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An insight into the reproductive biology of the bearded goby *Sufflogobius bibarbatus*

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Abstract
Preliminary results obtained from histological analyses of the male reproductive organs, supplemented with field and behavioural data, indicate that *Sufflogobius bibarbatus*, a small, slow growing gobiid exhibiting low fecundity, which plays an important role in the food web off Namibia, where large areas of the shelf are hypoxic, spawns demersally. Large males defend benthic nests, possibly at the edge of the hypoxic shelf. Male reproductive strategy appears to be flexible, and tentative evidence to suggest that polygyny and sneaking may also occur is presented.

The Namibian shelf is dominated by organic-rich mud where intense decay processes create a surface sediment with high concentrations of hydrogen sulphide and methane (Lavik *et al* ., 2009). Hypoxic conditions (<1 μM dissolved oxygen) cover >50% of the shelf area (Lavik *et al*., 2009).

The goby *Sufflogobius bibarbatus* (von Bonde 1923) occurs across the shelf off south-west Africa, and is found from Angola to South Africa, being most common off central Namibia (Cruickshank *et al* ., 1980). *Sufflogobius bibarbatus* is a slow growing species (13 cm standard length at 6 years) with late sexual maturation (2 – 3 years) and low fecundity (Melo & le Clus, 2005). Despite its low productivity and the hostile environment it inhabits, it is very successful in the region and has assumed a central position as a key prey in the food web following the collapse of sardines *Sardinops sagax* (Jenyns 1842) (Cury & Shannon, 2004). The success of *S. bibarbatus* can be attributed to a suite of unusual dietary, behavioural and physiological adaptations: adults shuttle between the hypoxic bottom waters to hide and feed during daylight and the more oxygenated pelagos to digest and re-oxygenate during darkness (Utne-Palm *et al* ., 2010). Although the larvae of *S. bibarbatus* can be found in the water column for much of the year, they peak in abundance during the austral summer and are least common in winter (O'Toole, 1978). Their eggs have never been recovered from plankton samples, suggesting that, despite the harsh conditions on the seafloor, this species might be a demersal spawner, as are most gobies (Miller, 1984). It is likely therefore that males build and defend nests and care for the eggs.

Males of demersal spawning gobies often lay sperm-containing mucous trails in their nests before and during spawning, which slowly dissolve and release active sperm for

several hours (Mazzoldi *et al* ., 2011). Seminal fluid mucins are produced by paired accessory sperm duct glands (SDGs) whose size and secretory activity vary in species where males perform alternative mating tactics (Mazzoldi *et al* ., 2011). In some gobies, in addition to large-sized and territory-holding males, there are also smaller, so called sneakers, which steal fertilizations from the territorial ones (Taborsky, 2008). Territorial males, which build nests and perform parental care, exhibit larger SDGs and more abundant secretions than sneaker ones (Mazzoldi *et al* ., 2011). Of the two male mating tactics, sneaking is less energetically costly and, although it has yet to be demonstrated, it might be expected that sneaking would be more prevalent under conditions of hypoxia.

Hypoxia has a negative impact on fish reproduction through, *e.g* . its impact on metabolism (Jobling, 1994) and endocrine disruption (Thomas *et al* ., 2007), as well as changing reproductive behaviour and mating tactics. Confronted with low oxygen water, male sand gobies *Pomatoschistus minutus* (Pallas 1770) build nests with larger openings (Lisså̊ker *et al* ., 2003), whilst the male dwarf Victoria mouthbrooder *Pseudocrenilabrus multicolor victoriae* (Seegers 1990), may reduce the total number of displays compared to males acclimated to high oxygen conditions (Gotanda *et al* ., 2011).

Here, the testes and SDGs of *S. bibarbatus* collected off Namibia were exam- ined histologically, supplemented with behavioural observations in the laboratory, in order to evaluate both the occurrence of demersal spawning and the possibility of alternative male mating tactics.

Sufflogobius bibarbatus were collected during a cross-shelf cruise conducted in April (austral autumn) 2008 off Walvis Bay between $23°\ 30^1$ S; $13°\ 40^1$ E and $23°\ 16^1$ S; $14°\ 23^1$ E. *Sufflogobius bibarbatus* were caught by demersal and pelagic trawls. Trawls were taken during day and night, catching *S. bibarbatus* both when in the pelagos and on the seabed. The physical and chemical environment were monitored using a Sea-Bird SBE 19 conductivity, temperature and depth sensor (CTD), fitted with an SBE 43 oxygen probe (www.seabird.com).

A maximum of 100 random *S. bibarbatus* specimens were measured (total length, L_T, ±1·0 mm) from each trawl, while the sex ratio was determined from further random sub-samples. Sex was determined from the shape of the genital papilla or by dissection and visual inspection of the gonads. Following dissection, mature testes and SDGs were excised and preserved in 4% neutral buffered formaldehyde solu- tion, before being weighed (M_{TEST} and M_{SDG}) to determine the gonado-somatic index (I_G) from $I_G = 100\ M_{\mathrm{TEST}}\ M_T^{-1}$ and the SDG somatic index (I_{SDG}) from $I_{\mathrm{SDG}} = 100\ M_{\mathrm{SDG}}\ M_T^{-1}$ ($n = 50$). Total mass (M_T) and not gutted mass was used, as gut and gut contents represent on average only 1 2% of M (95% c.i., 1 1–1 4%, $n = 274$) in fish between 62 and 123 mm L_T and SDGs of 25 males between 89 and 147 mm L_T were then embedded in paraplast, serially sectioned transversely, and mounted on slides for histological examina- tion. Sections were first stained with haematoxylin and eosin; polysaccharides were detected using the reaction of periodic acid-Schiff; sulphated and

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non-sulphated mucins by the alcian blue method at pH 1·0 and 2·5, respectively; and proteins were stained using the mercury bromophenol blue method (Pearse, 1985).

The cross-shelf CTD transect revealed a hypoxic zone extending between 20 and 60 m above the seabed. In this zone, oxygen levels dropped from 10 to <2% oxygen saturation at the bottom [Utne-Palm *et al*. (2010), Supplementary information Figs S1 and S2]. On the hypoxic shelf (60 – 150 m depth) *S. bibarbatus* between 70 and 90 mm L_T dominated the population: significantly smaller individuals were recorded in the pelagos (80·04 ± 9·54 mm, mean ± *s.d.*) than epibenthos (88·87 ± 5·79 mm) (*t*-test, $t = 7·69$, $P < 0·001$). At the deeper (180 – 200 m depth) and more oxy- genated shelf edge (>7% oxygen saturation), there was a bimodal size distribution with smaller juveniles (45·33 ± 10·55 mm) dominating the pelagos and larger adults (98·69 ± 18·45 mm) dominating the epibenthos (*t*-test, $t = 50·17$, $P < 0·001$).

Visual inspection of dissected gonads indicated that those of immature fish (<60 mm L_T) were transparent, thin and string-like, while maturing (>60 mm L_T) males were enlarged and increasingly white in colour. Mature males (>88 mm L_T) and mature females were found exclusively at the outer, more oxygenated shelf edge, whilst maturing specimens of both sexes inhabited the more hypoxic shelf. The M:F was 1·35 over the shelf (0·9, pelagos; 1·8, epibenthos) and 1·2 in the epibenthos at the shelf edge (all pelagic caught fish here were juveniles).

Larger males had significantly larger testes ($F_{1,43} = 4·445$, $P < 0·05$, $r^2 = 0·094$) [Fig. 1(a)] and SDGs ($F_{1,43} = 14·29$, $P < 0·001$, $r^2 = 0·249$) [Fig. 1(b)] than smaller males. All 25 males whose reproductive organs were histologically examined were sexually mature with paired testes, suspended from the dorsal wall of the coelomic cavity by a mesorchium. The sperm transport system consisted of two main sperm ducts, fusing into a common duct from which, before reaching the urogenital opening, a pair of solid, wing-like accessory structures grew out. Testes were organized into lobules, of the unrestricted spermatogonial type (Grier, 1981), whose walls were lined with germinal epithelium, presenting all the different stages of spermatogenesis: lumina full of sperm were observed in ripe males [Fig. 2(a), (b)]. The SDGs were multi-chambered, as commonly observed in gobies (Mazzoldi *et al*., 2011). The chamber wall consisted of an internal single layer of epithelial cells, a basal lamina and a thin external layer of connective tissue [Fig. 2(c)]. The chamber lumen did not contain sperm but was filled with a substance that reacted positively to sialoglycoproteins [Fig. 2(d), (e)], which have been noted as the dominant component of seminal fluid in other demersal spawning gobies (Mazzoldi *et al*., 2011).

The morphology of the male reproductive tract, and the generally positive rela- tionship between male size and both I_G and I_{SDG} suggests that, like other gobies (Breder & Rosen, 1966), *S. bibarbatus* is a demersal spawner. It also probably builds nests that it defends. SDG size is influenced by nest type and environment (Maz- zoldi *et al*., 2005). Given that *S. bibarbatus* seems to prefer muddy, over sandy, substrata (Salvanes *et al.*, 2011), it is also possible that the very large quantities of mucus secreted could be used to compact nest walls or to minimize bacterial infections, as observed for other gobies

(Mazzoldi *et al*., 2011). Evidence of territoriality between male *S. bibarbatus* was obtained from laboratory observations on pairs of large (92 – 102 mm L_T) healthy fish collected at the shelf edge. These males were kept in well-aerated, flow-through aquaria (100 cm × 100 cm × 40 cm), and it was noted that the larger individual attacked the smaller, and displayed territorial behaviour (attacking, biting, chasing, erecting fins and inflating mouth).
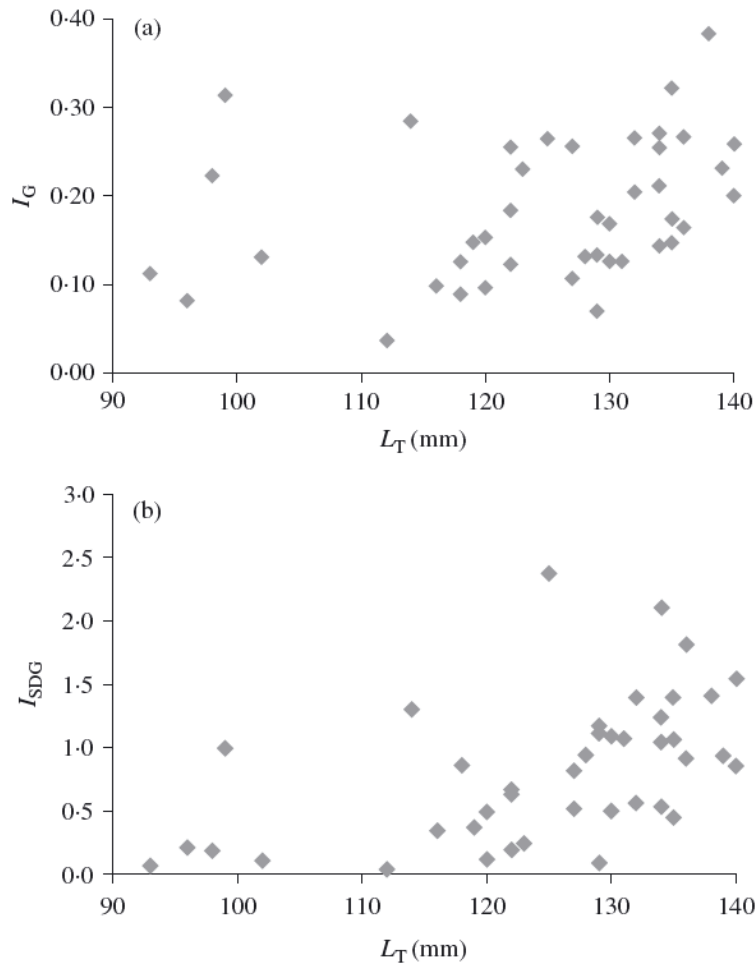

FIG. 1. Relationship between (a) gonado-somatic index (I_G) and (b) sperm duct gland index (I_{SDG}) and male total length (L_T) ($n = 50$) of *Sufflogobius bibarbatus*. ◆, individual measurements.

Whilst larger males generally had larger SDGs than smaller males, some of the latter had comparatively large testes and small, sperm-free SDGs (Fig. 1). This suggests that some males may be, or may have been, sneakers, although the small sample size precludes firm conclusions. In some other gobies, males may change their reproductive strategy depending on social context, *i.e.* male competition and female availability (Immler *et al*., 2004; Scaggiante *et al*., 2004).

The SDGs of *S. bibarbatus* are four times heavier than the testes, and this ratio is much higher than observed in territorial males of most other gobies, which generally invest equally in both gonads and accessory organs (Mazzoldi *et al*., 2005). This extraordinary investment in SDGs can also be interpreted in terms of polygyny, the levels of which are known to influence SDG development (Mazzoldi *et al*., 2005). Preliminary observations on *S. bibarbatus* spawning behaviour in captivity showed that a single male

mated and cared for the eggs that were released by three females (A. Kotze, H. Skrypzeck & B. Currie, unpubl. data).

To what extent either sneaking or polygyny are prevalent in field populations of *S. bibarbatus* is presently unknown, and only a slightly male biased M:F = 1
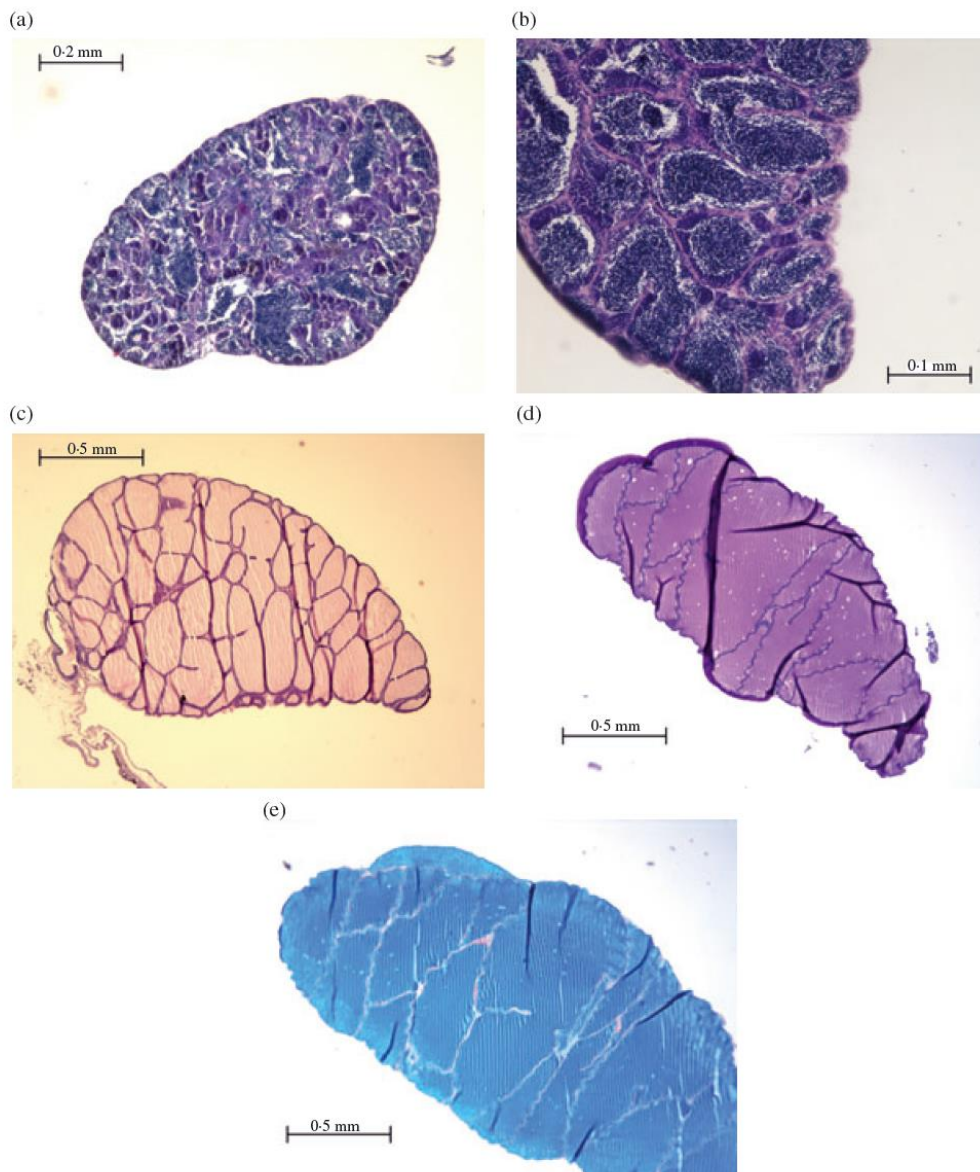

FIG. 2. Cross sections of sperm duct gland (SDG) and testis of male *Sufflogobius bibarbatus*. Cross-sections of (a) whole testis, (b) part of testis showing spermatogonia and cysts of spermatocytes; secondary spermatocytes and spermatids are present in the lobule walls, while sperm fill the lobule lumina. Haematoxylin and eosin. (c) SDG of a 98 mm L_T male. Chambers are highly extended and their lumina filled with secretion. Haematoxylin and eosin. (d) SDG of a 140 mm L_T male. Chambers are highly extended and their lumina filled with secretion. Both epithelial cells and secretion react positively to periodic acid-Schiff (PAS) staining. (e) SDG of a 140 mm L_T male. Chambers are highly extended and their lumina filled with secretion. Secretion reacts positively to alcian blue-PAS (pH 2·5).

Although a male biased sex ratio was observed over the hypoxic shelf, it should be remembered that males in this region were smaller than those at the shelf-edge and were maturing, not mature. Sex ratio can, however, vary in time and space due to both biotic and abiotic factors (Forsgren *et al*., 2004; Baroiller *et al*., 2009), and given that the present observations were made at the end of the active spawning season, it is likely

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that it would change through time with, *e.g* . changes in hypoxia and sulphide levels (Thomas *et al* ., 2007). As a consequence, it is likely that a male's choice of reproductive tactic would similarly change through the year. Also presence of males in the catches can decrease during the reproductive season, particularly if a nest burrowing and tending activity is present.

Perhaps unsurprisingly, it seems that *S. bibarbatus* spawns and rears its eggs in the more oxygen-rich waters located towards the edge of the shelf off Namibia. Benthic spawning in a species with low fecundity occurring in an upwelling area such as the Benguela is more advantageous than pelagic spawning as larvae are less likely to be lost from the system by Ekman transport (Stenevik *et al* ., 2007). In other words, benthic spawning might help to retain recruitment over the shelf, because whilst larvae have been observed across its width, highest concentrations occur at shallow depths where food is most abundant (O'Toole, 1978). The flexibility in the mating tactics of *S. bibarbatus* hinted at by the present observations (territoriality, sneaking and polygyny) are in line with its behavioural and trophic flexibilities, and its extraordinary physiology (Utne-Palm *et al* ., 2010), and help towards a better understanding of its unlikely success in such a hostile system over recent time. That said, clearly more work on this intriguing species is still needed.

References

Baroiller, J. F., D'Cotta, H. & Saillant, E. (2009). Environmental effects on fish sex determi- nation and differentiation. *Sexual Development* **3**, 118–135.

Breder, C. M. & Rosen, D. E. (1966). *Modes of Reproduction in Fishes*. Neptune City, NJ: T.F.H. Publications.

Cedras, R. B., Salvanes, A. G. V. & Gibbons, M. J. (2011). Investigations into the diet and feeding ecology of the bearded goby, *Sufflogobius bibarbatus*, off Namibia. *South African Journal of Marine Science* **33**, 313–320.

Cruickshank, R. A., Cooper, J. & Hampton, I. (1980). Extension to the geographical distribu- tion of pelagic goby *Sufflogobius bibarbatus* off South West Africa and some mensural and energetic information. *Fisheries Bulletin of South Africa* **13**, 77–82.

Cury, P. & Shannon, L. (2004). Regime shifts in upwelling ecosystems: observed changes and possible mechanisms in the northern and southern Benguela. *Progress in Oceanography* **60**, 223–243.

Forsgren, E., Amundsen, T., Borg, Å. & Bjelvenmark, J. (2004). Unusually dynamic sex roles in a fish. *Nature* **429**, 551–554.

Gotanda, K. M., Reardon, E. E. & Chapman, L. J. (2011). Hypoxia and male behaviour in an African cichlid *Pseudocrenilabrus multicolor victoriae*. *Journal of Fish Biology* **78**, 2085–2092.

Grier, H. J. (1981). Cellular organization of the testis and spermatogenesis in fishes. *American Zoologist* **21**, 345–357.

Immler, S., Mazzoldi, C. & Rasotto, M. B. (2004). From sneaker to parental male: change of reproductive traits in the black goby, *Gobius niger* (Teleostei, Gobiidae). *The Journal of Experimental Zoology* **301A**, 177–185.

Jobling, M. (1994). *Fish Bioenergetics*. London: Chapman & Hall.

Lavik, G., Stü̈hrmann, T., Brü̈chert, V., Van der Plas, A., Mohrholz, V., Lam, P., Mußmann, M., Fuchs, B. M., Amann, R., Lass, U. & Kuypers, M. M. M. (2009). Detoxification of sulphidic African shelf waters by blooming chemolithotrophs. *Nature* **457**, 581–584.

Lissåker, M., Kvarnemo, C. & Svensson, O. (2003). Effects of a low oxygen environment on parental effort and filial cannibalism in the male sand goby, *Pomatoschistus minutus*. *Behaviour Ecology* **14**, 374–381.

Mazzoldi, C., Petersen, C. W. & Rasotto, M. B. (2005). The influence of mating system on seminal vesicle variability among gobies (Teleostei, Gobiidae). *Journal of Zoological Systematics and Evolutionary Research* **43**, 307–314.

Mazzoldi, C., Patzner, R. & Rasotto, M. B. (2011). Morphological organization and variability of the reproductive apparatus in gobies. In *The Biology of Gobies* (Patzner, R., Van Tassell, J. L., Kovacic, M. & Kapoor, B. J., eds), pp. 367–402. Enfield, NH: Science Publishers.

Melo, Y. C. & Le Clus, F. (2005). Growth and reproduction of the pelagic goby *Sufflogobius bibarbatus* off the Orange River, southern Africa. *African Journal of Marine Science* **27**, 265–273.

Miller, P. J. (1984). The topology of gobioid fishes. In *Fish Reproduction: Strategies and Tactics* (Potts, W. & Wootton, R. J., eds), pp. 119–153. London: Academic Press.

O'Toole, M.J. (1978). Development, distribution and relative abundance of the larvae and early juveniles of the pelagic goby *Sufflogobius bibarbatus* (von Bonde) off South West Africa 1972-1974. *Sea Fisheries Branch Investigation Report* , *Cape Town. No.* **116**.

Pearse, A. G. E. (1985). *Histochemistry: Theoretical and Applied* . London: Churchill Living- stone.

Salvanes, A. G. V., Utne-Palm, A. C., Currie, B. & Braithwaite, V. A. (2011). Behavioural and physiological adaptations of the bearded goby: a key fish species of the extreme environment of Benguela. *Marine Ecology Progress Series* **425**, 193 − 202.

Scaggiante, M., Grober, M. S., Lorenzi, V. & Rasotto, M. B. (2004). Changes along the male reproductive axis in response to social context in a gonochoristic gobiid, *Zosterisessor ophiocephalus* (Teleostei, Gobiidae), with alternative mating tactics. *Hormones and Behavior* **46**, 607−617.

Stenevik, E. K., Sundby, S. & Cloete, R. (2007). Diel vertical migration of anchovy *Engraulis encrasicolus* larvae in the northern Benguela. *African Journal of Marine Science* **29**, 127−136.

Taborsky, M. (2008). Alternative reproductive tactics in fish. In *Alternative Reproductive Tactics. An Integrative Approach* (Oliveira, R. F., Taborsky, M. & Brockmann, H. J., eds). Cambridge: Cambridge University Press.

Thomas, T., Rahman, S., Khan, I. A. & Kummer, J. A. (2007). Widespread endocrine disrup- tion and reproductive impairment in an estuarine fish population exposed to seasonal hypoxia. *Proceedings of the Royal Society B* **274**, 2693−2702.

Utne-Palm, A. C., Salvanes, A. G. V., Currie, B., Kaartvedt, S., Nilsson, G. E., Braithwaite, V., Stecyk, J. A. W., Hundt, M., Flynn, B., van der Bank, M., Peard, K. R., Lunde, I. G., Sandvik, G. K., Klevjer, T. A., Pittman, K., Sweetman, A., Strandabø, R. A. U. (2010). Tropic structure and community stability in an overfished ecosystem. *Science* **329**, 333−336.