine pathways that control osmoregulation in more basal euryhaline vertebrates have yet to be elucidated. In this study we have analyzed the function of the GH/IGF1 axis in the control of osmoregulatory mechanisms in a representative of the base of the vertebrate lineage, the sea lamprey, Petromyzon marinus. Sea lampreys are an anadromous and semelparous species that undergo metamorphosis in preparation for their downstream migration and a parasitic, marine trophic phase. In this study, a seasonal effect on gh mRNA levels in the pituitary was observed and a large increase in liver igf1 but not ghr mRNA abundance was detected during metamorphosis. This suggests that IGF1 produced by the liver plays a key role in the metamorphic development and seawater tolerance of sea lamprey. A clear increased in gill igf1 mRNA levels during metamorphosis was also observed along with increased NKA and NKCC1, suggesting a paracrine action of IGF1 in the gill. Salinity challenges on postmetamorphic juveniles did not show changes in gh, ghr, and igf1. This together with a slight increase in plasma chloride concentrations after 1 day of seawater exposure supports the hypothesis that upon completion of metamorphosis, all physiological preparations for seawater entry are complete. Our results indicate that GH/IGF1 axis in lamprey has an analogous function to that of teleosts in controlling seawater acclimation and may also have important effects on other physiological and metamorphic changes during metamorphosis.

ABSTRACT N° ICBF22-512

EFFECTS OF A BLOOD DIET AND HABITAT ON THE GILL ION TRANSPORT MACHINERY OF PARASITIC AND NON-PARASITIC LAMPREYS.

Michael P Wilkie* [1], Julia Sunga[1], Margaret F Docker[2], Jonathan M Wilson[1]

[1]Wilfrid Laurier University, Waterloo,
[2]University of Manitoba, Winnipeg, Canada

Abstract:
Sea lamprey (Petromyzon marinus) and silver lamprey (Ichthyomyzon unicuspis) enter a blood-feeding, parasitic phase following metamorphosis. While juvenile parasitic sea lampreys are anadromous, parasitic silver lamprey are exclusively freshwater residents, as is the non-parasitic Northern brook lamprey (Ichthyoyzon fossor), which proceeds directly to the non-feeding adult stage following metamorphosis. The goal of our study was to determine if these differences in feeding behaviour and habitat were reflected in the functional organization of the gills. Using immunohistochemistry, we: (i) determined if feeding type influenced the distribution or abundance of key transporters related to ammonia excretion and ion regulation in the gills, and (ii) contrasted the gills of juvenile silver lamprey to those of anadromous sea lamprey. In sea lamprey, there was an abundance of Rhesus glycoproteins (Rhcg-like) concentrated within gill saltwater (SW) ionocytes found between lamellae, consistent with a high capacity to excrete ammonia. In contrast, Rhcg-like expression was much lower with a lamellar distribution in silver and northern brook lampreys, which lacked discrete SW ionocytes. In sea lamprey, branchial Na+/K+-ATPase was highly concentrated in SW ionocytes, a likely adaptation for seawater acclimatization not observed in silver lamprey. In silver and northern brook lampreys, V-ATPase transporters were concentrated in intercalated ionocytes found in interlamellar spaces, a feature consistent with active H+ excretion, indirectly coupled to Na+ uptake in fresh water. In sea lamprey, V-ATPase was absent. We conclude that the functional organization of lamprey gills is much more influenced by the presence or absence of FW-SW migration, than by diet.

ABSTRACT N° ICBF22-255

UNDERSTANDING THE PHOTOPERIOD-DRIVEN SEASONAL CLOCK OF FISH: EVIDENCE FOR A ROLE OF THYROID STIMULATING HORMONE, DEIODINASE AND THYROID HORMONE IN THE PITUITARY AND BRAIN.

Stephen Mccormick* [1], Daniel Hall[1], Thrandur Bjornsson[2]

[1]University of Massachusetts, Amherst, Amherst, United States,
[2]University of Gothenburg, Gothenburg, Sweden

Abstract:
Seasonal timing is important for many critical life history events of vertebrates, and photoperiod is often used as a reliable seasonal cue. In mammals and birds it has been established that a photoperiod driven seasonal clock resides in the brain and pituitary, and is driven by increased levels of thyroid stimulating hormone (TSH) and deiodinase (dio2) which leads to local increases in triiodothyronine (T3). We used anadromous (migratory) Atlantic salmon that utilize photoperiod to cue downstream migration and the development of salinity tolerance as a model species to examine photoperiod signaling in fish. After being held on short days (LD8:16) for several weeks, exposure to increased daylength (LD16:8) for 20 days resulted in increased plasma growth hormone (GH), gill Na/K-ATPase (NKA) activity and gill nka a1b mRNA levels that normally accompany increased salinity tolerance of salmon in spring. Pituitary tshBb mRNA levels increased within 2 days of exposure to increased daylength and were sustained at high levels for 30 days. Increased daylength also resulted in increases in dio2 mRNA and T3 levels in the brain. A threshold of 12 hours of daylength was necessary to stimulate pituitary tshBb, brain dio2 and plasma GH. Intracerebral T3 treatment resulted in elevated levels of plasma GH. The results provide evidence that the photoperiod-driven seasonal clock in fish includes pituitary production of TSH and elevated levels of brain dio2 and T3.

ABSTRACT N° ICBF22-261

THE EFFECT OF PHOTOPERIOD MANIPULATION IN FRESHWATER ON ACID-BASE REGULATION, SUBSEQUENT SEAWATER TRANSFER AND HYPOXIA TOLERANCE IN ATLANTIC SALMON (SALMON SALAR)

Gam Le* [1], Daniel Montgomery[1], Rachael Mackinnon[1], Daniel Laronde[1], Jeff Richards[1], Colin Brauner [1]

[1]Department of Zoology, The University of British Columbia, Vancouver, BC, Canada

Abstract:

Photoperiod manipulation (PM) and CO2 levels affect to fish physiology and growth performance in recirculating aquaculture systems (RAS), typically smoltification in the transfer from freshwater (FW) to seawater (SW).

Smolts are transferred into marine net-pens for a 2 year grow-out phase to yield market-sized fish. A major industry goal is to produce larger, more robust smolts with higher marine net-pen growth rates and survival, ultimately reducing the time farmed Atlantic salmon spend in the marine environment. Large smolts can be generated by PM in RAS, but little is known about how this procedure affects susceptibility to elevated CO2 and hypoxia. To address this, Atlantic salmon were reared from hatch in FW 3ppt under continuous light (24:00) with or without PM when fish reached one of three different sizes (230g, 580g of 1000g). The PM consisted of an 8 week exposure to (12:12 light:dark) followed by 4 week return 24:00, a treatment used in industry to induce smoltification. PM and non-PM fish were then exposed to 0 or 1.5% CO2 (hypercapnia) for 96h in FW (where blood and red blood cell pH was significantly reduced with no effect of PM) and then transferred to air equilibrated (SW, 35ppt) for 7 days. Fish were sampled at 24h and 7 days in SW for measurement of blood ion/acid-base status, muscle water content and gill and kidney Na+/K+ ATPase activity. Blood pH, plasma Na+, Cl- and osmolality increased significantly from FW to SW but were stable at 7 days in SW in all treatments and fish sizes. Overall, there were no negative effects of elevated CO2 in FW RAS to ion and acid-base regulation following SW transfer. Fish exposed to 0% CO2 in FW were also transferred to SW for measurement of hypoxia tolerance. Interestingly, hypoxia tolerance of the PM fish was higher than non-PM fish following 7 days in SW at 230g, but this was reversed when fish reached 580g.

ABSTRACT N° ICBF22-440

HYPOXIA MODIFIED THE OSMOTIC RESPONSE OF COHO SALMON ONCORHYNCHUS KISUTCH

Luis Vargas-Chacoff* [1, 2], Ricardo Oyarzún-Salazar[1], Pedro Pablo Cortes[3, 4], Oscar de Lázaro[3, 5], Kurt Paschke[2, 3]

[1]Institute of Marine and Limnological Sciences, Austral University of Chile,
[2]Fondap Center for High Latitude Research (IDEAL), and Millennium Institute Biodiversity of Antarctic and Subantarctic Ecosystems, BASE, University Austral of Chile, Valdivia,
[3]Aquaculture Institute, Austral University of Chile,
[4]Graduate School, Master in Aquatic Nutrition, Austral University of Chile,
[5]Graduate School, PhD in Aquaculture Sciences, Austral University of Chile, Puerto Montt, Chile

Abstract:
During the farm protocols (handling, sanitary etc) the hypoxia is one of the environmental variables that has increased the most in last times, and the main effect is decreasing the fitness and in some cases being lethal to fish. The aim of this research was to evaluate the osmotic response during 3 weeks hypoxia on juveniles of Oncorhynchus kisutch “Pacific salmon or Coho salmon”. For this, 25 juveniles were randomly arranged in 5 experimental conditions of 100 (control-normoxia), 60, 50, 35 and 25% oxygen saturation and after four weeks, the fish were sampled, and plasma, gills, intestine, kidney, muscle samples were taken. Results of this experiment presented mortality at 25% oxygen saturation. In plasma, different ions were evaluated, where the chlorine and calcium levels increased at 25% oxygen saturation. The osmolality levels present highest levels at 25% oxygen saturation. Meanwhile the NKA activity in all tissues were modified with decreasing of oxygen saturation. In muscle, water content decreased to higher hypoxia. Our results suggest an ionic imbalance, and modification of NKA activity due to hypoxia, and probably the mortality of coho salmon is due to osmotic failure. Acknowledgments: Fondecyt project 1160877 and 1190857, Fondap Ideal Center 15150003, ANID-Millennium Science Initiative Program-Center code “ICN2021_002”, and Master program in Aquatic Nutrition and VIDCA, all of them from UACH, for allowing the financing of this study.

ABSTRACT N° ICBF22-290

ACID PRODUCTION AND THE ROLE OF CO2 IN THE EEL SWIMBLADDER

Bernd Pelster* [1]

[1]Institute of Zoology, Innsbruck, Austria

Abstract:
Gas gland cells are known to acidify the blood during passage of the swimbladder in order to switch on the Root effect. The resulting increase in oxygen partial pressure is required to establish gas partial pressure gradients necessary for swimbladder inflation. Glucose is mainly converted into lactic acid, but some glucose is also metabolized in the pentose phosphate shunt, resulting in the production of CO2. The role of the pentose phosphate shunt for acid secretion has not yet been evaluated. In addition, analysis of the transcriptome of gas gland cells consistently revealed presence of aquaporin transcripts, and aquaporin has been shown to facilitate CO2 diffusion. Using immunohistochemistry the two aquaporin 1 genes Aqp1aa and Aqp1ab could be localized in endothelial cells of swimbladder capillaries as well as in basolateral membranes of gas gland cells. In addition, Aqp1ab was present in apical membranes of swimbladder gas gland cells suggesting that diffusion of CO2 into the blood and into the swimbladder lumen is facilitated by aquaporin. Simultaneous measurements of acid secretion and of oxygen consumption in isolated gas gland cells revealed that CO2 production in the pentose phosphate shunt significantly contributed to acid secretion of the cells. Collectively these data underline the pivotal importance of CO2 for swimbladder function by supporting blood acidification and by contributing to gas secretion. Oxygen consumption of isolated gas gland cells in a glucose free medium was consistently higher than in the presence of glucose.
Supported by FWF (P26363-25; I12984-B25)

ABSTRACT N° ICBF22-405

TEMPERATURE EFFECTS ON CARDIAC MITOCHONDRIA AND SKELETAL MUSCLE METABOLISM IN FISHES FROM THE RIO NEGRO

Grant Mcclelland* [1], Vera M.F. Almeida-Val[2], Adalberto Val[2]

[1]McMaster University, Hamilton, Canada,
[2]Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil

Abstract:
Warming waters of the Amazon basin may have detrimental effects on native fish species by negatively impacting muscle metabolism. To explore this issue, we sampled several species of the Rio Negro to understand the effect of elevated temperature on skeletal and cardiac muscle.

Five fish species were collected in the Anavilhanas Archipelago of the Rio Negro over two research expeditions. In Potamorhina pristigaster, skeletal muscle was sampled at rest, after exhaustive swimming and 30 min into recover at ambient temperature (30oC) and [metabolites] determined. The apparent Vmax of key metabolic enzymes were determined between 25-40oC. In 4 species (Geophagus proximus, Leporinus fasciatus, Hoplerythrinus unitaeniatus, and Hoplias malabaricus) permeabilized ventricle muscle fibres were prepared to determine leak, complex (CI), and CIV respiration at 25oC to 40oC.

We found that P. pristigaster showed rapid metabolic recovery from exercise at 30oC. Muscle enzymes showed only modest sensitivity to assay temperature except at the highest (40oC). This high assay temperature had a species-specific effect on ventricle mitochondrial leak and CI respiration. However, all species showed a decline in the ratio of CI/leak respiration suggesting a temperature-dependent uncoupling of mitochondria, even at ambient river temperatures. We also found some species showing a large excess in CIV compared to CI respiration.

Species differences in temperature sensitivity of enzyme activity and mitochondrial function suggests possibly variation in susceptibility to predicted river warming.

14-Tropical Fishes: state of the art and knowledge gaps

ABSTRACT N° ICBF22-498

REMODELING OF THE CARDIO-RESPIRATORY SYSTEM IN A CORAL REEF FISH LIVING ON THE WORLD'S HOTTEST REEF

Grace Vaughan* [1, 2], Jacob Johansen[3], Dain McParland[1], Bridget Evans[2], Lena Kolumba[1], John Burt[1], Holly Shiels[2]

[1]New York University Abu Dhabi, Abu Dhabi, United Arab Emirates,
[2]University of Manchester, Manchester, United Kingdom,
[3]University of Hawaii, Honolulu, United States

Abstract:

The Arabian Gulf (AG) experiences summer maxima >35°C, making it the hottest reef environment on earth, and creating a present-day example of end-of-century predictions. Here, we use AG reefs as natural laboratories for climate change to investigate temperature induced cardiac and gill remodeling as an adaptive strategy in meeting increased oxygen demands in the common coral reef fish the Arabian monacle bream (Scolopsis ghanam).

Gill and heart structure was extensively analysed in S.ghanam from AG, and the more benign Gulf of Oman (GO)(annual SST 22-32°C) at five temperatures representing the existing seasonal AG thermal range (18, 22, 27, 31.5, 35.5°C). Fishes were collected throughout the year when SST matched those of investigation, allowing an accurate representation of naturally occurring remodeling. As conditions in GO do not reach the same seasonal extremes, fishes from this region were acclimated for >3 weeks to 18°C and 35.5°C.

Elastin and collagen content within the heart, as well as spongey myocardium cell bundle size differed across temperatures, and between populations. Specifically, collagen content was significantly greater in fishes from AG compared to those from GO at higher temperatures, representative of thermal adaptation. Significant differences in gill structure were found between the two populations, and fishes from AG showed significant differences in lamellar structure as temperatures increased. The presence of an interlamellar cell mass (ILCM) was detected in both populations at lower temperatures, reducing as the temperature increased. This represents a novel finding within the field of reef fish gill remodeling.

ABSTRACT N° ICBF22-217

PHYSIOLOGICAL CONSEQUENCES OF CHRONIC HYPOXIA IN COLOSSOMA MACROPOMUM (PISCES, SERRASALMIDAE) ACCLIMATED TO DIFFERENT AMAZONIAN WATERS

Ignacio Ruiz-Jarabo* [1], Renan Amanajás [2], Derek Campos[2], Arlan Paz[2], Bernardo Baldisserotto [3], Juan Miguel Mancera [4], Adalberto Val[2]

[1]University Complutense of Madrid, Madrid, Spain,
[2]Instituto Nacional de Pesquisas da Amazonia (INPA) , Manaus,
[3]Universidade Federal de Santa Maria, Santa Maria, Brazil,
[4]University of Cadiz , Cadiz , Spain

Abstract:
Tropical rivers due to their high temperatures, amount of organic acids, and differentiated ion composition, result a challenge to teleost fish inhabiting them. Some waters are prone to chronic hypoxia such as certain areas in the Amazon. In this environment two types of water occur: Rio Negro (RN) water rich in humic acids and poor in ions, and groundwater (IG) with no humic acids and higher ions concentration. The physiological responses to chronic hypoxia in RN and IG waters are unknown. The present study demonstrates that a tropical species, tambaqui (Colossoma macropomum), modifies its basal homeostasis depending on the water conditions, with a great influence of dissolved oxygen levels. Chronic hypoxia induces lower oxygen consumption accompanied by the mobilization of energy metabolites (glucose and proteins) in both RN and IG waters. Na+ and K+ fluxes also present differentiated responses depending on the environmental water. Thus, RN-acclimated fish show a net loss of ions to the environment, while IG fish have a net gain of them. In liver, mitochondrial respiration is highly affected by RN water, as well as catalase and glutathione-S-transferase activities. Our results demonstrate that C. macropomum modifies its physiological responses to hypoxia depending on the environmental water. According to what is described here, it would be interesting to carry out comparative studies with other fish species acclimatized to these waters. These approaches can be valuable when managing environments as fragile as those in the Amazon.

ABSTRACT N° ICBF22-358

IS TRAP FISHING FOR THE ORNAMENTAL FISH TRADE PHENOTYPICALLY SELECTIVE? WE WILL ANSWER IT WITH CARDINAL

Daiani Kochhann*[1], Derek Campos[2], Bruna Osterno[2], Davide Thambithurai[3], Shaun Killen[3], Adalberto Val[2]

[1]Universidade Estadual Vale do Acaraú, Sobral,
[2]Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil,
[3]University of Glasgow, Glasgow, United Kingdom

Abstract:
Humans are incredibly effective predators and the harvest of animals during hunting and fishing is usually non-random. Commercial and recreational fishing pressures are a prime example in which individual fish with specific morphological, behavioural, or physiological traits may be more likely to be captured and removed from the population. It is being increasingly recognised that this form of selection may be causing evolutionary change to a range of traits in a phenomenon known as "fisheries-induced evolution". Importantly, there has so far been no effort examine how the selective effects of fishing may extend to the wild harvest associated with the ornamental fish trade. This is despite the fact the capture of ornamental fishes uses many of the same methods as recreational and commercial food fisheries and is therefore likely to select on the same traits. Indeed, as compared to large-bodied fishes targeted for food, evolution is likely to occur more rapidly in wild populations of ornamental fishes given their small body size and rapid generation times. The Amazon basin is a key supplier of wild freshwater fishes to the multi-billion-dollar global aquarium market and the mais exploited species is the cardinal (Paracheirodon axelrodi). We will investigate if there are any physiological and behavioural traits that make individuals fish more vulnerable to be harvest. To study this, we will run fishing simulations of wild populations of cardinal. After the fishing simulations we will measure metabolic and behavioural parameters and see if there are any phenotypes that increase capture vulnerability in this species.

ABSTRACT N° ICBF22-421

THE EFFECTS OF SEDIMENTATION ON HAWAIIAN HERBIVOROUS REEF FISH

Mathias Soerensen* [1], Jeroen Brijs[1], Jacob Johansen[1]

[1]University of Hawaii, Honolulu, United States

Abstract:
Sedimentation in near-shore coral reef ecosystems is increasing worldwide due to human activities including dredging, farming, deforestation, and urbanization. The direct impacts of sedimentation on corals and other benthic organisms are known to cause declining coral cover and health along with increased algal coverage and reduced diversity and abundance of reef fish. It is often assumed that observed changes in fish assemblages in sedimented habitats are due to declining coral health (i.e., bottom-up effects). However, recent evidence suggests that observed changes to fish assemblages may, in fact, be caused by direct impacts of sediments on the fishes themselves, and particularly herbivorous reef fish are affected at a larger proportion than other functional groups. Herbivorous fishes are often the last bastion against uncontrolled algal growth and serve as a vital trophic link for the persistence and resilience of coral-dominated reefs. To examine the direct impacts of sedimentation on Hawaiian herbivorous reef fish abundance and algal grazing rates, we are using a combination of field surveys and behavioral observations and innovative in-lab behavioral experiments examining their avoidance and foraging behavior under exposure to suspended sediment. Ultimately this project will address the capacity of herbivorous fishes to maintain top-down algal control on reefs exposed to sedimentation, as well as likely long-term consequences for herbivore biomass and coral-algal dynamics.

ABSTRACT N° ICBF22-367

COPPER AND CADMIUM IMPAIR THE SPERM FROM THE AMAZONIAN FISH COLOSSOMA MACROPOMUM

Gustavo PINTO[1], Jonatas CASTRO[2], Adalberto VAL*[2]

[1]Federal University of Santa Catarina, Florianópolis, SC,
[2]INPA - Brazilian National Institute for Research of the Amazon, Manaus, AM, Brazil

Abstract:
The contamination of aquatic environment by transition metals impair the reproductive process of several organisms. This study aimed to evaluate the effect of cadmium and copper on the sperm of tambaqui (Colossoma macropomum). Male and female specimens of C. macropomum were hormonally induced for spermiation and ovulation, and the sperm was activated in solutions containing cadmium (CdCl2) and copper (CuCl2). Sperm quality was assessed using motility time (s), percentage of mobile cells (%), activities of superoxide dismutase (SOD) and glutathione-S-transferase (GST), levels of lipoperoxidation (LPO), and morphological characteristics. In parallel, the effect of these metals on the rate of fertilization and hatching of oocytes was also evaluated. The duration and motility percentage of sperm were longer in the control treatment (85.7 ± 11.0 s; 90.0 ± 0.1%) and progressively decreased in the higher concentrations of CdCl2 and CuCl2. Exposure to copper did not cause changes in the activities of the SOD and GST enzymes and there were no significant changes in the levels of LPO, contrasting to exposure to higher concentration of cadmium which caused an increase in SOD activity and in the levels of LPO. The fertilization and hatching has been severely impaired in the presence of Cd and Cu. These data indicate that environments contaminated with cadmium and copper have a negative effect on the gametes of C. macropomum. (INCT ADAPTA: CNPq, FAPEAM, CAPES)

ABSTRACT N° ICBF22-246

IS IT BETTER TO BE LOVED OR FEARED? MANIPULATING CHEMICAL INFORMATION REGARDING PREDATION RISK AND MATING OPPORTUNITIES TO IMPROVE MANAGEMENT OF INVASIVE SEA LAMPREY IN THE LAURENTIAN GREAT LAKES

Michael Wagner* [1], John Hume[1], Tom Luhring[2], Jason Bals[1], Mikaela Hanson[1], Gregory Byford[1], Anne Scott[1], Mark Luehring[3]

[1]Michigan State University, East Lansing, Michigan,
[2]Wichita State University, Wichita, Kansas,
[3]The Great Lakes Indian Fish & Wildlife Commission, Odanah, Wisconsin, United States

Abstract:
A number of pervasive conservation challenges in aquatic ecosystems could be improved with the ability to manipulate the movement decisions of invasive species. Individual movement decisions are informed by sensory systems that have evolved to detect cues explicitly associated with fitness-impacting circumstances (i.e., the detection of food, predators, and mates). In aquatic ecosystems, this information often takes the form of semiochemicals: dissolved substances that either directly communicate or inadvertently advertise the locations of opportunities and risks. These substances function as potent attractants or repellents and have great appeal for use in conservation, as they typically are taxon specific, and have few unintended negative consequences. Here, we report the results of a series of field and laboratory experiments designed to manipulate sea lamprey movements through combinations of an anti-predator (alarm) cue and a mating pheromone. The experiments take place in a number of conservation scenarios including guiding migrating lampreys towards traps, streams targeted for pesticide control, and a selective fish passage device that excludes sea lamprey from other passing fishes. We also report data on how habituation occurs in response to alarm cue, and suggest means to delay its onset in management applications.

ABSTRACT N° ICBF22-253

NON-TARGET EFFECTS IN THE MANAGEMENT OF INVASIVE FISH SPECIES IN THE NORTH AMERICAN GREAT LAKES

Oana Birceanu*[1], Heather Bauman[2], Darren Foubister[2], Christopher White[2], Michael Wilkie[2]

[1]University of Western Ontario, London,
[2]Wilfrid Laurier University, Waterloo, Canada

Abstract:
The application of lampricides, which are chemicals used to control the invasive sea lamprey population in the North American Great Lakes, has contributed to the success of the Sea Lamprey Control Program. Sea lamprey population levels are at a historical low, ensuring economically and culturally important fishes, such as lake sturgeon, lake trout and whitefish, are minimally impacted by these invasive predators. The ongoing application of lampricides to rivers and streams remains a key factor in the continuous suppression of sea lampreys in the Great Lakes. However, there is a growing need to account for effects on non-target fishes, which, unlike lampreys, can effectively detoxify lampricides and withstand the exposure. Summarizing years of work that our group has done on the effects of lampricide exposure in non-target fishes, this presentation will highlight findings in lake sturgeon, rainbow trout, white suckers and blue gills, to provide a detailed picture on the impacts that these chemicals may have on these species. Here, we will focus on detoxification and tissue specific energy reserves, to better understand how lampricides affect fish physiology. Our work will propose future research that can be done to contribute to the development of robust adverse outcome pathways for lampricide effects, to mitigate impacts on on-targets.

ABSTRACT N° ICBF22-165

THE TRANSCRIPTOMIC BASIS OF 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) SENSITIVITY IN FISHES

Michael Lawrence* [1], Phil Grayson[1], Jennifer Jeffrey[1], Margaret Docker[1], Colin Garroway[1], Johnathan Wilson[2], Richard Manzon[3], Michael Wilkie[2], Kenneth Jeffries[1]

[1]University of Manitoba, Winnipeg,
[2]Wilfrid Laurier University, Waterloo,
[3]University of Regina, Regina, Canada

Abstract:
In the early 20th century, sea lamprey (Petromyzon marinus) successfully invaded the Laurentian Great Lakes of North America resulting in severe ecological and economic damage. Lampricide applications in rivers, specifically 3-trifluoromethyl-4-nitrophenol (TFM), have been the principal sea lamprey control method. The main mechanism of toxicity of TFM in fishes is through an uncoupling of oxidative phosphorylation in the mitochondria. While TFM is relatively specific against sea lamprey, its toxicity can vary amongst fishes, which likely stems from the variability in the types of biotransformation genes in the genome, notably UDP-glucuronosyltransferases (UGT). However, the mechanism behind the differences in TFM sensitivity, particularly at the gene level, is poorly characterized. Thus, we sought to identify the potential molecular mechanisms underlying TFM toxicity and detoxification using a whole transcriptome approach (RNA-Seq) to examine differential expression of biotransformation gene targets in sea lamprey (i.e., target species) and bluegill sunfish (native species) exposed to TFM (species-specific 24h LC10: lamprey = 2.21 mg L-1, bluegill = 22.06 mg L-1) over a 24 h period. We found that bluegill had a larger breadth of UGT gene families present in their transcriptome, relative to sea lamprey, and differential expression of UGTs was found only in TFM exposed bluegill. Differential expression of phase I, II, and III biotransformation genes were more diverse in bluegill when compared to sea lamprey. We also identified a broader mechanism of TFM toxicity at the transcript level, which included an arrest of cell growth, higher apoptosis, and immune responses in both species. Together, these data suggest that differences in TFM sensitivity in fishes may be linked to the species' transcriptomic response to exposure.

ABSTRACT N° ICBF22-514

BEHAVIOUR, HORMONAL PROFILES AND ATTEMPTED CHEMICAL CASTRATION OF THE INVASIVE CHAMELEON CICHLID AUSTRALOHEROS FACETUS

Flavia Baduy[1], Joao L. Saraiva[1], Gonçalo Nunes[1], Gonçalo Oliveira[1], Peter Hubbard[1], Adelino Canario[1], Pedro M. Guerreiro*[1]

[1]CCMAR- Centre of Marine Sciences, University of Algarve, Faro, Portugal

Abstract:
Invasive species are a growing concern for habitats worldwide. The chanchito, Australoheros facetus, a neotropical cichlid with marked social behaviors and aggressive parental care, is currently found in southern Portugal in small streams. We studied its endocrine physiology in the context of hierarchy formation and reproductive behavior and aimed to disrupt reproduction by chemical castration.

Behaviour is highly and rapidily affected by temperature changes, with territorial aggression ensuing within hours above threshold temperatures. Social groups were followed at different periods of the year. Dominance indexes and fish size are highly correlated and even very small size differences can account for social ranking, but no correlation to sex, GSI or HIS was found. Significant differences in cortisol and 11K-testosterone(11KT) levels occurred among dominant and subordinates males but not in females of different status. Monogamous pairs establish and defend breeding territories. Members of the pair perform parental duties during offspring development but males spend more time patrolling while females care for the young. Whether such behaviors are under hormonal/pheromonal control is currently under investigation. EOG in naïve fish shows higher nerve activity in response to fluids of dominant than subordinate fish. Injection of carbenoxolone in dominant males does not alter status or reproductive behavior but it is effective in reducing 11KT. It remains to be clarified if the treatment impairs fertility, thus resulting in possible tool to control populations.

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ABSTRACT N° ICBF22-537

GENETIC CONTROL OF INVASIVE SEA LAMPREY IN THE GREAT LAKES

Diogo Ferreira-Martins* [1], Jackson Champer[2, 3], David McCauley[4], Zhe Zhang[5], Margaret Docker[6]

[1]Department of Biology, University of Massachusetts UMASS, Amherst, United States,
[2]Center for Bioinformatics, School of Life Sciences, Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China,
[3]Department of Computational Biology and Department of Molecular Biology & Genetics, Cornell University, Ithaca,
[4]Department of Biology at the University of Oklahoma, Norman,
[5]Department of Fisheries and Wildlife, Michigan State University, East Lansing , United States,
[6]Department of Biological Sciences, Winnipeg, Canada

Abstract:
The sea lamprey, Petromyzon marinus, is the subject of conservation efforts in its native range on the western coast of Europe and eastern coast of North America. In contrast, efforts aim to control invasive sea lamprey in the Great Lakes of North America because it was a significant factor in the collapse of Great Lakes fish stocks and still threatens a multibillion-dollar fishing industry. Substantial resources are invested annually on sea lamprey control methods such as lamprey-specific biocides and physical barriers that block adult lamprey migration. Although these methods reduced sea lamprey in the Great Lakes by 90%, maintaining suppression of persistent populations and achieving targets for control in some lakes are major challenges; hence, more effective control measures are needed. Genetic control tools are theoretically powerful and effective pest control options, though with uncertain sociopolitical support. Here, an overview of genetic approaches classified as self-limiting (heritable sex ratio ratchet, Trojan gene, split gene drive) and self-sustaining (gene drive-based sex ratio distortion, homing suppression gene drive, toxin-antidote gene drives, and modification-type gene drives to aid suppression) will be provided jointly with a discussion on the potential applications, technical aspects, and challenges with particular focus on gene drives. The use of this technology to control invasive species for ecosystem conservation is a hot topic of debate. To minimize the risk of undesired spread of deleterious alleles to Atlantic populations, self-limiting genetic control methods and confined gene drives are likely the preferred options for sea lamprey control in the Great Lakes.

ABSTRACT N° ICBF22-402

IMPLEMENTING EDNA METABARCODING FOR THE DETECTION OF INVASIVE FISHES AT A BASIN LEVEL.

Manuel Curto* [1], Sofia Batista[1], Ana Veríssimo[2], Filipe Ribeiro[1], Carlos D. Santos[3], Sissel Jentoft[4], Maria J. Alves[5], Hugo F. Gante[6, 7]

[1]MARE, Marine and Environmental Sciences Center, Faculty of Sciences, University of Lisbon, Lisbon,
[2] CIBIO—Research Center in Biodiversity and Genetic Resources, Vairão,
[3]Centre for Environmental and Marine Studies, Faculty of Sciences, University of Lisbon, Lisbon, Portugal,
[4]Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, Oslo, Norway,
[5]cE3c - Center for Ecology, Evolution and Environmental Changes & Museu Nacional de História Natural e da Ciência,Universidade de Lisboa, Lisbon, Portugal,
[6]Department of Biology, KU Leuven, Leuven,
[7]Royal Museum for Central Africa, Section Vertebrates, Tervuren, Belgium

Abstract:
The implementation of metabarcoding for sequencing environmental DNA (eDNA) is a powerful tool for the detection of freshwater fish species. Theoretically all species present at a given site can be detected if the right primer combination is used. This characteristic is useful for detecting invasive fish species, especially at earlier stages of invasion when abundances are low and traditional monitoring techniques, like electrofishing, tend to be ineffective. Nevertheless, the primers used in metabarcoding preferably bind to certain taxa resulting in biases of relative abundance assessment and in some cases to false negatives. In this study, we tested the ability of eDNA metabarcoding to describe the fish fauna in the Portuguese section of the Tagus river with the aim of recently introduced fish species. The fish community detected by eDNA metabarcoding is similar to the one described using electrofishing and seine nets. However, eDNA metabarcoding detects taxa overlooked by traditional methods. This includes the first detection of two invasive fishes in this basin: a south American cichlid - Australoheros facetus an European leuciscid: Squalius cephalus) and improving of native fish distribution knowledge. Such detections are consistent with reports from anglers (citizen science) and new inter-drainage water connections. In turn, traditional methods were more effective in describing fish diversity at some sites. These results show the importance of the implementation of integrative approaches for monitoring fish invasions, where the information from both traditional methods and eDNA metabarcoding is combined.

ABSTRACT N° ICBF22-344

NON-NATIVE FISH ASSEMBLAGES DISPLAY POTENTIAL COMPETITIVE ADVANTAGES IN TWO PROTECTED SMALL AND SHALLOW LAKES OF NORTHERN ITALY

Vanessa De Santis* [1], Davide Cicala[1], Ilaria Baneschi[2], Chiara Boschi[2], Stefano Brignone[1], Mattia Iaia [1], Silvia Zaupa[1], Pietro Volta[1]

[1]Water Research Institute - National Research Council of Italy, Verbania,
[2]Institute of Geosciences and Earth Resources - National Research Council of Italy, Pisa, Italy

Abstract:
Italian fresh waters host 57 established non-native fish species but ecological and physiological conditions of fish assemblages are seldom evaluated, particularly in small and shallow lakes. Here, traditional fish surveys (gillnetting and electrofishing) and stable isotopes analysis (SIA) of carbon and nitrogen were combined to assess fish community composition, to evaluate fish health status and to characterize the interspecific trophic interactions (as isotopic niches and their relative asymmetric overlap) of the native and non-native species (NS and NNS, respectively) found in two protected small and shallow lakes, San Michele and Campagna (northern Italy). In San Michele, 92 % of the fish caught were NNS and both the individual and biomass per unit effort were dominated by NNS, particularly by the NNS Lepomis gibbosus and Ictalurus punctatus. In Campagna, 5.6 % of the fish in the total catches belonged to NNS and the number and biomass of the assemblage was dominated by the NS Alburnus alborella. SIA revealed that NNS had greater trophic structure and were exploiting a wider range of resources with a higher asymmetric overlap than NS assemblages in both lakes. In both lakes, the NNS Ameiurus melas had the broadest isotopic niche and the highest asymmetric overlap suggesting a potential competitive advantage over NS. In Campagna, the NNS A. melas and L. gibbosus had better body condition than expected while in San Michele all the most abundant fishes had worst condition than expected. The results sustain the hypothesis that eco-physiological plasticity favors the successful invasion of new habitats.

ABSTRACT N° ICBF22-505

A GIANT ON THE WATER: FROM PREDATION PRESSURE TO POPULATION CONTROL OF THE EUROPEAN CATFISH (SILURUS GLANIS)

Filipe Ribeiro*[1], Stéphanie Boulêtreau[2], Martin Cech[3], Manuel Curto[1], Maria Filomena Magalhães[4], Tiago Marques[5,6], Rui Martins[6], Bernardo Quintella[1], Diogo Ribeiro[7], Fréderic Santoul[8], Gil Saraiva-Santos[1], Lukas Vejrik[3], João Gago[7]

[1]MARE - Centro de Ciências do Mar e do Ambiente - Univ. Lisboa, Lisboa, Portugal,
[2]Université Toulouse III Paul Sabatier - Laboratoire Ecologie Fonctionnelle et Environnement, Toulouse, France,
[3]Institute of Hydrobiology, Ceske Budejovice, Czech Republic,
[4]Universidade de Lisboa - CE3C - Centre for Ecology and Evolution and Environmental Sciences, Lisboa, Portugal,
[5]Centre for Research into Ecological and Environmental Modelling, University of St Andrews, Saint Andrews, United Kingdom,
[6]Centro de Estatística e Aplicações, University of Lisboa, Lisboa,
[7]Instituto Politécnico de Santarém - Escola Agrária de Santarém, Santarém, Portugal,
[8]Université Toulouse III Paul Sabatier - Laboratoire Évolution & Diversité Biologique, Toulouse, France

Abstract:
The European catfish (Silurus glanis) has recently arrived to the Lower Tagus river (Portugal). This large predator has a high putative impact on the local fish communities given the high endemic level of the Iberian rivers. In spite of its wide damaging potential, the impacts of large predatory fish introduced to Iberian Peninsula remain understudied, particularly to diadromous fishes - highly valuable fisheries resource. This work will present the outline of the MEGAPREDATOR project that aims to answer the following questions: a) what is the predatory pressure of European catfish on native fauna, particularly on diadromous fish? b) what are the foraging movements and predatory behaviour of the European catfish in a recently invaded system? c) what kind of habitats do European catfish prefer in lentic systems and in lotic habitats? d) which is the timing of European catfish aggregations and which environmental factors can explain this behaviour; e) what kind of habitats are used during the aggregation? f) can we identify and predict new aggregation sites? g) how can we use all this information to develop control strategies that will mitigate the impact of the European catfish on native aquatic communities?
The success of this project will rely on building solid scientific knowledge on the trophic and spatial ecology of this species, allowing the development of new approaches for population control and close cooperation with both administration and fishermen.

ABSTRACT N° ICBF22-178

POTENTIAL IMPACT OF THE NON-NATIVE EUROPEAN CATFISH (SILURUS GLANIS) ON ANADROMOUS SPECIES – SYNTHESIS OF CURRENT KNOWLEDGE AND PERSPECTIVE

Frédéric Santoul* [1], Stéphanie Boulêtreau[2]

[1]Laboratoire EDB,
[2]Laboratoire Ecologie Fonctionnelle et Environnement, Toulouse, France

Abstract:
Due to their strong association with human activities, freshwater ecosystems have been the recipients of numerous non-native species and fish are the most frequently introduced organisms. Predatory fish introductions are known to impact native fish populations and to modify prey community and food web structure. The European catfish, the largest non-native freshwater fish predator in Europe, was introduced in Western Europe at the end of 19th century, and its spread across Southern Europe, North Africa, Asia and South Africa accelerated in the early 1990s. European catfish invasion biology and interactions with native biota have only recently been studied. In this communication, we will present our investigations on the trophic ecology and the behaviour of this species, especially focusing on catfish impact on anadromous fish species. Potential management strategies of this species will be also discussed.

ABSTRACT N° ICBF22-473

ADAPTING TO A NEW LIFE HISTORY: INVASIVE SEA LAMPREY IN THE LAURENTIAN GREAT LAKES

Jaanus Suurväli* [1], Phil Grayson[2], Meghan L. Mahoney[3], Jessie L. Ogden[1], Eleana Karachaliou[1], Michael J. Lawrence[1], Kenneth M. Jeffries[1], Margaret F. Docker[1], Colin J. Garroway[1]

[1]University of Manitoba, Winnipeg, MB, Canada,
[2]Harvard Medical School, Boston, MA,
[3]Colorado State University, Fort Collins, CO, United States

Abstract:
The sea lamprey (Petromyzon marinus) is a jawless fish that spawns and rears in freshwater, but usually feeds as a parasite in the Atlantic Ocean. In North America the species has become invasive and, by the late 1800s, had established freshwater-resident populations in Lake Ontario and the adjacent Finger Lakes (NY, USA). The construction of a shipping canal that bypasses Niagara Falls allowed it to invade the other four Laurentian Great Lakes (Erie, Huron, Michigan and Superior) by the 1930s. Unlike anadromous sea lamprey, Great Lakes sea lamprey spend their entire lives in freshwater environments.

Here we present our results from whole genome re-sequencing of 262 sea lamprey collected from the Great Lakes, Finger Lakes, and the North American anadromous population. The majority of sea lamprey captured in the Great Lakes area represent a single large population that originates from a recent genetic bottleneck. This freshwater population has evolved to be genetically distinct from the contemporary anadromous populations; there appears to be little or no genetic exchange between the two. Genomic scans for loci involved in local adaptation revealed an enrichment of genes involved in cell adhesion and synaptic signalling, among others. In my presentation I will explore evidence for selection on genes associated with physiological responses to lampricide and the potential utility of putatively adaptive loci for genetic control in the invasive population.

ABSTRACT N° ICBF22-506

LARVAL SEA LAMPREY DON'T MIND THE HEAT – IMPLICATIONS FOR SEA LAMPREY CONTROL IN A WARMING WORLD?

Hugo Flávio*[1], Dejana Mitrovic[2], Jessica Bell[1], Milica Koledin[1], Michael Wilkie[1]

[1]Wilfrid Laurier University,
[2]University of Waterloo, Waterloo, Canada

Abstract:
Sea lamprey (Petromyzon marinus) invaded the Laurentian Great Lakes in the early 20th century and remain a threat to fisheries. However, populations are controlled by a highly successful control program that mainly relies on the application of the lampricide 3-trifluoromethyl-4'-nitrophenol (TFM) to streams infested with larval sea lamprey. The effectiveness of TFM is affected by abiotic factors such as pH and alkalinity. Our recent findings indicate that temperature also profoundly influences TFM effectiveness, with the toxicity to larval sea lamprey decreasing with increasing temperature. This could undermine sea lamprey control efforts at warmer temperatures due to the enhanced ability of larvae to survive TFM applications and ultimately transition to the juvenile parasitic phase. Increasing TFM application rates to compensate for the higher tolerance of larval sea lamprey could also increase the risk of non-target mortality. To better understand how temperature influences larval sea lamprey physiology and their capacity to detoxify TFM, we generated a thermal performance curve for sea lamprey larvae using intermittent-flow respirometry. Our results indicate that larval sea lamprey have an unexpectedly wide thermal range with an optimal temperature nearing 28°C. We also performed TFM toxicity tests at various temperatures up to 28°C, revealing increasing LC50 concentrations with temperature up to the highest temperature tested. These findings suggest that larval sea lamprey tolerance to TFM will likely continue to increase as water temperatures in the Great Lakes continue to rise due to climate change. Non-target species with lower thermal tolerance could simultaneously become more vulnerable to TFM.

ABSTRACT N° ICBF22-518

PLASTIC PHYSIOLOGY AND WIDE ABIOTIC TOLERANCE IN THE CICHLID AUSTRALOHEROS FACETUS INCREASE ITS INVASIVENESS POTENTIAL UNDER CLIMATE CHANGE SCENARIOS IN SOUTHERN PORTUGAL

Flavia Baduy[1], Joao L. Saraiva[1], Beatriz Oliveira[1], Nuno Jesus[1], Adelino Canario[1], Pedro M. Guerreiro[*1]

[1]CCMAR- Centre of Marine Sciences, University of Algarve, Faro, Portugal

Abstract:

Australoheros facetus is a neotropical cichlid that was widely used in ornamental aquaria. It is originally from the slow-moving streams and lakes of eastern south America, from southern Brazil to the Buenos Aires province in Argentina, and is also now present in the wild in Southern Portugal and Spain, as well as in central Chile. The species displays high tolerance to abiotic factors and is found in small Mediterranean streams with striking seasonal variations in temperature and hydrological regimes.

Fish were exposed to a range of temperature, oxygen levels and salinities during short and long-term trials simulating seasonal and/or estuarine conditions. CTmax and CTmin were determined, showing wide thermal tolerance, with LOE ensuing as below as 2°C and as high as 39°C. Acclimation to 12°C or 24°C caused a approximately 4°C in CTmin and CTmax, The expression of metabolic and stress genes showed important differences in heat-shock and hypoxia related factors. Metabolic rates were determined in fish acclimated to 8C, 18C and 28C, showing wide amplitude and individual variation, but elevated aerobic scope throughout.

Tolerance to salinity appears reduced above the isosmotic levels, with marked up-regulation of gill NKA activity and increased cortisol levels at 15ppt. Growth and social behavior are abolished in fish at 18ppt. Summer conditions are characterized by discontinuous rivers featuring isolated ponds where fish accumulate under hypoxic conditions. Critical oxygen levels and metabolic rates for A. facetus are under determination. These features will be discussed in the context of winner and losers in comparison with native species and concerning the ability of A- facetus to colonize new areas.

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ABSTRACT N° ICBF22-502

PHYSIOLOGICAL PERFORMANCE OF THE INVASIVE ROUND GOBY NEOGOBIUS MELANOSTOMUS IN THE BALTIC SEA

Jane W. Behrens* [1], Iren Tabak[1], Mikael van Deurs[1], Tommy Norin[1], Emil A. F. Christensen[2]

[1]DTU Aqua, Kgs. Lyngby, Denmark,
[2]University of Glasgow, Glasgow, United Kingdom

Abstract:
Native to the Ponto-Caspian region, the round goby Neogobius melanostomus has for three decades been invasive to the North American Great Lakes and the Baltic Sea, including also several European inlet waters. Round goby is now considered one of the most impactful invasive fish worldwide. I will give an overview of the knowledge and insight we have derived from our field and experimental work with Baltic round goby, elucidating specifically the physiological performance of the fish in relation to salinity and temperature, and how this relates to its success as an invader. The data on thermal tolerance and preference is feeding into a physiology based model and integrated with oceanographic data. With this the aim is to identify current and future thermal habitat suitability of the species. This is presented in the subsequent talk by P. Domenici.

ABSTRACT N° ICBF22-480

HOW TO PREDICT THERMAL HABITAT SUITABILITY OF INVASIVE SPECIES USING METABOLIC SCOPE AND OCEANOGRAPHIC MODELING

Paolo Domenici* [1], Giovanni Quattrocchi[1], Matteo Sinerchia[1], Andrea Cucco[1], Stefano Marras[1], Emil A. F. Christensen[2], Jane W. Behrens[3]

[1]IAS CNR, Oristano, Italy,
[2]University of Glasgow, Glasgow, United Kingdom,
[3]DTU Aqua, Lyngby, Denmark

Abstract:
Effective conservation management requires models that make projections beyond the range of available ecological data. One way to deal with such extrapolations is to use a mechanistic approach based on physiological processes underlying climate change effects on organisms. Using physiological data of invasive species and their native counterparts, we illustrate a simple model based on aerobic scope integrated with oceanographic data of current and future scenarios, to characterize fish habitat suitability. We will discuss the example of the round goby (Neogobius melanostomus), one of the world's most impactful invasive fish species. The round goby is originally from Ponto-Caspian region, and it has spread in many temperate ecosystems in Northern Europe and North America, likely as a result of its wide environmental tolerance. Here we will focus on its invasion of the Baltic Sea. The potential effect of sea surface temperature (SST) on the metabolic scope of the round goby was estimated based on 14-years sightings in the Baltic Sea. The area within which round goby and other species (i.e. juvenile cod Gadus morhua) may co-occur was estimated for the past 40 years and for the future scenarios (up to 2100) of SST. The results were used to identify multi-decadal changes in spatial and temporal increase of unsuitable habitat in the Baltic Sea. Specifically, the SST dataset based on the past 40 years displayed a diminishing trend of the dimensions of unsuitable habitat, suggesting that the spread of the round goby in the Baltic Sea will increase in the future.

ABSTRACT N° ICBF22-433

DIFFERENCES IN PHENOTYPIC FLEXIBILITY DETERMINE SURVIVAL CHANCES OF TWO FISH SPECIES IN EUTROPHIC WATERS ON A HOT SUMMER DAY.

Gudrun De Boeck* [1], April Grace R Opinion[1], Essie M Rodgers[2]

[1]University of Antwerp, Antwerp, Belgium,
[2]University of Canterbury, Christchurch, New Zealand

Abstract:
Stressors can interact in unanticipated ways, where exposure to one stressor may influence resilience to another stressor. We examined the interaction between nitrate pollution and temperature increase onphysiological performance in a salmonid, the European grayling (Thymallus thymallus), and a cyprinid, the common carp (Cyprinus carpio). Fish were exposed to nitrate pollution (0, 50 or 200 mg $NO3^{-}$/L) at two acclimation temperatures (18°C and 22°C or 26°C). We assessed hypoxia and heat tolerance (CTMax), aerobic scope (AS) and cardiorespiratory attributes (haemoglobin HB, haematocrit HCT, relative ventricle mass RVM, somatic spleen index SSI).

In grayling, nitrate-exposed fish were significantly more susceptible to acute hypoxia at both acclimation temperatures. Effect of nitrate on heat tolerance depended on temperature. Elevated temperature increased AS and the improvement was stronger with nitrate exposure, indicating a positive synergistic interaction. HB was reduced by nitrate exposure. Temperature induced remodeling of key elements of the cardiorespiratory system. RVM was higher in fish exposed to 22°C but was independent of nitrate exposure. SSI was independent of temperature but was higher in fish exposed to nitrate. In common carp, warm acclimation also increased AS due to the increased maximum metabolic rate, and this improvement seemed greater at higher nitrate concentration. Warm-acclimated fish exposed to 200 mg NO3- were less susceptible to acute hypoxia, and fish acclimated at higher temperature exhibited improved heat tolerance by 5 C.

Taken together, these results highlight that simultaneous exposure to elevated temperatures and nitrate pollution can offer cross-tolerance benefits, especially in common carp, which may be underscored by cardiorespiratory remodelling. However, for grayling, nightly hypoxic event could lead to mass mortality.

12-Phenotypic flexibility? Coping with environmental change over developmental time

ABSTRACT N° ICBF22-443

HOW DIET CAN MEDIATE PHENOTYPIC FLEXIBILITY IN FISHES

Erika Eliason* [1], Emily Hardison[1]

[1]University of California, Santa Barbara, Santa Barbara, United States

Abstract:
Phenotypic plasticity is a key mechanism enabling fish to respond to environmental perturbations. Acclimatization processes require both energy and nutrients from the diet, yet, climate change is altering food availability, diet quality and diet preferences for fishes. Our research is examining how diet can influence thermal performance and tolerance limits in marine fishes. An integrative study on an omnivorous marine fish, opaleye, discovered that diet did impact thermal acclimation responses, but in trait-specific ways. Some physiological traits were highly sensitive to diet and temperature (e.g. cardiac performance, standard metabolic rate) while others were insensitive (e.g. sprint speed). Another study on invasive lionfish suggests that digestion capacity may be a key factor influencing their invasion success, and that they may actually consume more prey as oceans warm. In contrast, work on predatory tropical hawkfish suggests that digestion processes become more costly as temperatures warm, and they may not have the phenotypic flexibility to keep pace with warming. All told, diet is an often overlooked but key mechanism that may allow fishes to regulate their performance under different environmental conditions.

ABSTRACT N° ICBF22-321

EFFECTS OF WATERBORNE TRIIODOTHYRONINE ON THE DEVELOPMENT OF GASTROINTESTINAL TRACT FORM AND FUNCTION IN THE DEVELOPING LAKE STURGEON (ACIPENSER FULVESCENS)

Alyssa Weinrauch* [1], Gary Anderson[1]

[1]University of Manitoba, Winnipeg, Canada

Abstract:
Larval fish survival depends in part upon both the development of the gastrointestinal tract (GIT) and the successful transition to exogenous feeding. Triiodothyronine (T3) has previously been shown to improve hatch and survivability of larval fish species, which could be particularly important in conservation aquaculture. The lake sturgeon (Acipenser fulvescens) are endangered across their native range and thus, conservation aquaculture practices are being utilized to mitigate loss and enhance sturgeon populations in the wild. Like many fish species, sturgeon larvae are not fully developed at hatch and undergo further GIT development prior to the onset of exogenous feeding. We aimed to determine if waterborne exposure to T3 would result in developmental plasticity leading to improved survival, growth and accelerated GIT development. Following 6 h exposure to T3 (3ppm), larvae were reared in either tumbling jars or on egg mats, and sampled every 4 days for the first 30 days post fertilization (dpf). Fish reared in T3 demonstrated significant increases in whole body T3 at 10 dpf that returned to control values by 30 dpf. T3 exposed larvae demonstrated significant increases in growth as well as reduced cumulative mortality rates compared to controls. We further examined the development of the lake sturgeon GIT, surveying the morphological progression alongside functional changes in mRNA expression of putative endocrine factors involved in the regulation of digestive function. The results suggest that larval T3 exposure can play an important role in regulating early larval metamorphosis, survival and growth.

ABSTRACT N° ICBF22-340

INFLUENCE OF ENVIRONMENTAL ENRICHMENT ON CARDIOVASCULAR DEVELOPMENTAL PLASTICITY OF LANDLOCKED SALMON

Katja Anttila* [1], Anna Papadopoulou[1], Eila Seppänen[2]

[1]University of Turku, Turku,
[2]Natural Resources Institute, Enonkoski, Finland

Abstract:
Stocking is one way to support the natural populations of salmonids that have suffered from anthropogenic activities. For these purposes the hatchery reared juvenile fish are released to nature. However, the survival prospects of these hatchery fish are, in general, low as the fish are not used to natural environment and its requirements. Furthermore, rearing fish in aquaculture has been shown to induce severe cardiac abnormalities which could affect the fish survival e.g. by impairing the swimming capacity. Environmental enrichment has been implied to hatcheries in an attempt to improve the post-stocking survival. Furthermore, the enrichment could improve the welfare of the fish in aquaculture. However, it is largely unknown if early development in enrichment environment could improve the cardiovascular fitness of fish later on in life, before they are stocked to nature. Therefore, the purpose of this is study was to evaluate how increase of water flow velocity and environmental enrichment influence the developmental plasticity of cardiovascular system of the hatchery reared landlocked salmon, an endangered species from Lake Saimaa, Finland. The fish were divided to enriched and control groups that were reared in regular circular aquaculture tanks since hatching and their cardiovascular capacities were evaluated 430 days later.

ABSTRACT N° ICBF22-341

PHYSIOLOGICAL CHANGES ASSOCIATED WITH OXYGEN TRANSPORT MEDIATES CONNECTIVITY OF CORAL REEF FISHES

Adam Downie[1], Sjannie LeFevre[2], Björn Illing[1, 3], Jessica Harris[1], Michael Jarrold[1, 4], Peter Cowman[1, 5], Mark McCormick[4], Göran Nilsson[2], Jodie Rummer*[1, 4]

[1]ARC Centre of Excellence for Coral Reef Studies, Townsville, Australia,
[2]University of Oslo, Oslo, Norway,
[3]Thünen Institute of Fisheries Ecology, Bremerhaven, Germany,
[4]James Cook University,
[5]Museum of Tropical Queensland, Townsville, Australia

Abstract:
Connectivity among coral reef fish populations is achieved via strong swimming performance and complex navigation behaviours of pelagic larvae to find a suitable reef on which to settle, metamorphose, and grow into healthy adults. Whilst in the pelagic environment, larvae must exhibit elite swimming capabilities; yet, upon settlement onto the reef and metamorphosis, coral reef fishes must be hypoxia tolerant to cope with the night time low-oxygen conditions experienced within the reef matrix, two seemingly opposing traits. Our aim was to determine the physiological changes over development in coral reef fishes that enable such a critical transition. We measured oxygen uptake rates supporting swimming performance, hypoxia tolerance, and gene expression of oxygen-carrying proteins (e.g., haemoglobin, myoglobin) in anemonefish (Amphiprion melanopus) from the pelagic larval phase until settlement to determine the ontogenetic modifications in these physiological traits that may support the transition from pelagic to reef habitats. Oxygen uptake rates that support swimming decrease mid-way through the larval phase, coinciding with a gradual increase in hypoxia tolerance. Hypoxia tolerance is greatest immediately upon settlement onto the reef, which also coincides with the highest expression of genes coding for various haemoglobin and myoglobin subunits. Our results show that the ecological processes that supply reefs with new fish larvae that support future populations are made successful due to distinct physiological changes in both oxygen demands and the expression of oxygen-carrying proteins during the larval phase.

12-Phenotypic flexibility? Coping with environmental change over developmental time

ABSTRACT N° ICBF22-343

MECHANICAL CONSTRAINT AND ITS EFFECT ON SHORT AND LONG TERM PERFORMANCE PLASTICITY

Emily Standen* [1]

[1]University of Ottawa, Ottawa, Canada

Abstract:
Moving from water to land involves a large change in the external forces experienced by an animal. Over the short term, given the appropriate form, animals can use existing body structures in novel ways to accommodate locomotion in new environments. Novel utilization leads to novel forces experienced by body structures and initiates a cascade of physiological responses that, if persistent, change the long term mechanical and physiological function of the tissues involved. We elicit novel amphibious behaviour in Polypterus senegalus, a basal fish model, to understand the immediate and long term plastic responses of behaviour, motor control, and cellular and sub-cellular tissue structure and function. Shifting between whole animal biomechanical performance and tissue level responses, we will attempt to build a model which explains how mechanical constraint, both abiotic (environmental) and biotic (anatomical), provide the infrastructure within which the basic rules of tissue ‘behavioural’ plasticity operate. This combination of external constraint and internal rule based tissue behaviour determine the performance plasticity of the entire system, ultimately driving whole animal functional performance on short and long time scales in systems transitioning between environments.

ABSTRACT N° ICBF22-254

IMPACTS OF NATURAL THERMAL VARIABILITY ON WILD ATLANTIC SALMON GROWTH AND PERFORMANCE

Sean Andrew[1], Stephanie McKenna[1], Sula Swart[1], Jenna Morissette[2], Carole-Anne Gillis[3], Tommi Linnansaari[4], Suzie Currie[5], Andrea Morash* [1]

[1]Mount Allison University, Sackville,
[2]Dalhousie University, Halifax,
[3]Gespe'gewaq Mi'gmaq Resource Council, Listuguj,
[4]University of New Brunswick, Fredericton,
[5]Acadian University, Wolfville, Canada

Abstract:
Thermal stress is a major concern for Atlantic salmon populations in the Atlantic provinces, with mortalities observed after several days of high, fluctuating summer temperatures. Current conservation practices use a single temperature threshold despite thermal differences between rivers. We sought to better understand how natural temperature cycles affect the growth and performance of wild Atlantic salmon in the parr and smolt life stages from two tributaries in distinct New Brunswick, Canada river systems – The Miramichi River (tributary - summer temperature range: 11-24°C) and the Restigouche River (tributary summer temperature range: 11.9-16.5°C). We collected wild Atlantic salmon parr from both streams and acclimated them to either 16-21°C or 19-24°C for several months. Growth rate and smoltification was monitored throughout the duration of the experiment. At each life stage, we also measured salmon performance (exhaustive exercise) and CTmax. Parr growth was unaffected by acclimation temperature, but was significantly lower overall in the Restigouche population. Restigouche smolts also grew slower when reared at 19-24°C, while there was no impact of this thermal profile on Miramichi smolts. Time to exhaustion was independent of acclimation temperature, but was affected by both river origin and life stage, with Restigouche smolts taking the longest time to exhaust. These data provide possible evidence of some local adaption between these populations and suggest that management policies may need to be locally adjusted to reflect the thermal profile and physiology of resident fish.

ABSTRACT N° ICBF22-426

EFFECTS OF THERMAL STOCHASTICITY ON PHYSIOLOGICAL PERFORMANCE IN THE SELF-FERTILIZNG MANGROVE RIVULUS (KRYPTOLEBIAS MARMORATUS)

Sarah Brown* [1], Gabrielle Rivard[1], Suzie Currie[1]

[1]Acadia University , Wolfville , Canada

Abstract:
Climate change has led to variation in global mean temperatures, as well as the intensity, frequency, and duration of more extreme temperature events. The processes by which animals cope with these thermal variations are not yet fully understood, but it has been suggested that an ectotherm's response to increases in rising stable, mean temperatures is different than their response to thermal variability. Using the hermaphroditic, amphibious Mangrove Rivulus as a model species, we hypothesized that unpredictable or stochastic thermal variability would affect acclimation capacity, as individuals would allocate the majority of energy to maintaining homeostasis in an unpredictable environment. To test this, we acclimated Mangrove Rivulus (Kryptolebias marmoratus) in three thermal groups: predictable (27°C-35°C) and unpredictable thermal fluctuations (27°C -35°C), and stable temperature (27°C). After 4 weeks of acclimation, we measured growth rate, thermal biology, and interlamellar cell mass (ILCM) of the gills, the latter a measure of gill plasticity. We found that fish acclimated to either thermal cycle (27°C-35°C) had a lower growth rate than control fish, with fish in the unpredictable group having the lowest growth rate. Thermal safety margin (TSM) was not impacted by either temperature cycle. Notably, fish acclimated to either predictable or unpredictable thermal cycle had significantly more ILCM and therefore reduced gill surface area than control fish, suggesting that the osmorespiratory compromise favours protection against ion loss here. Collectively, our data reveal that the stochasticity of thermal fluctuations is an important factor to consider when studying physiological effects of thermal variation.

12-Phenotypic flexibility? Coping with environmental change over developmental time

ABSTRACT N° ICBF22-167

RAPID ACCLIMATION CAPACITY AT THE TISSUE LEVEL IN A SALMONID

Kim Birnie-Gauvin*[1, 2], Jacey Van Wert[2], Noa B. Mayer[3], Brian Hendriks[3], Scott G. Hinch[3], Erika J. Eliason[2]

[1]Technical University of Denmark, Silkeborg, Denmark,
[2]University of California Santa Barbara, Santa Barbara, United States,
[3]University of British Columbia, Vancouver, Canada

Abstract:
The capacity of animals to acclimate to environmental changes plays an important role in determining how well they are likely to fare with climate change. Most research has focused on the absolute acclimation capacity; however, the ability for an animal to rapidly acclimate to a change in their environment is also critical. We explored phenotypic plasticity at the tissue level in adult and juvenile kokanee salmon (Oncorhynchus nerka), an overlooked species of Pacific salmon residing solely in fresh water. We demonstrate rapid acclimation (approximately 40 hours) in enzyme activities in cardiac, white and red muscle, though each tissue differed in its pattern of acclimation. To date, comparisons of phenotypic plasticity across life stages and sexes of the same species remain rare. Our results provide insights into the mechanisms of acclimation, and contribute to our understanding of how these mechanisms may differ between males and females, and across life stages.

ABSTRACT N° ICBF22-425

TEMPERATURE ACCLIMATION INDUCES TISSUE-SPECIFIC METABOLIC REMODELING IN THREESPINE STICKLEBACK

Louise Cominassi* [1], Kirsten Ressel[1], Patrick Marbacher[1], Kristin O'Brien[1]

[1]Institute of Arctic Biology, UAF, Fairbanks, United States

Abstract:
Eurythermal fish living in thermally variable environments employ multiple strategies to maintain performance and offset the thermodynamic effect of temperature on metabolic rate. How thermal compensation differs among tissues, however, remains unclear. The authors hypothesized that tissues respond differently to temperature acclimation with some contributing more to changes in whole-organismal metabolic rate than others. Threespine stickleback were acclimated to 5, 12 and 20°C for 20–32 weeks. Maximal activities of citrate synthase (CS) and lactate dehydrogenase (LDH) were quantified in liver and oxidative and glycolytic skeletal muscles; mitochondrial respiration rates were measured in liver, oxidative skeletal and cardiac muscles; and resting and maximal metabolic rates were quantified to determine aerobic scope at each acclimation temperature. Absolute aerobic scope was different among all temperature groups and increased with acclimation temperature. Similarly, rates of mitochondrial oxidative phosphorylation and leak in the presence of oligomycin increased with acclimation temperature in liver, oxidative skeletal muscle, and heart. Rates of uncoupled respiration increased with acclimation temperature in liver and heart but was similar among all acclimation groups in oxidative skeletal muscle. Maximal activity of CS increased with acclimation temperature in glycolytic muscle and liver but remained unchanged in oxidative skeletal muscle, and the maximal activity of LDH increased with acclimation temperature only in liver. Overall, temperature-induced changes in metabolic rate of stickleback are associated with changes in aerobic metabolism in liver and glycolytic and cardiac muscles, but less so in oxidative skeletal muscle.

ABSTRACT N° ICBF22-173

STUCK BETWEEN A ROCK AND A HOT PLACE: HOW DOES SOCIABILITY IMPACT ADAPTIVE RESPONSES TO WARM TEMPERATURE?

Chloé Melanson* [1, 2], Simon Lamarre[1], Suzie Currie[2]

[1]Université de Moncton, Moncton,
[2]Acadia University, Wolfville, Canada

Abstract:
Understanding factors affecting ectothermic fishes' capacity to cope with warming temperature is critical given predicted climate change scenarios. We know that a fish's social environment introduces plasticity in how it responds to high temperature. However, the magnitude of this plasticity and the mechanisms underlying socially-mediated thermal responses are unknown. Using the amphibious, selfing hermaphroditic mangrove rivulus fish (Kryptolebias marmoratus) as a model, we tested the hypotheses that 1) the presence of other fish before and during a thermal stress would negatively influence their thermal tolerance and response to rising temperature, and 2) a desensitization of thermal sensation is responsible for the socially-mediated thermal response. To test these, we measured the temperature at which fish emerged (i.e. pejus temperature) with acute warming with socially naïve and socially experienced fish. We used temperature (hypothesis 1) and thermal receptor agonist concentrations (capsaicin; hypothesis 2) to create warming and chemical warming, respectively. Prior social experience resulted in fish emerging at a higher temperature and at greater capsaicin concentrations than socially naïve fish thus reducing their thermal safety margin. The presence of conspecifics during warming did not influence the emersion temperature of socially naïve fish, but did remove the influence of social experience, suggesting social buffering during the thermal stress. Collectively, our data suggest past and present social experiences interact to impact the behavioural response of fish to high temperature. We also provide evidence that social history impacts the capacity of fish to perceive temperature.

ABSTRACT N° ICBF22-264

EXERCISE TRAINING ENHANCES THE CARDIOVASCULAR PERFORMANCE OF RAINBOW TROUT (ONCORHYNCHUS MYKISS) AT HIGH TEMPERATURE WITHOUT BENEFITS FROM FUNCTIONAL FEEDS

Anna Papadopoulou* [1], Luca Pettinau[1], Eila Seppänen[2], Katja Anttila[1]

[1]University of Turku, Turku,
[2]Natural Resources Institute, Enonkoski, Finland

Abstract:
The cardiac plasticity has the potential to protect the fish from environmental changes e.g. temperature fluctuations. However, the hearts of fish reared in aquaculture might be less plastic to cope with changes than the ones of the wild fish, since domestication has led to severe cardiovascular abnormalities. The aim of this study was to reveal whether functional feeding and physical activity could improve the function of cardiovascular system of farmed rainbow trout during acute warming. To investigate this, rainbow trout were trained at water velocity of 1 body length per second for 6h per day, either alone or in combination with one of two functional feed-supplements, allicin and fucoidan. After 6 weeks of exercise training and feeding, maximum heart rate and the temperature coefficient of heart rate were significantly higher in the trained fish as compared to untrained ones. There was a slight increase in hematocrit in trained control fish reared on a normal diet compared to untrained fish fed with the same diet. This implies that exercise training enhanced oxygen delivery to trout tissues via an increase of cardiac blood flow in warm water. Allicin supplement caused a significant reduction in the maximum heart rate and the temperature coefficient of heart rate, especially in trained fish, while fucoidan supplement did not cause any effect on heart rate. We suggest further investigations to understand the antagonistic effect of allicin supplemental feeding and exercise training on cardiovascular performance.

ABSTRACT N° ICBF22-403

THE EFFECT OF DEVELOPMENTAL STRESS ON RESPONSES TO NOVEL PREDATORS AND BEHAVIORAL VARIANCE

Amelia Munson* [1], Andy Sih[2]

[1]University of Glasgow, Glasgow, United Kingdom,
[2]University of California, Davis, Davis, United States

Abstract:
Animals increasingly must respond to novel stressors – often including a mix of biotic and abiotic challenges. Particularly when environmental stressors vary unpredictably, among-individual variance in responses may be crucially important for determining population persistence. Similarly, despite benefits of behaving consistently, increased within-individual variation in response to environmental change may allow the individual to sample different responses and locate an appropriate behavioral strategy. While variation in developmental experience with one stressor has previously been shown to influence behavioral variance, the relationship between developmental stress and repeatability in multiple contexts is less well understood. Using the Western mosquitofish (Gambusia affinis), we examined how developmental experience with pulses of predator threat or warm water experienced either singularly or alternating with each other influenced later responses to novel and known predators and behavioral repeatability when assayed at either a standard or elevated temperature. Responses to predators depended on a three-way interaction between predator identity, developmental experience, and test temperature. Early exposure to any environmental stressor increased individual behavioral consistency of activity when assayed in non-stressful conditions but tended to increase among-individual variation in activity when assayed in stressful conditions. Our results suggest that developmental stress may be important for understanding differences in both responses to stressors later in life and variation within- and among-individuals but that this relationship is influenced by the environmental context.

ABSTRACT N° ICBF22-318

INFLUENCE OF ENVIRONMENTAL STRESSORS ON BODY CONDITION, POPULATION PARAMETERS, AND OTOLITH FLUCTUATING ASYMMETRY IN THE DRAA BARBEL LUCIOBARBUS LEPINEYI (PELLEGRIN, 1939) FROM THE ARID DRAA RIVER BASIN, MOROCCO.

Souhaib Hanfouri[1, 2], Abdelghani Guechchani [1, 2], Mohamed Naimi[2, 3], Mohammed Znari* [1, 2]

[1]Faculty of science semlalia, department of biology, cadi ayyad university ,
[2]The natural history museum of marrakech, cadi ayyad university, Marrakech,
[3]Polydisciplinary faculty, Sultan Mulay Sliman , Beni mellal, Morocco

Abstract:

The Draa barbel Luciobarbus lepineyi is a cyprinid fish endemic to the arid Draa river basin, southern Morocco. In the intermittent and ephemeral streams of this basin, ichthyofauna is faced with natural and anthropogenic multiple stressors. We assessed the impact of these stressors on body condition, growth and population parameters in barbels along a salinity gradient (0.40-10.3 ppt). Captured fish were weighed and measured for body dimensions, sexed and aged using otoliths. Scaled-Mass index (SMi), based on mass–length relationships, was used to analyze the effects of environmental factors, especially water salinization, have on the body condition. We estimated the growth parameters of Von Bertalanffy equation using Beverton-Holt method. We also investigated developmental instability using otolith fluctuating asymmetry (OFA) as an indicator of stress. The SMi declined sharply with increased salinity and remained constant beyond a high salinity threshold. The growth constant rate k (year-1) increased from 0.079 to 0.150. Conversely, the asymptotic body size L_∞ (mm) decreased from 477.2 to 126.4mm and the growth performance Φ was reduced from 4.26 to 2.95. Estimated longevity (years) decreased from 23.7 to 11.2, while the natural mortality rate (M) increased from 44% to 62%. In contrast to otolith mass and biometric measurements, a significant difference between the right and left otoliths shapes, based on the analysis of Elliptic Fourier descriptors, was only found at the highest salinity level, but significant discrimination was detected among the studied populations. Our findings can help in developing an effective management strategy for this endemic desert fish species.

ABSTRACT N° ICBF22-160

GENOME-WIDE DNA METHYLATION IN EUROPEAN SEA BASS CHALLENGED TO FRESH WATER AND ITS RELATION TO GENE EXPRESSION.

Eva Blondeau-Bidet* [1], Céline Cosseau[2], Ghizlane Banousse[3], Thibaut L'Honoré[4], Emilie Farcy[1], Catherine Lorin-Nebel[1]

[1]MARBEC Univ Montpellier, CNRS, Ifremer, IRD, Montpellier,
[2]IHPE UMR 5244 Université de Perpignan via Domitia, Perpignan, France,
[3]Universite du Quebec, Rimouski, Canada,
[4]CUFR Mayotte, Dembéni, France

Abstract:
Salinity is a main factor influencing plasticity and adaptive evolutionary processes through genetic and epigenetic mechanisms. The European sea bass undertakes seasonal migrations to lagoons and estuaries characterized by strong salinity fluctuations from fresh to hypersaline water. Freshwater (FW) tolerance requires powerful hyper-osmoregulatory mechanisms mainly involving gills. An intraspecific variation in the capacity to tolerate FW has been shown in previous studies. Following FW transfer, a decrease in the capacity to maintain hydromineral balance in around 30% of fish was followed by death whereas the other fish could tolerate FW for several months. This difference in the capacity to tolerate FW enabled to distinguish two phenotypes after a two-week FW transfer: FW tolerant (FWt) and FW intolerant (FWi) fish. Gills from both phenotypes were sampled and compared using whole genome bisulfite sequencing to investigate DNA methylation differences between FWt and seawater individuals (SW) as well as between FWi and FWt. To investigate the function of DNA methylation, we performed RNAseq in gills of the same individuals and assessed DNA methylome-transcriptome correlations. We have shown a negative correlation between gene expression levels and methylation levels of promoters, 1st exons and 1st introns. Moreover, a significant effect of salinity on DNA methylation patterns with an overall DNA hypomethylation in the FW transferred groups, more strikingly in FWi, points to a role of DNA methylation in salinity acclimation. Comparing the two salinity conditions, functional annotation showed that numerous differentially methylated genes are involved in functions linked to metabolism, ion transport and transepithelial permeability.

ABSTRACT N° ICBF22-409

IMPLICATIONS OF CHRONIC HYPOXIA DURING A CRITICAL DEVELOPMENTAL WINDOW IN RED DRUM

Benjamin Negrete, Jr.* [1], Andrew J Esbaugh[1]

[1]The University of Texas at Austin Marine Science Institute, Port Aransas, TX, United States

Abstract:
Respiratory plasticity is a beneficial response to chronic hypoxia in adult fish. Red drum, a teleost that commonly experiences hypoxia in the Gulf of Mexico, have shown the ability to increase oxygen binding in sub-lethal hypoxia, but implications of hypoxic exposure during development are unknown. We exposed red drum spawns (n=4) to hypoxia (40% air saturation) or normoxia (100% air saturation) during a critical developmental window that encompasses hatching and first feeding. Larval morphology at 24 hours post fertilization showed that hypoxia-exposed fish have larger pericardial area than control fish. Larvae were moved into normoxia after 3 days post fertilization (dpf) and sampled for gene expression at 3 dpf, 7 dpf, 14 dpf, and 21 dpf for changes of key respiratory genes. Fish were grown out in normoxia and measured for aerobic performance for implications of hypoxic exposure on critical metabolic parameters. We used a cross design wherein fish from normoxia (N=24) were swam in Blazka swim tunnels in both hypoxia (40%, n=12) and normoxia (100%, n=12), and likewise for hypoxia-exposed fish. Oxygen consumption, critical swim speed (Ucrit), maximum metabolic rate, and critical oxygen threshold (Pcrit) were measured. Results show that hypoxia-exposed fish have higher aerobic scope, and Ucrit when swam in normoxia, relative to control fish. Interestingly, hypoxia-exposed fish show decreased hypoxia tolerance (higher Pcrit), and recruit burst swimming at lower swim speeds relative to control fish when swam in hypoxia. These parameters, along with gene expression, will give insight into the implications of hypoxia during critical development periods.

12-Phenotypic flexibility? Coping with environmental change over developmental time

ABSTRACT N° ICBF22-352

MORPHOMETRIC DISCRIMINATION OF STOCKS AND VARIATION IN GROWTH PARAMETERS IN SARDINA PILCHARDUS (TELEOSTEI: CLUPEIDAE) OFF THE MOROCCAN ATLANTIC COASTAL WATERS

Naimi Mohamed*[1, 2], Abdelaziz Mounir[2, 3], Hayat Elmghazli[2, 3], Mohammed Znari[2, 3]

[1]Research unit: Polyvalent for Research and Development, Polydisciplinary Faculty, Sultant Moulay Slimane University, Beni-Mellal,
[2]The Natural History Museum of Marrakech, Cadi Ayyad University,
[3]Laboratory ‘Water, Biodiversity and Climate Change, Research unit: Ecology, Conservation Biology and Valorization of Animal Resources, Cadi Ayyad University, Faculty of Sciences-Semlalia, Marrakech, Morocco

Abstract:
This study was aimed at identifying potential morphologically-based stocks of the Sardine, Sardina pilchardus, and verifying if the specimens belonging to the stocks defined by FAO (1977; 2003) off the Moroccan Atlantic coastal waters, correspond to different morphotypes. In addition, potential variation in growth parameters is also investigated. Morphometric analyses of truss variables and landmark data on sardines collected at four geographical localities along the Moroccan Atlantic coast were performed using multivariate and geometric approaches. Individual were aged from reading otoliths, and Von Bertalanffy growth parameters determined using FISAT II software. The principal component analysis (PCA) of the truss variables and the clustering analysis of the mean shape of the studied specimens revealed the existence of three distinct morphotypes: [Larache], [Safi-Tantan] and [Dakhla]. These correspond to the stocks initially defined by the FAO. On the other hand, the published data on genetic variability are divergent and indicate the particularity of the Safi population that suffered from a bottle-neck due to over-exploitation. According to their linear growth rate, the specimens studied can be separated into three zones, from north to south: 'Larache', 'Safi' and 'Dakhla' with body size, growth rate and performance increasing southwards. This study has clearly shown the existence of three morphologically-based stocks with increasing growth performance southwards. The morphometric and growth variation observed could be explained by taking into consideration the regional hydrodynamic and trophic characteristics. Finally, the validity of the obtained results should be justified by additional studies taking into account seasonal variations and transition zones.

ABSTRACT N° ICBF22-323

STOCK DISCRIMINATION AND VARIATION IN GROWTH PARAMETERS IN THE ENDANGERED COMMON HORSE MACKEREL TRACHURUS TRACHURUS (TELEOSTEI: CARANGIDAE) OFF THE MOROCCAN ATLANTIC COASTAL WATERS

Mohammed Znari* [1], Hayat Elmghazli[1], Abdelaziz Mounir[1, 2], Mohamed Naimi[3, 4]

[1]1Laboratory 'Water, Biodiversity and Climate Change', [WBCC], Research unit: Ecology, Conservation Biology and Valorization of Animal Resources [EcoBioVal], Cadi Ayyad University, Faculty of Sciences-Semlalia, Department of Biology,
[2]Natural History Museum of Marrakech, Cadi Ayyad University,
[3]Laboratory 'Water, Biodiversity and Climate Change', [WBCC], Research unit: Ecology, Conservation Biology and Valorization of Animal Resources [EcoBioVal], Cadi Ayyad University, Faculty of Sciences-Semlalia, Department of Biology, Marrakech,
[4]Polydisciplinary Faculty, Department of Biology, Sultan Mulay Slimane University, Béni-Mellal, Morocco

Abstract:
To differentiate and characterize potential population units of the endangered Atlantic horse mackerel Trachurus trachurus, within the putative Moroccan–Saharan stock, we investigated the variation in morphology and growth parameters in this species from three fishing areas (north to south: Larache, Safi, and Dakhla) in the Moroccan Atlantic coastal waters. We used a geometric morphometric approach based on 30 truss measures derived from 14 landmarks in 165 fish specimens. We used a multivariate comparative analysis including discriminant analysis and Procrustes technique. Age was estimated from otoliths and Von Bertalanffy growth parameters calculated using the Beverton-Holt method. We found indications of at least two subunits: a differentiated subgroup in the north, probably a self-recruiting population or a locally adapted morphotype, and two slightly overlapped subunits, in the central and southern zones. The contribution of each morphometric variable to canonical functions indicated that differences among samples is associated with morphological changes in the anterior and posterior parts of the body, which may indicate adaptations to local environmental conditions and habitat use. Growth constant rate (year-1) and asymptotic body length L_∞ (mm) differed among stock units, with the highest k and lowest L_∞ values recorded off the Safi area and the reserve situation in the North. Growth performance (Φ) decreases southwards. An auximetric analysis revealed that variation in k accounts for only 11% of that of body length; other intrinsic and extrinsic factors could be involved. The obtained results on such a vulnerable declining small pelagic fish are of great importance for regional stock assessment and management.

ABSTRACT N° ICBF22-187

REGULATION OF CHOLESTEROL BIOSYNTHESIS BY THE GLUCOSE-SENSING TRANSCRIPTION FACTOR MONDOA IS REQUIRED FOR ZEBRAFISH EPIBOLY

Thomas Dickmeis* [1], Meltem Weger[2], Benjamin Weger[2], Andrea Schink[1], Masanari Takamiya[1], Johannes Stegmaier[3], Cédric Gobet[4], Alice Parisi[5], Andrei Yu. Kobitski[6], Jonas Mertes[7], Nils Krone[8], Uwe Strähle[1], G. Ulrich Nienhaus[6], Ralf Mikut[9], Frédéric Gachon[10], Philipp Gut[5]

[1]IBCS-BIP/KIT, Eggenstein-Leopoldshafen, Germany,
[2]Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Australia,
[3]Institute of Imaging and Computer Vision, RWTH Aachen University, Aachen, Germany,
[4]Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne,
[5]Nestlé Institute of Health Sciences SA, Lausanne, Switzerland,
[6]Institute of Applied Physics/KIT, Karlsruhe, Germany,
[7]Technobis tft-fos, Alkmaar, Netherlands,
[8]Department of Oncology & Metabolism, University of Sheffield, Sheffield, United Kingdom,
[9]Institute for Automation and Applied Informatics/KIT, Karlsruhe,
[10]Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Germany

Abstract:
The glucose-sensing transcription factors of the Mondo family regulate expression of metabolic genes in mammals. Upon activation by glucose metabolites, MondoA heterodimerizes with its Cofactor Mlx and binds ChoRE enhancer elements in the DNA of its target genes. By examining the zebrafish Mondo pathway, we have revealed an unexpected role of this pathway in vertebrate embryonic development. We showed that knock-down of mondoa impaired the early morphogenetic movement of epiboly in zebrafish embryos. Transcriptome analysis revealed that expression of nsdhl, an enzyme of cholesterol synthesis, was strongly reduced in these embryos. Loss of Nsdhl function likewise impaired epiboly and led to microtubule cytoskeleton defects in the embryo, similar to MondoA loss of function. Yolk syncytial layer specific knockdown of both genes revealed that this embryonic structure is a major site of their function in epiboly processes. Among other cellular roles, cholesterol serves as a substrate for synthesis of the steroid hormone pregnenolone. Consistent with MondoA impacting on cholesterol/steroid biogenesis, both epiboly and microtubule cytoskeleton defects were restored by pregnenolone treatment. Gene disruption of mondoa perturbed epiboly with only partial penetrance. Transcriptome analysis indicates that this is due to compensatory changes in the expression of cholesterol/steroid metabolism genes. Collectively, our results show a novel role for MondoA in the regulation of early vertebrate development, connecting glucose, cholesterol and steroid hormone metabolism with early embryonic cell movements.

ABSTRACT N° ICBF22-528

STEROIDOGENESIS DURING CHRONIC SOCIAL STRESS IN RAINBOW TROUT

Carol Best* [1], Kathleen Gilmour[1]

[1]University of Ottawa, Ottawa, Canada

Abstract:
Status in rainbow trout social hierarchies is associated with differences in endocrine stress axis activity. Subordinate rainbow trout exhibit chronically elevated cortisol levels, and a blunted cortisol response to additional acute stressors. We hypothesized that chronic social stress alters steroidogenesis in interrenal cells of the head kidney, and that these changes contribute to the observed differences in whole-animal cortisol dynamics. Juvenile, size-matched rainbow trout were allowed to interact in pairs for 4 days. The impact of chronic social stress on steroidogenesis was investigated by measuring abundance of key transcripts and proteins in the head kidney. To assess steroidogenic capacity, we also measured cortisol production from isolated head kidney preparations in vitro, in response to ACTH and the cAMP analogue, dibutyryl-cAMP (db-cAMP). Chronic social stress elevated plasma cortisol levels and resulted in higher abundance of several key components of steroidogenesis, including the melanocortin receptor accessory protein (mrap), a paralog of steroidogenic factor 1 (ff1d), p450 side chain cleavage (p450scc) and steroidogenic acute regulatory protein (star) transcripts. Isolated head kidney preparations reflected cortisol dynamics in the whole animal; subordinate fish had higher baseline cortisol production but blunted responses to ACTH and db-cAMP. Collectively, the results demonstrate that chronic social stress modifies cortisol steroidogenesis; subordinates exhibit increased baseline capacity for cortisol production, and their reduced capacity to respond to additional acute stressors arises at least in part downstream of cAMP production.

ABSTRACT N° ICBF22-142

REGULATION OF THE CORTISOL STRESS RESPONSE DURING AND AFTER CHRONIC SEVERE HYPOXIA EXPOSURE IN CRUCIAN CARP AND GOLDFISH

Nicholas Bernier* [1], Michael Alcorn[1], Michael Lim[1], Brett Culbert[1], Göran Nilsson[2]

[1]University of Guelph, Guelph, Canada,
[2]University of Oslo, Oslo, Norway

Abstract:
Severe hypoxia exposure in fish is known to stimulate the hypothalamic-pituitary-interrenal (HPI) axis and the production of the catabolic hormone cortisol. In contrast, a key strategy for sustained survival without oxygen in crucian carp and goldfish is to reduce energy utilization. In this study, to characterize the activity and regulation of the HPI axis in hypoxia-tolerant species, crucian carp and goldfish were exposed to chronic severe hypoxic conditions for 6 days and either sampled immediately or recovered in normoxia for 1 or 6 days. While crucian carp transiently reduced plasma cortisol levels in response to anoxia, severe hypoxia and early reoxygenation elicited modest increases in the circulating levels of cortisol in goldfish. Reoxygenation in goldfish was also characterized by transient increases in preoptic area corticotropin-releasing factor (CRF), pituitary CRF receptor type 1, and pituitary pro-opiomelanocortin mRNA levels. In both species, the first 24 h of recovery were characterized by a general reduction in head kidney steroidogenic enzyme expression. In contrast, recovery was associated with marked increases in the expression of genes involved in cortisol breakdown and in the mRNA levels of liver leptin, a known inhibitory cytokine of the HPI axis. Together, consistent with a hypoxia survival strategy that minimizes energy expenditure, our results suggest that crucian carp and goldfish utilize several mechanisms to blunt the activity of the HPI axis both during and immediately after severe hypoxia exposure.

ABSTRACT N° ICBF22-319

CORTISOL MODULATES SKELETAL MUSCLE GLUCOSE REGULATION IN ZEBRAFISH

Femilarani Antomagesh* [1], Jithine Jayakumar Rajeswari[1], Mathilakath M. Vijayan[1]

[1]University of Calgary, Calgary, Canada

Abstract:
In mammals, insulin-mediated skeletal muscle glucose disposal is critical for the tight regulation of circulating glucose levels. During stress, glucocorticoids (GCs) inhibit insulin-stimulated skeletal muscle glucose uptake to transiently sustain stress hyperglycemia. However, chronic stress leads to systemic insulin resistance in humans. In teleosts, despite a conserved insulin response to hyperglycemia, insulin-mediated glucose clearance is relatively inefficient compared to mammals. However, the mechanisms are unclear. Here we investigated whether elevated cortisol levels, mimicking a chronic stress, suppress skeletal muscle glucose uptake using zebrafish (Danio rerio). The chronic cortisol elevation resulted in an impairment in plasma glucose clearance when subjected to a glucose tolerance test, despite the glucose induced elevated whole-body insulin expression. To test the effect of cortisol on skeletal muscle glucose regulation we subjected the fish to exogenous insulin stimulation. Chronic cortisol exposure reduced skeletal muscle glucose uptake, and this corresponded with lower glycogen content. Cortisol treatment also reduced insulin-stimulated glucose clearance and glycogen synthesis. Cortisol group showed an increased insulin-induced phosphorylation of AKT in the skeletal muscle, but reduced phosphorylation of S6 kinase, a downstream target of AKT. Moreover, the transcript abundance of glut1b, the most abundant GLUT identified in the zebrafish skeletal muscle, was upregulated with insulin treatment only in the cortisol treated group. In summary, chronic cortisol stimulation restricts skeletal muscle glucose uptake and alter glucose metabolism, and this may involve disrupted insulin signaling downstream of AKT activation in zebrafish. Acknowledgement: Natural Sciences and Engineering Research Council of Canada Discovery Grant to MMV supported this study.

ABSTRACT N° ICBF22-532

INTEGRATIVE PROTEOMICS AND METABOLOMICS PROFILING OF FARMED GILTHEAD SEABREAM HEPATIC STRESS RESPONSE

Cláudia Raposo De Magalhães* [1, 2], Gavin Blackburn[3], Phil Whitfield[3], Ana Paula Farinha[1, 2], Raquel Carrilho[1, 2], Denise Schrama[1, 2], Marco Cerqueira[1, 2], Pedro Miguel Rodrigues[1, 2]

[1]Centre of Marine Sciences (CCMAR),
[2]Universidade do Algarve, Faro, Portugal,
[3]Glasgow Polyomics, University of Glasgow, Glasgow, United Kingdom

Abstract:
Aquaculture is crucial to keep pace with the increasing fish demand, however, the industry faces its own sustainability challenges to attain such endeavor. Managing fish stress is crucial to avoid negative impacts on fish health/welfare, and ultimately, on the aquaculture productivity. In this study, an integrated proteomics and metabolomics characterization of the fish liver, a central organ during stress adaptation, was performed to provide a holistic assessment of the molecular stress response at different organizational levels. Widen our understanding into the physiological changes occurring in the fish organism during stress exposure/adaptation can leverage the industry with the scientific knowledge for forthcoming species-specific welfare assessment protocols.

Sparus aurata was exposed to different suboptimal rearing conditions in three separate trials: overcrowding, net handling, and hypoxia, using fish reared under optimal conditions for the species, as control. Extracts from liver samples were prepared for further proteomics and metabolomics analysis by LC-MS/MS. Pairwise comparisons between identified proteins and metabolites, were achieved by a student's t-test, $p < 0.05$; FDR controlled at 0.05.

A total of 397 proteins and 121 metabolites were differentially regulated between challenged and control fish across the three trials, mostly implicated in the same KEGG pathways, namely amino acid and carbohydrate metabolism-related, inflammatory response, protein folding processes, among others. A STITCH protein-metabolism interaction network revealed a coordinated response of the hepatic system to the different challenges. These results shed light on the dynamics and extent of this species' metabolic reprogramming under stress, supporting future studies on stress markers' discovery and fish welfare research.

ABSTRACT N° ICBF22-464

GENE EXPRESSION AND PHYSIOLOGICAL CHANGES REFLECT DIFFERING STRATEGIES FOR THERMAL TOLERANCE IN JUVENILE RAINBOW TROUT (ONCORHYNCHUS MYKISS)

Leah Turner* [1], Moira Ferguson[1], Roy Danzmann[1]

[1]University of Guelph, Guelph, Canada

Abstract:
Rainbow trout (Oncorhynchus mykiss) are a key freshwater aquaculture species in Canada. Trout raised in net pens in the Great Lakes experience longer and more intense periods of thermal stress from June to September that manifests itself with loss of appetite and reduced growth leading to financial losses.

To better understand the genomic and physiological underpinnings of thermal tolerance in O. mykiss, we subjected juveniles from aquaculture strains of rainbow trout to acute warming trials (0.8C/hour). RNA-seq on hepatic tissue and enzyme activities of key aerobic and anaerobic genes in the liver, gill and white muscle, alongside blood metabolites were analyzed in fish with the best and worst thermal tolerance compared to controls.

Understanding short-term physiological and longer-term evolutionary responses to rising temperatures will be key to developing policy, and to mitigating the effects of climate change on rainbow trout aquaculture on a global scale.

ABSTRACT N° ICBF22-185

INTERACTIVE CROSS-TALK BETWEEN ENDOCRINE AND IMMUNE SYSTEMS IN FISH REGULATORY ORGANS UNDER STRESS

Lluis Tort Bardolet* [1], F. Reyes-Lopez[1, 2], A.R. Khansari[1], X. Liu[1, 3], E. Vallejos-Vidal[1, 2], M. Teles[1], J.C. Balasch[1]

[1]Dpt. Cell Biology, Physiology and Immunology, Univ. Autonoma de Barcelona, Barcelona, Spain,
[2]Centro de Biotecnología Acuícola, Facultad de Química y Biología, Univ. de Santiago de Chile, Santiago de Chile, Chile,
[3]3Key Laboratory of Aquatic Science of Chongqing, School of Life Sciences, Southwest University, Chongqing, China

Abstract:
The stress response in vertebrates involves many physiological compartments and mechanisms from genes and metabolism to behavioural reactions. In particular, regulatory organs and systems are implicated since the non-specific response to the stress challenge mobilises resources and mechanisms from the main regulatory neural, endocrine and immune systems. Our work shows that either brain, pituitary and head kidney, the main regulatory organs in the organisation of the stress response, are sensing and responding to the stress challenges regardless the type of stimulus (physical, chemical or biotic) Therefore, generating a complex and interactive response.

Our results also confirm that pituitary shows a significant immune response. Thus, pituitaries of rainbow trout cultured in vitro, incubated with bacterin, or bacterin plus CRH or cortisol, showed significant regulation of inflammatory genes (proinflammatory and anti-inflammatory genes). When fish were subjected to vaccination plus air exposure or vaccination without air exposure, a significant immune response was observed in the pituitary tissue in both cases, although less intense under vaccination than in fish subjected to air exposure.

ABSTRACT N° ICBF22-267

GLUCOCORTICOID MODULATION OF THE IMMUNE SYSTEM IN ZEBRAFISH

Marcel Schaaf* [1]

[1]Institute of Biology, Leiden University, Leiden, Netherlands

Abstract:
Glucocorticoids are steroid hormones that are secreted after stress and they have strong anti-inflammatory effects. Therefore, synthetic analogs of these hormones are widely used to treat various immune-related disorders. Glucocorticoid effects are mediated by the Glucocorticoid receptor (Gr), which acts as a transcription factor upon ligand activation. We study the molecular mechanisms of Gr action on the immune system in vivo, using the zebrafish as an animal model. As a model for inflammation, we use tail fin amputation in 3 day old larvae. We have demonstrated that the migration of neutrophils towards the amputated site is attenuated upon glucocorticoid treatment, but the migration of macrophages appears to be unaffected. Furthermore, we have shown that glucocorticoids inhibit the differentiation of macrophages towards a pro-inflammatory phenotype, by blocking almost the entire transcriptional response to the amputation. Interestingly, the transcriptional response to an infection with Mycobacterium marinum appears to be resistant to glucocorticoid modulation. We are currently using the zebrafish model for the development of novel glucocorticoid therapies with reduced side effects, and we focus on targeting of GCs to inflamed tissue by encapsulation in liposomes and on a class of glucocorticoids that are present in ginseng, a traditional Chinese herbal medicine.

ABSTRACT N° ICBF22-472

THE ROLE OF THE MINERALOCORTICOID RECEPTOR IN THE INNATE IMMUNE SYSTEM OF ZEBRAFISH

Erin Faught* [1], Marcel Schaaf[1]

[1]University of Leiden , Leiden , Netherlands

Abstract:
Stress and the attendant rise in glucocorticoids (GCs) results in a potent suppression of the immune system. Indeed, the anti-inflammatory properties of GCs are well established and have been exploited therapeutically for decades. Cortisol, the primary GC in both fish and humans, signals through two corticosteroid receptors, the low-affinity glucocorticoid receptor (GR), activated during high cortisol conditions, and the high-affinity mineralocorticoid receptor (MR), which is constitutively active. To date, the anti-inflammatory role of GCs has primarily been attributed to cortisol-GR signalling, while the role of MR is largely unknown. Here we tested the hypothesis that while GR promotes anti-inflammatory properties, MR activation will mediate pro-inflammatory responses. We first established a ubiquitous MR knockout line of zebrafish with fluorescently labeled leukocytes. Loss of MR resulted in altered macrophage distribution and a global reduction in macrophage numbers during primitive myelopoiesis. To further evaluate the role of MR during inflammation, we next employed the tail fin amputation assay in zebrafish larvae, whereby migration of fluorescently labeled leukocytes towards the wound site is visualized and quantified. Here we allowed myelopoiesis to proceed normally and subsequently treated larvae with an MR antagonist. This pharmacological blockade of MR reduced migration of macrophages towards the wound and qPCR analysis confirmed that the responsivity of macrophages was impaired due to downregulation of chemokine receptors. Taken together, our work suggests that MR signalling is necessary for adequate macrophage distribution and function and is thereby acting as a key modulator of immune system function in fish.

ABSTRACT N° ICBF22-230

EFFECT OF OXYTETRACYCLINE ON IMMUNE INTESTINAL BIOMARKER EXPRESSION IN COHO SALMON (ONCORHYNCHUS KISUTCH); IN-VIVO AND IN-VITRO EXPERIMENTS.

Daniela Nualart *[1, 2], Luis Vargas-Chacoff[1, 2], Jose Luis Muñoz[1, 3]

[1]Universidad Austral de Chile,
[2]Fondap-IDEAL , Valdivia,
[3]Universidad de Los Lagos, Puerto montt, Chile

Abstract:
The Oxytetracycline (OTC) has been used as an antibiotic for many kinds of bacterial diseases in cultured fish, but excessive dose of OTC are known to cause side effects in fish and can have negative effects on their environment. The objective of this work was to evaluate by qRT-PCR the expression levels of immune markers as TLR-1, TLR-2, IKB-a, MyD-88, NFK-b, INF-g and IL-6 of intestinal cells primary culture (foregut, midgut and hindgut) and in addition on in vivo response to different doses of OTC in coho salmon at different times. The expression levels of all genes increased significantly at 1 day and 1h in high dose of OTC compared with control conditions in all tissues in vivo and in vitro respectively. However, the transcription were decreased at 3, 6,12 hours in vitro and day 3 in vivo experiment. In conclusion the transcriptional responses were differently modulated by OTC on the three portions of intestine in both experimental conditions (in vivo and in vitro). These results are the first demonstration that expression of immune genes using primary cell culture fish, suggesting that immune biomarker expression of all tissues induce a differential response of these genes depending on the concentration of OTC and kinetics of time. This research provides significant information that may be useful for improving aquaculture, optimum doses of drugs, and fish health. Acknowledgments: This work was funded by Fondecyt Regular Nº1190857, Nº1180957 and Fondap-Ideal Grant Nº15150003

ABSTRACT N° ICBF22-222

PERSONALITY AND THE CLOCK

Christian Tudorache* [1]

[1]Leiden University, Leiden, Netherlands

Abstract:
Many physiological processes in our body are controlled by the biological clock and show a diel (day and night) rhythmicity. This rhythmicity is assumed to be robust for all healthy individuals of a population. In a previous study, however, we have observed a remarkable individual variation in rhythmicity in a wildtype zebrafish population, on various levels of biological function: clock gene expression, endocrine regulation and locomotor activity. These clock phenotypes were correlated with coping styles, varying along a continuum between proactive and reactive extremes. Adult proactive fish displayed a strong diel rhythm while reactive fish showed a complete absence of rhythmicity. In order to further explore the role of the glucocorticoid receptor (GR) in both, stress coping and the rhythmicity of the biological clock, we tested mutant larval zebrafish with a non-functional GR (grs357). Interestingly, when compared to wildtype fish, mutants displayed weaker rhythms of erratic behaviour and melatonin production, but surprisingly not of clock gene expression. This suggest that GR affects the diel rhythmicity of zebrafish larvae at the behavioural and endocrine level, but not their mediation by clock gene expression. Finally, we tested the hypothesis that coping styles are correlated with diel hatching time, during night- or day-time. Larvae, hatched during day-time, had a stronger behavioural response to a light dark challenge assay in locomotion related parameters and a weaker response in directionality related parameters, than larvae hatched during night-time. Together, these results suggest that diel hatching time may determine the behavioural phenotype of an individual. We conclude that variation in diel rhythmicity is naturally present in wildtype populations, as an integral part of a coping style, and interacts with various levels of biological function.

ABSTRACT N° ICBF22-238

BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES OF SEA BREAM TO DIFFERENT AQUACULTURE PRACTICES DEPENDING ON STRESS COPING STYLES

Sébastien Alfonso* [1], Walter Zupa[1], Amedeo Manfrin[2], Eleonora Fiocchi[2], Tobia Pretto[2], Maria Teresa Spedicato[1], Giuseppe Lembo[1], Pierluigi Carbonara[1]

[1]Coispa Tecnologia & Ricerca, Bari,
[2]Istituto Zooprofilattico delle Venezie, Legnaro, Italy

Abstract:
Stress coping style (SCS) is defined as a coherent set of individual physiological and behavioural differences in stress responses that are consistent across context and over time. SCS could have direct implications for aquaculture productivity and fish welfare. In this study we interested about one of the main farmed species of the European aquaculture, the sea bream (Sparus aurata), and investigated (1) correlation between boldness behaviour, hypoxia and level of stress physiological indicators (e.g., cortisol, glucose, lactate); (2) the behavioural and physiological responses to stocking density (low density, LD: 15 kg/m3 and high density, HD: 30 kg/m3). We (1) observed that the behavioural response in the hypoxia test was not consistent over time, while the behavioural response was consistent in the risk-taking test. Moreover, no behavioural consistency was observed between these two tests. Also, among fish that exited the safe area during risk taking test, bolder individuals displayed lower basal levels of cortisol, noradrenaline, and lactate while no correlation was found between those physiological parameters and the behavioural response in the hypoxia test. Then, in response to different stocking density (2), the level of some stress physiological parameters (e.g. glucose, lactate) vary. Mostly, swimming activity, indicative of energy expenditure, was found to be greater in bold individuals than shy ones at HD, while the opposite pattern was observed at LD. Altogether, these results are relevant to improve both health/welfare and productivity of farmed sea breams by selective robust fish, better adapted to farming conditions even if further research is needed.

ABSTRACT N° ICBF22-304

COHABITATION WITH ATLANTIC SALMON (SALMO SALAR) AFFECTS BRAIN NEUROMODULATORS BUT NOT WELFARE INDICATORS IN LUMPFISH (CYCLOPTERUS LUMPUS)

Fredrik R Staven* [1, 2], Manuel Gesto[3], Martin H Iversen[1], Per Andersen[4], Deepti M Patel[1], Jarle T Nordeide[1], Torstein Kristensen[1]

[1]Faculty of Biosciences and Aquaculture, Nord University, Bodø,
[2]Department of Research and Development, Aqua Kompetanse AS, Flatanger, Norway,
[3]Section for Aquaculture, Technical University of Denmark, Hirtshals, Denmark,
[4]Marinkonsulent AS, Flatanger, Norway

Abstract:
Lumpfish are utilized to combat ectoparasitic epidemics in salmon farming. Research gaps on both cleaning behavior and client preferences in a natural environment, emphasizes the need to investigate the physiological impacts on lumpfish during cohabitation with piscivorous Atlantic salmon. Lumpfish (39.9 g, S.D ± 8.98) were arranged in duplicate tanks (n = 40 per treatment) and exposed to Live Atlantic salmon (245.7 g, S.D ± 25.05), salmon Olfaction or lifelike salmon Models for 6 weeks. Growth and health scores were measured every second week. In addition, the final sampling included measurements of neuromodulators, body color and plasma cortisol. A stimulation and suppression test of the hypothalamic-pituitary-interrenal (HPI) axis was used for chronic stress assessment. Results showed that growth, health scores and body color remained unaffected by treatments. Significant reductions in levels of brain dopamine and norepinephrine were observed in Live compared to Control. Plasma cortisol was low in all treatments, while the stimulation and suppression test of the HPI axis revealed no indications of chronic stress. This study presents novel findings on the impact on neuromodulators from Atlantic salmon interaction in the lumpfish brain. We argue that the downregulation of dopamine and norepinephrine indicate plastic adjustments to cohabitation with no negative effect on the species. This is in accordance with no observed deviations in welfare measurements, including growth, health scores, body color and stress. We conclude that exposure to salmon or salmon cues did not impact the welfare of the species in our laboratory setup, and that neuromodulators are affected by heterospecific interaction.

ABSTRACT N° ICBF22-243

MITIGATING THE EFFECTS OF STRESS ON TRANSPORTED ORNAMENTAL FISH

Megan Jones*[1], Mhairi Alexander[1], Donna Snellgrove[2], Peter Smith[3], Sam Bramhall[4], Peter Carey[4, 5], Fiona Henriquez[1], Iain McLellan[1], Katherine Sloman[1]

[1]University of the West of Scotland, Paisley,
[2]Waltham Petcare Science Institute, Waltham on the Wolds,
[3]Aquasense UK Ltd, High Wycombe,
[4]Pets at Home Ltd, Handforth,
[5]CASCO Pet, Broadridge Heath, United Kingdom

Abstract:
Ornamental fishes are one of the most commonly owned companion animals globally. During the acquisition process, fishes are often transported over long distances from their origin to destination country, exposing them to biotic and abiotic conditions that increase stress and compromise welfare. Standard practices to reduce negative effects arising from transportation include water quality monitoring, using packaging to reduce mechanical disturbance and transporting at appropriate stocking densities. A large amount of research has been conducted on how these practices affect behaviour, however, the benefits of social complexity have received limited scientific attention. An alternative method to mitigate transport stress may include identifying optimum housing conditions for fish post-transport. Here we investigated two things (i) whether altering tank composition and (ii) whether the presence/absence of being able to see acclimated fish in adjacent tanks mitigated stress-like behaviours post-transport. We measured stress-like behaviours post-transport to identify whether altering a fish's environment post-transport alleviated any negative behaviours. The social composition that fish were placed into post-transport, affected behaviour. We observed an overall reduction in the frequency of stress-related behaviours in fish that were placed into empty tanks or into tanks with heterospecifics compared to fish placed into tanks where conspecifics were already present. In conclusion, altering the environment following transport, can help mitigate some of the effects of transport-induced stress and improve the welfare of fish in the ornamental trade. These results could potentially change standard practices within the ornamental fish supply chain, with benefit for both fish welfare and economic sustainability.

ABSTRACT N° ICBF22-530

HIGH EGG CORTISOL CONTENT AFFECTS EARLY DEVELOPMENT IN WILD COHO SALMON

Analisa Lazaro-Cote* [1], Mathilakath M. Vijayan[1]

[1]University of Calgary, Calgary, Canada

Abstract:
Female Pacific salmonids have relatively high circulating levels of cortisol during their spawning migration. Cortisol can enter the oocytes during vitellogenesis, and studies have shown that exposure to maternally derived cortisol in the egg may shape offspring phenotype. Cortisol is essential for the proper development of the offspring, but cortisol that is in excess may alter their developmental trajectory. In this study, we tested the hypothesis that the progeny from eggs with more cortisol may exhibit poor growth and stress performance. To test this, wild coho salmon (Oncorhynchus kisutch) females were collected from three rivers in British Columbia, Canada. Unfertilized eggs and plasma were taken from 60 females to quantify cortisol levels and egg size. We also measured the transcript abundance of corticosteroid receptors (MR, GR1, GR2), and the energy substrates (lipids, proteins, carbohydrates) in the eggs. The remaining eggs were fertilized and reared in a flowthrough vertical incubator to assess the linkages between egg cortisol content and offspring development. The results reveal that females with high plasma cortisol levels deposited more cortisol into their eggs, which resulted in reduced survival of the offspring to the eyed stage. Also, high cortisol content in the eggs decreased the condition factor at the fry stage. The results suggest that higher egg cortisol content affects offspring metabolism in wild coho salmon; however, whether egg MR and/or GR orchestrates these developmental changes remains to be elucidated.

ABSTRACT N° ICBF22-381

ENVIRONMENTAL STRESS IN ATLANTIC SALMON: EFFECTS OF SALINITY CHANGE AND ITS DIETARY MITIGATION POTENTIAL

Jonas Mueller* [1, 2], Doret van Muliekom[3], Matteo Pauly[2], Jacqueline Lindemeyer[4], Thekla Schultheiß[4], Alexander Rebl[3], Henrike Seibel[2], Carsten Schulz[1, 2]

[1]Institute of Animal Breeding and Husbandry, Department for Marine Aquaculture, Christian-Albrechts University, Kiel,
[2]Fraunhofer Research Institution for Individualized and Cell-Based Medical Engineering IMTE, Aquaculture Research Station, Büsum,
[3]Institute for Farm Animal Biology (FBN), Institute of Genome Biology, Fish Genetics Unit, Dummerstorf,
[4]Institute of Toxicology and Pharmacology for Natural Scientists, University Medical School Schleswig-Holstein, Kiel, Germany

Abstract:
A change in environmental salinity is part of the anadromous life-cycle of Atlantic salmon and a critical step during the production of this species. Besides an acute stress response following seawater transfer of Atlantic salmon smolts, a systemic downregulation of immune pathways has been described. Studies investigating salinity change independently from that of the smoltification process are however lacking. There is emerging interest into developing dietary mitigation strategies of stress in aquaculture settings. Functional diets based on microalgae have shown great potential to interact with immune and stress responses of various species, but information on their stress mitigating effects in Atlantic salmon is limited.

We investigated how Atlantic salmon post-smolt cope with a change in salinity and further explored whether functional diets enriched with microalgae can mitigate stress and immune related responses following salinity change. To do so groups of Atlantic salmon feed with six different diets were subjected to a change in salinity from brackish water (12 psu) to full strength seawater. A control group was kept in brackish water. We monitored feed intake and assessed plasma parameters, gene expression of stress- and immune-related genes in head kidney and gill and protein abundance of myeloperoxidase and superoxide dismutase 1 in liver after 20 h and 14 days in seawater.

The present talk will give new insights into how Atlantic salmon post-smolts cope with changing salinity from both a molecular as well as whole animal perspective. The role of the different functional diets in stress mitigation will further be discussed.

ABSTRACT N° ICBF22-401

REGULATION OF THE GASTROINTESTINAL CORTICOTROPIN-RELEASING FACTOR (CRF) SYSTEM DURING OSMOTIC STRESS IN SALMONID FISHES

Brett Culbert*[1], Stephen McCormick[2,3], Nicholas Bernier[1]

[1]University of Guelph, Guelph, Canada,
[2]U.S. Geological Survey, Eastern Ecological Science Center, Turners Falls,
[3]University of Massachusetts Amherst, Amherst, United States

Abstract:
Changes in salinity are typically stressful for fishes and several endocrine pathways aid in re-establishing homeostasis when the osmotic environment is altered. While the osmoregulatory roles of several hormones are well established (e.g., cortisol and growth hormone), the involvement of many other endocrine systems is less certain. For instance, the corticotropin-releasing factor (CRF) system is primarily thought to contribute to osmoregulation by influencing activity of the hypothalamic-pituitary-interrenal axis in teleosts, but studies in invertebrates and mammals suggest that the CRF system may also directly influence osmoregulatory processes in the gastrointestinal tract of teleosts. Therefore, we initially characterized the gastrointestinal CRF system and then evaluated the potential osmoregulatory role of the CRF system in the gastrointestinal tract of two salmonid fishes: anadromous Atlantic salmon (Salmo salar) and non-anadromous rainbow trout (Oncorhynchus mykiss). Several components of the CRF system were expressed throughout the gastrointestinal tract in both species and transcriptional regulation of these components was comparable when either species was transferred from freshwater to seawater. Using in situ hybridization we found that transcripts of CRF receptors and binding proteins in the intestine are primarily expressed in enterocytes, which is consistent with their proposed osmoregulatory function. Finally, we are currently conducting in vitro tissue culture experiments to determine the specific mechanism(s) by which the CRF system influences osmoregulatory processes within the intestine. Together, our data suggest that the CRF system likely has a direct osmoregulatory role in the teleost intestine.

ABSTRACT N° ICBF22-533

REGENERATION OF GILL FILAMENTS IN LABORATORY-REARED ATLANTIC SALMON (SALMO SALAR)

Ensiyeh Ghanizadeh-Kazerouni* [1], Phillip R. Morrison[1], Jonathan M. Wilson[2], Simon R. M. Jones[3], Colin J. Brauner[1]

[1]University of British Columbia, Vancouver,
[2]Wilfrid Laurier University, Waterloo,
[3]Fisheries and Oceans Canada, Nanaimo, Canada

Abstract:
Fish gills' direct contact with the aquatic environment exposes them to infectious and non-infectious stressors which can cause physical damage and tissue loss contributing to "complex gill disease" (CGD), an emerging threat in finfish aquaculture. CGD-induced pathology can compromise respiratory gas exchange and osmoregulation. Regeneration of damaged gill tissue has been reported for some species, however it is not known for Atlantic salmon, which is an economically important aquaculture species. Here, we investigated changes in the morphology of gill filaments and lamellae following resection in a laboratory population of Atlantic salmon held in freshwater. Two levels of resection severity were investigated: 30% or 50% of filament length from the most distal part of 16 filaments from the first branchial arch. Filament length was then measured in the same 8-12 individuals at 1, 2, 4, 8, and 12 weeks post-resection (wpr). Additional fish were terminally sampled at these times for gill immunohistochemistry. Filament regeneration was evident at all time points, but was most notable from 4- to 12- wpr. At 12 wpr, 10-15% of the resected filament length was regenerated. Regeneration rate was significantly different among individuals, but was not correlated with resection severity, initial body size, condition factor, or overall growth rate. Our results demonstrate significant gill regeneration in Atlantic salmon following resection which has important implications for recovery from CGD in aquaculture.

This work was funded in part by the Government of Canada through Genome Canada and Genome Atlantic, and by Cargill Inc., Cermaq Canada and Grieg Seafoods BC Ltd.

ABSTRACT N° ICBF22-249

CHRONIC STRESS ALTERS THERMAL TOLERANCE IN RAINBOW TROUT (ONCORHYNCHUS MYKISS)

Brittany Bard* [1], Steven Cooke[2], Kathleen Gilmour[1]

[1]University of Ottawa,
[2]Carleton University, Ottawa, Canada

Abstract:
Salmonid fishes are of ecological, cultural, and economic significance but are under threat from a variety of environmental and anthropogenic factors including climate change, over-exploitation, habitat loss, and water quality degradation. In a changing climate, salmonids face increasing temperatures along with other stressors, and therefore it is important to understand how chronic stress influences thermal tolerance. We addressed this question in a series of experiments that measured thermal tolerance and the cortisol response to elevated temperature in rainbow trout (Oncorhynchus mykiss) subjected to chronic social stress or cortisol treatment. Subordinate trout experiencing chronic social stress exhibited high baseline cortisol values and reduced thermal tolerance measured as the critical thermal maximum (CTmax). The decrease in CTmax was alleviated by recovery from social stress, which lowered cortisol levels, and restored when cortisol levels were maintained at a high level during recovery. Trout treated with intraperitoneal implants to elevate cortisol showed similar reductions in CTmax. Co-treatment with the glucocorticoid receptor (GR) antagonist RU486 prevented the decrease in CTmax, suggesting that the effect is GR-dependent. Finally, rainbow trout were subjected to increases in water temperature to determine whether the temperature threshold for an acute cortisol response differed between control and cortisol-treated individuals. Taken together, the findings demonstrate that cortisol plays a role in determining the thermal tolerance of rainbow trout.

ABSTRACT N° ICBF22-355

ELEVATED TEMPERATURE SHORTENS THE SEAWATER TOLERANCE WINDOW IN LARGE UNDER-YEARLING ATLANTIC SALMON SMOLTS

Lars Eirik Myklatun* [1], Angelico Madaro[2], Antony J. Prabhu[3], Per Gunnar Fjelldal[4]

[1]Department of Animal Welfare, Institue of Marine Research, Matre,
[2]Department of Animal Welfare, Institue of Marine Research,
[3]Department of Feed and Nutrition, Institue of Marine Research, Bergen,
[4]Department of Reproduction, Institue of Marine Research, Matre, Norway

Abstract:
Currently, the salmon farming industry are adopting new rearing practices for inducing seawater tolerance in large (>120 g) under-yearling (0+) salmon, aiming for a more effective regulation of the production time in freshwater tanks vs. sea-cages. However, knowledge about how these new farming practices impacts the development of hypo-osmoregulatory ability and its duration, is largely unknown. In two different experiments, we followed +0 salmon through several different rearing regimes designed to stimulate hypo-osmoregulatory ability. The fish were subjected to 24h 34ppt seawater challenge tests over a period of 725 degree-days (dd) to explore if and when hypo-osmoregulatory ability developed. In the first experiment, we used a 2x2 factorial design to investigate the effect of two different light regimes, either a regime where 12 h daylight and 12 h dark per day for a period of six weeks was followed by continuous light (LDLL), or a regime with continuous light throughout (LL), combined with two different experimental feeds with either elevated (SF) or normal (NF) salt content. The temperature was 12˚C throughout the experiment. Results show that LL-fish where able to hypo-osmoregulate at all time points (starting 50 dd after the LDLL fish were changed to continuous light), while LDLL fish were only able to regulate from 130 to 725 dd. SF had no impact on the timing of the development of hypo-osmoregulatory ability. In the second experiment, we subjected 0+ salmon reared at LDLL or LL in combination with 8˚C or 16˚C water temperature to 24h 34 ppt seawater challenge tests over a period of 1000 dd (same 'dd start point' as 1st experiment). Here, hypo-osmoregulatory ability had developed at all time points in LL-8C fish, from 272 to 1000 dd in LDLL-8C, from 272 to 850 dd in LDLL-16C, and from 384 to 1000 dd in LL-16C fish.

ABSTRACT N° ICBF22-140

EFFECT OF TOXIC CYANOBACTERIA EXPOSURE ON JUVENILE SALMON

Ryan Shartau* [1, 2], Lenora Turcotte[1], Hein Snyman[3], Julia Bradshaw[1], Stewart Johnson[1]

[1]Fisheries and Oceans Canada, Nanaimo, Canada,
[2]The University of Texas at Tyler, Tyler, United States,
[3]University of Guelph, Guelph, Canada

Abstract:
Farmed Atlantic Salmon (Salmo salar) and Chinook Salmon (Oncorhynchus tshawytscha) develop a severe liver disease called net-pen liver disease (NPLD), which is characterized by hepatic lesions that include prominent megalocytosis and loss of gross liver structure. NPLD is believed to be caused by microcystin (MC) exposure, a hepatotoxin produced by cyanobacteria. The etiology of NPLD remains uncertain despite the putative link with MC; furthermore, it is not clear if farmed salmon are naturally exposed to MC at farm sites. This was investigated by assessing the presence of MC in coastal southwest Canada and examining effect of sub-lethal MC exposure in Atlantic and Chinook Salmon.

Sampling over a two-year period at several sites revealed the presence of MC at all sites year-round; other algal toxins were also identified. The effect of sub-lethal MC exposure was investigated by orally gavaging post-smolt salmon with MC-producing cyanobacteria. Sampling over a 2-week period post-gavage collected tissues for histopathology and gene expression. In both species, lesions appeared at 6h but were resolved 2-weeks post-exposure. In liver and head kidney, upregulation of genes associated with stress and inflammatory responses occurred at 6h; these returned to control levels 2-weeks post-exposure in both species. Despite the changes observed in the liver, there was no evidence for NPLD due to the absence of hepatic megalocytosis and suggests the development of NPLD is not due to acute MC exposure. Instead NPLD may be associated with higher MC concentration occurring in food, chronic exposure through drinking of contaminated seawater, and/or as a result of other marine toxins detected.

ABSTRACT N° ICBF22-491

HOW THE PASSAGE THROUGH A BYPASS INSTALLED IN HYDROPOWER PLANT AFFECTS THE PHYSIOLOGICAL AND HEALTH STATUS OF ATLANTIC SALMON SMOLTS

julie Lucas* [1], Patrick kestemont[1]

[1]Université de Namur, Namur, Belgium

Abstract:
Atlantic salmon is anadromous species migrating from the upper-reach nursery in rivers to the oceanic feeding areas at smolt stage and inversely at adult stage requiring unimpeded migration routes. In many river systems such as in Meuse River (Belgium), Atlantic salmon are confronted to many hydroelectric power plants (HPP) which disrupt river connectivity and affect fish movement and survival. One possibility to reduce the impact of HPP is to install bypass to facilitate fish downstream migration. The aim of this study was to assess if the passage through Grands Malades bypass (Meuse River, Belgium) can affect survival, the physiological and immune status of Atlantic salmon smolts. Various key stress and immune biomarkers were studied at 24h, 72h and 120h after passage. We hypothetized that the passage through the bypass had a reduce impact on physiological and health status of fish

ABSTRACT N° ICBF22-460

PHYSIOLOGICAL INSIGHTS FOR CULTURE: SWIMMING CAPACITY AND EFFICIENCY, AND METABOLIC SCOPE FOR ACTIVITY IN COJINOBA SERIOLELLA VIOLACEA

Peter Allen* [1], Katherina Brokordt[2], Marcia Oliva[2], Katherine Alveal[2], Hector Flores[2], Claudio Alvarez[3]

[1]Mississippi State University, Mississippi State, United States,
[2]Universidad Catolica del Norte, Coquimbo,
[3]Centro de Estudios Avanzados en Zonas Aridas, La Serena, Chile

Abstract:
Aquaculture diversification relies on an understanding of the physiology of a species to optimize culture practices. The cojinoba (Seriolella violacea; Centrolophidae), a coastal pelagic marine fish, is a species of growing importance for aquaculture in South America. Although rearing practices have been established, little is known about the physiology of this species. Therefore, measures of metabolism, swimming capacity and efficiency, red:white muscle ratios, and aerobic and anaerobic enzyme activities (i.e., pyruvate kinase, citrate synthase, electron transport system), were measured. For 22 cm body length (BL) cojinoba at 15°C, critical swimming velocity (Ucrit) was approximately 80 cm/s (3.8 BL/s), and optimal swimming velocity (Uopt) was 50 cm/s (2.3 BL/s). Aerobic scope for metabolism was 300 mg O2/kg/hr, with Mo2 max 3.4x greater than standard metabolic rate. Swimming was facilitated by linear increases in tail beat frequency until near Ucrit. At low velocities, large (~20% BL) pectoral fins were used for propulsion and maneuverability. A greater proportion of red muscle in the caudal peduncle presumably corresponded to facilitation of sustained pelagic swimming. Plasma glucose and lactate, and white muscle anaerobic enzyme activity were elevated at Ucrit indicating mobilization of anaerobic energy sources at fatigue. Aerobic enzyme activities were higher in red muscle, decreasing at fatigue. The cojinoba has a moderate metabolic rate compared to other pelagic species, displays shoaling behavior, is maneuverable at low velocities due to pectoral fin use, but has optimal swimming efficiencies at higher velocities; factors beneficial for designing culture systems to promote exercise benefits for growth and welfare.

ABSTRACT N° ICBF22-306

SARCOLIPIN AND CALCIUM PUMP GENE EXPRESSION IN COLD-CHALLENGED MEDAKA FISH

Jens Franck* [1], Fatima Almoumen[1], Jayden Borley[2], Sean Robinson[1]

[1]University of Winnipeg, Winnipeg,
[2]University of Winnipeg, Winnpeg, Canada

Abstract:
Sarcolipin (SLN) is a small transmembrane protein that uncouples the calcium ATPase pump resulting in futile calcium cycling and endothermic heat, a form of non-shivering thermogenesis (NST). First characterized in mice, we have recently described sarcolipin's potential role as the molecular basis for heat generation in the endothermic Opah (Lampris incognitus). Sarcolipin transcript levels measured by quantitative PCR (qPCR) revealed up to three-fold higher expression in deep red pectoral muscle tissues of opah. In the current study we quantified by qPCR both sarcolipin (sln) and calcium ATPase (serca1) pump transcript levels in a cold-challenged ectothermic fish species, medaka (Oryzias latipes). Groups of fish were systematically cooled progressing from 24 degrees Celsius to 18, 12, and 8 degrees Celsius. Fish were maintained for five days at each temperature before performing RNA extractions and qPCR. The sln and serca1 transcript levels were assayed relative to the expression of the ef1alpha housekeeping gene. RNA was extracted from both white and red muscle tissues. Transcript levels for both sln and serca1 increased with decreasing temperature and showed the greatest increase in red muscle tissues. The freshwater medaka is known to tolerate large temperature extremes from 4 to 40 degrees Celsius. The ability to adapt to cold temperatures may be aided by inducing the futile calcium cycling NST mechanism as described in endothermic teleost species.

ABSTRACT N° ICBF22-360

CHARACTERIZATION OF THE LACTOTROPH POPULATION IN THE TELEOST PITUITARY USING THE JAPANESE MEDAKA AS A MODEL ORGANSIM

Muhammad Rahmad Royan[1], Khadeeja Siddique[1], Rasoul Nourizadeh-Lillabadi[1], Finn-Arne Weltzien[1], Christiaan Henkel[1], Romain Fontaine[* 1]

[1]Faculty of Veterinary medicine - Norwegian University of Life Sciences, Ås, Norway

Abstract:
In fish, lactotropes, which produce prolactin, are mainly located the most anterior part of the pituitary, the rostral pas distalis (RPD), and play an essential role in osmoregulation. However, in several species, smaller satellite lactotroph populations have been described in other parts of the pituitary. In addition, the recent single cell transcriptome of the Japanese medaka pituitary revealed the existence of two distinct cell types expressing the prolactin encoding gene. Therefore, in this study, we use Japanese medaka, which is a salinity tolerant fish, as a model organism to characterize the lactotroph populations, and to determine whether their cell composition and role differ. Transcriptomic analysis using single cell RNA sequencing data shows that one of the two lactotroph cell types is closely related to pituitary progenitor cells. Using fluorescent in situ hybridization, we confirm the existence of the two lactotroph cell types and demonstrate their location in both the satellite and the RPD populations. Therefore, the two lactotroph populations are heterogenous but have similar cell composition. We also show that both the RPD and satellite populations arise before puberty and grow throughout life, regardless of the sex of the animal. However, while the expression of prolactin is completely abolished in the RPD lactotrophs when the fish are raised in salt water, some prolactin expression remains in the satellite populations. This suggests that the RPD and satellite lactotroph populations have mainly the same role, osmoregulation, but are regulated differently.

ABSTRACT N° ICBF22-389

CONTRAST-ENHANCED ULTRASOUND IMAGING OF BOWEL INFLAMMATION IN RAINBOW TROUT

Marianna Horn* [1], Una Goncin[1], Ron Geyer[1], Ahmed El Kaffas[2], Helmut Segner[3], Markus Brinkmann[1], Steven Machtaler[1]

[1]University of Saskatchewan, Saskatoon, Canada,
[2]Stanford University, Stanford, United States,
[3]Universität Bern, Bern, Switzerland

Abstract:
Rainbow trout sensitivity to various toxins and food sources may result in bowel inflammation. In fish, this is typically quantified through histological analysis / scoring after euthanasia. Non-invasive tools to monitor bowel inflammation in fish are lacking and would be valuable for longitudinal studies, environmental monitoring, and to study species at risk. We propose that contrast-enhanced ultrasound imaging, a modality which is used clinically to quantitatively measure blood flow, can be applied to fish to perform minimally invasive quantification of inflammatory responses in the bowel through assessment of perfusion. Here, we established a model of bowel inflammation in rainbow trout using 2,4,6-trinitrobenzene sulfonic acid (TNBS), and verified it using histological analysis. Twenty-four hours after treatment, trout treated with TNBS developed classic signs of bowel inflammation, including epithelial damage, submucosal inflammation, and increased villi thickness. We then used contrast-enhanced ultrasound imaging to both visualize and quantify bowel inflammation induced in trout using TNBS. We constructed and intravenously administered microbubbles, a contrast agent used with ultrasound, and quantified vascular perfusion in the bowel using a preclinical, high-resolution ultrasound system. Analysis shows that trout bowels are acoustically visible, and that peak enhancement, a clinically important parameter used to measure bowel inflammation, is directly impacted by TNBS-induced inflammation. These data demonstrate that contrast-enhanced ultrasound imaging may be a valuable tool for non-invasive assessment of fish bowel inflammation.

ABSTRACT N° ICBF22-239

THE POTENTIAL OF IN VITRO MODELS IN FISH RESEARCH

Bianka Grunow[1], Philipp Lutze* [1, 2]

[1]Research Institute for Farm Animal Biology, Dummerstorf,
[2]Institute of Pathophysiology, University Medicine Greifswald, Greifswald, Germany

Abstract:
The fish is a popular model in animal research. They are second only to mice and rats, accounting for 14% of all animal experiments in the UK, and in Germany for 12%.
However, this number does not include fish used for research without prior testing (e.g. for organ or tissue removal or taxonomic determinations). Thus, the number of fish actually used in research is much higher.
Is it the case that all experiments need to be done directly on the organism? Why are fish cell cultures not routinely used for initial testing? In other disciplines, such as in human research, cell cultures have already become standard.
In this study, we will provide an overview of alternative in vitro models for fish research. Cell cultures can be used to explore many questions under controlled exogenous conditions. We will demonstrate the potential of in vitro models of different fish species (Acipenser oxyrinchus, Coregonus maraena, Oncorhynchus mykiss, etc.) to study for example, the effects of increasing temperatures and lower oxygen levels on cell physiological level. In addition, we will present the possibility to differentiate fish cells in vitro to obtain e.g. functional heart or nerve cells. In particular, in cardiac research, we could demonstrate the possibility to develop long-term spontaneously contracting aggregates for various applications, e.g. for questions on physiological changes due to climate change or also as a model system in fish virus research. To follow the efforts of the 3Rs (reduce, refine and replace of animal experiments) in fish research, we are sure that fish cells are the way forward.

ABSTRACT N° ICBF22-431

NON-LETHAL SAMPLING METHOD FOR THE ANALYSIS OF FATTY ACID PROFILES IN EUROPEAN SEA BASS

Mickaël Peron* [1], Jean-Baptiste Quemeneur[1], Victor Simon[1], Fabienne Le Grand[1], David Mazurais[1], Philippe Soudant[1], Marie Vagner[1]

[1]LEMAR, Plouzané, France

Abstract:
Sampling tissue on fish is usually carried out with the lethal collection of organic material (i.e. liver, brain, muscle…). Analysis of the fatty acid (FA) composition of fish tissue can provide information about nutritional quality, trophic behavior and is widespread in aquaculture research. Here we provide insight on an innovative non-lethal sampling method for FA analysis in European sea bass (Dicentrarchus labrax) juveniles. We performed six replicates of subcutaneous white muscle biopsy (between 1.5 and 15mg) on six individuals. White muscle was also collected on the same individuals following a classical lethal sampling method to be compared with biopsy sampling. FA of both reserve lipids (RL) and membrane lipids (ML) were analyzed. FA profiles of ML were similar in biopsy and the classical sampling, and showed repeatability among biopsy samples collected on the same individual. RL FA profiles showed high variability between biopsy samples taken on the same individual compared to ML. Lipid droplets located between white muscle and the fish skin could explain this difference. This sampling method could then prevent sacrifice of fish by using small quantities of tissue for FA analysis and be applied in various research areas. Special caution has to be taken during the sampling to avoid lipid droplets near the skin. Further investigation should be considered to assess the later effects of the biopsy on fish.

ABSTRACT N° ICBF22-435

PROLYL HYDROXYLASE DOMAINS (PHDS) IN THE ANOXIA-TOLERANT CRUCIAN CARP: INSIGHTS INTO REGULATION OF THE HYPOXIA-INDUCIBLE FACTOR (HIF) RESPONSE TO ANOXIA-REOXYGENATION

Lucie Gerber[* 1], Tellef Helle-Valle[1], Julien Resseguier[1], May-Kristin Torp[1], Helge-Andre Dahl[1], Göran E. Nilsson[1], Sjannie Lefevre[1]

[1]University of Oslo, Oslo, Norway

Abstract:
The hypoxia-inducible factor (HIF) is considered key in the transcriptional response to low oxygen availability. Yet, stabilization of HIF-α and thereby activation of HIF under anoxic conditions in the anoxia-tolerant crucian carp (Carassius carassius) remains unclear. Mounting a general HIF response would be counterproductive in a species such as crucian carp that experience only a short hypoxic window in the onset of anoxia (i.e. due to high O2 affinities). We therefore hypothesized that expression of prolyl hydroxylase domains, PHDs (the enzymes responsible for hydroxylation of HIF-α and targeting it for degradation) are upregulated to circumvent an energy-costly activation of HIF and in preparation for reoxygenation. The three isoforms PHD1, PHD2 and PHD3 are coded for by multiple paralogs of the genes egln2, egln1 and egln3, respectively. We quantified mRNA and protein expression using qPCR and western blotting, respectively, in brain of crucian carp exposed to 5 days normoxia, 5 days anoxia, and 5 days anoxia followed by 3 or 24 hours re-oxygenation. The mRNA expression of most egln paralogs were upregulated in anoxia; up to 20-fold for egln3 paralogs. There was also an increase in protein expression of PHD3. Both mRNA and proteins levels were increased after 24hrs of reoxygenation, suggesting a preconditioning for the reoxygenation period. Localization of PHDs in the brain, using immunohistochemistry, is under investigation to help characterize their function. Overall, our results confirm that upregulation of PHDs is part of the anoxia-reoxygenation response of crucian carp.

ABSTRACT N° ICBF22-497

VENTRICULAR PLASTICITY AFTER MYOCARDIAL INFARCTION IN RAINBOW TROUT, ONCORHYNCHUS MYKISS

Lucas Zena* [1], Andreas Ekström[1], Daniel Morgenroth[2], Albin Gräns[2], Catharina Olsson[1], Michael Axelsson[1], Henrik Sundh[1], Erik Sandblom[1]

[1]University of Gothenburg, Gothenburg,
[2]Swedish University of Agricultural Sciences, Skara, Sweden

Abstract:
Coronary arteriosclerosis appears to be an ongoing condition in many salmonid fishes, which may lead to e.g., myocardial ischemia. The potential of salmonids to recover, and the time course for such recovery from potential myocardial injury is essential for fish regularly exposed to stress, e.g., in iteroparous salmonids species and fish in aquaculture. Yet, the plasticity and capacity for myocardial restructuring and recovery following myocardial ischemia is poorly understood in salmonids. Here, we aimed to investigate the consequences of coronary occlusion on cardiac morphological characteristics and in vivo cardiac function in juvenile rainbow trout (Oncorhynchus mykiss) allowed to recover for 3 days and from at least 3 months. Acute coronary occlusion resulted in elevated resting heart rate along with reduced cholinergic tone on the heart relative to sham-operated fish, in which the coronary artery was not occluded. Moreover, we observed markedly abnormal patterns in the electrocardiogram (ECG), such as a reduction in the QRS wave amplitude and apparent fragmentation of the QRS complex. These are considered predictors of sudden cardiac death in human subjects with ischemic heart disease. Fish allowed to recover from coronary occlusion for at least 3 months exhibited normalized resting heart rate along with restored cholinergic tone relative to sham-operated trout. Taken together, we demonstrate that rainbow trout may cope with the aversive effects caused by coronary artery obstruction through plastic ventricular remodeling, which over time alleviates the adverse effects on cardiac function.

ABSTRACT N° ICBF22-330

MONITORING FISH WELFARE USING HEART RATE BIO-LOGGERS IN FARMED ATLANTIC SALMON (SALMO SALAR L.): AN INSIGHT INTO THE SURGICAL RECOVERY

Muhammad Naveed Yousaf* [1], Øyvind Røn[1], Patrycja plebaniak Hagen[1], Charles McGurk[1]

[1]Skretting Aquaculture Innovation, Stavanger, Norway

Abstract:
Farmed Atlantic salmon are one of the most studied cultured species, still continuous fish welfare monitoring is a challenge during farming operations. Although, mortality is the terminal indicator of fish welfare, but without further explanation, this parameter is of limited use. Fish welfare on-site can be monitored by using implantable bio-loggers that record heart rate, electrocardiography and temperature.

These heart rate loggers gave us the opportunity to monitor fish welfare under common farm handlings in free-swimming farmed Atlantic salmon (~1kg). Three different stress tests such as crowding (test 1), grading (test 2) & vaccination (test 3) were applied on 3 fish groups. Heart rate and biochemical parameters (blood plasma and mucous) were measured and compared in pre-stressed (T0), stressed (T1) and post-stressed (T48) fish. Two different post-surgical wound healing periods (1 vs 3 weeks), modified logger-anchoring techniques, surgical anesthesia dose and suture thickness were evaluated.

Following surgery, heart rate (fH) was elevated (70 beats min-1) which stabilized 3-10 days post-surgery. All three stress tests inflicted tachycardia suggesting aquaculture practices incur stress in farmed Atlantic salmon. Heart rate was raised at stress as compared to pre-stress levels that corresponds to 42-77 % elevation in all three tests. Cortisol levels were upregulated following tests but only remained significant in test 3. Upon handling, fish should be given enough time (48 h) to stabilize fH levels before commencing to other tests/handlings. In conclusion, heart rate can be used for continuous fish welfare monitoring under farming conditions.

ABSTRACT N° ICBF22-305

ENERGY BALANCE IN AN ELASMOBRANCH FISH POST-AIR EXPOSURE.

Alexandra Schoen[1], Alyssa Weinrauch[1], W. Gary Anderson* [1]

[1]University of Manitoba, Winnipeg, Canada

Abstract:
Energy production and usage is altered during periods of increased energy demand. One such situation is activation of the stress axis, which demands mobilization of energy to reestablish internal equilibrium. The dominant energy metabolite during the stress response in vertebrates is glucose, although there is recent evidence that suggests elasmobranchs may utilize ketone bodies as well, particularly beta-hydroxybuterate (BHB). In the present study, we describe energy regulation following a stress event in an elasmobranch, the Spiny dogfish (Squalus suckleyi). Fish were air exposed intermittently for 10 minutes and then sacrificed after 30 minutes, 1-hour, or 7 days. Non-air exposed control fish were sacrificed after 0 minutes or 7 days. Plasma samples were collected for corticosteroid (1alpha-hydroxycorticosterone, corticosterone, and cortisol) and energy metabolite (glucose and BHB) analysis, and tissue samples (liver, heart, white muscle, brain, and rectal gland) were collected and analysed for metabolic enzyme activity. There was an increase in plasma BHB and associated enzyme activity 7 days following air exposure as compared to controls. Interestingly, there was a decrease in BHB dehydrogenase in liver tissue 30 minutes post-air exposure, which is the primary site of BHB production. There was a decrease in plasma glucose 7 days following air exposure, but an increase in hexokinase activity in heart and rectal gland. There was no change in plasma glucose and associated enzyme activity 30 minutes and 1-hour post-air exposure. Results will be discussed in the context of energy mobilization following a stressor in the spiny dogfish.

ABSTRACT N° ICBF22-504

USE OF ECHOCARDIOGRAPHY FOR NON-INVASIVE ASSESSMENT OF CARDIAC MORPHOLOGY AND FUNCTION IN ATLANTIC SALMON (SALMO SALAR L.)

Victoria Becker* [1], Simona Kavaliauskiene[1], Ida B. Johansen[2], Ivar Sjaastad[1], William E. Louch[1], Michael Frisk[1]

[1]Institute for Experimental Medical Research, University of Oslo and Oslo University Hospital Ullevål, Oslo,
[2]Department of Preclinical Sciences and Pathology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway

Abstract:
Non-lethal tools for examination of cardiac morphology and function in fish are scarce and current methodologies are for the majority invasive, time-consuming, and stationary. Echocardiography has previously been examined as a non-invasive, quick and transportable alternative tool, but the presence of spongious myocardium in most species has hindered its usability. However, technical improvements during the last decade have enabled more refined functional assessment and hold promising potential for application in fish.

Thus, we examined application of echocardiography in 22 anaesthetized Atlantic salmon (Salmo salar L.) ranging from 345-2800 grams and 31-62 cm at 10. To facilitate versatility and transportability, a compact system (GE Vivid iq) was employed using two different probes: a linear (12L, 5-13 MHz) and Phased Array dedicated cardiac probe (12S, 4-12 MHz). Several protocols and projections were tested and intra- and inter-variation were assessed for evaluation of accuracy. In addition, cardiac dimensions were verified by comparison with excised hearts.

We observed that measurements of cardiac dimensions (ventricle, atrium and bulbus) were equally robust using both probes, where low variability and high accuracy were evident. However, the cardiac probe was favourable for capturing detailed functional parameters, such as ejection fraction, fractional shortening, strain, and strain rates. Like for morphological measures, functional assessment was precise and robust regarding both diastolic and systolic function.

In conclusion, we found that echocardiography is a powerful, non-invasive tool that produces reliable and reproducible results. Importantly, the transportability and versatility of the system tested enables cardiac assessment in otherwise inaccessible locations.

ABSTRACT N° ICBF22-349

L-PLASTIN LEVELS ARE ASSOCIATED WITH MORTALITY DURING CARDIOMYOPATHY SYNDROME IN FARMED ATLANTIC SALMON (SALMO SALAR L.)

Simona Kavaliauskiene*[1, 2], Victoria Becker[1, 2], Baojian Sun[3], Alf S. Dalum[4], Marco A. Vindas[3], Hege Lund[3], Ida B. Johansen[3], Michael Frisk[1, 2]

[1]Institute for Experimental Medical Research, University of Oslo and Oslo University Hospital Ulleval,
[2]K.G. JebsenCenter for Cardiac Research, University of Oslo, Oslo,
[3]Department of Preclinical Sciences and Pathology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences,
[4]Nofima, Norwegian Institute of Food Fisheries and Aquaculture Research, Ås, Norway

Abstract:
Cardiomyopathy syndrome (CMS) is a severe infectious cardiac disease affecting Atlantic salmon (Salmo salar L.). The disease causes sudden death and poses serious economical and fish welfare challenges in salmon production. Currently, there are no treatments or effective preventive tools available, and prognostic measures are lacking. CMS prevalence and costs associated with the disease are increasing and may become unmanageable within a few years. Thus, there is an urgent need for prognostic tools and treatment alternatives. To achieve this, an in-depth understanding of the biological mechanisms leading to CMS is first required. Proteomic-based blood plasma analysis of inflammatory and immune response proteins recently identified L-plastin as an acute response protein in salmon. Consequently, we investigated whether circulating L-plastin levels are associated with Piscine Myocarditis virus (PMCV) infection, the prime trigger of CMS. By comparing heart and blood plasma samples from surviving and deceased fish from a confirmed CMS outbreak site, we observed that L-plastin expression was elevated in cardiac tissue while circulating free L-plastin levels were reduced in deceased fish. Interestingly, a higher molecular weight band positive for L-plastin was detected in plasma from deceased fish and correlated with L-plastin expression in the heart. In addition, cardiac L-plastin levels correlated with cardiac histopathological changes, mononuclear cell infiltration and necrosis of cardiomyocytes in the heart. Our findings set the base for further investigation of L-plastin as a potential biomarker for PMCV infection and for understanding the course of CMS in farmed salmon.

ABSTRACT N° ICBF22-236

OSMOREGULATORY COMPROMISE IN LARVAL O. BETA WHEN FACING HYPERSALINE AND HYPERTHERMAL CONDITIONS

LeeAnn Frank* [1], Joesph Serafy[1, 2], Martin Grosell[1]

[1]Rosentiel School of Marine and Atmospheric Science, University of Miami,
[2]NOAA Southeast Fisheries Science Center, Miami, United States

Abstract:
Oxygen demands in hypoxia were examined in larval Opsanus beta under both thermal and salinity stress, conditions they frequently face in their natural habitat. Metabolic rates were measured at 27 and 33°C in seawater and 42 parts per thousand using intermittent resting respirometry. Closed respirometers allowed depletion of oxygen to 13.7 ± 2.9% O2 and were then flushed to measure recovery. Standard metabolic rate increased with temperature, but not salinity, but maximum metabolic rate and aerobic scope were not depressed in high temperatures or salinities. O. beta showed poor regulation of metabolic rates as oxygen was depleted with a regulation index of <0.25 across all treatments. However, the regulation index had a trend of increasing with both increasing salinity and temperature, while apparent oxygen affinity increased with salinity. Hypoxia exposure led to excessive post-hypoxia oxygen consumption in all treatments and increased in both hyperthermal and hypersaline conditions. Routine metabolic rate recovered 3.3- 5.8 h after return to normoxia with a significantly longer recovery time in hypersalinity. Combined salinity and thermal stress did not depress the aerobic scope in O. beta, but hypersalinity lessens the ability to extract oxygen at lower tensions, resulting in a larger oxygen debt, and a longer time to recover.

ABSTRACT N° ICBF22-190

EVALUATING THE POTENTIAL OF FISH SCALE HORMONE CONCENTRATIONS IN THE ASSESSMENT OF LONG-TERM STRESS IN TELEOST FISHES

Emily Kennedy* [1], David Janz[2]

[1]University of Saskatchewan Toxicology Graduate Program,
[2]University of Saskatchewan Western College of Veterinary Medicine and Toxicology Centre, Saskatoon, Canada

Abstract:
The aquatic environment is replete with anthropogenic stressors capable of negatively impacting fish populations. Recent efforts have provided convincing evidence for the use of fish scale cortisol (F) concentration in the assessment of long-term stress in fishes. However, F alone is not sufficient to fully describe this state of long-term stress. Dehydroepiandrosterone (DHEA) is an androgen and precursor steroid with actions that oppose those of F in mammals. Additionally, in some vertebrates high ratios of F to DHEA are considered indicative of chronic stress. Although not fully understood, the means by which DHEA negates the effects of F is thought to occur in part via changes in the metabolism of F to cortisone (E). The quantitation of F as well as DHEA and E could therefore provide a more complete picture of the overall state of stress. As DHEA and E have yet to be quantified within the fish scale our first objective was to ensure our sample processing protocol for the extraction and quantitation of F was applicable to these additional hormones. Following this, we induced a state of long-term stress in goldfish (Carassius auratus) and rainbow trout (Oncorhynchus mykiss) to determine changes in scale and circulating F, E and DHEA concentrations. Scale concentrations of F, E and DHEA were elevated in stressed fish compared to unstressed controls, however these elevations were not reflected in all serum samples. While there are many knowledge gaps left to be filled, this study supports the use of fish scale hormone concentrations in the assessment of long-term stress in teleost fishes.

ABSTRACT N° ICBF22-211

USING 15N TO DETERMINE THE METABOLIC FATE OF DIETARY NITROGEN IN SPINY DOGFISH

J. Lisa Hoogenboom* [1, 2], W. Gary Anderson[1, 2]

[1]University of Manitoba, Winnipeg,
[2]Bamfield Marine Sciences Centre, Bamfield, Canada

Abstract:
Nitrogen is a biologically important molecule essential for life, with acquisition necessary for somatic processes. Most aquatic organisms eliminate excess “waste” nitrogen, usually as ammonia, rather than retaining it. For ureosmotic marine elasmobranchs (sharks, skates, rays), nitrogen is essential for both somatic purposes and for synthesizing large concentrations of urea (> 300 mM) to balance their internal osmotic pressure with the external environment. This is the first study to examine how these animals incorporate dietary nitrogen into different compartments for osmoregulatory and somatic purposes. We fed North Pacific spiny dogfish (Squalus acanthias suckleyi) 7 mM 15NH4Cl in a 2% ration-by-body-weight of fish-slurry via gavage, and allowed them to digest for 20, 48, 72, and 168 h. The uptake and incorporation of 15N as ammonia, urea, glutamine, bulk amino acids, and protein was examined in the anterior, mid, and posterior intestinal spiral valve, plasma, liver, and skeletal muscle. Within 20 h post-feeding, 15N was taken-up from the gastrointestinal tract and found in all the nitrogenous compounds and tissues examined. The anterior spiral valve had the highest enriched 15N values by 20 h post-feeding, suggesting that it was a major region of 15N uptake. The presence of both 15N-glutamine and 15N-urea in all three intestinal regions indicates the likely synthesis of urea within these tissues. Throughout the 168 h digestion period, dogfish maintained enriched 15N values in all the nitrogenous compounds and tissues examined, highlighting their ability to incorporate and retain dietary nitrogen for both osmoregulatory and somatic processes.

ABSTRACT N° ICBF22-538

SEROTONIN DYNAMICS IN MAJOR BLOOD VESSELS AND BLOOD CELLS OF THE GULF TOADFISH, OPSANUS BETA

Mary Shay*[1], John Sebastiani[1], M. Danielle McDonald[1]

[1]University of Miami, Miami, United States

Abstract:
Serotonin (5-HT) is an ancient signaling molecule in the animal kingdom that is involved in controlling a variety of physiological processes including vascular resistance. The transport of 5-HT into cells can be attributed to the serotonin transporter (SERT), which is expressed in many tissues, including the heart and the gill. The inhibition of SERT-mediated 5-HT uptake leads to higher concentrations of extracellular 5-HT that can cause resting vascular tone to deviate, resulting in changes in blood pressure and blood flow. Therefore, circulating 5-HT in the blood needs to be tightly controlled. The objective of this study was to investigate whether SERT in major blood vessels and blood cells plays a role in controlling circulating 5-HT in fish by analyzing SERT mRNA expression, SERT-mediated [3H] 5-HT uptake, and uptake of [3H] counts in blood cells. We hypothesized there will be significant SERT mRNA expression and uptake within major blood vessels and that [3H] counts would be significant in the cellular compartment of the blood. Our results indicate that SERT mRNA expression was detectable but variable across major blood vessels and that there was significant 5-HT uptake by all major blood vessels with the highest uptake measured in the enteric portal vessels and hepatic vein. [3H] counts were significantly higher in the plasma compartment of the blood compared to the cellular compartment. These findings suggests that some blood vessels may have a role in controlling circulating 5-HT, which could have implications with respect to vascular resistance, blood pressure and flow.

ABSTRACT N° ICBF22-478

IMPLANTED BIOIMPEDANCE SPECTROCOPY FOR IN-VIVO PHYSIOLOGICAL MONITORING : INFLUENCE OF FISH ACTIVITY ON MEASUREMENT ACCURACY

Vincent Kerzerho* [1], Serge Bernard[1], Sylvain Bonhommeau[2], Tristan Rouyer[3], Stéphane Lallement[4], Alain Vergnet[4], David McKenzie[5]

[1]University Montpellier / CNRS/ LIRMM, Montpellier,
[2]IFREMER /DOI, Le Port,
[3]IFREMER/MARBEC, Sète,
[4]IFREMER/MARBEC, Palavas-les-Flots,
[5]University Montpellier/CNRS/MARBEC, Sète, France

Abstract:
Bioimpedance is an electrical measurement technique developed for medical application. Thanks to an electrical current flowing through the biological tissue under measurement, it provides an electrical parameter which is related to the tissue composition. For almost 15 years, it has been adapted to the fish biology analysis. Regular measurement set-up imposes the individual under measurement to be inert to limit disturbances and to guaranty measurement repeatability. We wish to break this rule to study the application of bioimpedance to the fish in vivo physiological monitoring. To reach this goal, we have set-up an experiment consisting in measuring bioimpedance spectroscopy in ten European sea bass (Dicentrarchus labrax). At first a four-contact electrode with a one-meter long cable for remote measurement has been implanted in the anaesthetized fish. After the surgery, the awakening phase was done in a swimming tunnel. After a 24-hour recovering phase in the swimming tunnel at 1bl/s speed, we have repeated a set of bioimpedance measurements at five swimming speeds (1, 1.25,1.5, 1.75, 2 bl/s). Thanks to this experiment, we have demonstrated that it is possible to measure bioimpedance spectroscopy in a moving animal. In addition, we have enlightened a potential new application of bioimpedance measurement which is the fish tail beat frequency measurement.

ABSTRACT N° ICBF22-474

TRANSCRIPTIONAL ACTIVATION OF THE INTESTINAL IMMUNE RESPONSE OF THE SUB-ANTARCTIC NOTOTHENIOID FISH ELEGINOPS MACLOVINUS CHALLENGED WITH FRANCISELLA NOATUNENSIS

Daniela Díaz Ibarrola* [1], Danixa Martinez[1], Carolina Vargas Lagos[1], Julia Saravia[1], Luis Vargas Chacoff[1]

[1]Universidad Austral de Chile, Valdivia, Chile

Abstract:
The lymphoid tissue associated with the intestinal mucosa has been the focus of research in procedures that seek to improve the immune response through the administration of functional foods, however, to date it is unknown whether the different portions of the intestine can activate the immune response differentially upon exposure to a bacterial pathogen. The objective of this study was to evaluate the host-pathogen interaction, through the transcription (qPCR) of markers involved in the innate and adaptive immune response in the intestine (Foregut, Midgut and Hindgut) of Eleginops maclovinus challenged intraperitoneally with Francisella noatunensis subsp. . noatunensis. The experimental treatments were: control only with bacterial culture medium, low dose, medium dose and high. The fish were sampled at days 1, 3, 7, 14, 21 and 28 post-injection. The expression profiles of TLR1, TLR5, TLR8, MHCI, MHCII and IgM were tissue-specific, dependent on the bacterial dose applied and the experimental time analyzed. Specifically, more transcription down-regulation could be observed in foregut, whereas in midgut and hindgut the transcription of these six immune markers was generally up-regulated. Francisella genetic material (DNA) was detected in all doses injected. The results suggest that participation in the immune response cannot be attributed to a specific portion of the intestine, at least at the transcriptional level, but rather that the cellular morphology of each segment (foregut, midgut and hindgut) could favor the entry of this pathogen in one portion more than another and that transcription of immune components is modulated by Francisella in all three portions of the E. maclovinus gut. This work was funded by VIDCA-UACH, Fondap-Ideal Nº15150003 and Fondecyt Regular Nº1160877

ABSTRACT N° ICBF22-313

REVISITING THE MODEL OF FISH OSMOREGULATION: IDENTIFICATION OF GILL IONOCYTES BY SINGLE-CELL RNA SEQUENCING

Jia-Jiun Yang[1], Ya-Ting Chuang[1], Ying-Jey Guh[1], Yung-Che Tseng[1], Pung-Pung Hwang[*1]

[1]Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan

Abstract:
The functions of gill ionocytes in seawater (SW) and freshwater (FW) fish have been studied for decades from morphological, physiological, and biochemical aspects to understand the molecular and cellular mechanisms of ion transport in different types of ionocytes. Given the recent progress in transcriptomics, we performed single-cell RNA sequencing (scRNA-seq) to compare cell types and their expression profiles in the gills between SW- and FW-acclimated Indian medaka (Oryzias melastigma). The integration analysis shows that the gills have more than 20 different cell types, including two types of ionocytes, NKA- (NKA-IC) and ECaC-ionocyte (ECaC-IC). ECaC-IC exists in both FW and SW gills. The function of ECaC-IC in FW gills may be associated with Ca2+ uptake, but that in SW gills is unknown. On the other hand, there are at least two sub-types of NKA-IC, Na+-Cl-cotransporter (NCC)-IC and Na+/H+ exchanger (NHE)-IC, in FW, but only one type of NKA-IC (Na+-K+-2Cl- cotransporter-IC) in SW. We identified novel conserved ionocyte markers expressed in both SW and FW and different ionocyte markers dominantly expressed in either SW or FW only. Further identification and functional analysis are ongoing

ABSTRACT N° ICBF22-191

MOLECULAR TARGETS OF PROLACTIN SIGNALING IN EURYHALINE MUMMICHOGS (FUNDULUS HETEROCLITUS)

Jason Breves* [1], Katie Puterbaugh[1], Serena Bradley[1], Annie Hageman[1], Adrian Verspyck[1], Lydia Shaw[1], Elizabeth Danielson[1], Yubo Hou[1]

[1]Skidmore College, Saratoga Springs, United States

Abstract:
Pioneering studies over 60 years ago employed mummichogs (Fundulus heteroclitus) to identify the fundamental activities of pituitary hormones in fishes, yet the cellular and molecular targets of prolactin (Prl) in this model teleost have remained unknown. Here, we conducted a phylogenetic analysis of two mummichog Prl receptor (prlr) genes, designated prlra and prlrb, prior to describing their organ- and salinity-dependent patterns of expression. We then administered ovine Prl (oPrl) to mummichogs held in brackish water and characterized the expression of genes associated with freshwater (FW)- and seawater (SW)-type ionocytes. Within FW-type ionocytes, oPrl stimulated the expression of Na+/Cl- cotransporter 2 (ncc2) and aquaporin 3 (aqp3). Alternatively, branchial Na+/H+ exchanger 2 and -3 expression did not respond to oPrl. Gene transcripts associated with SW-type ionocytes, including Na+/K+/2Cl- cotransporter 1, cystic fibrosis transmembrane regulator 1, and claudin 10f were reduced by oPrl. Isolated gill filaments incubated with oPrl in vitro exhibited elevated ncc2 and prlra expression. Given the role of Aqps in supporting gastrointestinal fluid absorption, we assessed whether several intestinal aqp transcripts were responsive to oPrl and found that aqp1a and -8 levels were reduced by oPrl. Our collective data indicate that Prl promotes FW-acclimation in mummichogs by orchestrating the expression of solute transporters/channels, water channels, and tight-junction proteins across multiple osmoregulatory organs. Supported by NSF [IOS-1755131].

ABSTRACT N° ICBF22-549

HOW TO GIVE A FRESHWATER FISH THE GUT PHENOTYPE OF A MARINE FISH

Alex Berry* [1], Harriet Goodrich[2], Rod Wilson[1]

[1]Biosciences Department, College of Life and Environmental Sciences, University of Exeter, Exeter, United Kingdom,
[2]Institute for Marine and Antarctic studies (IMAS), College of Sciences and Engineering, University of Tasmania, Launceston, Australia

Abstract:
Marine teleosts drink seawater to prevent dehydration. They therefore use intestinal ion transport mechanisms to support water uptake, despite the ingested fluid having a much higher osmolality than their blood. One component involves high rates of intestinal bicarbonate secretion, leading to elevated intestinal fluid pH (8.3-9.2) and [HCO3-] (30-100 mM). This alkaline environment precipitates calcium- and magnesium-rich carbonate minerals which reduce gut fluid osmolality, facilitating water absorption. This also reduces the need to excrete excess calcium via either the gills or kidney, which incur energetic costs and risk nephrocalcinosis (kidney stones).
By contrast, freshwater teleosts mostly avoid drinking and have neutral pH and low bicarbonate intestinal fluid. However, ingested diets can have a high calcium content, especially in predators that consume whole skeletons of fish (calcium phosphate) and shelled invertebrates (calcium carbonate), which can exceed their calcium requirements for growth. Dietary calcium minerals are dissolved in the acidic stomach, requiring additional gastric acid (with energetic implications), providing an abundance of free calcium ions in the chyme entering the intestine.
We investigated the physiological effects of pelleted diets supplemented with either calcium carbonate, calcium phosphate or calcium chloride in freshwater rainbow trout. We measured acid-base and ionic chemistry in the blood and gut fluids, net acid-base and ion fluxes to/from the external water, and faecal precipitate composition following feeding. All three diets resulted in intestinal fluid with high pH and bicarbonate concentration and substantial calcium mineral precipitates were excreted, challenging the idea that this phenomenon is exclusive to marine teleosts.

ABSTRACT N° ICBF22-455

RESPONSES TO HYPERSALINITY IN TWO EUROPEAN SEA BASS GENETIC LINEAGES

Catherine Lorin-Nebel* [1], Quanquan Cao[1], Eva Blondeau-Bidet[2]

[1]Montpellier University,
[2]CNRS, Montpellier, France

Abstract:
European sea bass (Dicentrarchus labrax) from the Atlantic Ocean and the Mediterranean Sea are two distinct genetic lineages. In their life cycle, D. labrax enter lagoons and estuaries where salinity fluctuates and sometimes reaches levels over 60 ‰, notably in Mediterranean lagoons. The Mediterranean Sea is expected to become warmer and saltier in the nearby future which could have an impact on fish populations. We investigated whether sea bass from Atlantic and West Mediterranean origin have distinct plastic responses when facing hypersalinity. We focused on the responses at intestinal level to address solute-coupled water uptake which is essential in fish facing high salinities in order to avoid dehydration. We also addressed vasotocinergic and isotocinergic systems that might be involved in triggering stress-related responses following salinity transfer. Fish were analyzed following a two-week transfer from seawater (SW, 36 ‰) to either seawater (SW, 36 ‰) or hypersaline water (HW, 55 ‰). Solute-coupled water uptake involves a set of ion transporters and aquaporins whose expression patterns inform about their role in the response to hypersalinity. Besides Na+/K+-ATPase and Na+/K+-2Cl cotransporter 2, several aquaporin paralogs (mainly AQP8ab and 8aa) were overexpressed in HW compared to SW. Among the multitude of arginine vasotocin receptors in D. labrax, v1a2-type receptor is the most expressed paralog and it is highly upregulated in HW compared to SW. Comparing sea bass lineages, gene expression patterns differ much more in seawater than in hypersaline conditions suggesting different water-conserving strategies in SW.

ABSTRACT N° ICBF22-444

INCREASING SURVIVABILITY OF JAPANESE MEDAKA DURING SEAWATER RE-ACCLIMATION IS COINCIDENT TO RETAINED EXPRESSION OF ION CHANNELS

Bo-Kai Liao* [1], Yun-Wei Lai[2], Sian-Tai Liu[3], Ming-Yi Chou[3]

[1]National Taiwan Ocean University, Keelung,
[2]Academia Sinica,
[3]National Taiwan University, Taipei, Taiwan

Abstract:
Euryhaline teleosts exhibit various acclimabilities to survive in environments alternating from hypotonic to hypertonic. This ability is well-known conferred by ion channels expressed on ionocytes, the iono-regulating cells in the gills or skin, but it is physiologically challenging for the switching between hypotonic and hypertonic since most of ion channels are unidirectional. A coordination between acute responses, e.g. gene expressions, and long-term responses, e.g. cell differentiation, is believed to crucially exert the adaptability. Moreover, the pre-acclimation to half seawater salinity was found to improve the survivability of Japanese medaka (Oryzias latipes) in direct transfer to seawater. Thus, it is conceptually intriguing as the ionocytes preserving hypertonic acclimability while performing hypotonic functions at the same time. Whether the acclimabilities are always entrainable in closed species and what is the potential mechanism in term of ion channel expressions are not well understood. In the present study, Japanese medaka pre-acclimated in brackish water showed higher survival rates and retained higher expression of three ion channels, ATP1a1a.1, ATP1b1b, and SLC12a2a. These gene retention can be maintained up to two weeks after transferring back to freshwater. Surprisingly, this entrained acclimability was not found in its close kin, Indian medaka (Oryzias dancena), which has natural habitats in brackish water. Japanese medaka surpass Indian medaka in seawater acclimability with experienced exposure to brackish water and this ability is coincident to the expression of the seawater-retention genes.

ABSTRACT N° ICBF22-129

EARLY REGULATION OF CORTICOSTEROID RECEPTORS AND OSMOREGULATION-RELATED GENES IN RAINBOW TROUT GILLS MEDIATED BY MEMBRANE-INITIATED CORTISOL SIGNALING

Jorge Aedo[*, 1, 2], Daniela Aravena-Canales[1, 2], Rodrigo Zuloaga[1, 2], Juan Valdes[1, 2], Alfredo Molina[1, 2]

[1]Universidad Andres Bello, Santiago,
[2]Interdisciplinary Center for Aquaculture Research (INCAR), Concepcion, Chile

Abstract:
Cortisol, acting as mineralocorticoid, is the main hormone involved in the regulation of osmoregulatory processes. The current paradigm of cortisol action is attributed to genomic/classic mechanisms which involve the interaction of the hormone with its intracellular glucocorticoid (GR) or mineralocorticoid (MR) receptor and subsequent modulation of target genes. However, cortisol also can interact with membrane components, activating rapid signaling pathways with unknown contribution in osmoregulatory responses. This novel cortisol mechanism is poorly understood and unlike other cell types, the presence of GR and/or MR in gills surface has not been reported.
Here, we evaluated the effects of cortisol and cortisol-BSA (exclusive inductor to membrane-initiated effects) on the early expression of corticosteroid receptors (gr1, gr2 and mr) and osmoregulation-related genes using rainbow trout gills (in vivo model) and RT-gills-W1 cells (in vitro model). After one hour of treatment, fish intraperitoneally administered with cortisol or cortisol-BSA showed a differential expression of key ion and water transporters (e.g., Na+/K+-ATPase, Na+/K+/2Cl cotransporter 1, and aquaporins) compared with sham group. In addition, cortisol or cortisol-BSA increased mRNA levels of gr2, but not gr1 and mr, in gills. This last result was confirmed using RT-gills-W1 cells stimulated with both cortisol analog. Finally, by using immunofluorescence, we observed for the first time the presence of GR and MR in the surface of RT-gills-W1 cells that could be involved in rapid responses to cortisol. Overall, our results suggest that rapid membrane-initiated cortisol actions could be involved in the early regulation of osmoregulatory responses in fish.
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ABSTRACT N° ICBF22-413

COMPARATIVE PHYSIOLOGY OF WILD-CAUGHT BROOK STICKLEBACK (CULAEA INCONSTANS) RESIDING IN ALKALINE AND NEUTRAL WATERS

Alex Zimmer* [1], Marina Giacomin[1], Chris Glover[2], Greg Goss[1]

[1]University of Alberta, Edmonton,
[2]Athabasca University, Athabasca, Canada

Abstract:
Many arid regions on Earth are home to endorheic lakes of elevated salinity and alkalinity. Despite the potentially challenging conditions of these environments, many of these lakes host abundant fish populations. Buffalo Lake, located in Alberta, Canada, is a sodium bicarbonate lake ([HCO3] = 20 mmol/L; pH = 9.3) that is home to many species including brook stickleback (Culaea inconstans). Exposure to elevated alkalinity in most fishes results in reduced ammonia excretion rates resulting from physiochemical changes in the apical gill microenvironment. We hypothesized that stickleback collected from Buffalo Lake possess a greater capacity for maintaining ammonia excretion rates in alkaline conditions compared to a population from a neutral reference site, Buck Lake (pH = 7.3). Fish collected from both lakes were acclimated to common facility conditions (pH = 8.2) for >2 months and then exposed to alkaline conditions mimicking those of Buffalo Lake ([HCO3] = 20 mmol/L; pH = 9.5) for 7 d. Stickleback from Buffalo Lake (alkaline site) experienced no mortalities over 7 d of exposure. In Buck Lake (neutral site) stickleback, survival in alkaline conditions was only 20% by 7 d, with most mortalities occurring between 4 and 7 d of exposure. Ammonia excretion was initially inhibited by alkaline conditions, however, contrary to our hypothesis, excretion rates were recovered by 4 d of exposure in both populations. Therefore, mortality of Buck Lake stickleback in alkaline conditions is not a function of reduced ammonia excretion capacity and ongoing experiments aim to uncover the lethal mode of action.

ABSTRACT N° ICBF22-279

EXTRA BRANCHIAL AMMONIA EXCRETORY ORGAN IN FISH

Salman Malakpourkolbadinezhad* [1, 2], Jonthan Wilson[3, 4]

[1]Coldwater Fisheries Research Center (CFRC), Iranian Fisheries Sciences Research Institute (IFSRI), Agricultural Research, Education and Extension Organization, , Tonekabon, Iran,
[2]2Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, , Porto, Portugal,
[3]Department of Biology, Wilfrid Laurier University, , Waterloo, Canada, Canada,
[4]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, , Porto, Portugal

Abstract:
The unique marine catfish, Plotosus lineatus belongs to the family Plotosidae possessing dendritic organ (DO), a specialized extra-branchial salt secreting anatomical structure. Additionally, this species has the ability to produce unusual hyperosmotic urine relative to plasma while typical marine teleosts are incapable of producing hyperosmotic urine and use the gill as their primary salt secreting organ.
More recently, it has been demonstrated that the DO parenchymal cells express high levels of Na^+/K^+-ATPase (NKA) and secretory $Na^+:K^+:2Cl^–$ cotransporter (NKCC1) and have an apical chloride channel CFTR Cl^- channel consistent with the secondary activity chloride secretion mechanism seen in other salt secreting epithelia in vertebrates including the gills of typical marine teleost fishes. The DO was central to hypoosmoregulation in the marine catfish and gill, kidney and intestine had a limited compensatory role. Thus, the DO represents a unique adaptation to hypo-osmoregulation in the Plotosidae catfishes which can be linked to their independent invasion of the marine environment by a freshwater siluriform ancestor. Furthermore, recently we demonstrated that DO of P. lineatus may have an extra physiological role in ammonia excretion although the gills are accepted as the main site of ammonia excretion in fishes.

05-Ion and acid-base regulation in fishes

ABSTRACT N° ICBF22-392

CONTRIBUTIONS OF THE GUT MICROBIOME TO NITROGEN HANDLING IN AN ELASMOBRANCH, THE PACIFIC SPINY DOGFISH (SQUALUS SUCKLEYI)

Jess MacPherson* [1, 2], Alyssa Weinrauch[1, 2], W. Gary Anderson[1, 2], Carol Bucking[2, 3]

[1]University of Manitoba, Winnipeg,
[2]Bamfield Marine Sciences Centre, Bamfield,
[3]York University, Toronto, Canada

Abstract:
Nitrogen recycling through the gut microbiome is an important mechanism used by animals to reclaim nitrogen trapped in urea, a metabolic end product, as useable ammonia. This process is known to be critical in animals with nitrogen-limited diets but whether it occurs in elasmobranchs, which are reported to be severely nitrogen-limited, is unknown. Nitrogen recycling may be especially beneficial to elasmobranchs as they rely on nitrogenous compounds not only for somatic growth and maintenance but also for energy metabolism and urea-based osmoregulation. With this in mind, we hypothesized that nitrogen recycling through the gut microbiome helps maintain overall nitrogen homeostasis in elasmobranchs and that a disruption to the gut microbial community would have implications on whole-body nitrogen stores. Antibiotics were used to deplete the gut microbiome of both fed and fasted Pacific spiny dogfish and changes in nitrogen handling were examined. In the fed animals, plasma urea, urea excretion, and ammonia excretion significantly decreased in the antibiotic-treated (ABX) individuals while plasma ammonia showed no change. Interestingly, the fasted individuals showed no significant differences in any of the measured parameters. ABX fed individuals also lost 4x more body mass than the fed controls over the course of the experiment, which was not observed in the fasted animals. These results suggest that compromising the gut microbiome significantly influences post-prandial nitrogen handling in spiny dogfish and that the recycling of urea-nitrogen may be vital to maintaining nitrogen balance in these fish.

ABSTRACT N° ICBF22-158

LOCALIZATION AND EXPRESSION OF AQUAPORIN WATER CHANNELS IN THE TISSUES OF THE SPINY DOGFISH (SQUALUS ACANTHIAS)

Christopher Cutler* [1], Tolulope Ojo[1]

[1]Georgia Southern University, Statesboro, United States

Abstract:
LOCALIZATION AND EXPRESSION OF AQUAPORIN WATER CHANNELS IN THE TISSUES OF THE SPINY DOGFISH (SQUALUS ACANTHIAS)

Christopher P Cutler and Tolulope Ojo, Georgia Southern University.

A study of all aquaporins in animals showed that elasmobranchs have a duplicate copy of aquaporin 3 water, glycerol and/or urea channel (AQP3; here called AQP3-2)1. The sequence of dogfish AQP3-2 and that of a splice variant were subsequently determined by a transcriptomics study2. The level of mRNA expression of AQP3-2 and its splice variant were measured using absolute QPCR in renal samples from dogfish acclimated to 120%, 100% and 75% seawater.

An antibody was made to the C-terminal portion of the AQP3-2 amino acid sequence. Initial immunohistochemistry experiments yielded no results but this was deemed to be due to relatively low levels of expression of AQP3-2. A tyramide kit was employed for (up to) 200x signal amplification and this enabled the localization of aquaporin 3-2 to various segments of the dogfish renal tubule.

Further results for AQP's 0, 1, 8 and 9 will be presented if time allows.

1. Finn, R.N., Chauvigné, F., Hlidberg, J.B., Cutler, C.P. and Cerdà, J. 2014. The lineage-specific evolution of aquaporin gene clusters facilitated tetrapod terrestrial adaptation. PLoS ONE 9, 1-38.

2. Chana-Munoz, A., Jendroszek, A., Sønnichsen, M., Kristiansen R., Jensen, J.K. Andreason,P.A., Bendixen, C. and Panitz, F. (2017). Multi-tissue RNA-seq and transcriptome characterization of the spiny dogfish shark (Squalus acanthias) provides a molecular tool for biological research and reveals new genes involved in osmoregulation. PLOS ONE 12(8), e0182756.

ABSTRACT N° ICBF22-157

LOCALIZATION AND EXPRESSION OF UREA TRANSPORTERS IN THE TISSUES OF THE SPINY DOGFISH (SQUALUS ACANTHIAS)

Christopher Cutler* [1], Tolulope Ojo[1]

[1]Georgia Southern University, Statesboro, United States

Abstract:
LOCALIZATION AND EXPRESSION OF UREA TRANSPORTERS IN THE TISSUES OF THE SPINY DOGFISH (SQUALUS ACANTHIAS)

Christopher P. Cutler and Tolulope Ojo, Georgia Southern University.

The localization and/or expression of UT-1 (shUT)1 urea transporters was explored in the dogfish. A transcriptomics study previously suggested the presence of a second UT paralog/isoform2. The partial sequence available (here called Brain UT) was used to produce a complete cDNA, a splice variant was identified and a tissue distribution was determined using PCR. The level of expression of UT-1 (long and short variants) and Brain UT was measured using absolute QPCR in renal samples from dogfish acclimated to 120%, 100% and 75% seawater.

Multiple antibodies were made against UT-1. For the initial antibody a tyramide kit was employed for (up to) 200x signal amplification and this enabled the localization of UT-1 proteins to various segments of the dogfish renal tubule. Further antibodies were used to corroborate the localization results. Western blotting was also performed with the antibodies. The results suggest the distribution of UT-1 in the dogfish may be somewhat different compared to that shown for other elasmobranchs.

1. Smith C.P. and Wright P.A. (1999). Molecular Characterization of an elasmobranch urea transporter. Am. J. Physiol. 276: R622-R626.

2. Chana-Munoz, A., Jendroszek, A., Sønnichsen, M., Kristiansen R., Jensen, J.K. Andreason,P.A., Bendixen, C. and Panitz, F. (2017). Multi-tissue RNA-seq and transcriptome characterization of the spiny dogfish shark (Squalus acanthias) provides a molecular tool for biological research and reveals new genes involved in osmoregulation. PLOS ONE 12(8), e0182756.

ABSTRACT N° ICBF22-286

FISH BRAIN FUNCTION IN A CARBONATED FUTURE

Göran E. Nilsson* [1]

[1]University of Oslo, Oslo, Norway

Abstract:
Over that last decade numerous studies have reported behavioural disturbances in fishes experimentally exposed to future CO2 levels. These disturbances are likely to be linked to altered ion gradients over brain cell membranes, leading to altered neurotransmitter function in the brain. Since the ionic changes may be relatively small, and since the induction of behavioural effects can days, altered gene transcription could play a key role in the mechanisms causing the disturbed behaviours. Indeed, it is now clear that also gene transcription in the brain of some fishes is significantly changed by exposure to elevated CO2, and based on such data we recently proposed that a vicious cycle can be triggered that amplifies the initial disturbance in neurotransmitter function. The proposed cycle is initiated by a reversal of the function of some inhibitory GABAA receptors, causing neural excitation, and then further amplified by adjustments in gene expression directed at suppressing excitation, but unfortunately having the opposite effect. A metabolic component may also feed into the cycle because increased metabolic production of CO2 by over-excited neurons is likely to elevate intracellular bicarbonate, and an increased out-flux of bicarbonate through GABAA receptors will act excitatory. Differences in pH regulation and in transcriptomal responses are factors that could explain why some species or life stages of fish are more sensitive to elevated environmental CO2 than others.

ABSTRACT N° ICBF22-256

OCEAN ACIDIFICATION EFFECTS IN JUVENILE PACIFIC SALMON

Andrea Frommel* [1]

[1]University of British Columbia, Vancouver, Canada

Abstract:
The Pacific Northwest is an area particularly impacted by climate change, with heatwaves and CO2 upwelling causing direct and indirect effects on the ecosystem. Many Pacific salmon populations in this region have been declining, with climate change a likely contributing factor to high mortality rates during their juvenile seaward migration. The ocean conditions in the main migration route of juvenile salmon between Vancouver Island and mainland BC are characterized by high sea surface CO2 levels caused by tidal upwelling. As a result, plankton productivity is reduced, resulting in low foraging success for juvenile salmon. In this talk, combined direct and indirect effects of ocean acidification on survival, condition and acid-base regulation in juvenile Pacific salmon will be discussed.

ABSTRACT N° ICBF22-545

THE IMPACT OF HIGH CO2 IN AQUACULTURE ON THE DIGESTIVE PHYSIOLOGY OF RAINBOW TROUT.

William G. Davison* [1], Rod W. Wilson[1]

[1]Biosciences Department, College of Life and Environmental Sciences, University of Exeter, Exeter, United Kingdom

Abstract:
Globally, aquaculture development has outstripped wild capture fisheries as the main source of fish for human consumption. However, for future growth to be maximised, a holistic understanding of the relationship between the farm environment and animal biology is required. Intensive aquaculture is synonymous with elevated dissolved carbon dioxide far beyond anything most fish would regularly experience in the wild. Exposure to elevated CO2 induces significant acid-base disturbance to fish, specifically a respiratory acidosis compensated by active retention of blood bicarbonate. However, feeding induces a blood “alkaline tide” that necessitates rapid excretion of excess bicarbonate to restore blood pH. A linear decline in growth as CO2 increases has been documented in salmonids and we hypothesised that the conflicting acid-base regulatory needs associated with high environmental CO2 and feeding may be a causative factor. Utilising a combination of intermittent flow respirometry to measure specific dynamic action (SDA), whole organism fluxes, and analysis of body fluid acid-base chemistry we investigated this idea in freshwater rainbow trout after feeding on a 3 % body mass meal. Contrary to our prediction we conclude that the reduced growth observed under high CO2 may be linked to impairment of nitrogen handling during digestion, with a 10% increase in cumulative ammonia excretion observed under elevated CO2. This influence on nitrogen handling may be a key factor in the reduced growth observed in studies using aquaculture relevant levels of elevated CO2 if greater loss of nitrogen as ammonia is symptomatic of reduced protein growth in tissues.

ABSTRACT N° ICBF22-228

ACID-BASE DISTURBANCES AND EFFECTS ON OXYGEN UPTAKE IN NILE TILAPIA FOLLOWING ACUTE AND PROLONGED HYPERCAPNIC EXPOSURE

Peter Vilhelm Skov* [1], Muumin Iddi Hamad[2]

[1]Technical University of Denmark, Hirtshals, Denmark,
[2]Sokoine University of Agriculture, Dodoma, Tanzania, United Republic of

Abstract:
High levels of dissolved carbon dioxide (CO2) is a daily occurring phenomenon in earthen ponds. Hypercapnic conditions lead to a respiratory acidosis, which fish buffer by an accumulation of HCO3-. The degree of hypercapnia that fish are able to recover appears to be species specific, but for Nile tilapia, a freshwater tropical teleost traditionally produced in earthen ponds, no information is available concerning the dissolved levels of CO2 it can tolerate. Here, we investigated the effects of three levels of dissolved CO2 (pCO2 5.4, 16.2, and 32.4 mmHg / 10, 30, and 60 mg L-1) against a normocapnic control (pCO2 0.3 mmHg) on the standard (SMR) and maximum metabolic rate (MMR), haematology, and extra- and intracellular acid-base status in Nile tilapia, following acute (1h) and prolonged (24h) exposure. All hypercapnic treatments resulted in modest but significant decreases in SMR that persisted for 24h. After 1h exposure to CO2, MMR decreased by up to >25%, which was recovered after 24h. Extra- and intracellular pH dropped by up to 0.5 units in proportion to pCO2, and only the lowest hypercapnic treatment (pCO2 5.4 mmHg) was able to fully recover within 24h. All haematological variables were affected by elevated pCO2 during short-term exposure of 1 hour but were fully or partially recovered after 24h. Although the Nile tilapia is generally considered a species able to adapt to and tolerate poor water quality, the modest or slow acid-base regulation following hypercapnic exposure suggests that it is quite sensitive to carbon dioxide exposure.

ABSTRACT N° ICBF22-546

A NOVEL METHOD FOR ACHIEVING ACCURATE BLOOD ACID-BASE CHEMISTRY IN FISH WITHOUT CANNULATION

William G. Davison* [1], Christophe A. Cooper[2], Katherine A. Sloman[3], Rod W. Wilson[1]

[1]Biosciences Department, College of Life and Environmental Sciences, University of Exeter, Exeter, United Kingdom,
[2]International Zinc Association, Avenue de Tervueren 168, Bruxelles, Belgium,
[3]Institute for Biomedical and Environmental Health Research, University of the West of Scotland, Paisley, United Kingdom

Abstract:
Phlebotomy of fishes has long been a vital tool in studying how fish respond to environmental stimuli. Two main methods are typically used: grab 'n' stab and cannulation. Grab 'n' stab involves rapidly removing a fish from the water, rendering it insensible, and then taking the blood sample from the vasculature. In contrast cannulation involves surgically implanting a cannula into the vasculature under anaesthesia, after sufficient recovery blood can be drawn. While both methods have their benefits, both have significant issues that prevent them being useful in various scenarios. The acute capture stress associated with grab 'n' stab renders blood gas and acid-base variables useless while cannulation is limited to sufficient body sizes, has certain technical requirements, and induces chronic stress that can influence typical behaviours such as feeding. Here we present a novel method involving gradual introduction of anaesthetic to a tank to render fish insensible without physical struggling and anaerobic muscle use, before transfer to a gill irrigation table to maintain adequate water flow across the gills. This method avoided the blood chemistry disturbances associated with grab 'n' stab (e.g. elevated catecholamines, lactate, pCO2 and metabolic acid, low pH and pO2) and generated results directly comparable to samples taken using cannulation. Crucially this method was also successfully applied to fish too small for cannulation (e.g. 10-30 g). This method therefore opens up a key avenue in the fields of comparative physiology, aquaculture and climate change, generating data that has thus far been unable to be captured.

ABSTRACT N° ICBF22-199

A UNIQUE MACHINERY OF AMMONIA PRODUCTION AND EXCRETION IN AMMONOTELIC TELEOSTS FOR COPING WITH ACIDIFIED ENVIRONMENT

Hsin-Ju Chuang* [1, 2], Ling Chiu[1], Jia-Jiun Yan[1], Ming-Yi Chou[2], Hon-Tsen Yu[2], Pung-Pung Hwang[1]

[1]Institute of Cellular and Organismic Biology, Academia Sinica,
[2]Department of Life Science, National Taiwan University, Taipei, Taiwan

Abstract:
Climate changes and anthropogenic activities cause environmental acidification in fresh water as in marine. Both ureotelic mammals and ammonotelic teleosts adopt ammonia excretion as the major way for net acid secretion. Teleosts generally excrete a much higher rate of ammonia in the gills than that in human kidney. It is reasonable to hypothesize that ammonotelic teleosts may have developed unique mechanisms for ammonia production and excretion in the gills, which are different from that in human kidney. Ammonia excretion and the mRNA expressions of ammonia transporters (nhe3, rhbg, and rhcg2) and glutaminase (GLS, gls) were induced in the gills by acidic exposure, similar to human kidney under acidosis. We identified a novel gill cell type (GLS cells), which are rich in GLS and mostly adjacent to the ammonia-excreting ionocytes (NHE cells). In the time-course experiments, ammonia excretion, GLS expression and the number of GLS and NHE cells cell were simultaneously increased several hours right after acidic stress, suggesting that GLS cells play a triggering role in ammonia production/excretion to cope with acidic stress. Ammonotelic teleosts developed the trait of labor division between GLS cell (for ammonia production) and NHE cell (for ammonia excretion) in the gills, which may benefit fish to efficiently and timely trigger ammonia production/excretion to overcome acidic environment during vertebrate evolution.

ABSTRACT N° ICBF22-262

AN IN VIVO ELECTROPHYSIOLOGICAL APPROACH TO STUDY ACID EXCRETION IN ADULT FISH GILLS

Shang-Wu Shih* [1, 2], Ming-Yi Chou[1], Pung-Pung Hwang[2]

[1]Department of Life Science, National Taiwan University,
[2]Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan

Abstract:
Molecular and physiological analyses in ionoregulatory organs (e.g. adult gills and embryonic skin) are essential for studying fish ion regulation. Most of the recent progress in fish ion regulation have been studied from the skin of fish embryos but not from the gills of adult fish. One of possible reasons is the lack of direct methods for in vivo functional assays in adult gills. Here, the present study aims to apply the scanning ion-selective electrode technique (SIET) in adult gills and investigate branchial acid-excreting functions in vivo. We removed the opercula from zebrafish and performed long-term acid acclimation experiments. The results showed that the expression of acid excretion-related genes and the number of H+-ATPase-rich ionocytes were increased in the gills under acidic situations. By using SIET, we proved that the H+ and NH4+ excretion capacities were indeed enhanced in the gills acclimated to acidic water. Besides, both H+-ATPase and Na+/H+ exchanger (NHE) inhibitors decreased branchial H+ excretion capacity, suggesting that H+ is excreted through H+-ATPase and NHE in zebrafish gills. These results suggested that SIET is competent for in vivo detection in fish gills. The SIET applied in the gills would be a new breakthrough to approach fish ion regulation physiology.

ABSTRACT N° ICBF22-168

THE KIDNEY IS KEY TO LIFE AT SEA IN THE ARIIDAE CATFISHES

Sophie Roth[1], Peter Allen[2], Wayne Vogl[3], Jonathan Wilson* [1, 4]

[1]Wilfrid Laurier University, Waterloo, Canada,
[2]Mississippi State University, Starkville, United States,
[3]University of British Columbia, Vancouver, Canada,
[4]CIIMAR-UP, Matosinhos, Portugal

Abstract:
Marine teleost fishes generally have a common strategy for water and ion balance that involves drinking seawater and excreting excess salt via their gills to compensate for osmotic water loss and passive salt loading. Unlike in tetrapods and freshwater fishes, the kidney generally has a minor role in osmoregulation in marine teleosts. To date, the sole exception appeared to be the marine catfishes belonging to the Plotosidae family, which are capable of forming a concentrated urine but also have an extrabranchial salt secreting (dendritic) organ. In the present study we find that the Ariidae catfishes, which independently invaded the marine environment and lack a dendritic organ, have unique renal adaptations for hyperosmotic urine production (three-fold higher than plasma) for marine osmoregulation. Microscopy techniques including light and electron microscopy as well as immunohistochemistry were used to study the gills and the renal architecture of two Ariidae species, Bagre marinus and Ariopsis felis. Their gills had sparse numbers of ionocytes [Na+/K+-ATPase (NKA)-rich cells] lacking NKCC1 (Na+:K+:2Cl-cotransporter-1) that are more typical of freshwater than seawater fishes. In the kidney, we observed the presence of a highly unusual arrangement in the late distal tubule consisting of radiating short NKA-rich cecae. This medusa-like structure, wrapped in the ciliated early distal tubule rich in NKCC1, was followed by an unusual actin-rich cone-shaped structure capping the entrance to the collecting duct. The presence of the medusa-like structure was not universal suggesting the potential for at least two nephron types. The significance of these findings will be discussed.

ABSTRACT N° ICBF22-195

MECHANISMS OF ACID-BASE REGULATION FOLLOWING RESPIRATORY ALKALOSIS IN RED DRUM

Joshua Lonthair* [1, 2], Andrew Esbaugh[1], Angelina Dichiera[1, 3]

[1]University of Texas at Austin - Marine Science Institute, Port Aransas,
[2]University of Massachusetts Amherst, Amherst, United States,
[3]The University of British Columbia, Vancouver, Canada

Abstract:
Respiratory acidosis and subsequent metabolic compensation are well-studied processes in fish exposed to elevated CO2 (hypercapnia). Yet, such exposures in the marine environmental are invariably accompanied by a return of environmental CO2 to atmospheric baselines. This understudied phenomenon has the potential to cause a respiratory alkalosis that would necessitate base excretion. Here we sought to explore this question and the associated physiological mechanisms that may accompany base excretions using the red drum (Sciaenops ocellatus), using a two-fold experimental design which allowed us to compare the impacts of both an acidosis and an alkalosis. As expected, when high pCO2 (nominal 15,000 µatm CO2) acclimated red drum were transferred to normal pCO2 their net H+ excretion shifted from 0.157 ± 0.044 to -0.606 ± 0.116 mol g-1 h-1 in the 2 h post-transfer period. Net H+ excretion returned to control rates during the 3 to 24 h flux period. Gene expression and enzyme activity assays demonstrated that while the acidosis resulted in significant changes in several relevant transporters, no significant changes accompanied the alkalosis phase. Confocal microscopy was used to assess translocation of V-type H+ ATPase (VHA) to the apical or basolateral membrane; however, no apparent translocation was observed. Overall, these data demonstrate that fluctuations in environmental CO2 result in both acidic and alkalotic respiratory disturbances; however, red drum maintain sufficient regulatory capacity to accommodate base excretion. Furthermore, this work does not support a role for basolateral VHA translocation in metabolic compensation from a systemic alkalosis in teleosts.

ABSTRACT N° ICBF22-317

THE ROLE OF CARBONIC ANHYDRASE IN HCO3- EXCRETION IN PACIFIC HAGFISH (EPTATRETUS STOUTII)

Marina Giacomin* [1], Jenna Drummond[2], Claudiu Supuran[3], Greg Goss[1]

[1]University of Alberta, Edmonton,
[2]University of Manitoba, Winnipeg, Canada,
[3]University of Florence, Florence, Italy

Abstract:
Pacific hagfish (Eptatretus stoutii) are scavengers that feed on decaying animal carrion by burrowing inside carcasses where they are exposed to several environmental stressors, including hypercapnia (high CO2). Hagfish possess a remarkable acid-base regulatory capacity and can tolerate prolonged exposures to hypercapnia via rapid alterations in plasma HCO3- concentrations. Our study aimed to characterize the relative roles of both membrane-bound and intracellular branchial carbonic anhydrase (CA) in CO2/HCO3- excretion in control and hypercapnia-exposed hagfish. We employed a dual perfusion technique of the individual gill pouch and [14C]HCO3- efflux was measured in fish exposed to control, hypercapnia (48 h), and recovery from hypercapnia conditions (6 h). Fluxes were assessed in the presence of two pharmacological inhibitors of CA, the membrane-impermeant C18 (targeting plasma accessible CA) and membrane-permeant acetazolamide (targeting all forms of CA, including extracellular and intracellular CAs). C18 resulted in a non-significant 13% reduction in HCO3- flux in control fish, whereas acetazolamide resulted in a 59% significant reduction. In hypercapnic fish, baseline HCO3- fluxes were much higher and the addition of acetazolamide caused a 53% reduction of HCO3- flux. The same pattern was observed for fish in recovery, where in all three experimental conditions, there was no significant inhibition of plasma accessible CA. In summary, our data suggests that plasma accessible CA plays only a minor role in HCO3- excretion, contrary to current models, and that this role does not change in response to hypercapnia.

ABSTRACT N° ICBF22-361

REGULATION AND ACTIONS OF INSULIN-LIKE GROWTH FACTORS IN THE ZEBRAFISH OVARY

Glen Van Der Kraak* [1], Nick Melnyk[1], Jacquie Matsumoto[1]

[1]University of Guelph, Guelph , Canada

Abstract:
There is growing evidence that insulin-like growth factors may be important autocrine and paracrine regulators of ovarian physiology in fish. Our research and that of others identified a complete ovarian IGF system in the zebrafish ovary that includes multiple ligands (igf2a, igf2b and igf3), receptors (igf1ra and igf1rb), and binding proteins. Of the ligands, igf3 expression was highest at the time of oocyte maturation and then decreased around ovulation. Exogenous IGFs induced maturational competence, oocyte maturation and steroid biosynthesis in zebrafish follicles. Our recent work using qPCR and ELISA showed that igf3 transcript and IGF3 protein levels are stimulated in full grown follicles by the addition of human chorionic gonadotropin, a Luteinizing hormone (LH) analog. In other studies, we showed that exogenous IGFs promoted the production of the prostaglandins PGE2 and PGF2a in full grown follicles and this action was mediated through an increased expression of cytosolic phospholipase A2 (pla2g4a) prostaglandin-endoperoxide synthase 2 (ptgs2). These studies confirm IGFs, and in particular IGF3, as part of the LH-mediated pathway controlling oocyte maturation and ovulation.

ABSTRACT N° ICBF22-523

ROLE OF NEUROPEPTIDES IN ENDOCRINE/PARACRINE CONTROL OF SPERMATOGENESIS IN THE ZEBRAFISH TESTIS

Hamid R Habibi* [1], HP fallah[1], Maira S Rodrigues[2], Maya Zanardini[1], Rafael H Nobrega[2]

[1]Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada,
[2]Department of Structural and Functional Biology, Sao Paulo State University, Botucatu, Brazil

Abstract:
Spermatogenesis in zebrafish starts with the mitotic division of undifferentiated spermatogonial stem cells and the generation of diploid spermatogonia (Spermatogonia A and B). This is followed by a meiotic phase and the generation of primary and secondary spermatocytes (haploid). The final stage is further differentiation into spermatids and eventually flagellated spermatozoa. Gonadotropin hormones are essential for the initiation and regulation of spermatogenesis in fish and other vertebrates. In the testis, gonadotropins, LH and FSH, act directly on Leydig cells to stimulate the production of hormones which in turn regulate spermatogenesis. Our studies demonstrate that LH and FSH interact with locally produced neurohormones, including gonadotropin-releasing hormone (GnRH) and gonadotropin-inhibitory hormone (GnIH) to regulate testicular function and spermatogenesis. We used isolated adult zebrafish testis in an ex-vivo culture system to demonstrate that GnRH and GnIH are expressed in the testis and exert direct actions on different stages of spermatogenesis, using histomorphometric, gene markers and immunohistochemistry approaches. The findings support the hypothesis that endogenous GnRH and GnIH working in concert with the pituitary gonadotropins are essential components of the multifactorial control of spermatogenesis in the zebrafish testis.

ABSTRACT N° ICBF22-427

INTERACTION BETWEEN DAY LENGTH AND STEROID FEEDBACK FOR THE DIFFERENTIAL REGULATION OF GONADOTROPES IN THE TELEOST FISH, MEDAKA

Muhammad Rahmad Royan* [1], Kjetil Hodne[1], Shinji Kanda[2], Daichi Kayo[3], Rasoul-Nourizadeh Lillabadi[1], Christiaan Henkel[1], Finn-Arne Weltzien[1], Romain Fontaine[1]

[1]Norwegian University of Life Sciences, Ås, Norway,
[2]Atmosphere and Ocean Research Institute, the University of Tokyo, Chiba,
[3]The University of Tokyo, Tokyo, Japan

Abstract:
Synchronization of gametogenesis with favorable environmental conditions is critical for ensuring perfect timing of reproduction. In vertebrates, gametogenesis is regulated by pituitary gonadotropes which produce the two gonadotropins, follicle-stimulating (FSH) and luteinizing hormone (LH). This study investigates the seasonal regulation of gonadotropes in the model fish medaka as this remains unclear in fishes.

We demonstrate that fish raised in long photoperiod (LP) reproduce, unlike fish raised in short photoperiod (SP). Fish raised in LP have higher gonadotropin transcript levels and gonadotrope cell number. We then show that a shift from SP to LP in adult fish increase not only gonadotropin expression but also gonadotrope proliferation. Interestingly, pituitary melatonin receptors show lower transcript levels in LP fish, and tshb mRNA levels also show photoperiod dependency, suggesting that the inhibition of gonadotrope activity in SP probably occurs through melatonin signal and TSH cells as previously reported in mammals. We demonstrate that folliculostelate cells (FS) are TSH sensitive and send extensions towards gonadotropes, suggesting that TSH might regulate gonadotropes via FS cells. Finally, using gonadectomies, we show that absence of sex steroids stimulates FSH proliferation and activity in LP fish, explaining why FSH production increases before LH.

To conclude, this study demonstrates that increasing photoperiod stimulates both gonadotropin production and gonadotrope proliferation in the pituitary, maybe via pituitary melatonin receptors, TSH, and FS cells. Finally, sex steroid feedbacks seem to drive the differential activity of FSH and LH cells in favorable conditions for reproduction.

ABSTRACT N° ICBF22-212

UNIQUE ROLES FOR TYROSINE KINASES AS PART OF GNRH-STIMULATED TRANSDUCTION NETWORKS WITHIN THE GOLDFISH PITUITARY

Enezi Khalid* [1], Joshua Pemberton[1, 2], John Chang[1]

[1]University of Alberta, Edmonton, Canada,
[2]National Institutes of Health, Bethesda, United States

Abstract:
In goldfish, gonadotropin-releasing hormones (GnRH) are major regulators of luteinizing hormone and growth hormone secretion from pituitary gonadotrophs and somatotrophs, respectively. Two native goldfish GnRHs (chicken GnRH-II, GnRH2; and salmon GnRH, GnRH3) differentially signal through a shared population of receptors (GnRHRs) that belong to the G protein-coupled receptor (GPCR) superfamily, to elicit distinct hormone secretion outcomes in a ligand- and cell type-specific fashion. Prior work has already outlined the ligand-selective involvement of intracellular effectors such as MEK-ERK, distinct PI3Ks, and unique Ca2+ stores downstream of GnRH-stabilized receptors. However, the processes by which goldfish GnRHRs direct these divergent transduction responses remains elusive. Receptor-proximal effectors, including heterotrimeric G proteins, -arrestins, and GPCR kinases (GRKs), are generally thought to facilitate ligand-selective transduction by GPCRs, and we have recently shown that these mechanisms underlie part of the observed GnRHR-mediated signalling bias in the goldfish pituitary. Alternatively, in this study, we investigated noncanonical GnRHR activation of intracellular tyrosine kinases (c-Src) and potential transactivation of transmembrane receptor tyrosine kinases (RTKs). Using established cell signalling endpoints, as well as relevant physiological outcomes, including acute GnRH-stimulated hormone release, we showed through transient pharmacological inhibition that both c-Src and RTK-activating matrix metalloproteases participate in mediating differential GnRH actions. These data indicate the unique involvement of growth factor receptors downstream of GnRHR activation in the pituitary, which enables the recruitment of non-canonical effectors, and represents novel avenues for understanding integrated GnRH-dependent signalling mechanisms in this system.

ABSTRACT N° ICBF22-137

THE HUNGER GAMES: DOES FASTING AFFECT SEX IN THE EUROPEAN SEA BASS (DICENTRARCHUS LABRAX)?

Alexander Goikoetxea* [1], Eva Blondeau-Bidet[2], Sophie Hermet[2], Anne-Sophie Martinez[3], Camille Houdelet[1], Marie-Odile Blanc[1], François Ruelle[1], Frédéric Clota[1, 4], Allan Bengue[1], Bastien Sadoul[5], François Allal[1], Marc Vandeputte[1, 4], Benjamin Geffroy[1]

[1]MARBEC Univ Montpellier, CNRS, Ifremer, IRD, Palavas-Les-Flots,
[2]MARBEC Univ Montpellier, CNRS, Ifremer, IRD, Montpellier,
[3]Université de Caen Normandie, Caen,
[4]Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas,
[5]ESE, Ecology and Ecosystem Health, Institut Agro, INRAE, Rennes, France

Abstract:
In some gonochoristic teleost fish, there are instances where specific external conditions can induce functional sex reversal in otherwise GSD species. Here, we examine the effect of thermal stress (measured as cortisol) and explore the potential role of energy mobilisation mechanisms (i.e., fasting) in mediating sex determination and differentiation in the European sea bass (Dicentrarchus labrax). To fulfil our aims, we used two experimental set-ups. In the first one, European sea bass larvae and juveniles were exposed to a range of temperatures (19 , 21 , 23 and 25) until fish in each group attained a size of 8 cm (when sex is presumably fixed). To track the interrenal hormonal changes blood samples were collected. We also obtained gonadal and brain tissue to conduct the histological analysis of gonadal morphology, and in situ hybridisation and qPCR expression analysis of classical sex- and stress-pathway genes (cyp19b, gr1, gr2, crf and mr). For our second experiment, European sea bass larvae and juveniles were subjected to fasting periods across different stages of early development to evaluate the effect of starving on sex ratios. We evaluated the expression of multiple growth-, energy- and sex-related genes in the trunk (piwi, vasa, igf, gh), hypothalamus (npy, avt) and gonads (cyp19a1a, gsdf2), as well as immunohistochemical analysis of germ cell proliferation indicator vasa. Overall, our results show that an association between cortisol and sex ratios does not exist in the European sea bass. We anticipate that this work will also enhance our understanding of the role of energy mobilisation mechanisms during sex reversal, improve our understanding of sex determination and differentiation across vertebrates, and may lead to new tools to control fish sex ratios in aquaculture.

ABSTRACT N° ICBF22-289

PRODUCTION OF PRECOCIOUS FEMALES VIA 17-ESTRADIOL EVAC IMPLANTS INDUCTION IN THE PROTANDROUS BARRAMUNDI (LATES CALCARIFER)

Ad Fine Idan[1], Berta Levavi-Sivan[2], Joseph Aizen[* 1]

[1]Faculty of Marine Sciences, Ruppin Academic Center, Mikhmoret,
[2]Department of Animal Sciences, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Abstract:
The Asian seabass, Barramundi (Lates calcarifer), is one of the most important commercial tropical aquaculture species. We describe herein induction methods to produce precocious females.Captive-reared barramundi age of 12-18 months were injected intramuscularly, monthly for 3 months with a single shot of E2 EVAc implants in three doses (0.5, 1, 1.5mg/kg BW), control groups were injected with an empty implant. Fish were sampled from day 0 every month for 6 months and after a year from the initial treatment for plasma E2 and 11KT levels and were biopsied for oocyte development. Treated fish with EVAc implants in all doses exhibited an increase in E2 plasma levels compared to the control group from day 28 towards day 52. All treated groups presented a peak of E2 levels at day 110 followed by a significant decrease at day 181. From day 77 oocyte diameter was recorded. Oocyte diameters showed correlation to E2 levels increase. Although E2 levels were decreasing on the day 181 the oocyte average size (90-100 micron) peaked in all treatment groups. 11KT levels were not significant between the control and treated group remaining low (10-20pg/ml), higher levels of 11KT was shown in the control fish and no oocytes were recorded in the biopsies. At day 181 the percentage of females in the treated group was 75-85%. To validate that the treatments were not reversible, oocyte diameter was recoded in day 400 and found to be between 350 to 430 microns. To further validate selected females were treated for spawning with GnRHa injections and all treated females spawned, and eggs were fertilized. Our work suggests that E2 EVAcs can be used to improve barramundi production via selective breeding and can help to reduce the high generation time in protandrous barramundi. Future work should focus on optimizing E2 administration in younger fish.

ABSTRACT N° ICBF22-244

DEVELOPMENT OF CRYOPRESERVATION OF IMMATURE TESTIS IN THE ASIAN SEA BASS (LATES CALCARIFER)

Somkiat Sreebun* [1], Kensuke Ichida[2], Ryosuke Yazawa[2], Goro Yoshizaki[2], Surintorn Boonanuntanasarn[1]

[1]Suranaree University of Technology, Nakhon Ratchasima, Thailand,
[2]Tokyo University of Marine Science and Technology, Tokyo, Japan

Abstract:
To develop long-term preservation of spermatogonia in the Asian sea bass (Lates calcarifer), this study investigated the optimum condition of cryopreservation of whole testis which contained high proportion of spermatogonia. First, histological study of testis of the Asian seabass at various growth stages were conducted, and the fish at size of 300 – 500 g body weight were used for testis sampling. In order to determine suitable extender and cryoprotectant, a 3*3 factorial design with three different extenders [Mounib's extender (ME), Non-Activating Medium (NAM) and Leibovitz's medium (L-15)] and 3 different of cryoprotectants [dimethyl sulfoxide (DMSO), ethylene glycol (EG) and propylene glycol (PG)] was employed in a randomized complete design with six replicates. Slow freezing method was performed, and viability of spermatogonia like cell were determined. The results showed that cryomedium containing L-15 and DMSO gave the highest viability rate. Therefore, we tested for the optimum concentration of DMSO by varying concentration of DMSO at 7.5, 10, 12.5 and 15%. The highest viability rate of frozen spermatogonia like cell was observed when using cryomedium containing L-15 and DMSO at 10 %. Subsequently, thawing process including temperature (10 °C and 28 °C) and time (4, 8 and 10 minutes) were optimized, and the results showed that thawing condition at 10 °C for 8 minutes gave the highest viability rate. Taken together, the optimum condition of cryopreservation of immature testis in the Asian sea bass using cryomedium containing L-15 and 10 % DMSO with thawing condition at 10 °C for 8 minutes.

ABSTRACT N° ICBF22-109

THE EFFECT OF FREE-RANGE CHICKEN EGG YOLK ON THE SPERMATOZOA MOTILITY AND ABNORMALITY OF THE KOI FISH AFTER FREEZING

Abinawanto Abinawanto* [1], Retno Lestari[1]

[1]Cellular and Molecular Mechanisms in Biological System (CEMBIOS) Research Group, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, Indonesia

Abstract:
Cryopreservation study using natural cryoprotectatnt such as skim milk, and honey solution have been conducted. However, the application of the free-range chicken egg yolk as a natural cryoprotectant on the spermatozoa quality is still limited. Accordingly, the aim of study was to evaluate the effect of free-range chicken egg yolk on the spermatozoa motility and abnormality of Koi fish, Cyprinus carpio Linnaeus 1758, 48 h after freezing. Sperm was collected by hand stripping method, and was diluted by a dilution solution (methanol 10 % and various concentrations of free range chicken egg yolk) with ratio 1:4. The given concentration of free range chicken egg yolk was 0 % (control), 5 %, 10 %, 15 %, 20 %, and 25 %. Freezing was carried out at -34 °C for 48 hours. Thawing was conducted at 40 °C for 90 sec. The one factor ANOVA showed that various concentration of chicken egg yolk had effect ($P<0.05$) between control and treatment. Free range chicken egg yolk at concentration 15 % was the optimum concentration for maintaining motility of koi fish sperm 48 hours after freezing, because it produced the highest percentage motility 75,57 ± 7,9%, and reduced the abnormality (23,7 ± 4,34%)

ABSTRACT N° ICBF22-285

THE POTENTIAL OF DATE PALM, PHOENIX DACTYLIFERA L. JUICE FOR CRYOPRESERVATION OF KOI FISH (CYPRINUS CARPIO LINNAEUS 1758) SPERMATOZOA

Retno Lestari*[1,2], Esther Purwitasari[1], Anom Bowolaksono[1,2], Abinawanto Abinawanto[1,2]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia,
[2]Cellular and Molecular Mechanisms in Biological System (CEMBIOS) Research Group, Department of Biology , Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, Indonesia

Abstract:

Abstract: Many natural cryoprotectant, namely skim milk, honey solution, and egg yolk have been used to preserve fish spermatozoa. While, the potency of date palm juice as a natural cryoprotectant in preserving fish spermatozoa was not as much as other synthetic cryoprotectant. Therefore, the objective of this study was to evaluate the effect of date palm juice (0%, 3%, 5%, 7%, and 9%) combined with 10% methanol on the Koi spermatozoa 48 h after kept frozen. The spermatozoa was collected through hand stripping. The samples were stored in -34°C for 48 hours. The post cryopreservation spermatozoa were evaluated by examining motility, viability, and abnormality,. The data was analyzed using a one-way ANOVA test and followed by the Tukey's multiple comparison test. The results showed that there was a significant difference ($P<0.05$) among control and treatment. The optimal concentration was discovered at 5% of date palm juice, which produced the highest motility (61,74 ± 4,47%), and viability (72,60 ± 2,96%), respectively. Besides, 5% of date palm juice combined with 10% methanol was also showed the lowest spermatozoa abnormality (22,00 ± 3,16%).

Keywords: Date palm juice, Koi fish, Spermatozoa quality

ABSTRACT N° ICBF22-481

VGLL3 IS ASSOCIATED WIH THE AGE OF PUBERTY IN FARMED ALL MALE ATLANTIC SALMON

Thomas Fraser* [1], Tom Hansen[1], Fernando Ayllon[2], Anna Wargelius[2], Per Gunnar Fjelldal[1]

[1]Institute of Marine Research, Matredal,
[2]Institute of Marine Research, Bergen, Norway

Abstract:
Atlantic salmon (Salmo salar) display a large degree of phenotypic plasticity regarding the age at puberty and this has been linked to the vgll3 locus. In the current study, we aimed to establish the extent to which the vgll3 alleles explain the likelihood of male salmon to enter puberty following environmental regimes that stimulate parr and/or post-smolt maturation. Initially we established an all-male line of Atlantic salmon using sex reversal to produce YY males that were used to fertilize XY eggs. We then conducted two experiments. Firstly, two YY males heterozygous for vgll3 were crossed with four females, two of which were homozygous for the early vgll3 maturation genotype and two were homozygous for the late vgll3 maturation genotype. Fish were reared on a photoperiod and temperature regime known to induce post-smolt maturation. In the second experiment, one YY male heterozygous for vgll3 was crossed with three females heterozygous for vgll3. These fish were reared on a regime that induced both parr then post-smolt maturation. In both parr and post-smolts studies, males with the early vgll3 maturation genotype were more likely to enter puberty as parr and/or post-smolts than those with the late maturation genotype, whereas those progeny heterozygous for vgll3 were intermediate between the early and late genotypes. In addition, we also found some phenotypic females within our all male line (40/1384), of which 5% were genetically male.

ABSTRACT N° ICBF22-218

THE INDUCTION OF OOCYTE MATURATION AND OVULATION IN EUROPEAN EEL (ANGUILLA ANGUILLA): IN VITRO AND IN VIVO COMPARISON OF PROGESTERONE WITH 17ALPHA,20BETA-DIHYDROXY-4-PREGNEN-3-ONE

Pauline Jehannet* [1], Arjan Palstra[1], Leon Heinsbroek[2], Miriam Meijerhof [1], Henk Schipper[1], Ron Dirks[3], Ignacio Gimenez Nebot[4], William Swinkels[5], Hans Komen[1]

[1]Wageningen University & Research,
[2]Wageningen Eel Reproduction Experts , Wageningen,
[3]Future Genomics, Leiden, Netherlands,
[4]Rara Avis, Valencia, Spain,
[5]Palingkwekerij Koolen BV, Bergeijk, Netherlands

Abstract:
Artificially matured female European eels are injected with 17alpha,20beta-dihydroxy-4-pregnen-3-one (DHP at 2 mg.kg-1) to induce oocyte maturation and ovulation. This study compared treatment of DHP with progesterone (P), as upstream precursor in the steroidogenic pathway: in vitro, to finetune dose effects, and in vivo, to validate the in vitro findings. For the in vitro trial, oocyte biopsies were incubated in culture plate wells containing hormone-free medium and medium supplemented with the treatment (P: 10, 100, 1000 ng.mL-1; DHP: 1, 10 and 100 ng.mL-1). Before and after incubation for 12 and 18 h, oocytes were sampled for microscopy and qPCR analysis. For the in vivo validation, females were either injected with P or DHP at a dose of 2 mg.kg-1 to assess their effects on reproductive success. At the moment of stripping, eggs were sampled for RNA-sequencing to compare differentially expressed genes involved in gamete quality aspects. Both P and DHP induced germinal vesicle breakdown in vitro (DHP: 100; P: 100 and 1,000 ng.mL-1). The expression of marker genes involved in oocyte maturation and ovulation was similar for both P and DHP treatment. RNAseq results reflected similar P and DHP effects on egg quality aspects. Females injected with either P or DHP were equally competent to produce larvae. In conclusion, P and DHP effects are identical, but using P is 5,000 times cheaper than using DHP.
The project received funding from DUPAN foundation; The Dutch Ministry of Economic Affairs and the European Union and European Maritime and Fisheries Fund.

ABSTRACT N° ICBF22-366

A COMPREHENSIVE 3D APPROACH TO UNRAVEL FOLLICULAR GROWTH DYNAMICS THAT GOVERNS FECUNDITY IN MEDAKA FISH (ORYZIAS LATIPES)

Manon Lesage* [1], Jerome Bugeon[1], Manon Thomas[1], Stephanie Gay[1], Thierry Pecot[2], Julien Bobe[1], Violette Thermes[1]

[1]INRAE, Fish Physiology and Genomics Institute,
[2]BIOSIT, SFR UMS CNRS 3480-INSERM 018, Rennes, France

Abstract:
In fish, female fecundity, generally defined as the number of eggs spawned, depends on the tightly regulated recruitment, growth and maturation of follicle-enclosed oocytes ranging from 20 µm to over 1000 µm in diameter. To better understand the regulatory mechanisms of fish fecundity, including the role of important novel players such as microRNAs, we still lack a comprehensive spatiotemporal model of follicular dynamics. Thus, our work aimed at developing a novel method to describe the follicular content and its organization in the entire ovary at different life stages of the medaka fish, a daily spawner with an asynchronous ovarian development.

Using a confocal microscopy approach, we developed a complete 3D imaging and analysis workflow that overcomes the methodological biases of classical stereological 2D approaches. First, we established an efficient permeabilization and clearing procedures that allows staining and imaging of the entire ovary at both adult and larval stages. To achieve reliable 3D quantitative image analysis, we took advantage of the recent deep-learning algorithm Cellpose for cell segmentation (Stringer et al, 2021), in combination with other image processing tools for enhancement, denoising, filtering and reconstruction. Additionally, we applied this method to decipher the role of miR-202 during oogenesis, a key regulator of fish fecundity (Gay et al, 2018).

This pipeline now allows a comprehensive and accurate assessment of ovarian content at different developmental stages, thus providing original data to help understanding the follicular growth dynamics and regulations at the whole-organ level.

ABSTRACT N° ICBF22-145

IDENTIFYING SEX MARKERS BY SEQUENCING CIRCULATING MIRNAS OF FOUR MARINE FISH SPECIES

Camille Houdelet* [1], Eva Blondeau-Bidet[2], Sophie Hermet[2], Benjamin Geffroy[1]

[1]MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Palavas-les-Flots,
[2]MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Montpellier, France

Abstract:
Identifying fish sex is crucial for understanding population dynamics. In many fish species, no sexual size dimorphism has been detected and it is most often impossible to identify fish sex based on external characteristics. In aquaculture, the identification of sex at the earliest stage is a predominant preoccupation for fish farmers. The development of new non-invasive technics to sex fish would be a clear asset in those domains. As part of the miSS (microRNA, Sex&Stress, FEAMP) project, we proposed to assess potential circulating microRNAs (miRNAs) detected in the blood, as sex markers. MiRNAs are short and conserve sequences of nucleotides (20-22nt) involved in the regulation of multiple biological processes, which includes sexual development.
Blood samples and gonads of four fish species (Dicentrarchus labrax, Sparus Aurata, Scophthalmus maximus and Sciaenops ocellatus) were sampled on immature and mature individuals. Total RNA of plasma samples was extracted and simultaneously, the sex of immature fishes was determined by observation of the histological sections of gonads.
The sequencing allowed identifying a list of miRNA differentially expressed between males and females at the different developmental stages as potential markers that will be used for the development of a specific miRNA biosensor.

ABSTRACT N° ICBF22-132

MICRORNA 202 IS REQUIRED FOR THE PRODUCTION OF FERTILIZABLE EGGS IN MEDAKA (ORYZIAS LATIPES) THROUGH THE REGULATION OF THE HIPPO PATHWAY

Mariana Roza de Abreu[1], Fernanda de Mello[1, 2], Cervin Guyomar[3], Thaovi Nguyen[1], Martin Pšenika[4], Amaury Herpin[1], Violette Thermes[1], Julien Bobe*[1]

[1]INRAE LPGP, Rennes, France,
[2]Instituto de Biociências, Universitade de São Paulo, Sao paulo, Brazil,
[3]INRAE SIGENAE, Castanet Tolosan, France,
[4]University of South Bohemia in eské Budjovice, Vodnany, Czech Republic

Abstract:
Micro RNA 202 (miR-202) is predominantly expressed in gonads in fish and vertebrates. In medaka (Oryzias latipes) miR-202 is involved in the regulation of follicular recruitment and growth and is necessary for female reproductive success (Gay et al. 2018). Eggs originating from miR202 -/- knock out (KO) females mated with either wild-type or mutant males exhibit a dramatically reduced fertilization success, while eggs originating from wild-type females mated with a mutant male can be fertilized but yield embryos exhibiting a developmental arrest during the first cleavage stages of embryonic development (Gay et al. 2018). In this context, the aim of our study was to understand why eggs produced by medaka (Oryzias latipes) lacking miR-202 exhibit a reduced fertilization success. Recent studies have showed that Taz, an effector of Hippo pathway signaling, is required either very early for the differentiation of the micropylar cell or for its specification, as well as for fertilization in zebrafish (Dingare et al. 2018; Yi et al. 2019). Here we show that several genes of the Hippo pathway are dysregulated in the ovary of mutant miR-202 -/- fish including genes that are predicted targets of miR-202. In addition, we have demonstrated that sperm does not enter the egg obtained from -/- females despite the presence of a micropyle on the chorion. Together, our data suggest that eggs produced by miR-202 -/- females exhibit a non-functional micropyle as a result of the dysregulation of the Hippo pathway in the ovary.

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ABSTRACT N° ICBF22-543

THE METHYLOME OF PATERNAL GAMETES IS SENSITIVE TO REARING TEMPERATURE DURING SPERMATOGENESIS IN RAINBOW TROUT

Aurélien Brionne[1], Anne-Sophie Goupil[1], Catherine Labbé[1], Jean-Jacques Lareyre[1], Stéphanie Kica[1], Audrey Laurent[*1]

[1]INRAE LPGP, Campus de Beaulieu, Rennes, France

Abstract:
During fertilization, the spermatozoon not only delivers its haploid genome into the oocyte, it also transmits important epigenetic information such as DNA methylation, protamines and histones post-translational modifications, mRNAs or ncRNAs. However epigenetic modifications are sensitive to the environment, and fish are particularly exposed. In the context of both climate change and innovative aquaculture practices, important questions remain regarding the exact nature of fish gametes epigenomes, their respective roles for embryonic development and their stability or sensitivity to temperature rise.

In this study, we aimed at characterizing the rainbow trout sperm methylome and its potential variations upon rearing temperature increase. In this purpose, we produced the first rainbow trout exhaustive methylomes by whole genome bisulfite sequencing, performed on three types of samples:

- sperm of rainbow trouts reared at 16°C during late gametogenesis
- sperm of rainbow trouts reared at 12°C (controls)
- muscle of rainbow trouts reared at 12°C.

The results of the analyses show that rainbow trout sperm methylome carries the typical double signature of vertebrate germinal cells with a specific hypomethylation of (i) promoters of genes implicated in spermatogenesis and (ii) promoters regulating pluripotency and genes instrumental for embryonic development.

In addition, we found that the rainbow trout sperm methylome was sensitive to temperature since we identified 2000 differentially methylated regions between sperm cells from fish reared at 16°C versus 12°C. Impacted genes seem to relate to spermiogenesis and spermatic function (pre- and peri-fertilization), while methylation of developmental genes does not seem to be affected.

ABSTRACT N° ICBF22-138

DEVELOPMENT OF NOVEL BIOCONTAINMENT METHODS USING ZEBRAFISH

Marc Ekker* [1], Amanda D'Attolico[1], Stephanie McMillan McKay[1], Robert Devlin[2]

[1]University of Ottawa, Ottawa,
[2]Fisheries and Oceans Canada, Vancouver, Canada

Abstract:
The increasing demand for fish in the food industry has resulted in extensive overfishing in wild fisheries and an increased reliance on aquaculture. However, the escape of farmed fish and their genetic interactions with wild conspecifics remains one of the greatest challenges to sustainable aquaculture. Confinement methods, such as induced triploidy to cause sterility, are not 100% reliable. We are using zebrafish as a proof-of-concept to develop novel biocontainment strategies based on transgenic methodologies or on CRISPR-Cas9 genome editing to impair germ cell formation. We have used a dual transgene approach to impair gamete development. The first transgene, zpc-cre, expressed the Cre recombinase under the control of the promoter from the zona pelucida gene zpc. The second transgene, loxP-caspase, contained a caspase coding region cassette flanked by loxP sites. We produced double-transgenic animals that have a high percentage of sterility. This was confirmed by morphological examination of ovaries and expression analysis of vasa, a gene involved in germ cell development. This approach can be combined to induced triploidy, as we have recently demonstrated. These novel sterility-causing approaches can be adapted to species of commercial interest, alone or in combination with currently existing ones, to better insure containment of fish in an aquaculture context.

ABSTRACT N° ICBF22-356

IMPACT OF LIGHT POLLUTION ON REPRODUCTIVE SUCCESS IN MALE MEDAKA

Lauren Closs* [1, 2], Dianne Baker[2], Amin Sayyari[1], Muhammad Rahmad Royan[1], Finn-Arne Weltzien[1], Romain Fontaine[1]

[1]Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway,
[2]University of Mary Washington, Fredericksburg, VA, United States

Abstract:
In many fish species, males form a dominance hierarchy that influences their reproductive success. This male reproductive competition can also be observed in the model fish, Japanese medaka (Oryzias latipes). However, the environmental factors and physiological mechanisms that determine the reproductive success of dominant males remain largely unexplored. Exposure to artificial light at night is of particular interest as light pollution disrupts wildlife that have optimized their reproduction according to diurnal and seasonal light rhythms. This study aims to investigate the reproductive success of dominant fish and the physiological mechanisms involved, in medaka exposed to different light regimes.
When two male medaka are paired with a female, one male establishes dominance and guards the female, limiting access of the subordinate male to the female. We observed that dominant males are significantly more aggressive, remain closer to the female, and spend ten times longer spawning than subordinates. By using males with different genotypes, we determined the paternity of the progeny by genotyping or screening embryos. We found that dominants and subordinates are equally successful at fertilizing eggs in normal light conditions. However, when exposed to light at night, dominant males fertilize more eggs. We then investigated whether this change was due to behavioral or physiological modifications. We measured behavioral parameters, and collected brains and pituitaries for qPCR analysis, blood samples for sex steroid ELISAs, semen for sperm quality analysis, and testes to determine gonadosomatic index. We found evidence that light pollution influences the brain-pituitary-gonad axis and fish fitness.

ABSTRACT N° ICBF22-445

FISHES ON THE WORLD'S HOTTEST CORAL REEF: ECOLOGICAL AND PHYSIOLOGICAL ADAPTATIONS FOR SURVIVAL

Jacob L Johansen* [1], Matthew D Mitchell[2], Grace O Vaughan[2], Daniel M Ripley[3], Holly A Shiels[3], John A Burt[2]

[1]University of Hawaii, Honolulu, United States,
[2]New York University Abu Dhabi, Abu Dhabi, United Arab Emirates,
[3]Manchester University, Manchester, United Kingdom

Abstract:
The hottest tropical coral reefs on earth are found in the Arabian Gulf (AG) where summer water temperatures reach >35°C. These water temperatures are comparable to business-as-usual ocean warming projections for tropical coral reefs by 2100, providing a natural present-day laboratory for climate change. Although AG reefs have been exposed to extreme temperatures for several thousand years, fewer than 50 known species of reef fish and 35 species of coral have managed to adapt to survive there.

In this study we reveal the adaptive changes and compromises that have occurred within two species of reef fishes (Lutjanus ehrenbergii and Scolopsis ghanam), allowing them to maintain ecological and physiological performance at >35°C and thrive where others cannot. Using Steffensen-type respirometers, we found significantly increased cardio-respiratory function and swimming performance in AG fishes across 27°C, 31.5°C and 35.5°C, signifying an increased capacity to supply energy for physiological maintenance and critical ecological activities. AG fishes also had higher thermal tolerance compared to fishes from more benign reefs, which had low survival at 35.5°C. Most strikingly, AG fishes also required significantly less energy to survive at 35.5°C as total oxygen consumption at rest and at maximal activity was reduced by up to 55%. This reduction in total energetic demand was primarily explained by size reductions, with AG fishes reaching less than 1/2 the mass of other regions. Notably, our results provide strong empirical evidence in support of the projected sharp declines in the maximal size and size-at-age of tropical fishes due to climate change.

ABSTRACT N° ICBF22-103

ENERGETICS AND CELLULAR STRESS RESPONSES IN THE RED MUSCLE OF THERMALLY STRESSED SPARUS AURATA

Ioannis Georgoulis* [1], Konstantinos Feidantsis[1], Ioannis Giantsis[2], Andreas Zachariou[1], Berrin Campaz[1], Marilena Christoforou[1], Hans O. Pörtner[3], Basile Michaelidis[1]

[1]Aristotle University of Thessaloniki, Thessaloniki,
[2]University of Western Macedonia, Florina, Greece,
[3]Alfred-Wegener-Institut, Bremenhaven, Germany

Abstract:
For a comprehensive understanding of fish responses to increasing thermal stress, we investigated tissue energetics, antioxidant levels, inflammatory and cell death responses in Sparus aurata red muscle during exposure to elevated temperatures compared to the control temperature of 18oC. Energetic aspects were assessed by determining lactate, glucose and lipids levels in blood plasma, ATP, ADP and AMP levels, and AMPK phosphorylation as an indicator of regulatory changes in energy metabolism. Oxidative defence was assessed by determining superoxide dismutase, catalase and glutathione reductase maximum activities. Moreover, xanthine levels were determined as an indicator of purine conversion to xanthine and associated ROS production. In the context of inflammatory response and cell death due to oxidative stress, pro-inflammatory cytokines (IkB phosphorylation, IL-6 and TNF) levels, and LC3 II/I ratio and SQSTM1/p62 as indicators of autophagic-lysosomal pathway were also determined. A recovery in the efficacy for ATP production after a marked decrease during the first days of exposure to 24oC is observed. This biphasic pattern is paralleled by antioxidant enzymes' activities and inflammatory and autophagy responses, indicating a close correlation between ATP turnover and stress responses, which may benefit tissue function and survival. However, exposure beyond 24oC caused tissue's antioxidant capacity loss, triggering the inflammatory and cell death response, leading to increased fish mortality. The results of the present study set the thermal limits of the gilthead seabream at 22oC – 24oC and establish the used cellular and metabolic indicators as tools for the definition of the extreme thermal limits in marine organisms.

ABSTRACT N° ICBF22-525

THE ROLE OF SEROTONIN UPTAKE AND DEGRADATION IN CARDIOVASCULAR AND RESPIRATORY RESPONSES TO HYPOXIA IN GULF TOADFISH

John Sebastiani* [1], M. Danielle McDonald[1]

[1]University of Miami - RSMAS, Miami, United States

Abstract:
Serotonin (5-hydroxytryptophan, 5-HT) is a signaling molecule found circulating in the plasma of vertebrates. 5-HT usually acts as a vasoconstrictor and exerts constant tension on the arterial wall, and is thought to contribute to maintenance of resting vascular tone. Previous studies with Gulf toadfish (Opsanus beta) have shown that during hypoxia, 5-HT is removed from the plasma via the 5-HT transporter SERT and either stored or degraded via monoamine oxidase. Inhibiting SERT-mediated uptake with fluoxetine has been shown to attenuate some cardiovascular reflexes to hypoxia at high doses (50 micrograms per gram). However, the extent to which non-SERT transporters and 5-HT degradation contribute to typical cardiorespiratory hypoxia reflexes remains unclear. Toadfish were given intraperitoneal implants containing 25 micrograms per gram fluoxetine in addition to 10 micrograms per gram bupropion and 0.1 micrograms per gram decynium-22 to inhibit non-specific 5-HT uptake into cells. Other toadfish were treated with 10 micrograms per gram clorgyline, which inhibits 5-HT degradation. Each fish was exposed to 15 min of hypoxia. While hypoxia caused control fish to experience a decreased ventral aortic and caudal arterial pressure, and increased ventilation amplitude, these effects were significantly attenuated in both transporter-inhibited and degradation-inhibited fish. Drug treatment had no effect on heart rate, pulse pressure, or ventilatory frequency. These data suggest that non-SERT mediated 5-HT uptake and degradation both significantly contribute to vascular and ventilatory responses to hypoxia.

ABSTRACT N° ICBF22-329

RESPONSE TO ACUTE HYDROGEN SULFIDE EXPOSURE IN ATLANTIC SALMON

Julie Hansen Bergstedt*[1], Peter Vilhelm Skov[1]

[1]Technical University of Denmark, Hirtshals, Denmark

Abstract:
The physiological effects of hydrogen sulfide (H2S) exposure in fishes is gaining increasing attention, due to the discovery of its role as a transmitter molecule. Prior to this, H2S was known for its high toxicity, even at low concentrations. The effects of the toxic gas have also had an impact on the aquaculture industry, leading to mass mortalities of Atlantic salmon (Salmo salar) in recirculating aquaculture systems (RAS). Particularly when operating under saline conditions, there is a risk of H2S production from the microbial degradation of organic material if anoxic conditions occur. Despite the large economic consequences of H2S occurrences, little is known about the tolerance to H2S exposure in Atlantic salmon, or their ability to recover following exposure. Using intermittent respirometry, we exposed Atlantic salmon to acute levels of H2S to examine the metabolic response during exposure and recovery. In H2S concentrations that exceed the oxidation rate of H2S in the mitochondria, H2S reduces the oxygen uptake (MO2) by inhibition of mitochondrial respiration. Based on the measured MO2 values the critical concentration thresholds for H2S were determined, as the point where MO2 decreased below SMR as a result of the increasing H2S concentrations, thus showing an impaired MO2. Furthermore, post exposure oxygen consumption was recorded to assess the metabolic recovery response to acute H2S exposure.

ABSTRACT N° ICBF22-383

SHUTTLE-BOX SYSTEMS FOR STUDYING PREFERRED ENVIRONMENTAL RANGES BY AQUATIC ANIMALS

Emil Christensen* [1]

[1]University of Glasgow, Glasgow, United Kingdom

Abstract:
Animals' selection of environments within a preferred range is key to understanding their habitat selection, tolerance to stressors and responses to environmental change. For aquatic animals, preferred environmental ranges can be studied in so-called shuttle-boxes, where an animal can choose its ambient environment by shuttling between separate choice chambers with differences in an environmental variable. The original shuttle-box was presented in Science in 1972 and its design modified and refined by Steffensen and colleagues since 1991. Based on a recent litterature review, I will present the use of shuttleboxes over the past 50 years, describe the current state-of-the-art with regards to setup, data analyses, experimental design, and study reporting.

ABSTRACT N° ICBF22-201

PROLONGED CORTISOL ELEVATION AFFECTS NUTRIENT DIGESTIBILITY, WHOLE BODY, AND TISSUE METABOLISM OF RAINBOW TROUT

Tilo Pfalzgraff* [1], Ivar Lund[1], Peter Vilhelm Skov[1]

[1]Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, Hirtshals, Denmark

Abstract:
The elevation of plasma cortisol levels in fish associated with stress is known to reduce growth rates by mobilizing fuels for energy-demanding processes, resulting in a higher cost of living. It has been shown that routine metabolic rates are higher in cortisol-treated rainbow trout compared to sham-treated control fish, and it was proposed that cortisol was responsible for the mobilization of lipid stores to fuel this additional energy requirement.

The present study was conducted to assess how long-term elevated plasma cortisol affects nutrient uptake and utilization and metabolic rates. Cortisol did not affect feed intake but cortisol treated fish showed significantly lower specific growth rates and higher feed conversion ratios compared to untreated fish. The reduced growth could partly be explained by the lower apparent digestibility coefficients of macronutrients conceivably due to the reduced relative digestive tissues mass. Moreover, standard metabolic rate (SMR) was elevated in fish with very high cortisol levels, confirming a higher cost of living even after several weeks of cortisol elevation. Likewise, mass-specific oxygen consumption of some metabolically active tissues was increased. Reduced lipid contents in the cortisol-treated fish compared to sham fish confirmed lipid as the main fuel for the elevated energy expenditure.

ABSTRACT N° ICBF22-227

INFLUENCE OF NUTRITIONAL STATUS ON THE METABOLIC RATE OF FISHES

Peter Vilhelm Skov* [1], Tilo Pfalzgraff[1]

[1]Technical University of Denmark, Hirtshals, Denmark

Abstract:
Why are there differences in the standard metabolic rate of conspecific fishes of similar size? During the past decades, there has been considerable research into the intraspecific variability in the metabolic rates in fishes. Differences in standard metabolic rate (SMR) have been used to assign behavioural phenotypes, assuming that high individuals with higher SMRs may be more combative or potentially have a higher feeding capacity. Groups of rainbow trout were maintained on different feeding regimes to create a range of growth trajectories ranging from negative to positive. These were subsequently used in respirometry measurements to determine SMR and MMR. While MMR was unaffected by nutritional status, SMR varied >3-fold. The masses of gastrointestinal tissues revealed a strong positive correlation between with growth rate and SMR. We show that nutritional status can explain a great deal of the variability that is observed when determining SMR in fishes, and that it is likely derived from the very high mass specific oxygen consumption of the digestive tissues. The high SMR in fish that were growing fast appeared quite plastic. When fish were subjected to fasting the relative masses of the digestive tissues was rapidly down-regulated, and SMR was reset within 9 days. These results show that rainbow trout can quickly respond to periods of high and low feed availability by either hyperplasia or atrophy of the digestive tissues, and that there is an obvious bioenergetic advantage to this.

ABSTRACT N° ICBF22-328

PARTITIONING OF OXYGEN UPTAKE IN AIR-BREATHING FISHES

Sjannie Lefevre* [1]

[1]University of Oslo, Oslo, Norway

Abstract:
Intermittent-flow respirometry is widely accepted as best practice in studies of the respiratory physiology and performance of fish – and all other aquatic animals for that matter. The earliest research I did involved optimising the system we had for measurements of oxygen uptake in air-breathing fish, which take up oxygen not only from the water phase but also from the air phase. This means that to get an accurate measure of total oxygen uptake, one has to not only use intermittent-closed respirometry over a suitable period of time, but also do it in both phases without getting water into the air phase and take diffusion between the phases into account, etc. etc. In this presentation, I want to talk about some of the cases where we learned something new by comparing oxygen uptake with and without air breathing where the use of bimodal respirometry gave particular insights. I have worked primarily with three species, the tropical striped catfish (Pangasius hypophthalmus) and striped snakehead (Channa striata) from Vietnam, and the Arctic Alaska blackfish (Dallia pectoralis). The studies have included the effects of hypoxia, swimming and digestion, in various combinations, and have examined the dependence on oxygen from the air as well as from the water.

ABSTRACT N° ICBF22-408

MITOCHONDRIAL FORM AND FUNCTION IN THE HEART OF THE WORLD'S LONGEST-LIVING VERTEBRATE

Pierre Delaroche*[1], Christian Pinali[1], David Smith[1], Samantha Forbes[1], Stephanie Church[1], Garth Cooper[2], John Fleng Steffensen[3], Peter Bushnell[4], Diego Bernal[5], Holly Shiels[1]

[1]The University of Manchester, Manchester, United Kingdom,
[2]University of Auckland, Auckland, New Zealand,
[3]Københavns Universitet, Copenhagen, Denmark,
[4]Indiana University South Bend, South Bend,
[5]University of Massachusetts Dartmouth, Dartmouth, United States

Abstract:
The life span of the Greenland shark (Somniosus microcephalus) is at least 272 years and may be as long as 500 years making this animal the longest living vertebrate on the planet. This extreme longevity is particularly interesting for cardiac studies, because aging is synonymous with heart disease in humans. Mitochondria are dynamic subcellular organelles whose individual shape, organisation, and function (including reactive oxygen species production) is known to change with age. Thus, the focus of this study was to combine electron microscopy imaging and machine learning approaches in cardiac tissue from Greenland shark, aged between ~30 and ~210 years, to determine how mitochondrial form and function change with age. Both two- and three-dimensional electron microscopy image analysis correlated in showing that mitochondrial volume densities were similar among individuals regardless of age. In contrast, image analysis showed a marked increase in inner mitochondrial membrane (IMM) content in larger (and thus presumably older) sharks. Metallomics showed that copper-levels were low in Greenland sharks compared with mammals and the catshark and did not change with age, which may indicate that the copper-based Complex IV of the mitochondrial respiratory-chain is less abundant in this species. Together these data could indicate that mitochondria of older Greenland sharks increase IMM as a mean to facilitate oxygen diffusion rather than to facilitate aerobic capacity. Functional studies are required to support this contention and to shed greater light on the role of mitochondria in this model of extreme aging.

ABSTRACT N° ICBF22-126

STRUCTURAL CHARACTERIZATION OF THE SWIMMING MUSCLES OF THE GREENLAND SHARK, SOMNIOSUS MICROCEPHALUS, AND EATING THE CORRECT HERRING.

Robert Shadwick* [1]

[1]University of British Columbia, Vancouver, Canada

Abstract:
Greenland sharks (Somniosus microcephalus) live in deep, cold water in high northern latitudes, grow to a large size (greater than 5m), have a lifespan of hundreds of years and appear to be very sluggish with little capacity for sustained high-speed swimming. To complement a study of the contractile properties of the lateral skeletal muscles we examined their structural features by light (LM) and electron (EM) microscopy. As is typical of other sharks, the majority of the myomeres are composed primarily of fast white muscle (WM) fibres, while slow red muscle (RM) fibres are located in lateral bands just below the skin. Cell diameters averaged 79 microns for red muscle and 260 microns for white. The most prominent feature in both muscle cell types was the large amount of lipid deposits within the cells. In RM cells these were observed as 3-6 micron vesicles located in a peripheral band as well as centrally among the myofibrils, and comprising about 25% of cell cross-sectional area. In WM cells, similar sized vesicles were more homogeneously distributed throughout the myofibrillar bundles, occupying about half of the cell area. Mitochondria were sparse in WM but very abundant in RM, residing primarily among the lipid vesicles. Sarcomere spacing averaged 1.5 microns in RM and 1.2 microns in WM.

ABSTRACT N° ICBF22-378

INCORPORATING PHYSIOLOGY INTO DESIGN OF INSTREAM RESTORATION STRUCTURES: THE INFLUENCE OF ALTERED FLOWS AND TURBULENCE ON FISH ENERGETICS AND POSITIONAL CHOICE

Katherine Strailey* [1], Rafael Tinoco[1], Piotr Cienciala[1], Bruce Rhoads[1], Cory Suski[1]

[1]University of Illinois at Urbana-Champaign, Champaign, United States

Abstract:
Habitat degradation and loss are among the factors leading to widespread declines in the abundance of freshwater fish. Instream restoration structures are frequently utilized to create and improve fish habitat in an effort to reverse population declines. Such structures alter the natural flow of a river and generate additional turbulence, but little is known about how generated turbulence impacts fish energy expenditure and habitat choice, particularly on small scales. The goal of this study was to quantify how simulated restoration structures alter turbulence and flow dynamics, and, subsequently, how this influences fish energetics and swimming behavior. To accomplish this goal, we first used accelerometers and an intermittent-flow respirometer to link energy expenditure to acceleration in two species, smallmouth bass (Micropterus dolomieu) and rainbow trout (Oncorhynchus mykiss), allowing for the estimation of oxygen consumption in different flow environments. We then swam implanted fish in a large racetrack flume with simulated restoration structures of multiple orientations and diameters across a range of flow velocities. Flow characteristics behind each simulated structure were quantified using particle image velocimetry. When combined, these two components allowed us to determine fish positional choice and energetics in response to simulated restoration structures and relate these responses to turbulence. Together, this information will serve to increase the success of restoration activities by identifying the characteristics of instream restoration structures that lead to flow conditions that are energetically beneficial for fish, ultimately giving managers additional tools for monitoring and evaluating the success of restoration projects that can further fish conservation.

ABSTRACT N° ICBF22-373

ACCELEROMETRY OF GILTHEAD SEABREAM IN SWIM-TUNNEL AND SEA-CAGE

Arjan Palstra* [1], Martin Lankheet[1], Ana Roque[2], Pablo Arechavala-Lopez[3]

[1]Wageningen University & Research, Wageningen, Netherlands,
[2]IRTA, Sant Carles de la Rapita, Spain,
[3]Centro de Ciencias do Mar , Faro, Portugal

Abstract:
Activity patterns of seabream were investigated by monitoring accelerations with acoustic transmitter tags in a sea-cage for a period of 6 weeks (Palstra et al., Front. Mar. Sci. 8:639608, 2021). Daily rhythms in accelerations under the experimental conditions were characterized by more active periods from 6 to 14 h and 18 to 0 h and less active periods from 0 to 6 h and 14 to 18 h. This W-shaped pattern remained over the experimental weeks, even with diurnal accelerations decreasing that was correlated to the dropping temperature. Acceleration was negatively correlated to heart and mesenteric fat mass, which was the exact contrary of our expectations for sustainedly swimming seabream. Our results raised questions on the output of the transmitter tags during sustained swimming exercise. Therefore, we compared their output with swimming performance and body motion in swim-tunnels at different flow speeds (Arechavala-Lopez et al., Front. Anim. Sci. 2:679848, 2021). Tag implantation in the abdominal cavity had no significant effects on swimming parameters. Accelerations, cost of transport and variations on head orientation were negatively related to flow speed in the tunnel, whereas oxygen consumption and frequencies of tail-beat and head movements increased with flow speed. These results show that the transmitter tags mainly recorded deviations from sustained swimming in the tunnel, due to spontaneous and explorative swimming at the lowest speeds or intermittent burst and coast actions to cope with water flow.

ABSTRACT N° ICBF22-154

CHANGING THE DIRECTION OF SWIM FLUME STUDIES: ENERGETICS IN AN OSCILLATING FLOW

Keith Korsmeyer* [1], Mathias Schakmann[2], Travis Marcoux[3], Christopher Fulton[4], Peter Bushnell[5], John Steffensen[6]

[1]Hawaii Pacific University,
[2]University of Hawaii, Honolulu, HI,
[3]Hawaii Uncharted Research Collective, Kailua-Kona, HI, United States,
[4]Australian National University, Canberra, Australia,
[5]Indiana University South Bend, South Bend, IN, United States,
[6]University of Copenhagen, Helsingor, Denmark

Abstract:
Studies of fish swimming physiology have traditionally used swim flumes, or tunnels, to induce the fish to swim at steady speeds in one direction. Natural behaviors, however, include turns and maneuvers, and many fish live in shallow, near-shore environments exposed to wave-induced water motions that cause oscillations in flow direction, or wave surge. Station-holding in these dynamic natural flow conditions involve continuous adjustments to swimming speed and direction. To examine the energetic costs of these activities, new methods and devices have recently been developed to allow respirometry during repeatable unsteady swimming behaviors with controlled frequencies of direction change or turning. These studies are helping to reveal the added swimming costs of turning in fishes and in relation to the level of exposure to wave-induced oscillatory flows over a range of wave periods and amplitudes. Field observations have shown that the extent of wave-exposure on reef habitats correlate with the fin morphology and swimming style of resident fishes. By comparing the swimming performance of diverse species in oscillatory flows, we can determine the if metabolic costs vary and what features may be adaptive in reducing the costs, allowing some species to exploit areas of high wave energy.

ABSTRACT N° ICBF22-430

THE INFLUENCE OF FISH SCHOOLING BEHAVIOUR ON THE ENERGETIC COSTS OF SWIMMING AND WITHIN-GROUP PHYSIOLOGICAL ASSORTMENT

Shaun Killen* [1], Stefano Marras[2], David McKenzie[3], Paolo Domenici[2], John Steffensen[4]

[1]University of Glasgow, Glasgow, United Kingdom,
[2]CNR, Torregrande, Italy,
[3]CNRS, Montpellier, France,
[4]University of Copenhagen, Copenhagen, Denmark

Abstract:
Many fish species exhibit group living and, much like John Steffensen, experience a variety of benefits associated with living a social lifestyle. Here, we review work showing that individual fish that move in schools can reduce their costs of locomotion by taking advantage of vortices produced by schoolmates. Early studies in this area suggested that individual fish that position themselves posterior to schoolmates use less energy to swim at a given speed. Interestingly, however, more recent work suggests that even individuals swimming near the front of schools save energy compared to those swimming in isolation. Not only does moving in a school help fish reduce the energetic costs of movement, but it also helps conserve aerobic scope for other physiological processes beyond locomotion. Indeed, when schools move at relatively fast speeds, a form of within-school physiological assortment can emerge whereby individuals with a higher aerobic scope and swimming capacity are located at the front of the school. While much of the work done in this area has been done with small fish schools swimming within flumes, we also discuss work examining whether individual mackerel swimming in large schools within a semi-natural environment – a large public aquarium – display similar locomotor benefits and within-school physiological assortment. Overall, results suggest that individual fish occupying particular spatial positions within schools may display different phenotypes associated with metabolism and locomotor capacity and may be exposed to differing selective pressures.

01- Fish environmental physiology: A tribute to John Fleng Steffensen

ABSTRACT N° ICBF22-454

FISH SWIMMING WITH JOHN STEFFENSEN

Paolo Domenici* [1], Jacob L. Johansen[2], John Fleng Steffensen[3]

[1]IAS CNR, Oristano, Italy,
[2]University of Hawaii, Kaneohe, United States,
[3]University of Copenhagen, Helsingor, Denmark

Abstract:
In a long-standing collaboration with John F. Steffensen and students of the Fish Swimming course held every other year at the Friday Harbor Laboratories (USA), we have carried out a number of studies on fish swimming ecophysiology, energetics and behaviour. Specifically, we will illustrate examples of studies focusing on (1) the effect of environmental factors such as hypoxia on swimming behaviour, schooling and energetics (2) the energetics of gait transition, and (3) environmental modulation of escape swimming. Various examples will be discussed to highlight the usefulness of studying fish swimming using an integrated approach based on ecophysiology, behaviour and kinematics.

01- Fish environmental physiology: A tribute to John Fleng Steffensen

ABSTRACT N° ICBF22-335

TRAVELS OF A SWIM TUNNEL

David McKenzie* [1]

[1]UMR MARBEC (CNRS, UM, Ifremer, IRD), Montpellier, France

Abstract:
I met John in 1985, we've collaborated on many projects since about 1992, always using his respirometry and/or behaviour setups. I'm the proud custodian of what must be one of the world's oldest Steffensen-type swim tunnels, constructed at the University of Birmingham in 1996 at the behest of Ted (E.W.) Taylor. Ted and I then drove it to a research facility near Milan, where we were working on EU projects. It immediately offered all sorts of new research avenues and opportunities. In the ensuing quarter century, it has travelled back and forth across Europe and has been used in the laboratory and in the field. It now sits comfortably in a research station near Montpellier in France where, with various refurbishments, it still works excellently. I'll take us through a (mercifully brief) talk about some studies that it was used for, plus some results of ongoing research.

ABSTRACT N° ICBF22-119

PARAMETER ESTIMATION FOR A WIDE RANGE OF AQUATIC SPECIES USING A WISCONSIN-TYPE BIOENERGETICS MODELLING APPROACH

David Deslauriers* [1], Brett Van Poorten[2], James Breck[3]

[1]Université du Québec à Rimouski, Rimouski,
[2]Simon Fraser University, Burnaby, Canada,
[3]University of Michigan, Ann Arbor, United States

Abstract:
Bioenergetics models are very useful in the prediction of fish growth and food consumption under known or anticipated environmental conditions. Bioenergetics models have been successfully used in assessing the impacts of invasive species, conservation stocking efforts, and the management of species with commercial interests. While the development and application of such models is useful, they are typically laborious to assemble as they require access to the species of interest, are time consuming, and often necessitate specialized equipment. This may be particularly challenging in rare or endangered species. In this presentation, we describe a novel approach to estimate bioenergetics parameters relying solely on simple growth studies. We show that, for Pallid Sturgeon, Scaphirhynchus albus, the statistical approach we use to derive parameters related to the metabolic rate of the fish agrees well with the known values that were used to simulate the growth of the fish. We go on to show how this modeling approach can be used on a wide variety of published studies and therefore increase the number of bioenergetics models for fish species of interest.

ABSTRACT N° ICBF22-428

INFLUENCES OF THERMAL VARIATION AND FEED RESTRICTION ON GROWTH AND THERMAL PHYSIOLOGY OF EARLY LIFE-STAGE CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA)

Cassidy Cooper[*1], Kenneth Zillig[1], Nann Fangue[1]

[1]University of California Davis, Davis, United States

Abstract:
California contains 21 evolutionarily significant units (ESUs) of at-risk Pacific Salmonids, of which 11 federally listed species are projected to be extinct within 50 years under current climate warming trends, including the Chinook salmon. Past research in salmonids has demonstrated that fish with abundant feed can thrive in warmer temperatures. However, limitation in food availability or optimal rearing temperatures in wild populations may result in energetic tradeoffs between performance and growth, impacting the ability of Chinook salmon to reach critical juvenile sizes for outmigration. Understanding this phenomenon may open up new management opportunities (e.g. food supplementation via floodplain restoration) to protect Chinook salmon and other salmonids from continued population decline in warmer, drier climates. Our research provides insight on the influence of temperature and feed/prey variation in context of life history; further, this research has significant potential toward informing practical-use management interventions during crucial periods of early development. We reared N=4,500 fall-run (fertilized November 30th, 2020) juvenile Chinook salmon under a full factorial cross of temperature (11, 16, and 20 °C) and feed ration (35%, 60% and 100% ad libitum of optimal feed rate) conditions. Individual growth rates of VIE tagged fish were tracked for 15 weeks (at 5 timepoints) for 30 fish per treatment. Aerobic scope data was collected for a subset of individuals at the end of the experimental period. Critical thermal maximum (CTmax) was measured for 12 fish per treatment at 5 timepoints throughout the experimental rearing period. Both temperature and ration have an effect on individual growth rate. However, the effect of ration on CTmax is minimal, and the influence of rearing temperature reflected classic thermal acclimation responses.

ABSTRACT N° ICBF22-406

THE EFFECT OF TEMPERATURE ON SPECIFIC DYNAMIC ACTION OF JUVENILE CHINOOK SALMON, ONCORHYNCHUS TSHAWYTSCHA

Vanessa Lo* [1], Benjamin Martin[2], Eric Danner[3], Dennis Cocherell[1], Joseph Cech[1], Nann Fangue[1]

[1]University of California, Davis, Davis, United States,
[2]University of Amsterdam, Amsterdam, Netherlands,
[3]NOAA Fisheries, Santa Cruz, United States

Abstract:
Fish must feed to obtain nutrients and energy essential for growth, maintenance, and reproduction, and the digestion and absorption of these nutrients requires physiological work. The resulting increase in oxygen consumption is used as a metric for measuring the metabolic cost of processing a meal – termed specific dynamic action (SDA) – and is thought to be affected by water temperature. We measured SDA variables, standard metabolic rate (SMR), and maximum metabolic rate (MMR) in juvenile Fall-run Chinook salmon (Oncrhynchus tshawytscha; mass: 27.6 ± 3.8 g, fork length: 13.3 ± .5 cm) acclimated to 16 and tested at 13, 16, 19, 22, and 24 after consumption of a meal of 2% by body weight. We detected a significant positive effect of temperature on SMR, but not on MMR or the difference between the two, termed absolute aerobic scope (AAS). There was no significant effect of acute temperature on the total O2 cost of digestion, duration, peak metabolic rate standardized to SMR, time to peak, percent of meal energy utilized, nor the ratio of peak O2 consumption to SMR. Peak O2 consumption represented 10.4 – 14.5% of AAS, leaving a large amount of aerobic capacity available for other activities. Thus, we conclude that juvenile Fall-run Chinook salmon exhibit thermal stability in their SDA response, which may play a role in maintaining homeostasis of digestive capability in a highly heterogeneous thermal environment where rapid growth is important for successful competition with conspecifics and for avoiding predation.

ABSTRACT N° ICBF22-207

HYPOTHALAMIC AMPK IS INVOLVED IN MODULATION OF PERIPHERAL ENERGY EXPENDITURE IN RAINBOW TROUT

Marta Conde-Sieira[1], Mauro Chivite[1], Sara Comesana[1], Ayelen M Blanco[1], Jessica Calo[1], Rosa Alvarez-Otero[1], Jose L Soengas* [1]

[1]Universidade de Vigo, Vigo, Spain

Abstract:
Central areas are involved in the regulation of peripheral metabolism. The autonomic nervous system modulation of peripheral organ activity via efferent nerves is a crucial component of an integrated adaptive response started in brain as a result of the integration of metabolic, hormonal and nervous afferent inputs. Appropriate regulation of autonomous outputs onto metabolically active tissues is required to finely orchestrate inter-organ communication during post-absorptive states. Thus, hypothalamic AMPK plays a major role in the regulation of whole-body metabolism and energy balance. We recently demonstrated in fish that inhibition of AMPK1 and AMPK2 in rainbow trout hypothalamus led to decreased capacity to use and synthesize glucose, lipid, and amino acid in liver. In mammals hypothalamic AMPK1 is also involved in the regulation of energy expenditure through enhanced thermogenesis in adipose tissue. Some studies pointed to increased oxidative capacity in fish muscle during adaptation to low temperature allowing us to hypothesize the existence of a central mechanism possibly dependent on hypothalamic AMPK involved in modulation of peripheral energy expenditure in fish not assessed yet. Accordingly, we evaluated in a first study the impact of low temperature on central control of food intake and peripheral (liver, muscle, adipose tissue and intestine) energy expenditure whereas in a second study we evaluated the role of central AMPK on those responses. The results are discussed in the context of central regulation of peripheral energy expenditure in fish.

Acknowledgments: Supported by Spanish AEI and European Fund of Regional Development (PID2019-103969RB-C31 and FEDER)

ABSTRACT N° ICBF22-150

JAPANESE MEDAKA UPREGULATE BOTH GLUTAMINOLYSIS AND LIPOGENESIS TO COPE WITH ACIDIC ENVIORNMENT

Ling Chiu[*1], Hsin-Ju Chuang[1, 2], Ming-Yi Chou[2], Yung-Che Tseng[1], Pung-Pung Hwang[1]

[1]Institue of Cellular and Organismic Biology, Acdamia Sinica,
[2]Department of Life Science, Taipei, Taiwan

Abstract:
Climate changes and anthropogenic activities cause acidification in aquatic environment. Excess acid disturbs body fluid acid-base homeostasis, which is essential for cell activities and physiological processes in both aquatic fishes and mammals. In human, metabolic acidosis is known to stimulate glutaminolysis and consequent gluconeogenesis in the kidneys, thereby increasing ammonia production/excretion and glucose supply to adjust the internal homeostasis. Teleost, an ammonotelic vertebrate, excretes much higher rates of ammonia through the gills. It is scientifically reasonable to wander if teleost have also evolved the same mechanism in the gills as in human kidney. The present study used Japanese medaka (Oryzias latipes) to test the proposed hypothesis by investigating the metabolic changes of medaka upon acid treatment. The acid-treated medaka lost more body weight and had a higher hepatosomatic index (HSI) compared with the control group. They showed increases in blood glucose and the glutaminolysis in the gills, liver, and muscle, while the genes related to gluconeogenesis were downregulated in the gills and liver by acid treatment. Additionally, lipogenesis was induced in the liver, and consequently the fat body content also increased. Comprehensively, our data showed different metabolic strategies adopted by teleosts and mammals to cope with acidic stress. Teleosts, same as mammals, upregulated glutaminolysis to enhance ammonia excretion function for acid-base homeostasis; however, teleosts induce consequent lipogenesis, rather than gluconeogenesis, probably to store energy to face the harsh environment

ABSTRACT N° ICBF22-404

IN VITRO AND IN VIVO ASSESSMENT OF GENISTEIN AND COUMESTROL EFFECTS ON MINERALIZED TISSUES FROM GILTHEAD SEA BREAM

Sara Balbuena-Pecino* [1], Vera Gomes[2], Joaquim Gutiérrez[1], Deborah M. Power[2], Isabel Navarro[1], Patrícia I.S. Pinto[2], Encarnación Capilla[1]

[1]Universitat de Barcelona, Barcelona, Spain,
[2]Centro de Ciencias do Mar (CCMAR), Faro, Portugal

Abstract:
In the context of the increased use of plant-derived ingredients in fish feeds, the physiological impact of their content in phytoestrogens on osteogenesis is not well known yet. With the ultimate goal of optimizing diet formulation, this study aimed to investigate the effects of genistein (GE) and coumestrol (COU) on mineralized tissues of gilthead sea bream (Sparus aurata). Primary vertebra bone-derived cells were incubated with GE or COU at 10 or 100 M, 17-estradiol (E2) at 1 M or 0.1% DMSO as control. Furthermore, juveniles received intraperitoneal injections of GE, COU or E2 at 5 µg/g body weight. In vitro, the lower dose of COU presented osteoinductive properties, indicated by increased cell proliferation and higher fibronectin mRNA levels. Contrarily, cells exposed to 10 µM GE and 100 µM COU showed reduced viability and higher mRNA levels of early markers of osteoblast differentiation (i.e., alkaline phosphatase and bone morphogenetic protein 4). This suggested an acceleration towards the maturation phase, although mineralization of the extracellular matrix (ECM) was not affected. In accordance, in vivo, both GE and COU significantly upregulated in vertebra the gene expression of matrix gla protein, a component of the ECM. Moreover, plasma mineral levels (P, Ca and Mg) were significantly increased after 3 and 5 days of E2 injection, validating the experimental approach. Overall, these findings provide new insights on the modulation of skeletal growth and development by dietary phytoestrogens in fish. Funded by: MICIUN (AGL2017-89436-R, PRE2018-085580); EU-H2020 ASSEMBLE Plus (grant agreement 730984-contract 11241); and FCT (UIDB/04326/2020, DL57/2016/CP1361/CT0015).

ABSTRACT N° ICBF22-213

PHENOTYPIC CHARACTERIZATION OF LEPTIN RECEPTOR DEFICIENT RAINBOW TROUT (ONCORHYNCHUS MYKISS)

Jamie Mankiewicz* [1], Beth Cleveland[1]

[1]USDA/ARS - National Center for Cool and Cold Water Aquaculture, Leetown, WV, United States

Abstract:
Leptin is a pleiotropic hormone known for regulating metabolism and suppressing appetite. In fishes, leptin is primarily produced in the liver and there are reports of the hormone regulating glucose homeostasis as well as lipid metabolism. Most fishes have only one leptin receptor (LepR), however, paralogs have recently been documented in a few species including rainbow trout. To further evaluate the role of leptin signaling in rainbow trout, we used CRISPR/Cas9 genome editing to disrupt both leptin receptor genes, lepra1 and lepra2. We compared wildtype (WT) and mutant fish that were either fed to satiation or feed deprived for 6-weeks. The LepR mutants exhibited hyperphagia, which led to heavier body weight, faster specific growth rate, increased viscero- and hepatosomatic indices, and greater condition factor. Muscle glycogen, plasma leptin, and leptin-a1 transcripts were also elevated in fed LepR mutant fish. Expression of several hypothalamic genes involved in feed regulation were analyzed (agrp, npy, orexin, cart-1, cart-2, pomc-a1, pomc-b). No differences were detected between fed WT and mutants except for pomc-b, where levels were 7.5-fold higher in LepR fed mutants, suggesting that pomc-b expression is regulated by leptin signaling. All detectable fatty acids (FA) were higher in muscle of fed mutant fish compared to WT, albeit not significant. However, fasted mutants exhibited significantly lower muscle FA content for virtually all FAs, suggesting that fasted LepR mutants exhibit increased FA mobilization during fasting. These data demonstrate a key role for leptin signaling in lipid and energy mobilization and storage in a teleost fish.

ABSTRACT N° ICBF22-196

PRODUCTION OF RECOMBINANT MASU SALMON INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-2B

Takuto Miura[1], Ryousuke Kizaki[2], Munetaka Shimizu* [3]

[1]Graduate School of Environmental Science, Hokkaido University,
[2]Graduate School of Fisheries Sciences, Hokkaido University,
[3]Field Science Center for Northern Biosphere, Hokkaido University, Hakodate, Japan

Abstract:
A family of six insulin-like growth factor binding proteins (IGFBPs) are important modulators of IGF actions in mammals, with IGFBP-3 being a major carrier of circulating IGFs. Three major IGFBPs at 20-25, 28-32 and 40-50 kDa are generally detected in plasma/serum of salmonids and have been identified as IGFBP-1b, -1a and -2b, respectively. Salmon IGFBP-2b is a major carrier of circulating IGF-1 and physiologically similar to mammalian IGFBP-3 based on its response to feeding and regulation by growth hormone (GH). However, its function is yet to be demonstrated in salmonids. In addition, there are up to four paralogs for each member of IGFBPs in salmonids due to extra-rounds of whole genome duplication. We expressed recombinant masu salmon (rs) IGFBP-2b1 with fusion partners, thioredoxin (Trx) and His-tag (Trx.His.rsIGFBP-2b1), by using a bacterial expression system. The fusion partners were removed by enzymatic digestion and rsIGFBP-2b1 was purified by reversed-phase HPLC. We next examined in vitro effects of recombinant human (rh) IGF-1 and rsIGFBP-2b1 on the secretion of GH using a primary masu salmon pituitary cell culture. Adding rhIGF-1 alone had no effect on the medium GH levels as measured by time-resolved fluoroimmunoassay. On the other hand, addition of rsIGFBP-2b1 increased the GH secretion with and without the presence of rhIGF-1. However, it is currently unknown if rsIGFBP-2b1 exhibited its action through modulating endogenous pituitary IGF-1 or independent of IGF-1. Availability of recombinant protein should facilitate functional analyses and re-establishment of the immunoassay for salmon IGFBP-2b.

ABSTRACT N° ICBF22-172

POST LARVAL RED MUSCLE GROWTH IN RAINBOW TROUT (ONCORHYNCHUS MYKISS)

Lucas Genevé* [1], Jérôme Bugeon[1], Béatrice Porcon[1], Jean-Charles Gabillard[1]

[1]INRAE, LPGP, 35000 Rennes, Rennes, France

Abstract:
The myotome of fish consists of red and white muscles which differ in function and metabolism. The red muscles are separated from white muscle and located as a band along the side of the fish and composed of slow myofibers with an aerobic metabolism. In contrast, the white muscles are located more in depth with fast myofibers of anaerobic metabolism, and correspond to the major part of the fish's musculature. To better understand the overall and relative growth of red muscles during the post larval development, its proportion in transversal cuts, the number and the size of the red myofibers from 10g to 2kg body weight trout was measured. Our results showed in ~35cm trout that the proportion of red muscles increased linearly on the antero-posterior axis from 1.5-2% (head) to 7% (caudal fin). Red muscle proportion at the anal fin position increased from 4,7% to 6% between 10g and 100g fish respectively, and then was maintained up to 2kg. Histology analysis revealed that red myofibre mean diameter only increased between 500g and 2kg fish (30 versus 45 µm respectively). In contrast the number of red myofibers dramatically increased between 10g and 500g fish (8260 versus 114 000 fibers), and then was maintained in fish up to 2kg (153 000 fibers). Altogether, these results indicate that from 10g up to 500g, red muscle growth occurs mainly by a mechanism of hyperplasia, and continue by hypertrophy at least up 2kg in trout.

ABSTRACT N° ICBF22-395

THE RESPONSE OF THE MUSCLE PROTEOLYTIC SYSTEMS IN DIFFERENT PHYSIOLOGICAL MODELS IN GILTHEAD SEA BREAM

Miquel Perelló-Amorós[1], Isabel García-Pérez[1], Aitor Otero-Tarrazón[1], Albert Sánchez-Moya[1], Adrià Robles-Briones[1], Emilio J. Vélez[2], Isabel Navarro[1], Encarnación Capilla[1], Jaume Fernádez-Borràs[1], Josefina Blasco[1], Joaquim Gutiérrez[* 1]

[1]Department of Cell Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona, Barcelona, Spain,
[2]Université de Pau et des Pays de l'Adour, E2S UPPA, INRAE, UMR 1419 Nutrition Métabolisme et Aquaculture, Saint-Pée-sur-Nivelle, France

Abstract:
The main endogenous proteolytic systems (PS) are calpains, cathepsins and ubiquitin-proteasome, and they play a remarkable role to adapt to the changes in nutrition and physical activity; both aspects can be applied to improve growth and flesh quality in aquaculture. This study compares the response of the PS in gilthead sea bream under different conditions. Juveniles were fasted for 21 days and refeed for 7 days, or injured in dorsal muscle (12G needle). Fingerlings were also exposed to 8 weeks of moderated and sustained exercise (5 Body Length/second). A second group of fingerlings under voluntary swimming or moderated and sustained exercise (2.5 BL/s), were fed a high-protein (HP) or high-fat diet (HE). Dorsal muscle samples were obtained and gene and protein expressions were analyzed. Calpains expression (CAPN1, CAPN2, CAPN3, CAPNS1A) decreased during fasting, while ubiquitin-proteasome (MAFBx, MURF, N3) increased. Refeeding reversed the situation. After a muscle injury, CAPN2, CTSL and MAFBx were progressively upregulated (at days 1, 2 and 8 post-injury, respectively); hence contributing to muscle regeneration process. Interestingly, exercise provoked an upregulation of almost all proteolytic systems. HE diet increased the expression of Calpains, UB and N3 whereas HP increased MAFBx and CTSDL. When fish fed with those diets were exposed to moderated and sustained exercise, almost all differences in PS expression disappeared. These results show the different roles of the PS in muscle, pointing out their potential as growth and quality markers for fish species. Supported by RTI2018-100757-B-I00. M.P-A and I. G-P were FPI fellows (BES-2016-078697 and PRE2019-089578 respectively).

ABSTRACT N° ICBF22-111

ON THE EXISTENCE OF CHAPERONE-MEDIATED AUTOPHAGY IN FISH

Laury Lescat[1], Vincent Veron[1], Brigitte Mourot[2], Sandrine Peron[2], Nathalie Chenais[2], Karine Dias[1], Natalia Riera-Heredia[1], Florian Beaumatin[1], Muriel Priault[3], Stephane Panserat[1], Benedicte Salin[3], Yann Guiguen[2], Julien Bobe[2], Amaury Herpin[2], Iban Seiliez* [1]

[1]UMR1419 NuMeA, INRAE-UPPA, St-Pée-sur-Nivelle,
[2]UR1037 LPGP, INRAE, Rennes,
[3]IBGC, UMR5095, CNRS, Bordeaux, France

Abstract:
Chaperone-Mediated Autophagy (CMA) is a major pathway of lysosomal proteolysis recognized as a key player in the control of cellular metabolism and homeostasis. To date, this function was presumed to be restricted to mammals and birds, due to the absence of an identifiable lysosome-associated membrane protein 2A (LAMP2A), a limiting and essential protein for CMA, in non-tetrapod species. However, we recently identified the existence of expressed sequences displaying high homology with the mammalian LAMP2A in several fish species, challenging that view and suggesting that CMA appeared much earlier during evolution than initially thought. In the present study, we first demonstrate that the gene LAMP2 appeared after the second whole genome duplication that occurred at the root of the vertebrate lineage approximately 500 million years ago. By using a fluorescent reporter previously used to track CMA in mammalian cells, we then revealed the existence of a CMA-like pathway in a fibroblast cell line of the fish medaka (Oryzias latipes). We show that this CMA reporter localizes, upon long-term starvation, to lysosome and late endosomes in a Lamp2a-dependent manner. Finally, to address the physiological role of Lamp2a in fish, we generated, medaka knockout for the splice variant lamp2a, and found severe alterations in carbohydrate and fat metabolisms, as previously demonstrated in mice deficient for CMA in liver. Altogether, our data provide the first evidence for a CMA-like pathway in fish, and thus bring significant conceptual changes in our understanding of the mechanisms involved in the control of metabolism in these species.

ABSTRACT N° ICBF22-124

FIRST IN VITRO ASSESSMENT OF CHAPERONE-MEDIATED AUTOPHAGY AND ITS METABOLIC IMPLICATIONS IN RAINBOW TROUT HEPATOCYTES

Emilio J Vélez* [1], Simon Schnebert[1], Maxime Goguet[1], Karine Dias[1], Linda Beauclair[1], Karine Pinel[1], Vincent Veron[1], Florian Beaumatin[1], Amaury Herpin[2], Iban Seiliez[1]

[1]Université de Pau et des Pays de l'Adour, E2S UPPA, INRAE, UMR1419 Nutrition Métabolisme et Aquaculture, Saint-Pée-sur-Nivelle,
[2]INRAE, UR1037 Laboratory of Fish Physiology and Genomics, Rennes, France

Abstract:
During the last years, the aquaculture industry has made extensive efforts toward increasing production and sustainability through reformulating fish diets. However, strategies aiming at replacing fishmeal with plant-based ingredients eventually face an inherent surge in the final proportion of carbohydrates. This deeply impacts carnivorous fish such as the rainbow trout, for which a high-carbohydrate (HC) diets induce sustained hyperglycemia, oxidative stress, and ends up affecting growth and production functions. In mammals, it is known that oxidative stresses upregulate a selective autophagy process, called Chaperone-Mediated Autophagy (CMA), and nowadays considered as a major pathway in controlling cellular metabolism. This process is notably responsible for the delivery of key metabolic enzymes involved in glycolysis (e.g., ENO1, ALDOA, GAPDH) and lipid storage (e.g., PLIN2, PLIN3) to lysosomes, ultimately controlling their degradation and cellular availability. Hence, tuning CMA activity could be an interesting strategy to develop the use of dietary carbohydrates for carnivorous species. Although we recently provided evidences for the existence of CMA in the medaka fish, it still remains unknown whether other fish species exert CMA activity. In the present study, we established for the first time a rainbow trout hepatoma cell line (RTH-149) stably expressing a CMA-reporter to monitor and characterize CMA activity. Preliminary results show that trout hepatocytes indeed exhibit CMA, and suggest that HC-induced oxidative stress definitely stimulates this process. Future studies will address the metabolic contribution of CMA and consider novel approaches for taking advantage of this process in the optimization of finfish aquaculture.

ABSTRACT N° ICBF22-424

SUPPRESSION OF GASTRIC ACID SECRETION BY OMEPRAZOLE TREATMENT RESULTS IN ALTERED DIGESTION AND GROWTH RATES IN NILE TILAPIA: IMPLICATIONS FOR THE DEVELOPMENT OF PHENOTYPIC STOMACH LOSS

Kelsy Moffatt[1], Jonathan M Wilson* [1, 2]

[1]Wilfrid Laurier University, Waterloo, ON, Canada,
[2]CIIMAR University of Porto, Matosinhos, Portugal

Abstract:
An acid-secreting stomach provides many selective advantages to fish and other vertebrates, yet phenotypic stomach loss has occurred independently multiple times and is linked to loss of both the gastric proton pump and the protease pepsinogen. Reasons underpinning stomach loss remain uncertain; however, understanding the importance of gastric acid-secretion to the metabolic costs of digestion and growth will provide key insights into drivers of loss of this key digestive structure. In this study, omeprazole, a well-characterized gastric proton pump inhibitor, was used to simulate the agastric phenotype by inhibiting gastric acidification in Nile tilapia, and an experimental diet was designed with an indigestible marker, yttrium oxide. Analysis of fecal samples after feeding allows for the determination of protein and phosphorus digestibility. Omeprazole (25mg/kg body mass (BM)/day) in the feed was found to reduce growth rates of fish fed fixed rations of 2-3%BM by 21% (2%BM) and nearly 55% (3% BM) but had no effect on food consumption. We observed the expected significant reduction in gastric acidification with omeprazole treatment, as well as a decrease in the duration and magnitude of the specific dynamic action but not the peak or time to peak. Gastric evacuation rate was also increased and together these data would suggest that the decrease in growth rate was the result of reduced nutrient digestion, uptake and/or assimilation. Pending nutrient digestibility data will address this question. Overall, this research helps improve our understanding of how much gastric digestion contributes to the digestion of key nutrients necessary for growth.

ABSTRACT N° ICBF22-223

A COMPREHENSIVE STUDY ON THE FATTY ACID SENSING MECHANISMS IN THE RAINBOW TROUT GUT

Ayelen Melisa Blanco* [1], Jessica Calo[1], Sara Comesaña[1], Marta Conde-Sieira[1], Gabriel Pérez-Tierra[1], Mauro Chivite[1], María Alborja[1], José Luis Soengas[1]

[1]Centro de Investigación Mariña, Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía, Universidade de Vigo, Vigo, Spain

Abstract:
In mammals, it is well established that the gastrointestinal tract (GIT) plays a key role in the homeostatic control of food intake and energy balance through the so called gut-brain axis. Such a role for the GIT is based on the ability of intestinal cells to sense the presence of nutrients (carbohydrates, lipids/fatty acids and proteins/amino acids) in the lumen, and respond to such information with the release of signaling molecules that ultimately modulate brain circuits governing appetite. However, gut nutrient sensing mechanisms and their implication in the control of food intake remain almost unexplored in fish. The present study characterized fatty acid (FA) sensing mechanisms in the GIT of a fish model species with great interest in aquaculture, the rainbow trout. Major results demonstrated that: (i) the trout GIT expresses mRNAs encoding several key FA transporters and receptors characterized in mammals, (ii) the identified receptors selectively respond to FAs of different length and degree of unsaturation, (iii) intragastrically-administered FAs modulate the gastrointestinal levels of intracellular signaling elements and appetite-regulating hormones, as well as the brain mRNA levels of key appetite-regulating neuropeptides, and (iv) luminal FA-derived central changes in neuropeptide mRNAs are not observed (or are lessened) in vagotomized fish. Together, our findings provide comprehensive evidence supporting the existence of a 'gut-brain' axis in fish. In addition, we observed important differences in fatty acid sensing mechanisms of rainbow trout compared with mammals, which may indicate evolutionary divergence between fish and mammals.

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ABSTRACT N° ICBF22-347

VEGETAL-BASED DIETS INDUCED MOLECULAR DYSREGULATIONS IN CARNIVOROUS FISH: FOCUS ON AMINO ACID TRANSPORTERS IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) CELL LINES

Guillaume Morin* [1], Karine Pinel[1, 2], Karine Dias[1], Iban Seiliez[1], Florian Beaumatin[1, 2]

[1]INRAE, Saint-Pée-sur-Nivelle,
[2]E2S UPPA, Pau, France

Abstract:
Plant-based diets (PBD) disturb rainbow trout (RT) physiology and growth owing to multifactorial reasons including amino acid (AA) imbalance. A careful in-depth analysis of transcriptional data from 2 previous independent studies showed that PBD dysregulate AA transporters among which some cationic AA transporters (CAAT) as well as targets of the integrated stress response (ISR) pathway. Since ISR is induced following AA restriction/starvation and was described in other species to regulate transcription of some AA transporters, we wondered if the observed dysregulations of CAAT expression could depend on AA limitation through the activation of the ISR pathway. To this end, we assessed, after identifying the whole subfamily of CAAT genes in RT genome, their specific transcriptional regulations according to AA availability as well as the potential involvement of the ISR in their regulation through an in vitro approach allowing the precise control of nutritive conditions: the use of RT cell lines.

CAAT genes analysis in RT genome reveal the existence of 42 orthologs of human CAAT genes among which 28 are expressed in selected tissues. Moreover, our results show that almost half of the CAAT expressed in RT cell lines tested are upregulated upon AA starvation and strongly suggest ISR involvement in their over-expressions. Finally, these results tend to demonstrate that AA homeostasis is compromised in fish fed PDB which could contribute, at least partially, to the observed growth retardation as also suggested by our results notably pointing dysregulations of the mTOR pathway, a master regulator of cell growth and proliferation.

ABSTRACT N° ICBF22-268

EFFECTS OF HIGH CARBOHYDRATE FEEDING STIMULUS FOR SHORT PERIOD DURING FRY STAGE ON CARBOHYDRATE METABOLISM IN JUVENILE NILE TILAPIA (OREOCHROMIS NILOTICUS)

Nattanan Srisakultiew* [1], Suksan Kumkhong[1], Lucie Marandel[2], Stephane Panserat[2], Surintorn Boonanuntanasarn[1]

[1]Suranaree University of Technology, Nakhon Ratchasima, Thailand,
[2]INRAE, Saint-Pée-sur-Nivelle, France

Abstract:
This study aimed to investigate the effect of early carbohydrate (CHO) feeding stimulus on CHO metabolism in Nile tilapia at later juvenile stage. The nutritional stimulus was conducted by feeding fry from first feeding with 1) low-CHO/high-protein (LC/HP) for 3 weeks (control), 2) high-CHO/low-protein (HC/LP) diet for 1 week (short HC/LP), or 3) HC/LP diet for 3 weeks (long HC/LP). The effects of nutritional stimuli on CHO metabolism were determined at mRNA levels, and the results showed that early feeding of HC/LP diet modulated CHO metabolism including induction of glycolysis and lipogenesis suppression glucose transport, gluconeogenesis and amino acid catabolism ($P<0.05$). Fish were fed with commercial diet through week 20 (juvenile), and long-term effects of early HC/LP stimuli history were evaluated. HC/LP diet stimuli history persisted it effects on modulation of CHO metabolism as well as increase in hepatic fat (only in long HC/LP) and triglyceride content ($P<0.05$). Subsequently, during week 21- 24, fish were challenged with high CHO diet (CHO-H). Our results showed that early HC/LP stimulus history improved growth performance. Early HC/LP stimulus history (both short and long) influenced blood metabolites, nutritive composition and CHO metabolism at molecular level which indicated induction of glycolysis and lipogenesis as well as suppression of amino acid catabolism and gluconeogenesis. Taken together, early HC/LP feeding in tilapia fry from first feeding could achieve metabolic programming, and 1-week HC/LP early feeding stimulus could accomplish nutritional intervention and programming its impacts at later in juvenile stage.

ABSTRACT N° ICBF22-500

HOW LACTATE SIGNALING AFFECTS CARBOHYDRATE AND LIPID MOBILZATION IN RAINBOW TROUT

Giancarlo Talarico*[1], Elisa Thoral[1,2], Elie Farhat[1], Loïc Teulier[2], Jan Mennigen[1], Jean-Michel Weber[1]

[1]University of Ottawa, Ottawa, Canada,
[2]Université Claude Bernard Lyon 1, Lyon, France

Abstract:
Lactate is now recognized as an important regulator of fuel selection in mammals because it inhibits lipolysis by binding to hydroxycarboxylic acid receptor 1 (HCAR-1). The goal of this study was to quantify the effects of exogenous lactate on glucose and fatty acid mobilization in rainbow trout and identify potential signaling mechanisms by monitoring the expression and activity of key glycolytic, gluconeogenic, lipolytic and beta-oxidation targets in liver and muscle. In vivo measurements of metabolic fuel kinetics show that lactate strongly reduces hepatic glucose production (16.4±2.0 to 8.9±1.2micromole kg1 min1), but does not affect lipolysis (no change in rate of appearance of glycerol: mean value of 7.3±0.5 micromole kg1 min1). The reduction in hepatic glucose production can be explained by the substitution of glucose by lactate as an oxidative fuel and suppression of gluconeogenesis through phosphoenolpyruvate carboxykinase (PCK) inhibition (24% and 60% decrease in protein and gene expression, respectively). In contrast to mammals, trout exhibited no lipolytic suppression during lactate administration, suggesting HCAR-1 signaling is weak in trout (no response of gene expression in white muscle and liver; inhibition in red muscle). We conclude that lactate regulates metabolic fuel selection of trout via preference for lactate over glucose and PCK-mediated suppression of gluconeogenesis. However, lactate signaling does not reduce lipolysis via HCAR-1 as it does in mammals.

ABSTRACT N° ICBF22-432

THE EARLY DEVELOPMENT OF PIKEPERCH – FILMING TIMES OF CHANGE

George Franz* [1], Katrin Toenissen[1], Bianka Grunow[1]

[1]FBN Dummerstorf, Dummerstorf, Germany

Abstract:
Pikeperch has become a new species of interest for European freshwater aquaculture. It is a popular sport fish and is liked by consumers. But as a relative newcomer in this industry, raising and husbandry are still facing phases with high mortality rates. Especially during the transition from embryo to larva, specimens die due to internal developmental changes and external pressures as the starting cannibalism.

We combined different methods to gain insights into this developmental shift. As a case study, the developmental and morphometric changes of pikeperch were observed during this ontogenetic phase. Following this, a software was developed to count specimens in a laboratory-scale setting. Additionally, we conducted a transcriptome analysis of muscular and skeletal developmental genes on specimens of these age stages. A change in the growth patterns was detected, which showed a pause in length growth. This concurred with a shift from skeletal and muscle tissue development to digestive system development. Subsequently, an increase in size variations was measured, highlighting the impact of cannibalism at that time. The developed software hereby presents a possible way to detect starting cannibalism in an aquaculture setting.

Concluding, general developmental patterns during the embryo-larval-threshold of pikeperch and the possible future uses of the developed software are discussed.