

# Evaluation of the influence of light conditions on crayfish welfare in intensive aquaculture

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## Abstract

During the last years, evidence has arisen for the ability of crustaceans to suffer from stress and noxious situations. This has led to an increasing public concern for crustacean welfare. Due to the interest in intensification of crayfish aquaculture, the question arises whether artificial culture conditions may impact these crustaceans' welfare.

We evaluated the effect of six light conditions on noble crayfish (*Astacus astacus*) behaviour in a recirculating aquaculture system. Crayfish were cultured under cool (5500 K), neutral (3800 K) or warm (2600 K) white light, using bright (761 lux) and weak (38 lux) intensity.

The crayfish's dark-light preferences were evaluated by observing their behaviour in a plus maze. Animals kept under 38 lux spent  $27.94 \pm 8.95$  % of their time in the light arms of the maze, while those kept under 761 lux only spent  $20.06 \pm 9.98$  % in the light arms. Thus, crayfish from bright light showed a slight trend to avoid the light arms of the maze ( $p = 0.098$ ), an indication of anxious behaviour. The light spectrum had no influence.

Following earlier preliminary signs of pain perception and neophobia in *A. astacus* these new observations invite to investigate further indications of a more intensive mental life in crustaceans. Human activities such as the catching, rearing, shipping and cooking of crustaceans may have a considerable impact on the welfare of these animals if these results are confirmed. We argue there are good ethical reasons to re-evaluate the omission of non-cephalopod invertebrates from European animal welfare legislation.

**Keywords:** *Astacus astacus*, dark-light preference, mental ability

## Introduction

Although non-cephalopod invertebrates are not protected by European animal welfare legislation, strong evidence has arisen during the last decade for the ability of crustaceans to suffer from stress and noxious situations (Magee & Elwood, 2013, Elwood & Adams, 2015, Vervaecke et al., 2015). In recent years, the public concern on crustacean welfare has been increasing (Yue, 2008).

There is an increasing interest in the intensive production of crayfish in Europe (Seemann et al., 2015; Abeel et al., 2014; Franke et al. 2013). Considering their detritivorous nature and high market potential, these animals are promising candidates for sustainable aquaculture. In intensive rearing systems, these decapods behave highly cannibalistically, hindering the development of commercially viable production (Abeel et al., 2014). The question arises if intensifying the production of these animals, and other decapod crustaceans (e.g. crabs, shrimps and lobsters), has consequences for their welfare.

Light conditions such as illuminance and photoperiod are known to influence growth, metabolism and behaviour in crayfish (Thomas et al., 2016; Franke et al., 2013; Delabbio, 2011; Fanjul-Moles et al., 1998) and are considered potential factors to improve production in crayfish rearing systems (Franke & Hörstgen-Schwark, 2015; Gonzalez et al., 2010). In this study, we

evaluated the effect of white light spectra and light intensity or illuminance in a recirculating aquaculture system (RAS) on noble crayfish behaviour.

To evaluate the welfare implications, we quantified negative affective states related to living in particular light conditions using the paradigm of a subaquatic dark-light plus maze, validated by Fossat et al. (2015). He assessed stress by placing red swamp crayfish (*Procambarus clarkii*) in a conflicting situation between its innate curiosity for novel environment and its aversion for light. Under natural circumstances crayfish frequently hide in dark places and explore new environments. Experimentally stressed animals however showed less explorative behaviour and preferentially remained in the dark arms, whereas unstressed crayfish also explore the illuminated arms (Fossat et al., 2014).

## Material & methods

### *Light conditions*

468 pond raised noble crayfish (*Astacus astacus* L.) summerlings were obtained from a German farm, and transferred to an indoor recirculating aquaculture system. They were housed under six different light conditions for 191 days. Average initial body weight was  $0.66 \pm 0.13$  g and mean occipital carapace length (OCL) was  $10.64 \pm 0.10$  mm (figure 1). The animals were divided over 18 tanks, resulting in a stocking density of 52 animals/m<sup>2</sup>, or 26 individuals per tank. Sex ratio was 50:50. During the experimental period, water temperature was kept at  $21.0 \pm 0.35$  °C, pH at  $8.75 \pm 0.07$  and dissolved oxygen level was  $7.25 \pm 0.51$  mg/l.

Three types of 36W T8 fluorescent lights were used to provide three spectrums of white light: cool, neutral and warm, with correlated colour temperature (CCT)  $\approx$  5500 K, 3800 K and 2600 K respectively. Each spectrum of white light was tested in six tanks. These tanks were further split up in two groups in which two light intensities were applied. For one half of the tanks, the lights were covered with a black cloth to lower illumination, creating two light intensities for each spectrum: bright light (761 lux) and weak light (38 lux). This way, we obtained six different light conditions (table 1). Three tanks with 26 crayfish were used for each treatment.

**Table 1. The six experimental light conditions, used in the recirculating aquaculture system**

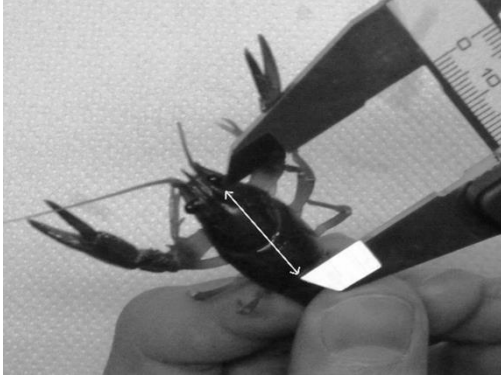
Light condition	Lamp type	Intensity	Spectrum
cool bright light	Philips T8 36W 865	761 lux	CCT $\approx$ 5500 K
neutral bright light	Philips T8 36W 840	761 lux	CCT $\approx$ 3800 K
warm bright light	Philips T8 36W 827	761 lux	CCT $\approx$ 2600 K
cool weak light	Philips T8 36W 865	38 lux	CCT $\approx$ 5500 K
neutral weak light	Philips T8 36W 840	38 lux	CCT $\approx$ 3800 K
warm weak light	Philips T8 36W 827	38 lux	CCT $\approx$ 2600 K

### *Dark/light preference*

A plus maze (figure 2) was designed and adapted to the crayfish's size, according to the protocol of Fossat et al. (2015). The maze had a 29 cm diameter and was nine cm high. Each arm had a surface dimension of 7 x 11 cm. The sides of the dark arms were covered with a black foil and the top was closed with an opaque lid.

After the crayfish spent six months under the experimental light conditions, two males and two females were randomly caught from each tank. Hence, we observed 72 crayfish in total, or twelve animals from each treatment. Each crayfish was put in the plus maze (figure 2) in order to determine its preference for dark or light. Illuminance and colour temperature of the lighting

in the plus maze were adapted in accordance with the experimental light conditions in the RAS. Animals from the bright light treatments, were exposed to 547 lux in the lit arms and six lux in the dark arms of the maze. For crayfish from the weak light treatments, illuminance in the lit and dark arms were 21 lux and 0 lux respectively. The maze was filled with water from the RAS. This water was changed after each observation.



**Figure 1. Occipital carapace length (OCL)**



**Figure 2. Dark/light preference test in a plus maze**

Each crayfish was caught separately, just before observation took place. The animals were caught from their tanks using a small fishing net. Catching was performed carefully, in order to avoid potentially stressing escape reactions such as tail flipping. After a crayfish was observed in the plus maze, it was placed in a separate container to assure no individuals were tested twice.

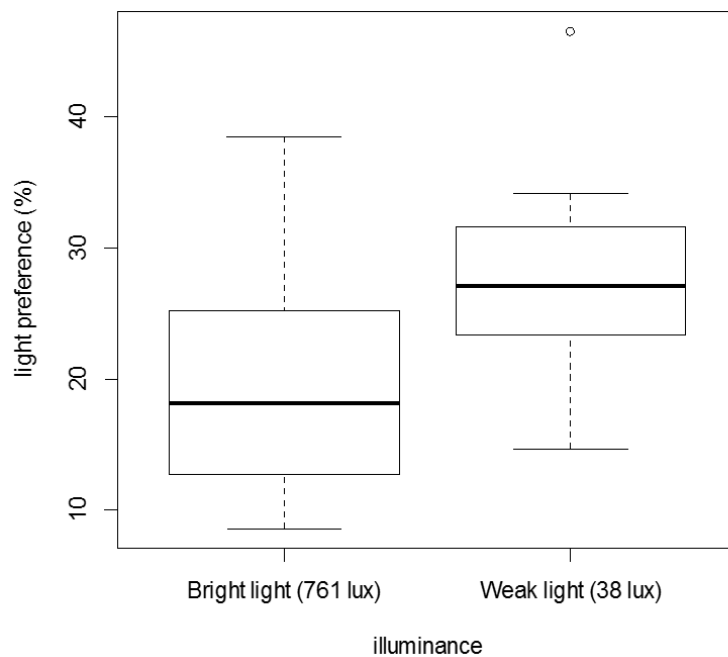
Before the observation, each crayfish was put in a opaque cage (inner dimensions: 6x6x10 cm) in the center of the plus maze during one minute for acclimation. The animal was subsequently released from the cage, allowing it to explore the entire maze for 10 minutes. During this period, the animal's location was scored every five seconds, resulting in 121 observations per individual. From these data, we calculated the percentage of time spent in the light and dark arms of the plus maze, in order to determine the crayfish's dark-light preference. We averaged over the 4 crayfish in each tank to have independent observations per treatment arm.

#### *Statistical analysis*

As the number of observations per treatment arm were small ( $n=3$ ) we used non-parametric tests. A Kruskal-Wallis test was performed to see if there was a difference in dark-light preference between the six treatments. As we were specifically interested in the effect of light intensity this was tested separately with a Mann-Whitney test and light spectrum with a Kruskal-Wallis test.

#### **Results**

There was no significant difference in time spent in the lit versus dark arms between the six treatments (ie. three levels of light spectrum combined with two levels of light intensity)  $p=0.209$ , Kruskal-Wallis test). Crayfish from weak light treatments showed a tendency ( $p = 0.098$ , Mann-Whitney-U test) to spend more time ( $27.94 \pm 8.95$  %, mean  $\pm$  SD) in the lit arms of the plus maze than crayfish cultured under bright light ( $20.06 \pm 9.98$  %). Thus, crayfish kept under bright conditions tended to avoid light, as shown in figure 3. Light spectrum did not influence the light-dark preference of the crayfish in the maze ( $p = 0.715$ , Kruskal-Wallis test).



**Figure 3. Light preference in the plus maze for crayfish from weak and bright light treatments**

## Discussion

Living in an environment with cool, neutral or warm white light did not affect the behaviour of crayfish in a dark-light preference maze. In weak light, the animals showed a tendency towards showing less anxious behavior ( $0,05 < p < 0,1$ ) in weak light. These crayfish generally explored the whole maze, including the illuminated arms. This natural exploratory activity under weak light conditions complies with Frasers et al.'s (1997) definition of welfare. This is consistent with the results of the zootechnical parameters under the same light conditions (unpublished data).

These results are comparable to the findings of Fanjúl-Moles et al. (1998), who found that high irradiance caused increased hemolymph lactate in *Procambarus clarkii* and *P. digueti*, indicating higher stress levels in these crayfish. While *P. clarkii* managed to cope with these artificial conditions, high irradiance caused high mortality in *P. digueti*. Prieto-Sagredo et al. (2000) found that these species showed a different metabolic response to high light intensity, illustrating the species-specific reaction to this stressor. In addition, Thomas et al. (2016) found that invasive signal crayfish (*Pacifastacus leniusculus*) hide in their shelters more often when exposed to simulated light pollution from a street light during the night.

Although the current observed trend does not allow any confident conclusions about the mental capacities of *A. astacus*, following earlier preliminary signs of pain perception and neophobia (Vervaecke et al., 2015), these new observations are a further invitation to investigate the intensity of mental life in crustaceans. Human activities such as catching, rearing, shipping and cooking of crustaceans all are potentially stressing or painful, and may have a considerable impact on the welfare of these animals. In that sense, the debate about crustaceans may now be at the same point as the fish welfare debate a few decades ago (see e.g. Braithwaite, 2010).

The European welfare legislation on farmed animals (European Union, 1998) is based on the European Convention for the Protection of Animals kept for Farming Purposes (Council of Europe, 1976) that states they should be provided with care that 'is appropriate to their physiological and ethological needs in accordance with established experience and scientific

knowledge'. The Lisbon Treaty (European Union, 2007) took this a step further by explicitly asserting 'animals are sentient beings', something that applied to agricultural as well as fisheries policies. From the experimental animals Directive (European Union, 2010), however, it is clear that European legislators are only referring to 'non-human vertebrate animals' and cephalopods.

This legislation clearly mirrors ethical arguments that can be rooted in the utilitarian tradition (suffering, sentience). From a legislative perspective there is still no 'established experience and scientific knowledge' that proves sentience in crustaceans, but the results provided here and in Vervaecke et al. (2015) provide a moral impetus to embark on a serious endeavour into the mental capacities of crustaceans, thereby re-evaluating the omission of non-cephalopod invertebrates from European animal welfare legislation.

A similar, and probably even stronger need for clarification arises when approaching this question from a 'subject of a life' approach as suggested by Regan (1983). If these preliminary findings are confirmed, this type of mental activity should be enough reason to include crustaceans (or at least *A. astacus*) in our moral circle of compassion.

## Conclusions

As the intensity of white light appears to affect behaviour and welfare of cultured noble crayfish, this factor should be taken into account when setting up a commercial production system. Weak light may lead to a better wellbeing of the cultured animals.

From an ethical as well as legislative view, the current results are a further indication of the need for in-depth research into the physical and mental abilities of crustaceans and possibly other non-cephalopod invertebrates. In relation to EU legislation confirmation would clearly have far-reaching implications, but it would also bring a sharper focus to the edges of the ethical circle of compassion.

## Acknowledgements

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## References

- Abeel, T., Adriaen, J., Meeus, W., Himpe, W., Roelant, E., Sannen, A., Laevens, H., Van Der Elst, J., Aerts, S. (2014). Eindrapport PWO-project: Technische en economische evaluatie van de productie van Europese rivierkreeft (*Astacus astacus*) in recirculatiesystemen. Odisee University College, Sint-Niklaas, Belgium.
- Braithwaite, V. (2010). Do fish feel pain? New York, United States: Oxford University Press.
- Council of Europe (1976). European Convention for the Protection of Animals kept for Farming Purposes. Strasbourg, France: Council of Europe. Available at: <http://www.coe.int/en/web/conventions/full-list/-/conventions/rms/0900001680076da6>.
- Delabbio, J. (2013) Method and system for enhancing growth and survivability of aquatic organisms. U.S. Patent US 2013/0174792 A1. Jul. 11, 2013.
- Elwood, R.W., Adams, L. 2015. Electric shock causes physiological stress responses in shore crabs, consistent with prediction of pain. *Biology Letters*. 11 (11) .
- European Union (1998). Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes. Official Journal of the European Union, L 221: 23-27 Available at: <http://eur-lex.europa.eu/eli/dir/1998/58/oj>.
- European Union (2007). Treaty of Lisbon amending the Treaty on European Union and the Treaty establishing the European Community, signed at Lisbon, 13 December 2007. Official

- Journal of the European Union, C 306: 1-271. Available at: <http://eur-lex.europa.eu/legal-content/EN/NOT/?uri=CELEX:12007L/TXT>.
- European Union (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union, L 276: 33-79. Available at: <http://data.europa.eu/eli/dir/2010/63/oj>.
- Fanjul-Moles, M.L., Bosques-Tistler, T., Prieto-Sagredo, J., Castañón-Cervantes, O., Fernández-Rivera-Río, L. (1998) Effect of Variation in Photoperiod and Light Intensity on Oxygen Consumption, Lactate Concentration and Behavior in Crayfish *Procambarus clarkii* and *Procambarus digueti*. Comparative Biochemistry and Physiology. Vol. 119A, No. 1, pp. 263–269. Elsevier Science Inc.
- Fossat, P., Bacque-Cazenave, J., De Deurwaerdere, P., Delbecque, J. P. and Cattaert, D. (2015). Measuring Anxiety-like Behavior in Crayfish by Using a Sub Aquatic Dark-light Plus Maze. *Bio-protocol* 5(3): e1396. <http://www.bio-protocol.org/e1396>
- Fossat, P., Bacque-Cazenave, J., De Deurwaerdere, P., Delbecque, J. P. and Cattaert, D. (2014). Comparative behavior. Anxiety-like behavior in crayfish is controlled by serotonin. *Science* 344(6189): 1293-1297.
- Franke, R., Wessels, S., Hörstgen-Schwark (2013) Enhancement of survival and growth in crowded groups: the road towards an intensive production of the noble crayfish *Astacus astacus* L. in indoor recirculation systems. *Aquaculture research*. 44, 451-461.
- Franke, R., Hörstgen-Schwark, G. (2015) Control of activity patterns in crowded groups of male noble crayfish *Astacus astacus* (Crustacea, Astacidea) by light regimes: A way to increase the efficiency of crayfish production? *Aquaculture*. Volume 446, Pages 103–110.
- Fraser, D., Weary, D.M., Pajor, E.A. and Milligan, B.N. (1997) A scientific conception of animal welfare that reflects ethical concerns. *Animal Welfare* 6, 187-205.
- Gonzalez, R., Celada, J.D., García, V., Carral, J.M., González, A., Sáez-Royuela, M. (2010) Shelter and lighting in the intensive rearing of juvenile crayfish (*Pacifastacus leniusculus*, Astacidae) from the onset of exogenous feeding. *Aquaculture Research*, 1-7.
- Magee, B., Elwood, R.W. 2013. Shock avoidance by discrimination learning in the shore crab (*Carcinus maenas*) is consistent with a key criterion for pain. *The Journal of Experimental Biology*, 216, 353-358.
- Prieto-Sagredo, J., Ricalde-Recchia, I., Durán-Lizarraga, M.E., Fanjul-Moles, M.L. 2000. Changes in Hemolymph Glutathione Status After Variation in Photoperiod and Light-irradiance in Crayfish *Procambarus clarkii* and *Procambarus digueti*. *Photochemistry and Photobiology*. 71(4), 487-92.
- Regan, T. (1983), *The Case for Animal Rights*, Berkeley, United States: University of California Press.
- Seemann, U.B., Lorkowski, K., Slater, M.J., Buchholz, F., Buck, B.H. (2015). Growth performance of Noble Crayfish *Astacus astacus* in recirculating aquaculture systems. *Aquaculture International*. DOI: 10.1007/s10499-014-9859-2.
- Thomas, J.R., James, J., Newman, R.C., Riley, W.D., Griffiths, S.W., Cable, J. (2016) The impact of streetlights on an aquatic invasive species: Artificial light at night alters signal crayfish behaviour. *Applied Animal Behaviour Science* 176: 143-149.
- Vervaecke, H., Roelant, E., Hendrycks, W., Abeel, T., Vermeersch, X., Aerts, S. (2015). Pain perception in crayfish (*Astacus astacus*): empirical observations and ethical consequences. In: *Know your food. Food ethics and innovation*. Wageningen, The Netherlands: Wageningen Academic Publishers, 157-162.
- Yue, S. (2008). An HSUS Report: The Welfare of Crustaceans at Slaughter. HSUS Reports: Farm Industry Impacts on Animals. Paper 4. Available at: [http://animalstudiesrepository.org/hsus\\_reps\\_impacts\\_on\\_animals/4](http://animalstudiesrepository.org/hsus_reps_impacts_on_animals/4)