**Variation in growth, morphology and reproduction of the bearded
goby (*Sufflogobius bibarbatus*) in varying oxygen environments of
northern Benguela**

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Running page head: Vital traits of gobies in varying oxygen environments

ABSTRACT

This paper examines variations in gender-specific body growth, morphology and reproduction of the bearded goby (*Sufflogobius bibarbatus*) across the Namibian shelf, built on previous work on male reproductive modes. The results indicate a spatial variation in the size composition, condition factor and maturation of gobies across the shelf area. Low oxygen ($< 0.5 \text{ ml L}^{-1} \text{ DO}$) did not hinder reproduction in the bearded goby and off Walvis Bay, maturing and mature females and males were found mostly at the outer shelf edge (150-200 m depth). The histological analysis of gonads validates the macroscopic scale applied for assessing maturity, and the mean number of maturing oocytes was $1060 \pm 690 \text{ g}^{-1}$ body weight. Males were smallest in the central area (where oxygen levels are known to be lowest), and relative condition increased latitudinal from north to south. The bearded goby displays clear sexual size dimorphism (males larger than females), and there is evidence that supports previous findings suggesting that males display alternative reproductive tactics. Assumed territorial males were older than assumed sneaker males, and all morphological measures, except eye diameter, were larger (absolutely and relatively), in territorial males compared to sneakers. The morphologic measures of sneakers did not differ from those of females suggesting female mimicry by sneakers. The role of unclassified males was less clear. The findings are discussed in view of environmental variation and behavioural ecology.

Keywords: bearded goby, reproductive behavior, fecundity, fish morphology, fish growth rate, reproduction in hypoxia

1. Introduction

Oxygen is a major limiting environmental factor in aquatic systems (Wu, 2002). The observed expansion of severely hypoxic water masses (here defined as $< 0.5 \text{ mL L}^{-1} \text{ DO}$; 87 mmol kg^{-1}) in many locations worldwide has become a major cause of concern (Diaz and Rosenberg,

2008). Not least because it compresses the habitable area for many marine organisms (Salvanes *et al.*, 2015), and leads to reduced growth and feeding, at least in fishes (Chabot and Claireaux, 2008). A reduction in oxygen may alter behaviour and trophic relations (Ekau *et al.*, 2010, Gallo and Levin, 2016), as well as generating shifts in the abundance and accessibility of commercially exploited species (Gilly *et al.*, 2013, Stramma *et al.*, 2011). Nutrient-rich upwelling areas with high productivity often produce sediments dominated by organic-rich mud (e.g. Compton and Bergh, 2016). Even sediment surface layers show intense decay processes creating hypoxic conditions with high concentrations of hydrogen sulphide and methane. Such environments are inhospitable to most forms of life and those organisms that can survive in these areas tend to be specialists that cope with anoxic or hypoxic conditions (Levin *et al.*, 2009) and sulphide-oxidising chemolithotrophic bacteria (Lavik *et al.*, 2009).

Such harsh environmental conditions are found on the continental shelf off the coast of Namibia, especially in the central regions. Although few organisms survive in the areas dominated by the extreme low oxygen conditions at the seafloor, the bearded goby *Sufflogobius bibarbatus* has recently been found to thrive in these waters (Salvanes *et al.*, 2011, 2015, Utne-Palm *et al.*, 2010). This species has a number of physiological and behavioural adaptations to the environment and effectively transfers “dead-end” resources back into the food chain through the consumption of jellyfish and sulphidic diatomaceous mud (Salvanes *et al.*, 2011, 2015, Utne-Palm *et al.*, 2010; van der Bank *et al.*, 2011). *Sufflogobius bibarbatus* is abundant and forms a major prey for large commercial fish populations (Crawford *et al.*, 1987). Following the decline of clupeid populations in the late 1960s, and their subsequent collapse in the 1980s, higher predators off Namibia have become increasingly dependent on *S. bibarbatus* as prey (Crawford *et al.*, 1987). Yet despite their

abundance and ecological importance, there are no estimates of the population size, spawning biomass and number of recruits of *S. bibrabatus*.

Within a species, males and females may use different tactics to maximise their reproductive output, and different tactics can appear in distinct frequencies (Gross, 1996). Owing to the widespread use of external fertilization in teleosts, sperm competition can shape the reproductive tactics displayed by males in some species (Taborsky, 1994; 1998). Bourgeois male morphs hold territories, build nests and care for eggs, whereas parasitic males undertake sneak fertilizations in the (temporarily) unguarded nests of territorial males (Immler *et al.*, 2004 Taborsky, 1994, Taborsky *et al.*, 2008). Bourgeois, paternal males cover their nest with trails of sperm-containing mucous (Mazzoldi *et al.*, 2005), which release the sperm slowly as the trails dissolve. The mucin is produced in sperm duct glands, also called seminal vesicles (SV; a term used hereafter). The SVs are accessory structures of the testes that vary in size and function according to male reproductive tactic (Drilling and Grober, 2005, Rasotto and Mazzoldi, 2002). In the following, these “morphs” (bourgeois and parasitic males) are referred to as assumed territorials and assumed sneakers, respectively, in addition to a third unclassified category. As assumed sneaker males are smaller (Seivåg *et al.*, 2016, Utne-Palm *et al.*, 2013) and are not capable of defending and keeping their own nests; they will benefit from traits that give them access to the territorials’ nests. Sneakers can thus benefit from being inconspicuous, or by resembling females morphologically and behaviourally, i.e. “female mimicry” (Dominey, 1980, Gross, 1996, Morris, 1952, Ruchon *et al.*, 1995, Taborsky, 1994). Female mimicry occurs in several goby species, including the black goby (*Gobius niger*) and the blue-banded goby (*Lythrypnus dalli*) (Drilling and Grober, 2005, Mazzoldi and Rasotto, 2002).

While territorial males tend to invest energy into body size and defence behaviour, sneaker males mostly allocate resources into developing gonads (Schütz *et al.*, 2010, Stockley

et al., 1997). Territory defense, nest building, and the guarding of eggs are energetically costly and are therefore more likely to be expressed by large individuals, which possess the necessary resources (see e.g. overview for fish by Drilling and Grober, 2005 [Table 3]). Consequently, the adoption of a certain reproductive behavior, if not strictly genetically predetermined, has been linked to body growth and size (Alonzo *et al.*, 2000). Here we study this by analysing size and growth in otoliths. Otoliths are composed of aragonite and a protein matrix and found in the inner ear and used ubiquitously in marine sciences to make inferences about life histories of fishes (e.g. Limburg *et al.*, 2014).

Previous studies on growth and reproduction have primarily been based on samples collected off southern Namibia (Melo and Le Clus, 2005), or they have focused on the reproductive biology of animals from the central shelf area (Seivåg *et al.*, 2016, Utne-Palm *et al.*, 2013). This paper presents basic life-history parameters for *Sufflogobius bibarbatus* collected from across the entire Namibian shelf area. Growth, morphology and reproduction are studied using a combination of histological and macroscopic examinations of the gonads, ageing using otoliths and morphological measurements. Spatial variation in body and organ size metrics and maturity stages and how these vary with ambient oxygen levels and temperature are evaluated. For males the relative investment into seminal vesicles and testes and variation in morphologic measurements are used as indicators for alternative reproductive tactics. Thus, our work represents an extensive expansion of a previous study of reproduction of male gobies (Seivåg *et al.* 2016).

2. MATERIALS AND METHODS

2.1 Study site and data overview

All frozen samples of the bearded goby were collected off northern, central and southern Namibia during 2008 (RV *G.O. Sars*) and 2012 (Commercial Vessel *Blue Sea*) (Figure 1,

Tables 1 and 2), while fresh samples for histology were collected by RV *Dr. Fridtjof Nansen* (October 2011 and February 2012).

Sampling during 2008 occurred between 31 March and 10 April, across the central shelf (23.33°S – 14.71°E to 23.66°S – 13.25°E), at the (presumed) end of the reproductive season for this species (Melo and Le Clus, 2005). Fish were collected using a combination of bottom trawls (14.5 m wide, 4.5 m high) and pelagic trawls (16 m wide, 12 m high); the latter fitted with a multi-sampler having three cod-ends that could be opened and closed at preferred depths. A total of 685 males and 504 females were analysed; all fish being frozen before processing. Salinity, temperature and dissolved oxygen (DO) profiles were determined immediately after each trawl operation and on the same site as the trawl using a SBE 9 PLUS CTD supplied with a calibrated SBE 43 oxygen sensor. The concentration of dissolved oxygen in the bottom water (here defined from samples collected at approximately 5 m above the seabed) varied between 0.10 – 0.52 ml L⁻¹ DO (1.7 – 8.7 % at 13°C). These data were subdivided by trawl location into “inner” 10 km offshore (50 – 100 m depth), “middle” 35 km offshore (100 – 150 m depth) and “outer” 60 km offshore (150 – 200 m depth) strata.

A second set of samples (Table 2) was collected as by-catch from the 2012 Namibian hake survey using the commercial vessel *Blue Sea* (13 January – 21 February) covering the entire Namibian shelf and slope area (28.67°S – 16.21°E to 18.71°S – 11.95°E). A total of 610 randomly selected males and 338 females were processed from an area of which bottom oxygen concentrations varied between 0.49 and 1.93 ml L⁻¹ DO (8.3 – 32.6 % oxygen saturation at 13°C). The oxygen data were collected using a calibrated Seabird 911plus CTD-O instrument. This survey included samples from severely hypoxic areas in the north (17 – 21 °S) and more oxygenated areas in the southern area (25 – 29 °S, Figure 1) (c.f. Bartholomae and van der Plas, 2007).

A third set of samples (not shown in Tables 1 & 2) was collected for histological purposes in October 2011 and February 2012 using the RV *Dr. Fridtjof Nansen*. One station (26.80°S 14.88°E) was sampled on 27 February (mid reproductive season) in severely hypoxic water (0.07 ml L⁻¹ DO; 1.2 % oxygen saturation at 13°C). Two other stations (25.97°S 14.68°E and 25.99°S 14.49°E) were sampled on 9 October (early reproductive season) in low-oxygen conditions (2.40 and 3.40 ml L⁻¹ DO; 40.5 and 57.4 % oxygen saturation at 13°C, respectively). Respective totals of 91 and 25 males and 76 and 14 females were fixed using phosphate-buffered 10% formalin (Bancroft and Stevens, 1996). A limited number of samples were also frozen and used for exploring size distributions, condition, growth and reproduction. Totally, 22 females were examined histologically in this paper and 24 males were examined histologically and described in Seivåg *et al.* (2016).

To examine morphological differences between assumed territorial, assumed sneaker and unclassified males, and females, 174 males and 113 females were examined. Specimens were caught at 180 m depth during the day, from the outer areas of the cross-shelf transect conducted in 2008 (Stations 76 and 83 of Table 1). The males were classified to tactic using the method described in Seivåg *et al.* (2016). The new data on reproductive tactic were combined with the previously published data (Fig. 3 in Seivåg *et al.*, 2016) with permission from the publisher.

For ageing analyses, random subsets (regarding spatial and temporal origin) of otoliths of males with assumed territorial and sneaker tactics were examined.

The distribution of bottom oxygen concentrations shown in Figure 1 represent the gridded average for all the hake summer (January – February) surveys, spanning the period 1990 – 2012.

2.2. Dissections

The sex of all dissected fish was determined by examining the shape of the urogenital papillae, following Melo and Le Clus (2005). Total and standard lengths were measured (mm). For individuals used for morphological measures, the eye diameter, head width, body height, length of urogenital papilla and both the length and depth of pectoral and caudal fins were measured (0.1 mm), and wet weight determined (0.1 g): (Table 3).

Fish were cut open on their ventral side and the gastrointestinal organs and swim bladder were removed. In the case of males, the testes and seminal vesicles were staged macroscopically (following the criteria outlined below), dissected and weighed separately to the nearest 0.00001 g (Sartorius Genuis ME 235S). Gonads were fixed in 10% neutralised formalin solution. In the case of females, the ovaries were dissected and weighed to the closest 0.00001 g, prior to fixation.

2.3. Macroscopic gonad maturity staging

Macroscopic maturity stages were based on visual characteristics of the gonads (Table 4). Relative investment into egg and sperm production was estimated using the gonadosomatic index (GSI). For males, relative investment into seminal vesicles was represented by the seminal vesicle-somatic index (SVSI), as outlined in Seivåg *et al.* (2016).

$$GSI = \frac{100 \times W_{SV,wet}}{W_{gonadfree}} \quad SVSI = \frac{100 \times W_{gonad}}{W_{gonadfree}} \quad [1]$$

Where W_{gonad} refers to the wet weight of the ovary or testes, and $W_{gonadfree}$ refers to the individual weight excluding gonads (both sexes) and seminal vesicles (males).

In individual males with prominent gonads, the allocation of resources into reproductive tissue was not directed equally into seminal vesicles and testes, indicating the presence of both territorial and sneaker males. Using the criteria defined in Seivåg *et al.* (2016) males investing relatively heavily into seminal vesicles (SVSI > 0.75) or testes (GSI > 0.60) were classified as assumed territorials and assumed sneakers, respectively.

2.4. Histology of gonads

Gonads were preserved to microscopically quantify fecundity (number of developing oocytes), and to evaluate if the corresponding macroscopic maturity stage used in the field was accurate. In the case of males, histology was also further used to evaluate the utility of macroscopic maturity staging as determined using a simple stereological approach, i.e. estimation of volume fraction (3D) of various sperm cells, which theoretically equals the corresponding measured area fraction (2D) in histological slides, cf. “Delesse principle”. This principle was employed for the first time on fish testes by Seivåg *et al.* (2016), studying the present species, but has in several cases been used on female gonads (e.g., Korta *et al.*, 2010, Kurita and Kjesbu, 2009).

The two lobes of the gonad were separated, examining thereafter one, randomly selected lobe in detail: initial serial sectioning indicated that the studied reproductive organs of males and females were homogeneous in structure. The tissue samples were dehydrated in progressing series of ethanol, embedded in Technovit® 7100 (2-hydroxyethyl methacrylate) and sectioned longitudinally. The 4-µm thick sections (n = 3 per organ, spaced 40 µm apart to avoid registering cells twice) were stained with 2% toluidine blue and 1% sodium tetraborate, prior to image analysis (volume fraction). Due to the homogenous nature, three slides from each organ (start, middle and end of organ) were considered sufficient. Studies on female gonads were complemented with an additional analysis of oocyte size and shape to estimate

fecundity using advanced oocyte packing density theory (Korta *et al.*, 2010, Kurita and Kjesbu, 2009), as outlined below.

2.4.1. Microscopic gonad maturity staging

Details (and data) of the staging procedure employed for males are provided in Seivåg *et al.*, (2016). Although data for spermatids and spermatozoa were pooled, the presence of mature spermatozoa was specially noted. Phases of sperm cell and seminal vesicle development were recorded and divided into three major stages (Table 5). For females, six stages of oocyte development were identified (Table 5), based on three females of each maturity stage 1, 2, 3 and 5, but only one of stage 4.

Assessment of fecundity

Oocyte production was detailed by stage according to methodology in Kurita and Kjesbu (2009):

$$\log(OPD_i) = \log[VV_i \times (1/\rho_o) \times \{(1+k)^{3/8} \times k\}] + 12.28 - 3 \times \log(ODV_i) \quad [2]$$

where OPD_i is oocyte packing density for each oocyte stage i , VV_i is corresponding volume fraction, ρ_o is ovarian specific gravity, k is the ratio of short and long axes of stage i oocytes and ODV_i the resulting volume-based mean oocyte diameter (μm), and, finally, the (absolute) number of oocytes in each stage in every gonad given as:

$$\text{Oocyte number} = OPD \text{ g}^{-1} \times \text{Gonad weight (g)}, \quad [3]$$

and the corresponding relative number as:

$$\text{Relative oocyte number} = \text{Oocyte number} / \text{Gutted body weight (g)} \quad [4]$$

Fecundity of each gonad was then calculated for sexually mature females as the sum of all developing oocyte stages found in the gonad, i.e. excluding previtellogenic oocyte stages (PVO 1 and 2 stages) (“reservoir”) but including the immediately following stage of cortical alveoli oocytes (and subsequent stages up to spawning).

2.5. Age and growth

In this study, only otoliths from males assigned to the two assumed morphs were used for age and growth estimations. Whole sagittal otoliths were soaked for 24 hours in freshwater, arranged on black plastic slides (in water) with the distal side up and viewed using a Leica dissecting microscope MZ9.5 at a variety of magnifications. Dozens of otoliths were viewed to get familiar with distinct features and the regularity of opaque and translucent increments, before actual data collection began. For the latter, increments of 171 male individuals were counted directly under the microscope. This procedure was corroborated by annotating increments on pictures of the otoliths taken with digital camera DS-Fi1 (Nikon), associated software NIS Elements F v2.3 (Nikon) and ImageJ (Rasband, 2013). Additionally, a confidence level describing the readability of the respective otolith on a scale from 1 - 4 was recorded:

- **Confidence 1:** Otolith very easy to read; clear structure patterns.
- **Confidence 2:** Otolith relatively easy to read; some interpretation of structure patterns necessary.
- **Confidence 3:** Otolith difficult to read; unclear structure patterns.

- **Confidence 4:** Reading almost impossible; otolith either very translucent or very opaque.

In order to validate fish age, knowledge of seasonality of otolith zone formation is important (Høie *et al.*, 2009). Therefore, the otolith edge type (opaque or translucent) was assessed and related to sampling time. As all otoliths analysed here and elsewhere (Christiansen *et al.*, unpublished) cover samples collected in the months January, February, April, September, and October, year-round coverage is still lacking. The available data in conjunction with results from Melo and Le Clus (2005, Table 2), however, strongly suggest that translucent zones are formed during the austral autumn/winter (April – July), while opaque otolith deposition occurs over the rest of the year mostly, which leads to opaque edges being recorded in fish sampled during that time of year. It is therefore assumed that one translucent zone approximates one year of age. Only otoliths with confidence levels 1 or 2 were eventually used for growth modelling using von Bertalanffy functions growth functions (Beverton, 1954; and Beverton and Holt, 1957):

$$E[L_t] = L_{\infty} \left(1 - e^{-K(t - t_0)} \right) \quad [5]$$

where $E[L_t]$ is the expected total length at time or age t , L_{∞} is the asymptotic average length, K is the growth rate coefficient and t_0 represents estimated length at time 0. The latter was significantly estimated ($p = 0.04$) from all data pooled by non-linear regression as $t_0 = -1.34$ and this value was used for both sneaker and territorial growth models.

2.6. Statistical analyses

Data were analyzed using R version 3.3.1 (R Core Team 2016, <http://www.r-project.org/>) and accompanying open source interface Rstudio v0.99.902 (RStudio 2016). Oxygen levels, mean

fecundity and mean relative fecundity across the central shelf transect and between northern, central and southern subareas were compared using linear modeling, assuming a normal distribution. A binary logistic regression (glm) was used to analyse the influence of oxygen levels on the probability of catching mature females. Differences in morphological measures between the three male morphs (assumed sneakers and territorials, and unclassified males) and females relative to their standard length were tested using a linear model (lm) on arcsine transformed data. Tukey multiple comparison tests were used to test for pair-wise differences.

The relationship between total and standard length (respectively L_t , L_s) was modelled through linear regression, thereby allowing the estimation of any missing data. Total length was subsequently visualized using empirical cumulative density functions as implemented in R package “ggplot2” (Wickham, 2009; function `stat_ecdf`) with the aid of R package “plyr” (Wickham, 2011) and R package “scales”. Such cumulative density plots were proposed earlier as useful tools to visualize potential differences in condition and size between sample groups (Folkvord *et al.*, 2009). In addition, statistically significant differences were identified using Kolmogorov-Smirnov tests with a significance threshold of $p < 0.05$, using R package `rgr` (Garrett, 2013; function `gx.ks.test`).

Condition was expressed as relative condition (K_{rel}) following Le Cren (1951). To estimate K_{rel} the relationship between length and weight was expressed as

$$W = a \cdot L_t^b \quad [6]$$

where total weight (W) in g is the product of coefficient a and total length in millimetre (L_t), with exponential coefficient b . Coefficients were obtained through linear regression after

logarithmic transformation of Equation [6]. Subsequently, estimated mass was derived and the ratio between estimated (W_{est}) and observed mass (W_{obs}) was calculated, that is $K_{\text{rel}} = W_{\text{obs}}/W_{\text{est}}$. Similarly to length data, K_{rel} between sample groups was visualized with cumulative density functions and tested for significance using Kolmogorov-Smirnov tests ($p < 0.05$). Growth was modelled using non-linear regressions with R package ‘nlstools’ using von Bertalanffy functions as described earlier.

3. RESULTS

The stations in the 2012 samples from the central Namibian shelf were mainly located offshore of the outer samples collected during the 2008.

3.1. Spatial variation in oxygen and temperature

Cross central shelf variation (2008 data): The bottom water across the entire transect was hypoxic/anoxic, and the highest concentrations of oxygen were observed over the outer shelf (60 km offshore, 150-200 m depth; outer vs inner: l_m , $t = 28.93$, $p < 0.001$; outer vs mid: l_m , $t = 33.83$, $p < 0.001$; Fig. 2a). The bottom oxygen concentrations did not differ between the inner (10 km offshore; 50 – 99 m depth) and the mid shelves (35 km offshore; 100 – 149 m depth; l_m , $t = 0.07$, $p = 0.99$; Fig. 2a), and in both these areas the oxygen levels were below 0.2 ml L^{-1} DO. There was a cross shelf decrease in bottom temperature from the inner to the outer shelf (Fig. 2c).

North – south variation (2012 data): The bottom oxygen concentrations ranged from anoxia to well-oxygenated levels ($> 1.5 \text{ ml L}^{-1}$ DO), with the southern region being better oxygenated

than the northern and central areas (south vs north: lm, $t = 8.73$, $p < 0.001$; south vs central: lm, $t = 6.73$, $p < 0.001$; Fig. 2b). Concentrations of oxygen did not differ between the northern and central areas (lm, $t = -1.44$, $p = 0.32$, Fig. 2b), though there was a decrease in temperature from north to south (Fig. 2d).

3.2. Distribution of maturity stages

Cross central shelf variation (2008 data): The sex ratio was biased towards males ($M/F=1.65$). Mature females were only found on the outer shelf (stage 4; 8%), and 52% of the females were maturing (stages 2 and 3), and 41% were immature (stage 1). No post-spawning females (stage 5) were found. The population over the outer shelf area (60 km offshore) contained the highest proportion of mature males ($> 28\%$), while the anoxic middle area (35 km offshore) contained the highest proportion of immature males ($> 60\%$). Pelagic samples in all three areas contained high proportions of immature males ($> 60\%$) (Seivåg *et al.* 2016).

Populations over the inner- and mid-shelf areas had the highest percentages of immature females (stage 1; 88%), and the lowest percentages of maturing females (stages 2 and 3). No mature or post-spawning females were observed on the inner- and mid-shelf areas. The highest proportion of mature males was also found over the outer shelf ($>28\%$, but see Seivåg *et al* (2016) for details). More than 60% of males on the mid-shelf were immature (Seivåg *et al* 2016). The stations 60 km offshore were the only ones where assumed sneakers were found on the bottom: the majority of the assumed territorials from the central shelf transect were also found here.

North –south variation (2012 data): The sex ratio was also biased towards males ($M/F = 2.12$). Over 50% of the females in all three areas were immature (stage 1), especially in the north

(68%). Maturing stages (stage 2 and 3) were relatively uncommon in the north (9%) but increased in proportional abundance in the central (31%) and southern areas (38%). None of the females were mature (stage 4) in the South. Some mature females were found in both the northern (9%) and central areas (14%). Post-spawning females were mainly found in the north (14%).

Probability of maturation: Oxygen concentrations had a positive effect on the probability of maturation in females in the northern (glm; $z = 2.11$; $p=0.035$) and central (glm; $z = 3.81$; $p < 0.001$) areas of the Namibian shelf, but not in the south (glm; $z= 0.64$; $p=0.52$). The pooled 2008 and 2012 data show similar results, and more than 50% of females were mature at 2 ml L⁻¹ DO (glm; $z = 2.54$; $p = 0.01$). Waters containing low levels of oxygen (down to 0.2 ml L⁻¹ DO) did not appear to limit reproduction in the bearded goby, since mature females were found in small percentages even in waters with 0.2 ml L⁻¹ DO. This was especially apparent over the central shelf both in 2008 (0.1 – 0.5 ml L⁻¹ DO) and 2012 (0.5 – 1.3 ml L⁻¹ DO). The probability of finding mature females in the samples increased from 10% at 0.2 ml L⁻¹ DO, and reached 98% at 0.8 ml L⁻¹ DO. Similar results were obtained for the northern subarea (0.5 – 1.0 ml L⁻¹ DO), where the probability of maturation increased from 22% (0.5 ml L⁻¹ DO) to more than 60% at 1.0 ml L⁻¹ DO. The data did not show any effect of oxygen concentration on the probability of finding mature females in the south (0.07– 2.0 ml L⁻¹ DO).

For males, a visual evaluation of the pooled data in Seivåg *et al.* (2016) showed that the proportion of mature males was relatively high in many of the samples collected at depths of between 100-190 m, compared to deeper and shallower samples. Large proportions of immature males were found in low oxygen conditions of <0.5 ml L⁻¹ DO. While a larger proportion of

mature and maturing males was found in more oxygenated waters ($>1 \text{ ml L}^{-1} \text{ DO}$), no apparent differences in maturity proportions were found in the different subareas.

Gonado-somatic index (GSI). The GSI of females increased with bottom depth (lme; $F_{1,14} = 10.49$; $p = 0.006$), while oxygen concentration had no effect on GSI (lme; $F_{1,15} = 1.99$; $p = 0.18$). Fifty percent of the females were mature at 0.57% GSI (lmer; $z = 8.91$; $p < 0.001$) and for condition 0.88 g m^{-3} (lmer; $z = 4.17$; $p < 0.001$). The GSI for males is described in detail by Seivåg *et al.* (2016), who report that both male GSI and SVSI increased with bottom depth and bottom oxygen concentration from 0.07 to $3.40 \text{ ml L}^{-1} \text{ DO}$.

3.3. Histology

Oocyte numbers: For each maturity stage there was a dominant type of oocytes, which was coherent with the macroscopic scale. With respect to oocyte numerical production standardized by body size, there was no significant difference in the abundance of early (PVO1) and late previtellogenic oocytes (PVO2), cortical alveoli oocytes (CA) or oocytes with yolk (Y) (see Table 5 for definition of oocyte stages) between females collected in the central ($Y=1060 \text{ g}^{-1} \text{ body weight}$) and southern ($Y=690 \text{ g}^{-1} \text{ body weight}$) shelf areas (t-test: $p \geq 0.321$, Fig. 3a). PVO1 were clearly more frequent than PVO2 both within the central and south area (t-test: $p < 0.001$), while the latter stage and subsequent stages being more equal in numbers (Fig. 3a). PVO2 were not detected in some stage-4 individuals.

Fecundity increased from CA to Y, but not significantly so (t-test: $p \geq 0.233$, Fig. 3b). However, M (migratory nucleus, defined in Table 5) were significantly fewer than Y, tested statistically on ovaries from the central shelf area (t-test: $p = 0.010$). The single M point for the southern shelf area indicated a similar drop in fecundity (Fig. 3b).

410

411 *Oocyte development:* The diameter of PVO2 oocytes were larger in the south (mean diameter
412 = 156.7 μm), than in the central area (mean diameter = 99.9 μm) (t-test: $p = 0.033$), stage-
413 specific oocyte (PVO1, CA and Y) diameters were similar in the central and southern shelf
414 areas (t-test: $p \geq 0.155$) (Fig. 3c). Oocyte size increased gradually from *c.* 75 μm for PVO1 to
415 *c.* 500 μm for M (Fig. 3c). PVO2 and Y clearly overlapped in this regard, i.e. no clear size
416 gap (hiatus) existed between these two oocyte stages (Fig. 4).

417

418 *Males:* Owing to the different patterns of resource allocation to gonads of assumed territorials
419 and sneakers, it was expected that “true” maturity would best be determined microscopically
420 through a combination of the estimates of lumina volume fraction (in SV) and stage III cell
421 volume fraction (in testes), as suggested by Seivåg *et al.* (2016). Using these criteria, some
422 macroscopically assessed Stage 2-(maturing) and Stage 3-(mature) individuals were found to
423 be microscopically similar (Fig. 5). However, Seivåg *et al.* (2016) showed that this
424 macroscopic key generally reflects histological findings.

425

426 **3.4. Spatial variation in length and condition**

427 Total length (L_t) and relative condition (K_{rel}) were compared across sampling regions, males
428 and females, and male reproductive tactic. All available data, except for 14 significant outliers
429 (t-test on lm with Bonferroni correction, $p < 0.05$), were used for these analyses: $N = 2216$
430 individuals. The subset size per sex and area ranged from $N = 82$ to $N = 909$ (Figs. 6 and 7),
431 with the subsets from the central area having largest N (Table 1). Female gobies from the
432 northern area were smaller than females from the central and southern areas, and those in the

central area were largest ($p < 0.001$; Table 6; Fig. 6a). Male gobies were largest in the south and smallest in the central area ($p < 0.001$; Table 6; Fig. 6b). Overall, male gobies (93.10 ± 19.27 mm) were larger than females (80.64 ± 11.11 mm) ($p < 0.001$; Table 6; Fig. 6c).

There was a significant latitudinal trend of increasing condition factor southwards in both sexes, with fish from the northern sampling area having the lowest condition (Table 6; Figs. 7a, b). Females showed only a slightly higher overall condition than males (Table 6; Fig. 7c).

3.5. Alternative reproductive tactics in males and reproductive investments

Within individual males, we expected the allocation of resources into reproductive tissue to be directed towards either seminal vesicles or testes, indicating a presence of territorial and sneaker males, following the criteria outlined by Seivåg *et al.* (2016). Assumed sneaker males were smaller than assumed territorials and unclassified males (Table 6; Fig. 6d), and males with an unclassified mating tactic were smaller than assumed territorials (Table 6; Fig. 6d). Assumed sneaker males had a higher condition than both unclassified and assumed territorial males (Table 6; Fig. 7d), and even higher than females ($D = 0.361$, $p < 0.001$; comparison not shown). Assumed territorial and unclassified males, by contrast, did not differ in condition (Table 6; Fig. 7d). However, when using the relative investment into seminal vesicles and testes as a criterion for reproductive tactic, we found that most of the males could not be classified as either sneakers or territorials (Fig. 8). Based on the extremes in this L-shaped scatterplot, Seivåg *et al.* (2016) classified individuals that stood out as having a high investment into seminal vesicles ($SVSI > 0.75$) or testes ($GSI > 0.60$) as assumed territorials and sneakers, respectively. Individuals with a high GSI were predominantly less than 100 mm TL. The males with the highest SVSI, on the other hand, were generally larger than 100 mm TL. Applying these limits to the combined data (Seivåg *et al.*, 2016 and the new data shown in

Tables 1 and 2: Fig. 8), the number of assumed territorials was, for both sets of data, substantially higher than the number of assumed sneakers, though the clear majority of the males remained unclassified.

3.6. Morphology of alternative reproductive tactics and females

All unstandardized morphological measurements of individuals classified as territorials were larger than those of sneakers, unclassified males and females (Fig. 9). With the exception of relative eye diameter, this was also consistent on a relative scale: relative eye diameter being smaller in territorials than in sneakers ($t = -6.23$, $p < 0.001$), females ($t = -8.09$, $p < 0.001$), and unclassified males ($t = -3.49$, $t = 0.003$). The relative length of the urogenital papilla did not differ between territorial and sneaker males ($t = 0.96$, $p = 0.76$), but it was longer in territorials ($t = 5.75$, $p < 0.001$) and sneakers ($t = 2.63$, $p = 0.04$) compared to unclassified males.

The relative head width of territorial males was larger than that of sneakers (lm , $t = 2.87$, $p = 0.02$), and the relative body height was larger for territorials than for sneakers ($t = 4.86$, $p < 0.001$), females ($t = 5.66$, $p < 0.001$), and unclassified males ($t = 4.85$, $p < 0.001$). The relative pectoral fin depth and fin length were larger in territorials than in sneakers (depth: $t = 4.76$, $p < 0.001$; length: $t = 3.15$, $p < 0.01$), females (depth: $t = 4.50$, $p < 0.001$; length: $t = 4.45$, $p < 0.001$), and unclassified males (depth: $t = 3.75$, $p = 0.001$; length: $t = 3.27$, $p = 0.006$). The relative caudal depth was also larger in territorials than in sneakers ($t = 5.65$, $p < 0.001$), females ($t = 7.64$, $p < 0.001$), and unclassified males ($t = 6.62$, $p < 0.001$).

All measurements relative to standard length of sneaker males were similar to the measurements of females, except the head width and the length of the urogenital papilla (Fig.

9). Sneakers had smaller relative head widths than females ($t = -2.84$, $p = 0.02$), and longer urogenital papillae ($t = 7.84$, $p < 0.001$).

3.7. Age and growth

While most whole otoliths showed a clear increment pattern, some otoliths proved difficult to read. Thus, only the data from high confidence readings (confidence 1 and 2; sneakers 19 out of 29, territorials 21 out of 44) have been used to infer the approximate age and growth functions of gobies. In this study, we focus on the difference between assumed sneaker and territorial males, with all but one sneaker male being younger than territorial males. Generally, the age estimates of sneakers ranged from 1 – 5 years, whilst territorials were aged between 5 – 7 years (Fig. 10). Sneaker males showed a different growth pattern than territorials, reaching a lower asymptotic average length ($L_{\infty} = 99.9 \pm 9.6$ mm) than territorials ($L_{\infty} = 158.2 \pm 25.7$ mm). Sneakers also appear to have higher growth rates ($K = 0.397 \pm 0.096$ yrs⁻¹ sneakers, $K = 0.205 \pm 0.075$ yrs⁻¹ territorials; Fig. 10). That said, caution should be exercised in the interpretation of the comparative von Bertalanffy parameters as the data ranges are almost non-overlapping owing to the younger age of sneakers than assumed territorials.

4. DISCUSSION

The bearded goby belongs to a family of fish (Gobiidae) that shows several consistent and characterizing features. Members of this family occupy diverse ecological niches facilitated by their extensive adaptations (Tornabene *et al.*, 2013, Yamada *et al.*, 2009). Gobies are all generally small, and often display paternal guarding of eggs, which are typically attached within a nest structure on the seabed (Mazzoldi *et al.*, 2012, Miller, 1984.). Preparation for

reproduction in gobies, as Miller (1984) has described, starts with the male choosing a territory and making a nest, often on a rather smooth and sheltered surface; e.g. on macroalgae, in crevices or under empty bivalves. After completion of nest preparation, courtship behaviour follows and subsequently a female lays her eggs in the nest in a single-layered patch attached to the nest's ceiling or wall. Usually she visits and releases several clutches of eggs in different nests. After all egg clutches are laid, the female's role in reproduction is over and the male takes care of the eggs by ventilating them as well as defending the nest and surrounding near area against intruders (Miller, 1984). Thus, females spend energy only on egg production and courtship, while males are additionally responsible for choosing a nest site in environments that allow him to ventilate and protect the egg clutch over several days without leaving the seabed. Skrypzeck *et al.* (2014) have shown that a batch of *Sufflogobius bibarbatus* eggs took seven days from fertilization until hatching at 19 °C, but it is expected to take longer at 13 °C: the temperature at the seabed where mature gobies were collected. The present study has not studied the reproductive behavior of the bearded goby *in situ*, but has utilised absolute and relative measures of the ovaries, testes and seminal vesicles from field collections to make inferences about reproductive biology.

This study has shown that while there is a spatial variation in bottom oxygen levels and temperature across the Namibian shelf, low oxygen ($< 0.5 \text{ ml L}^{-1} \text{ DO}$) did not hinder reproduction in the bearded goby, though maturing and mature individuals were found mostly at the outer shelf edge (150-200 m depth) along a transect off Walvis Bay. The mean number of maturing oocytes was $1060 \pm 690 \text{ g}^{-1}$, and no spatial difference in fecundity was found. Individuals were smallest in the north where bottom oxygen concentrations were lowest and there was a latitudinal trend of increasing relative condition of southwards. The data also demonstrate sexual size dimorphism with males larger than females, and they support the findings of Seivåg *et al.* (2016), who report alternative reproductive tactics in males. Our new

data suggest that the alternative reproductive tactics are possibly associated with differences in morphology. Assumed territorial males were larger and older than assumed sneaker males, and all morphological measures, except eye diameter, were larger both absolutely and relatively, in territorial than sneaker males. The data also show that assumed sneaker size and morphology were similar to females. There was also a large group of males that could not be classified to any tactic; they were smaller than assumed territorials, but had similar condition.

4.1. Bottom oxygen and reproduction

Previous studies in the region have shown that the volume of hypoxic deep waters over the shelf areas fluctuates with warming events (Shannon *et al.*, 1986, Gammelsrød *et al.*, 1998). And with the advection of hypoxic waters from north of the Angola-Benguela-Front (e.g., Boyd *et al.*, 1987, Gammelsrød *et al.*, 1998, Rouault *et al.*, 2007, Rouault, 2012, Shannon *et al.*, 1988). The bearded goby and two of its major predators, hake (*Merluccius* spp) and horse mackerel (*Trachurus trachurus capensis*), were found to distribute themselves differently in relation to warming events (Salvanes *et al.*, 2015), with gobies and hake expanding, and horse mackerel contracting, their distributions. The present study shows that low oxygen waters do not appear to limit the distribution of gobies, and that low oxygen ($< 0.2 \text{ ml L}^{-1} \text{ DO}$) does not limit reproduction, but that it does reduce growth. Size and condition were lowest in the northern and central shelf areas where DO concentrations were lowest (Table 6; Figs, 6 and 7). Previous studies have demonstrated that the bearded goby has a higher physiological tolerance to anoxia than hake (outside the reproductive season: Utne-Palm *et al.*, 2010), and that gobies recover quickly after prolonged exposure to anoxia once returned to more normal seawater (Salvanes *et al.*, 2011, Utne-Palm *et al.*, 2010). Outside the reproductive season, these fish can cope with prolonged exposure to low oxygen (and hydrogen sulphide) due, in part, to their display of diel vertical migration (DVM). That is, fish move from day-time

depths at the severe hypoxic seabed to shallower, more oxygen-rich depths during the night (Utne-Palm *et al.*, 2010). All this said, however, reproduction would appear to occur predominantly at the shelf edge, which is outside of the areas where hypoxia is most severe (see also Seivåg *et al.*, 2016).

The bottom oxygen concentrations were particularly low in the northern and central areas, but varied also across the sampling transect conducted over the central shelf area off Walvis Bay. Here, the most mature individuals were found on the outer shelf where DO was highest, though in 2008 $< 0.5 \text{ ml L}^{-1}$ DO) at 150- 200 m depth. Low oxygen levels were the most important factor limiting spawning activity in territorial males (Seivåg *et al.* 2016). Our data and the data of Seivåg *et al.* (2016) suggest the existence of a spawning site on the outer Namibian shelf in the central region, where the bottom water is hypoxic (oxygen saturation of 6.2–6.7%), but not as severely so as over the middle central shelf area (oxygen saturation of 1.7–2.9%), where no mature males were found. Nevertheless, spawning sites will vary with location, and our data do not cover all inshore and offshore sites of the continental shelf, so future studies will be needed to identify all major spawning sites for the bearded goby. These findings are consistent with observations from studies on the distribution of fish larvae conducted in the mid 1980s by Olivar *et al.* (1992) and Olivar & Shelton (1993). These authors found goby larvae over the entire shelf of the northern Benguela ecosystem for most of the year, with the smallest larvae located in water masses between the 100 and 200 m isobath. Based on this, Olivar *et al.* (1992) and Olivar & Shelton (1993) concluded that the spawning areas of the bearded goby were present in those shelf areas.

Coastal upwelling areas, such as the northern Benguela, are characterized by wind-driven advective transport, which affects the distribution of planktonic eggs and larvae in the shallow Ekman layer (Stenevik *et al.*, 2003). Goby larvae are generally distributed between 20-60 m depth (Olivar *et al.*, 1992) and are thus below the offshore moving Ekman layer

(Stenevik *et al.*, 2003). This is a depth where water masses typically move onshore and/or northwards (c.f. the cross section modelling results shown on Fig 5 of Stenevik *et al.*, 2003). The youngest larvae are typically found on the outer shelf (O'Toole, 1978; Olivar *et al.*, 1992), suggesting perhaps that they spawn there. In the case of the bearded goby, we know that males care for egg clutches (Skrypzeck *et al.*, 2014) and it is not unreasonable to suppose that eggs attached to seabed substrata will suffer lower mortality than shallow planktonic eggs for a fish species that naturally is adapted to low oxygen. As eggs hatch, larvae will become pelagic and be moved onshore by currents (Sundby *et al.*, 2001). Sundby *et al.* (2001) report that the mesopelagic *Merluccius capensis* spawns over the outer shelf area and, based on fieldwork and experiments on the buoyancy of eggs and larvae and on larval behavior. These authors conclude that spawning on the outer shelf is beneficial for larval survival. The European hake (*Merluccius merluccius*) (Alvarez *et al.*, 2001) and the hake (*Merluccius productus*) found in the California Current and the Puget Sound (Bailey 1982), spawn also on outer shelf areas. Sundby *et al.*, (2001) speculate whether the spawning behavior and the physical properties of hake eggs have developed as ecological adaptations to enhance the transport of the spawning products inshore in upwelling regions.

The data presented here show that the bearded goby can grow and invest in gonads under severely hypoxic conditions (0.2 – 1.0 ml L⁻¹ DO). Although some coral-dwelling and freshwater gobies are known to tolerate hypoxia (Nilsson *et al.*, 2004, 2007, Svetovidov, 1964), the bearded goby is the first marine goby that has been found to mature at such extreme and low oxygen concentrations as those found on the Namibian continental shelf. Similar traits have been reported for the Dover sole (*Microstomus pacificus*) in the California upwelling region, where more sexually mature individuals occur in the deeper oxygen minimum zone (OMZ) and in severe hypoxic waters than in shallower and better oxygenated waters (Hunter *et al.*, 1990). The latter authors suggested that the fish's adaptation could be

related to the lower temperatures of the deep than shallow habitats, since this reduces the metabolic rate and oxygen needs (Gallo and Levin, 2016). For the bearded goby, the adaptation is probably both physiological and behavioural, and is made possible by their DVM (Salvanes *et al.* 2011, Utne-Palm *et al.* 2010). The bearded gobies do feed whilst on the seabed during the day, but can digest their food only at night when up in shallower and more oxygenated part of the water column (Utne-Palm *et al.* 2010, Hundt *et al.* 2011). However, in the more oxygen rich spawning area, only the larger mature individuals (60-140 mm) were caught on the bottom, while smaller individuals (< 60 mm) were caught in the pelagos, throughout a 48 h survey (Hunt *et al.*, 2011, Fig. 2). This suggests perhaps that the large, nest-holding males in this area were not leaving the bottom.

4.2. Bottom oxygen and variation in growth and condition

The physiological process of growth in fish depends strongly on temperature, life-history stage and food availability, and reduced concentrations of dissolved oxygen may alter behaviour and trophic relations (Ekau *et al.*, 2010; Gallo and Levin, 2016). In Atlantic cod (*Gadus morhua*), growth is reduced in hypoxic conditions (Chabot and Dutil, 1999), and environmental hypoxia represents a metabolic constraint (Chabot and Claireaux, 2008). There is a latitudinal (north to south) increase in goby condition off Namibia (Fig. 7 a, b) that reflects increasing bottom oxygen concentrations and decreasing temperature (Fig. 2b & d). A comparison of the length frequency distribution of individuals collected across the shelf (Fig 6a & b) indicates that males from the central area were smaller than those from either the north or the south (Figure 6b), suggesting a higher proportion of sneakers in the central subarea. Whilst previous studies have shown that smaller and immature fish mainly occur over the middle and inshore central shelf (Seivåg *et al.*, 2016), the outer central area represents a possible spawning site. If so, and given the relatively high proportion of sneaker

males there, this would reduce the size of males in the central area. Although it could be argued that the result is an artifact of the pooling of samples from the cross-shelf transect in 2008 (which included samples from shallower stations (Table 1) where smaller fish were common), with those from the north south transect in 2012 (Table 2), excluding the shallow samples from the pooled data did not alter the result. This suggests that since the males comprised a higher proportion of small individuals on the central shelf, the sneaker reproductive tactic could be present in higher frequency here.

4.3. Sexual size dimorphism

Sexual size dimorphism (SSD) is common in many organisms, not only teleosts. In some species, females are larger than males, whilst in others the male becomes much larger (Ota *et al.*, 2010, Schütz and Taborsky, 2000). In birds and mammals, it has been suggested that males have evolved to grow larger because sexual selection is stronger than fecundity selection (Blanckenhorn, 2005). In teleosts and invertebrates, however, SSD with larger females is the common pattern (Parker, 1992). Only species with parental care and particularly aggressive male-male competition and/or male territory guarding often show male-biased SSD (Pyron, 1994).

The data presented here clearly demonstrate sexual size dimorphism in the bearded goby, as also reported by Melo and Le Clus, (2005). Female gobies were both smaller and lighter than males, suggesting a slower growth rate in females. The otoliths of females tended to be larger than those of similarly sized males (Melo and Le Clus, 2005, Christiansen *et al.*, unpublished), and females were significantly smaller than males of the same age (Christiansen *et al.*, unpublished).

4.4. Fecundity

Bearded gobies have a low fecundity. The low fecundity is due to their small body size; a similar level of relative fecundities is commonly seen for marine teleosts, i.e. when considering oocyte production per gram body mass (Kjesbu 2016). They have relatively large eggs and spawn repeatedly in batches each year (Melo and Le Clus, 2005). Compared to species from other ecosystems, the reproductive investment is low. Since the eggs are laid on benthic surfaces, and are cared for by territorial males (Skrypzeck *et al.*, 2014), egg mortality is likely to be lower than for pelagic-spawned eggs. Therefore, substantial investment in large eggs and parental care will likely result in comparably larger larvae in good condition that have a higher survival rate in their planktonic phase. O'Toole (1978) reported the larvae of goby and sardine (*Sardinops sagax*) to co-occur in the pelagic implying that competition for food may occur if the latter is in limited supply. Therefore, an investment in large eggs and paternal care will likely result in high-quality larvae that will enjoy an advantage in the plankton. The extended reproductive season and repeated batch spawning is typically an adaptation to intra-annual variability in environmental conditions (Cushing, 1990) – a prominent feature of the Benguela ecosystem (Hutchings *et al.*, 2009).

The relative numbers of smaller oocytes (PVO1) generally exceed those of larger oocytes (Y), implying that the bearded goby has a rich “reservoir” (standing stock) of immature oocytes for subsequent spawning seasons. This is typical of many teleosts (Korta *et al.*, 2010). More important, the M stage was represented with much fewer oocytes than the Y stage, indicating that the species is a batch-spawner producing several batches of eggs during a single spawning season (Kjesbu, 2016). The number of batches actually released per individual requires further study due to a likely body-size dependent batch size (Kjesbu, 2016), and the possibility of *de novo* recruitment of developing oocytes during the length of the spawning season, i.e. so-called indeterminate fecundity (Ganias, 2013). Adopting an

indeterminate strategy seems logical in this fluctuating environment as this enables fast increases in fecundity under improved ambient conditions. Specifically, the existence of this strategy was presently indicated by a significant overlap in PVO2 and Y oocyte frequency distributions, one of several criteria (“lines of evidence”) to address this complex issue (Ganias 2013).

4.5. Reproductive tactics

The L-shaped curve for male investments into testes vs seminal vesicles (Fig. 8), and the pattern of morphological variations of males clearly indicates alternative reproductive tactics in male bearded gobies. Fully mature male gobies were either: (1) small individuals with large GSI in combination with small SVSI (heavy investment in testes), (2) large individuals with small GSI and large SVSI (heavy investment in seminal vesicles) or (3) small and large individuals that have both small GSI and SVSI.

Alternative reproductive tactics can be genetically fixed, be a response to exposure to certain environmental factors in early life, or they can change ontogenetically (Taborsky, 1994). Rasotto and Mazzoldi (2002) report for the black goby (*Gobius niger*) that small males (1-year old) adopt a sneaking tactic, while the larger individuals (3-year old) adopt a parental tactic. The males of intermediate size and age (2-year old) express an intermediate investment in both GSI and SVSI. Rasotto and Mazzoldi (2002) suggest that these males may be too small to gain a nest though competing with larger males, but they are large enough to compete opportunistically for vacated territories. Thus, depending on opportunities they may become either territorials or sneakers. For an ontogenetic shift to take place in bearded gobies, sneaker males would need to exhibit a growth boost around age 4 - 5 in order to reach the size of territorials. Moreover, this would include a dramatic shift from investing in large testes to

investing in large seminal vesicles (Figure 8). In addition, the observed differences in morphology may seem unlikely to be able to be attained by a former sneaker male in one season.

The different growth patterns observed for sneaker and territorial males (Fig. 10) suggest it is unlikely that sneakers will become territorials after they have spent their first years of life as sneakers. Territorial males grew larger but with a smaller growth coefficient K . The data ranges to which the growth models were fitted for sneaker and territorial males are largely non-overlapping, meaning it is difficult to definitively conclude how the growth patterns may change over their complete life cycle. Because sneakers were consistently younger and smaller, it may seem intuitive that these two tactics are consecutive. However, it is important to realize that such a pattern could also result from an increased mortality, or faster senescence, of sneakers. On the other hand, conditional tactic change has been documented in other phylogenetically relatively close species (*Gobius niger*, *Bathygobius fuscus*; Immler *et al.*, 2004; Takegaki *et al.*, 2012), and a genetically fixed strategy in *Sufflogobius bibarbatus*, would be unexpected. Until direct evidence from aquarium experiments is available, it remains unclear whether male bearded gobies can change reproductive tactic, and what factors would trigger such a change.

4.6. Morphology and secondary sex traits

Territorial males of many species, for example the peacock blenny (*Salaria pavo*) and the black goby (*Gobius niger*), invest much into secondary sex traits such as colouration or specifically accentuated body parts in order to attract females (Goncalves *et al.*, 1996, Gross, 1984; Ruchon *et al.*, 1995). For territorial males, parental care and nest defence will be required. Ventilation of the nest provides the eggs with sufficient oxygen, and is conducted

using both pectoral and caudal fins (Jones and Reynolds, 1999; Skolbekken and Palm, 2001). Large fin areas will be beneficial for efficient fanning and will benefit nest owners, but not necessarily sneakers. Further, fish that defend nests, and threaten and chase away intruders would benefit from large body size. Males that engage in male-male competition benefit from an investment in somatic growth, because larger individuals are more likely to win contests (Parker, 1992). The development of morphologies that are beneficial for winning male-male contests is, therefore, expected in territorial males.

In the case of sneaker males, however, access to fertilization does not appear to through large size, but by having a morphology that is similar to females. Such a strategy is not uncommon in organisms competing for fertilization by attempting to gain access to eggs of females that are attracted to territorial males, as it is thought to reduce male aggression (Dominey, 1980). Female mimicry is a well-known phenomenon that has been observed in more than 30 species from 10 teleost families (Taborsky, 1998, Drilling and Grober, 2005, Mazzoldi and Rasotto, 2002, Goncalves *et al.*, 1996, Ruchon *et al.*, 1995).

4.7. Size and condition of unclassified males

Depending on the timing of sampling and of the reproductive season, unclassified males that have small seminal vesicles and small testes could represent individuals that have completed their reproduction, with both testes and seminal vesicles spent. The fact that the urogenital papilla of unclassified males was significantly shorter than that of territorial or sneaker males suggest that the papilla expands and shrinks with reproductive readiness this. Alternatively, unclassified males could represent individuals that have skipped the season's reproduction (skip-spawners), as they had not yet reached their capacity to become territorial. Or, they could be animals in transition between sneaker and territorials. The growth curves for sneaker

and territorial males (Fig. 10) do not support an ontogenetic shift in tactic. The cumulative relative length distribution curve for males (Fig. 6c), however, clearly show bimodality with the first mode reflecting the sneakers and the second mode the territorial males. A similar pattern of bimodality is found for the unclassified males (Fig. 6d). This suggests that unclassified males were a mixture of sneakers and territorials that have already reproduced, or that have not yet reproduced. Furthermore, the cumulative distribution curve of relative condition (Fig. 7d) of the unclassified males is similar to that of the territorials, which could mean there were more territorials than sneakers among the unclassified. However, all measures of morphology (Fig. 9) hint that there could have been more sneakers among the unclassified males since most morphological measures put unclassified individuals between territorial and sneaker morphs, although the variation in the group of unclassified males is larger as well. Hence, it is still not clear what the exact reproductive role or stage of unclassified males is. Future studies to resolve this could for example employ genomic or transcriptomic approaches to uncover shared or differentiated adaptive signals between sneaker, territorial and unclassified males, respectively.

5. CONCLUSION

The results of the present study, when taken together with Seivåg *et al.* (2016) provide some much needed basic life-history parameters of the bearded goby off Namibia. Whilst the data are insufficient for a full exploration of the species' population dynamics, they represent essential knowledge that will be useful for population modelling. Perhaps the biggest surprise for us is the fact that alternative mating tactics are now shown for a species that is distributed over a large and oxygen-poor continental shelf, as these have hitherto been shown only for species in shallow sublittoral areas and in freshwater (e.g. Gobiidae, Miller 1984; Labridae, Uglem *et al.* 2000; Salmonidae, Fleming 1996). We suggest that the reproductive strategy of

nest building and alternative reproductive tactics in males are likely adaptations to reduce egg mortality and to compensate for low, absolute fecundity. The question that then arises though is whether benthic spawning can help retain recruitment over the shelf, as bottom hatched compared to pelagic hatched larvae are less likely to be lost by Ekman transport (Stenevik *et al.*, 2007), due to their prolonged association with the seabed at the egg stage. If so, then this emphasizes the importance of the outer shelf benthic habitat to the breeding success of the bearded goby. Population dynamic modelling of fish populations are typically developed for species that do not display parental care. How parental care reproductive strategies affect recruitment and population dynamic modelling has to be studied further since it has been shown for other species that alternative reproductive tactics can be beneficial by increasing effective population size and decreasing inbreeding (e.g. Perrier *et al.*, 2014).

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FIGURE CAPTIONS

Fig. 1. Distribution of sampling stations for bearded goby (*Sufflogobius bibarbatus*) in the north, central and south Namibian shelf areas in 2008 and 2012. |Green circles refers to the 2008 stations, blue circles to 2012 stations and white squares to stations where samples for histology were collected. The bottom oxygen is the gridded average for all the hake summer (January – February) surveys of 1990 – 2012. Isolines show the 100 m, 150 m and 200 m depth profiles.

Fig. 2. (a) The bottom water (5m above seabed) oxygen concentration (ml L⁻¹ DO) along the cross-shelf transect from inner (50-90 m depth, 10 km off shore), mid (100-149 m depth; 35 km offshore) and outer (150-199 m depth, 60 km offshore) in 2008. (b) The bottom water oxygen concentration (ml L⁻¹ DO) in the north (N), central (C) and south (S) shelf areas on the Namibian continental shelf in 2012.

Fig. 3. Relative oocyte number and corresponding oocyte diameter (both measured as mean $\pm$ SD) at different oocyte stages in central and southern shelf areas. (a) Oocyte production split into “reservoir”, i.e. previtellogenic oocytes (PVO1 and PVO2), and “fecundity” (developing oocytes) (CA, Y and M). (b) Zooming-in on developing oocytes, and (c) growth in oocyte size with oocyte stage. ns: $P > 0.05$; **: $0.01 < P < 0.05$; PVO1: early vitellogenic oocytes; PVO2; late previtellogenic oocytes; CA: cortical alveoli oocytes; Y: oocytes containing yolk (vitellogenin); M: migratory nucleus oocytes. The intermediate phase CA+Y was presently included as Y.

Fig. 4. Oocyte diameter frequency distributions of PVO2 and Y in central and southern shelf areas. Note that Y overlapped with the larger fraction of PVO2. PVO2; late previtellogenic oocytes; Y: oocytes containing yolk (vitellogenin).

Fig. 5. Comparing the macroscopic maturity staging procedures for male *Sufflogobius bibarbatus* against the microscopic maturity determined by stereological analyses. Standard procedure considers testes only, while the new key includes visual examination of seminal vesicles. In both procedures, stages are converted to fit into categories 1=immature/barely maturing, 2=maturing, 3=mature/ripe. Lumina volume fraction represents microscopic maturity of seminal vesicles, while volume fraction of stage III cells represents microscopic maturity of testes.

Fig. 6: Total length (L_t) in mm of *Sufflogobius bibarbatus* caught off Namibia, visualized as cumulative density functions by sampling area for a) females, and b) males, c) by sex (all areas pooled), and d) by reproductive tactic (all areas pooled, but only males). The sampling areas are north (N), central (C), and south (S); the reproductive tactics are sneaker (S), territorial (T), and unclassified (U). Sample sizes (N) are shown in brackets.

Fig. 7: Relative condition (K_{rel}) of *Sufflogobius bibarbatus* caught off Namibia and visualized as cumulative density functions by sampling area for a) females, and b) males, c) by sex (all areas pooled), and d) by reproductive tactic (all areas pooled, but only males). The sampling areas are north (N), central (C), and south (S); the reproductive tactics are sneaker (S), territorial (T), and unclassified (U). Sample sizes (N) are shown in brackets.

Fig. 8. Classification of assumed territorial, assumed sneaker and unclassified *Sufflogobius bibarbatus* males (N = 1296) using relative investment into seminal vesicles and testes as criteria. Modified with permission from Seivåg et al. (2016) where “old” refers to the reused data points.

Fig. 9. Means of morphological measurements of territorial, sneaker, unclassified males and females of *Sufflogobius bibarbatus*. a) Total Weight; b) Standard length; c) Head width; d) Body height ; e) Pectoral fin length; f) Pectoral fin depth; g) Caudal fin length; h) Caudal

1072 fin depth; i) Eye diameter; and j) Urogenital papilla length. All measurements are in mm and
1073 error terms are standard deviations (SD). The sample size consists of 174 males and 113
1074 females from the central area in 2008.

1075 **Fig. 10:** Relation of total length (L_t) and otolith-derived age of *Sufflogobius bibarbatus* males
1076 as modelled with von Bertalanffy growth functions. Age corresponds to the number of opaque
1077 zones which is approximate to years; data has been jittered (small noise introduced) in R to
1078 reduce overlap of the plotting symbols and thus enhance clarity. Males with assumed sneaker
1079 (diamonds, S) and territorial (circles, T) reproductive tactic show different growth patterns
1080 with lower asymptotic average length (L_∞) and higher growth coefficient (K) in sneakers.
1081 Colour of the symbols indicates spatial origin of the samples, i.e., black represents the central,
1082 blue the north, and purple the south area.

Table 1: Overview of data collected in 2008 from cross-shelf transect (23.0-23.5°S); showing the selected zone in this area (“Inner” 10 km offshore (50-99m depth), “Middle” 35 km offshore (100-149m depth) and ”Outer” 60 km offshore (150-200m depth) along with their stations’ number, series, geographical position (decimal degrees), bottom and sampling depth, bottom oxygen and fishing depth oxygen concentration, type of trawl (P is pelagic and B is bottom trawl) used, total number of fish (N), number of females and males for each station. Modified and expanded from Seivåg et al. (2016) with permission.

Zone	Station (series)	Latitude (°S)	Longitude (°E)	Bottom depth (m)	Sampling depth (m)	Bottom oxygen (ml L ⁻¹)	Fishing depth oxygen (ml L ⁻¹)	Trawl	N	F	M
Inner	72 (196)	23.23	14.36	56	56	0.52	0.52	B	52	7	45
Inner	86 (225)	23.25	14.31	82	31	0.11	2.19	P	58	25	33
Inner	86 (226)	23.25	14.3	82	28	0.11	3.83	P	47	14	33
Inner	86 (227)	23.25	14.3	82	34	0.11	1.79	P	64	27	37
Middle	68 (186)	23.31	14.2	118	81	0.17	0.18	P	69	36	33
Middle	68 (187)	23.33	14.2	118	84	0.17	0.17	P	96	36	60
Middle	99 (246)	23.35	14.18	123	123	0.1	0.10	B	82	26	56
Middle	100 (247)	23.35	14.2	121	121	0.14	0.14	B	92	26	66
Outer	73 (197)	23.5	13.71	176	176	0.38	0.38	B	40	19	21
Outer	75 (199)	23.5	13.71	175	175	0.39	0.39	B	149	73	76
Outer	77 (203)	23.51	13.71	177	177	0.38	0.38	B	14	2	12
Outer	104 (255)	23.38	13.96	159	130	0.4	0.73	P	48	13	35
Outer	104 (256)	23.38	13.96	159	96	0.4	1.70	P	45	20	25
Outer	104 (257)	23.36	13.96	159	NA	0.4	0.40	P	46	16	30
Outer	76 (200)	23.00	14.00	176	176	0.39	0.39	B	130	60	70
Outer	83 (216)	23.00	14.00	177	177	0.38	0.38	B	157	104	53
Total									1189	504	685

Table 2: Information about all data collected by bottom trawl from the entire Namibian shelf and slope area (2012 data) showing each subarea, stations' number and their geographical position, bottom depth, bottom oxygen concentration, type of trawl used, time of collection, total number of fish, number of females and males for each station. All stations were made available by the vessel Blue Sea, except for station number 253, which was made available by Dr. Fridtjof Nansen. Modified and expanded from Seivåg et al. (2016) with permission. Geographical positions in decimal degrees.

Zone	Station	Latitude (°S)	Longitude (°E)	Bottom depth (m)	Bottom oxygen (ml L ⁻¹)	N	Females	Males
South shallow	15	27.96	15.53	86	1.93	149	70	79
South deep	53	26	14.46	190	1.2	179	33	146
South (Nansen)	253	26.8	14.88	163	0.07	102	43	59
Central shallow	85	24	14.08	165	0.48	100	35	65
Central deep	86	24	13.84	238	0.71	83	40	43
Central deep	87	24	13.59	272	0.75	20	8	12
Central deep	98	23.67	13.59	228	1.27	55	26	29
North Shallow	179	19.35	12.37	128	0.49	86	14	72
North Shallow	202	17.93	11.61	132	0.68	15	7	8
North deep	215	18.95	11.59	275	0.67	9	5	4
North deep	167	20.19	12.24	280	0.53	117	50	67
North deep	158	20.84	12.42	404	1	33	7	26
Total						948	338	610

Table 3: Parameters measured on individual *Sufflogobius bibarbatus*.

Parameter	Unit	Comment
Total Body Length	mm	nose until end of caudal fin
Standard Body Length	mm	nose until beginning of caudal fin
Eye Diameter	mm	left eye
Head Width	mm	just behind the eyes
Body Height	mm	at the beginning of the dorsal fin
Urogenital Papillae Length	mm	
Pectoral Fin Length	mm	left pectoral fin
Pectoral Fin Depth	mm	left pectoral fin
Caudal Fin Length	mm	
Caudal Fin Depth	mm	
Total Body Weight	g	
Testes Weight	g	
Seminal Vesicle Length	mm	one seminal vesicle measured
Seminal Vesicle Weight	g	
Ovary Weight	g	
Gut-free Weight	g	weight excluding the guts

Table 4. Overview of macroscale for maturity stages for female and male bearded goby (*Sufflogobius bibarbatus*). Male characteristics are from Seivåg et al. (2016) and female characteristics are not published, but presented in Taha (2013).

Stage	Description of visual characteristics
Females:	
1 Immature	Ovaries range from transparent to light yellow. No oocytes visible.
2 Early maturing	Ovaries range from light yellow to light orange with presence of few orange to red dots. No distinct oocytes present.
3 Late maturing	Orange ovaries. Oocytes are clearly seen yet they are small and ovaries are not fully packed.
4 Mature	Orange ovaries. Oocytes are relatively large and packed to the point ovaries are bursting with oocytes. Some oocytes could be lost while extracting this stage.
5 Post spawning	Ovaries are yellowish to orange in colour. Can be easily mistaken with stage 2, however, the ovary is more stretched, empty and can easily be broken when extracted. Sometimes a few developing oocytes were found inside, other times the ovaries were full of liquid.
Males:	
0 Immature	Non-extractable, very elastic, hair-thin testes, which are hard to spot (transparent) and fragile. Seminal vesicles are tiny or appear missing.
1 Barely maturing	Testes are transparent, elastic and slim, and difficult to remove in one piece without stretching and tearing. The seminal vesicles are usually less than $\frac{1}{4}$ of testis length and can be hard to spot.
2 Maturing	Testes are thicker (>1 mm wide), opaquely white in colour; not transparent. They are more brittle than elastic. Either seminal vesicles or testes can be large relative to the other component, but neither dominates the abdominal cavity. The seminal vesicles are no more than $\frac{1}{2}$ of the testis length, but are clearly visible.
3 Mature	Testes are milky-white, somewhat bulging and brittle, but thickness varies. The seminal vesicles are often large, sometimes >0.5 testis length with clear veins. Testes are relatively larger in typical sneaker males, whereas territorials have relatively larger seminal vesicles.

Table 5. Overview of microscale for maturity stages for female and male bearded goby (*Sufflogobius bibarbatus*) obtained from histology. Male characteristics are from Seivåg et al. (2016) and female characteristics are not published, but presented in Taha (2013).

Stage		
Females:	Oocyte development	
Early previtellogenic oocyte (PVO1)	In this oocyte stage no yolk is present and the cytoplasm is homogenous; dark blue in color.	
Late previtellogenic oocyte (PVO2)	This oocyte shows different coloration in the cytoplasm; dark around the nucleus and light at the periphery.	
Cortical alveoli (CA)	The cortical alveoli start to appear in the cytoplasm as small white dots scattered in the cytoplasm.	
Cortical alveoli and yolk (CA & Y)	The protein yolk starts to appear in the cytoplasm as very small light green dots, while the cortical alveoli are pushed to the peripheral of the cytoplasm. The presence of yolk means the oocyte is starting to mature, i.e. vitellogenic oocyte.	
Yolk	Here the yolk fills most of the cytoplasm and the oocyte is large compared to the previous types.	
Migratory nucleus (M)	Same characteristics as the yolk oocyte but the nucleus is starting to migrate to the periphery of the oocyte. The nucleus has a crescent shape and the cytoplasm around the nucleus is different from the rest of the cytoplasm.	
Atresia	A process of degradation and reabsorption that some developing oocytes go through (Kjesbu, 1991).	
Males:	Sperm cell development	Seminal vesicle developments
I	Early spermatogonia	Thin lobule wall including epithelium
II	Late spermatogonia and spermatocytes	Mucin-filled lumina
III	Spermatids and spermatozoa	Mucus-producing cells (Fig. 2).

Table 6. Variation in mean total length (L_t) and relative condition (K_{rel}) of female (F) and male (M) *Sufflogobius bibarbatus* by sampling areas off Namibia (Central (C) North (N) and South (S)), by sex (all areas pooled) and by reproductive tactic (repr.type; all areas pooled, but only males; sneaker (S), territorial (T) and unclassified (U) tactic). Sample size (N), mean and standard deviation (SD) are given. Differences within each group (by area, females; by area, males; by sex; by reproductive tactic) were tested using Kolmogorov-Smirnov tests with $P < 0.05$ as significance threshold (marked in **bold**). The Bonferroni adjustments are used to account for multiple comparisons with $\alpha = 0.017$.

Sex	Area		N	$L_t \pm SD$ (mm)	Test statistic	$K_{rel} \pm SD$	Test statistic
F	C		545	81.62 ± 10.93	C vs. N, D = 0.31, p < 0.001	1.01 ± 0.09	C vs. N, D = 0.36, p < 0.001
F	N		82	76.57 ± 15.21	N vs. S, D = 0.35, p < 0.001	0.94 ± 0.10	N vs. S, D = 0.59, p < 0.001
F	S		144	79.48 ± 7.96	C vs. S, D = 0.13, p = 0.038	1.06 ± 0.09	C vs. S, D = 0.31, p < 0.001
M	C		909	90.09 ± 17.25	C vs. N, D = 0.28, p < 0.001	0.99 ± 0.09	C vs. N, D = 0.29, p < 0.001
M	N		177	94.22 ± 23.16	N vs. S, D = 0.19, p < 0.001	0.93 ± 0.09	N vs. S, D = 0.56, p < 0.001
M	S		359	100.28 ± 20.04	C vs. S, D = 0.28, p < 0.001	1.05 ± 0.08	C vs. S, D = 0.32, p < 0.001
F			771	80.64 ± 11.11	F vs. M, D = 0.33, p < 0.001	1.02 ± 0.10	F vs. M, D = 0.10, p < 0.001
M			1445	93.10 ± 19.27		1.00 ± 0.10	
		Tactic					
M		Assumed sneaker	45	82.51 ± 10.75	S vs. T, D = 0.93, p < 0.001	1.08 ± 0.08	S vs. T, D = 0.47, p < 0.001
M		assumed territorial	130	122.18 ± 9.01	T vs. U, D = 0.74, p < 0.001	0.98 ± 0.10	T vs. U, D = 0.07, p = 0.571
M		Unclassified	1218	91.28 ± 17.33	S vs. U, D = 0.23, p = 0.024	1.00 ± 0.10	S vs. U, D = 0.43, p < 0.001