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Reproductive trade-offs in a temperate reef fish under high pCO_2 levels

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ABSTRACT

Fishes are currently facing novel types of anthropogenic stressors that have never experienced in their evolutionary history, such as ocean acidification. Under these stressful conditions, energetically costly processes, such as reproduction, may be sacrificed for increased chances of survival. This trade-off does not only affect the organism itself but may result in reduced offspring fitness. In the present study, the effects of exposure to high pCO_2 levels were tested on the reproductive performance of a temperate species, the two-spotted goby, *Gobiusculus flavescens*. Breeding pairs were kept under control (~600 µatm, pH~ 8.05) and high pCO_2 levels (~2300 µatm, pH~ 7.60) conditions for a 4-month period. Additionally, oxidative stress and energy metabolism-related biomarkers were measured. Results suggest that reproductive activity is stimulated under high pCO_2 levels. Parental pairs in the simulated ocean acidification conditions exhibited increased reproductive output, with 50% more clutches and 44% more eggs per clutch than pairs under control conditions. However, there was an apparent trade-off between offspring number and size, as larvae of parental pairs under high pCO_2 levels hatched significantly smaller, suggesting differences in parental provisioning, which could be related to the fact that these females produce more eggs. Moreover, results support the hypothesis of different energy allocation strategies used by females under high pCO_2 conditions. These changes might, ultimately, affect individual fitness and population replenishment.

1. Introduction

Reproduction is a very energetically expensive process due to the time and energy parents invest towards producing offspring. In many coastal reef fish, while males typically expend large amounts of energy on courtship, nest defense, and offspring care (Gillooly and Baylis, 1999; Mackereth et al., 1999; Cox et al., 2010), females may have a greater influence on the offspring phenotype by altering the number, size, energy content, or yolk biochemical composition of their eggs, depending on the available energy to invest in offspring (Bernardo, 1996).

Due to the associated physiological costs, fish reproduction generally occurs within a narrow range of environmental conditions, set to favour offspring. However, variation in the environmental conditions experienced by parents may influence their body condition, potentially affecting offspring phenotype (Leatherland et al., 2010; Meylan et al., 2012). In addition to the natural ecological stressors, such as predation and habitat availability, fishes are currently facing novel types of anthropogenic stressors that are varying in ways outside their evolutionary history (Sih et al., 2011), such as climate change. Under these circumstances, energetically costly processes such as reproduction may be sacrificed for increased chances of survival. This trade-off may affect the organism, as well as induce changes in offspring fitness, with consequences for population replenishment. The predicted increase in temperature is well known to affect reproductive processes from gamete development, spawning, to embryogenesis and hatching (reviewed by Pankhurst and Munday, 2011). However, the impacts of climate change are not limited to increasing temperature. Rising atmospheric CO_2 levels are also driving an increased CO_2 ocean uptake, with concomitant changes in the carbonate chemistry of seawater (Caldeira and Wickett, 2003; Orr et al., 2005; Gattuso et al., 2015). It is generally assumed that adult fish can maintain their internal pH within an optimal range and are therefore relatively tolerant to changes in ambient CO_2 concentrations (Ishimatsu et al., 2004, 2008; Melzner et al., 2009), suggesting that effects of exposure to high CO_2 can be compensated. Still, compensation can be metabolically costly and may reduce the amount of energy available for other critical processes, such as reproduction (Pörtner et al., 2004; Kroeker et al., 2010). The exposure to specific stressors, including environmental stress, may increase individuals' internal imbalance, with subsequent negative

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consequences on a variety of biological processes and systems (i.e. molecular and metabolic disruptions, cellular damage), which can impair the physiological condition of an organism (Depledge, 1993; Metcalfe and Alonso-Alvarez, 2010). To address these changes, biomarkers associated with stress-related biochemical responses have been widely applied as early-warning tools to assess an individual's condition (Van der Oost et al., 2003; Wu et al., 2005; Silva et al., 2016; Alves et al., 2016; Kühnhold et al., 2017).

To date, only few studies have examined the potential effects of exposure to high CO_2 in fish reproductive performance, but have yielded contradictory results. While Miller et al. (2013) described positive effects (more clutches and more eggs) of elevated CO_2 on reproductive performance in the cinnamon anemonefish, *Amphiprion melanopus*, in a posterior study on the same species, no effect of high CO_2 on reproduction was detected (Miller et al., 2015). For another anemonefish, *A. percula*, Welch and Munday (2016) demonstrated an increased reproductive output. However, for a close related species, the damselfish *Acanthochromis polyacanthus*, the same authors reported decreased number of clutches produced under high CO_2. On a temperate fish species, the three-spined sticklebacks, *Gasterosteus aculeatus*, Schade et al. (2014) provided evidence of increased clutch size and increased survival and growth rates in juveniles. These mixed results reinforce the need for further investigation on the impacts of future ocean acidification scenarios on the reproductive success of marine species.

In the present study, the effects of exposure to high CO_2 levels were tested on the reproductive performance of a temperate species, the two-spotted goby, *Gobiusculus flavescens*. Breeding pairs were kept under control (~600 µatm, pH~ 8.05) and high pCO_2 (~2300 µatm, pH~ 7.60) conditions for a 4-month period, which included the breeding season and 1 month of CO_2 conditioning before breeding begun. Reproductive performance was evaluated by measuring the number of clutches produced by breeding pairs, the average number of eggs, average egg area, larval size at hatching, and adult body condition. Additionally, oxidative stress and energy metabolism-related biomarkers were measured for both males and females, as increased stress due to exposure to high CO_2 may increase metabolic costs, and affect sexes differently.

2. Material and methods

2.1. Study species and collection

The two-spotted goby, *Gobiusculus flavescens*, is a small (adult 35–55 mm), sexually dimorphic, marine fish that lives along the coast of Europe, from Portugal to northern Norway (Miller, 1986). It typically lives for only 1 year (Johnsen, 1945), but can reproduce repeatedly during the breeding season (Mobley et al., 2009), which in the present study area can last from February to July. It is a substrate brooder, with males providing all parental care, for a period of 1–3 weeks depending on water temperature (Bjelvenmark and Forsgren, 2003; Skolbekken and Utne-Palm, 2001). According to the literature, the two-spotted goby exhibits sexual size dimorphism, with males on average slightly larger than females (Pélabon et al., 2003; Forsgren et al., 2004). However, the species may show some size dependent plasticity, as another recent study has reported a population showing a reversal of this trend, with males on average being smaller than females (Utne-Palm et al., 2015).

Adult males and females *G. flavescens* were collected by SCUBA diving at the Arrábida Marine Park, Portugal (38° 28′ N; 8° 59’ W), during early March 2016. Fish were immediately transported to the laboratory and transferred to a 100 L tank with a continuous supply of recirculating seawater, and left for one week to recover from handling. Subsequently, breeding pairs were randomly assigned to 35 L tanks (one pair per tank). All individuals were weighed (wet weight; ww) and standard length and total length (mm) measured immediately before being placed into treatment tanks, ensuring that there was an even distribution of weights and sizes among treatment groups. Nine breeding pairs were randomly assigned to each of the two treatment groups - control (~600 µatm, pH~ 8.05) and high pCO_2 (~2300 µatm, pH~ 7.60) - and maintained at those conditions until the end of the breeding season, in July. Within sex, weight and size did not differ between control and high pCO_2 treatments (males, weight: $F_{1,16} = 0.03$, $p = 0.86$; males, standard length: $F_{1,16} = 0.00$, $p = 1.01$; females, weight: $F_{1,16} = 0.36$, $p = 0.55$; females, standard length: $F_{1,16} = 0.00$, $p = 1.01$). Furthermore, within each treatment, initial weight and size did not differ between males and females (control, weight: $F_{1,16} = 3.24$, $p = 0.09$; control, standard length: $F_{1,16} = 3.45$, $p = 0.08$; high, weight: $F_{1,16} = 3.53$, $p = 0.08$; high, standard length: $F_{1,16} = 2.29$, $p = 0.15$).

Pairs were maintained under temperature and salinity conditions matching conditions at the field site (~16 °C, 35 PSU), with a summer light cycle of 14h light: 10h dark simulated using fluorescent lights, and fed with *Artemia* nauplii twice a day.

2.2. pCO_2 treatment

The high pCO_2 treatment was chosen as an extreme condition, which is not expected to occur in the open ocean until 2300 (Hartin et al., 2016); however, the two-spotted goby inhabits nearshore regions frequently exposed to CO_2 fluctuations, where pCO_2 values up to 1900 µatm have been recorded during seasonal upwelling events (Oliveira et al., 2012). The amplifying effects of anthropogenic ocean acidification can result in pCO_2 levels greater than those projected to occur in open ocean environments (Melzner et al., 2013; Shaw et al., 2013).

Artificial seawater used in the experiments was adjusted to a salinity of 35 by blending a commercial salt mixture (Tropic Marin®) with filtered freshwater (reverse osmosis system). pCO_2 in the high pCO_2 treatment (pH_{NBS} ~7.6) was slowly adjusted over a 3-week period to the desired level, by CO_2 injection into a 200 L sump, regulated with a pH-controller (Tunze Aquarientechnik, Germany). A second 200 L sump was maintained at ambient pCO_2 levels (pH_{NBS} ~8.0). Each sump, equipped with biological, mechanical, chemical, and ultraviolet filtration, supplied seawater into nine 35 L tanks at a flowrate of ~700 mL min^{-1}. Rearing tanks were sealed with a clear glass lid to limit CO_2 exchange with the atmosphere. Seawater pH, on NBS scale (pH_{NBS}), was measured twice daily, using a portable, hand-held pH meter (SevenGo Pro pH/Ion, Mettler Toledo) and glass electrode (InLab1413 S8, Mettler Toledo) calibrated with certified reference materials for NBS consisting of pH_{NBS} 4 and 7 buffer solutions (Mettler Toledo, two-point calibration). Temperature and salinity were also measured twice daily, using the same portable meter. Oxygen levels were maintained above 90% saturation by the mixing action of the diffusion pumps in the sumps. Samples for total alkalinity (TA) determination were weekly collected from experimental tanks, placed in air-tight containers without air space, stabilized by mercuric chloride poisoning and kept at +4 °C until further analysis. Analyses were performed using automated Gran titrations, with certified reference material supplied by A. Dickson (Scripps Institutions of Oceanography, San Diego). pCO_2 was calculated from the *in situ* temperature, TA and pH, using the carbonic acid dissociation constants given by Millero et al. (2006) and the CO_2 solubility coefficient of Weiss (1974). Estimated seawater parameters are shown in Table 1.

2.3. Experimental design and data collection

Breeding pairs were provided with a PVC pipe (10 cm long, Ø1.3 cm) as a shelter and breeding substrate. Each pipe was lined inside with a removable acetate sheet, where spawning females could attach their eggs. The presence of egg clutches was checked daily and in the presence of clutches, the acetate sheet with the clutch was carefully

Table 1
Mean (± SD) seawater parameters in the experimental systems.

pCO$_2$ condition	pH$_{NBS}$	T (°C)	Salinity	TA μmol kg^{-1}	pCO$_2$ μatm
Control	8.06 ± 0.07	15.87 ± 0.32	34.47 ± 1.02	2811.259 ± 99.108	597.345 ± 61.50
High	7.58 ± 0.03	16.12 ± 0.28	34.32 ± 1.15	2836.861 ± 106.734	2332.383 ± 247.93

removed from the nest, placed in a Petri dish with sea water, photographed to assess the number of eggs laid, and gently placed back into the nest. Parental males seemed not to be affected by the short-time removal of the clutch. A random sample of 10 eggs was also taken, and photographed under a stereomicroscope directly after removal from the clutch, to calculate egg area (to the nearest 0.01 mm^2).

Reproductive output, per clutch, was calculated by multiplying the total number of eggs in the clutch by the average individual egg area for that clutch, to give an overall area (mm^2) of eggs, and therefore an estimate of maternal investment per clutch. The day before hatching, egg clutches were transferred to small rearing boxes (~ 50 mL), with a mesh bottom to allow water recirculation, and placed in the same tank as the parental pair. At hatching, a sample of 10–15 larvae was collected, larvae euthanized using an overdose of MS222, and immediately photographed under a stereomicroscope. Larvae standard length was determined to the nearest 0.01 mm. All photographs were analyzed using ImageJ software (National Institute of Health, Bethesda, MD, USA).

At the end of the experiment, all adult individuals were weighed (ww) and measured (standard and total length) for determination of Fulton's K condition factor (body condition), using the formula $K = 100*(W/L^3)$ where W is the wet weight in grams and L is the standard length in centimeters. Afterward, fish were euthanized by rapid cooling and immediately stored at −80 °C for subsequent biomarkers analysis.

2.4. *Biomarker analysis*

Muscle from 35 fish, 17 males and 18 females, was dissected for biochemical measurements. Visceral content, as well as egg sacs, were discarded to avoid biochemical variations, since most of females were pregnant by the time they were sacrificed. Approximately 0.5 g of muscle (ww) was homogenized, using a mechanical homogenizer (Ystral X10/25, Germany), in a 1:12 proportion (m:v) of potassium-phosphate buffer (0.1 M, pH 7.4). To further analyze the presence of the superoxide anion radical (O_2^-) [reactive oxygen species (ROS) proxy], an additional 0.05 g (± 0.01) of tissue was separated from the total muscle available. Oxidative stress (superoxide anion, superoxide dismutase, catalase), oxidative damage (DNA damage, lipid peroxidation) and energy metabolism (lactate dehydrogenase, isocitrate dehydrogenase, carbohydrates, lipids, proteins and electron transport system) protocols were conducted following Silva et al. (2016, 2017) procedures. All parameters were determined in triplicate, together with blanks (potassium-phosphate buffer 0.1 M, pH 7.4), using a Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Vermont, USA).

2.5. *Data analysis*

Validity of assumptions that underlie the data analysis was verified and data were standardized and log (x + 1) transformed, when applicable. A one-way ANOVA tested for differences in fish size and weight, within sex and treatment, at the start and end of the experiment, and an ANCOVA was used to compare body condition of adults between treatments, at the start and end of experiment, with weight as the dependent variable and length as the covariant. A Chi-square test for independence was used to compare the total number of clutches produced in control and high CO$_2$ treatments. A one-way ANOVA was then used to compare the average number of clutches per reproductive pair between treatments. The mean number of eggs per clutch and average egg area per clutch were analyzed with nested ANOVAs, with parental pair nested within CO$_2$ treatment. For egg area, the average of the 10 measurements per clutch was used as the unit of replication in the analysis. A nested ANOVA, with parental pair nested within CO$_2$ treatment, was used to test for effects of exposure to high CO$_2$ on size at hatching. The effect of CO$_2$ treatment on biochemical parameters was addressed by a two-way ANOVA, using the sex and treatment as factors, followed by Tukey Unequal N HSD post-hoc test for multiple comparisons.

Statistical tests were performed using Statistics13.0 ™ software. All data is presented as mean ± SD, and the significance level was set at $p \le 0.05$.

2.6. *Ethics statement*

This study was performed in strict accordance with the recommendations of the Animal Care and Use Committee of ISPA - Instituto Universitário, and undertaken under the supervision of an accredited expert in laboratory animal science (following FELASA category C recommendations). Permission for capturing fish at the field site was granted by the Instituto da Conservação da Natureza e das Florestas (ICNF).

3. Results

Fish weight (0.73 ± 0.15 g) and size (3.80 ± 0.20 cm) at the start of the experiment did not differ between treatments (weight: $F_{1,32} = 0.19$, $p = 0.669$; standard length: $F_{1,32} = 0.01$, $p = 0.935$). Eight of nine pairs reproduced in control treatment, while all nine pairs reproduced in the high CO$_2$ treatment (the non-breeding pair from the control treatment was excluded from the reproductive output analysis). Over the breeding season, control pairs of two-spotted goby produced a total of 26 clutches, while pairs under high CO$_2$ conditions produced a total of 40 clutches, but this 50% increase was non-significant ($\chi^2_1 = 2.97$, $p = 0.085$). Likewise, there was a trend towards more clutches per pair in the high CO$_2$ treatment (4.44 ± 2.00 *vs.* 2.89 ± 1.69), although also non-significant (Fig. 1a; $F_{1,16} = 3.16$, $p = 0.09$).

Embryonic duration did not change with increasing CO$_2$, varying from 10 to 11 days. The average number of eggs per clutch increased with increasing CO$_2$ (Fig. 1b; $F_{1,46} = 15.60$, $p < 0.001$), and differed among parental pairs ($F_{14,46} = 4.43$, $p < 0.001$). The control group produced 531 ± 254 eggs per clutch, while breeding pairs under high CO$_2$ produced, on average, almost 44% more eggs per clutch (763 ± 253). Egg area did not differ between control and high CO$_2$ treatments (0.43 ± 0.03 *vs.* 0.44 ± 0.04 mm^2) (Fig. 1c; $F_{1,46} = 0.343$, $p = 0.561$), but the overall reproductive output (mean egg area*-number of eggs) was 40% higher under high CO$_2$ conditions (Fig. 1d; $F_{1,46} = 11.90$, $p < 0.001$), and differed among parental pairs ($F_{14,46} = 4.17$, $p < 0.001$).

Despite the lack of difference in egg area between treatments, pairs under high CO$_2$ produced significantly smaller larvae at hatching (Fig. 1e; $F_{1,207} = 16.26$, $p < 0.001$), and larval size differed among parental pairs ($F_{8,207} = 4.92$, $p < 0.001$). In the control treatment larvae hatched with 2.89 ± 0.21 mm, while under high CO$_2$ larvae hatched with 2.79 ± 0.21 mm.

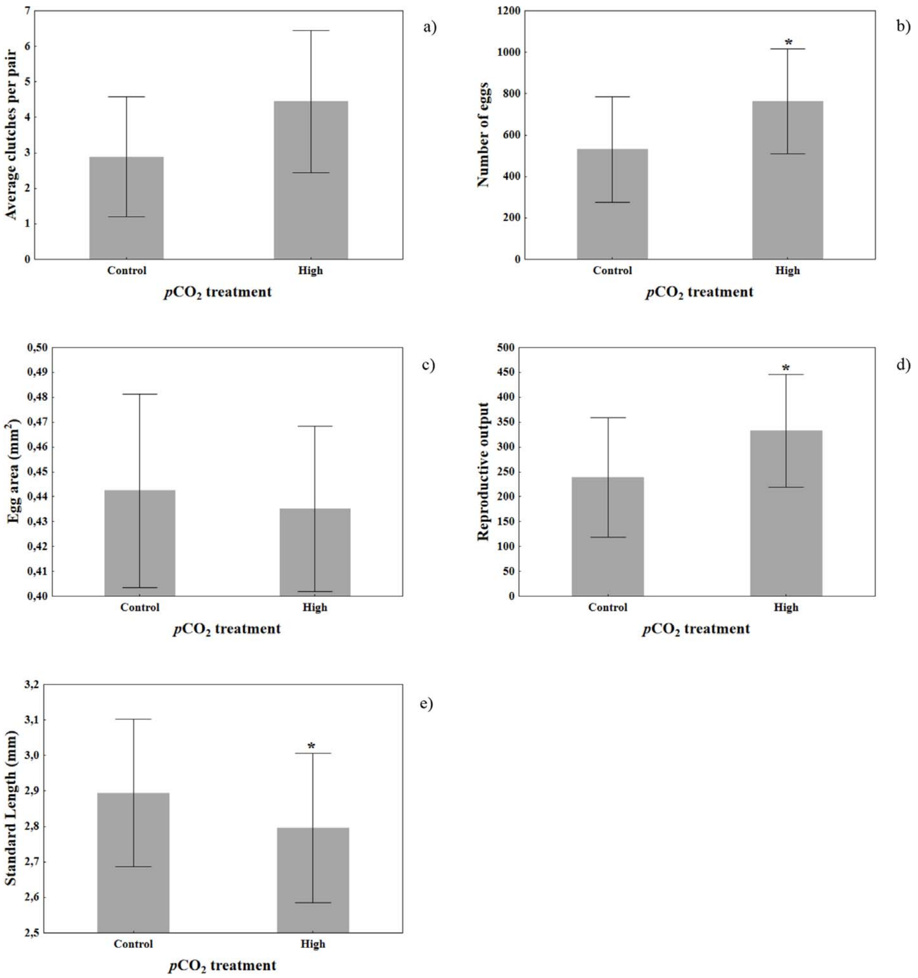

Fig. 1. Effect of high CO_2 exposure on reproductive characteristics of *Gobiusculus flavescens*. a) average clutches per pair; b) number of eggs; c) egg area; d) reproductive output; e) standard length of larvae at hatch. Data are means ± S.D.; * indicate statistical differences between treatments.

The higher reproductive output under high CO_2 conditions did not appear to come at a cost to adult body condition, as no significant difference in Fulton's K body condition factor between treatments was detected (0.94 ± 0.09 *vs*. 0.92 ± 0.09, for control and high CO2 treatments, respectively) ($F_{1,31} = 0.43$, $p = 0.517$). Moreover, within each sex, no differences in weight and size between treatments were detected by the end of the breeding season (males, weight: $F_{1,16} = 0.26$, $p = 0.61$; males, standard length: $F_{1,16} = 0.002$, $p = 0.96$; females, weight: $F_{1,16} = 0.56$, $p = 0.47$; females, standard length: $F_{1,16} = 0.10$, $p = 0.75$). However, by the end of the experiment, females from each treatment were significantly smaller and weighting less than males (control, weight: $F_{1,16} = 18.11$, $p < 0.001$; control, standard length: $F_{1,16} = 7.31$, $p = 0.02$; high, weight: $F_{1,16} = 13.08$, $p = 0.002$; high, standard length: $F_{1,16} = 14.75$, $p = 0.002$), likely highlighting females' greater reproductive effort throughout the breeding season.

The reactive oxygen species (ROS) levels, measured by means of superoxide anion production, differed between CO_2 treatment and sex (pCO_2 treatment: $F_{1,30} = 4.877$, $p = 0.035$; sex: $F_{1,30} = 15.301$, $p < 0.001$), with females from control conditions presenting lower ROS levels when compared to control and high CO_2 exposed males (Fig. 2a). There was, however, no significant interaction between the two factors ($F_{1,30} = 0.423$, $p = 0.521$), contrary to the observed for the levels of lipid peroxidation (LPO), where exposure to high levels of CO_2 affected sexes differently ($F_{1,30} = 5.752$, $p = 0.023$). Despite the lack of a treatment or sex effect on LPO levels (CO_2 treatment: $F_{1,30} = 2.877$, $p = 0.100$; sex: $F_{1,30} = 0.749$, $p = 0.349$) (Fig. 2b), there was a tendency for lower levels in females under high CO_2 levels. Other than ROS levels, no significant differences were detected between treatments for the remaining measured biomarkers. On the other hand, differences between sexes were more commonly observed, with males presenting significant higher levels of CAT ($F_{1,30} = 4.314$, $p = 0.046$), SOD ($F_{1,30} = 4.580$, $p = 0.041$) and ETS ($F_{1,30} = 4.512$, $p = 0.042$), and a similar tendency (not significant) for the other biomarkers, except for carbohydrates (Fig. S1 and Table SI – Supplementary material).

4. Discussion

Results suggest that reproductive activity of the two-spotted goby is stimulated under high CO_2 levels. Parental pairs in the simulated ocean acidification conditions exhibited increased reproductive output, with 50% more clutches and 44% more eggs per clutch than pairs under control conditions. These results contrast with the ones by Forsgren et al. (2013) on the same species, where elevated CO_2 did not affect number of eggs. However, in that study, authors exposed adult fish to treatment shortly before spawning, whereas in the present study fish were exposed to elevated CO_2 conditions before the onset of the breeding season, potentially affecting processes such as gametogenesis. Moreover, the elevated CO_2 condition in that study was lower than the pCO_2 levels in the present study (1400 µatm vs. 2300 µatm, respectively), which may explain the different results. However, other studies exposing adult fish to lower pCO_2 levels than the ones used in the current study have also reported increased reproductive activity. The temperate marine three-spined stickleback *Gasterosteus aculeatus*, acclimated for a 3-month period to control (~400 µatm) and

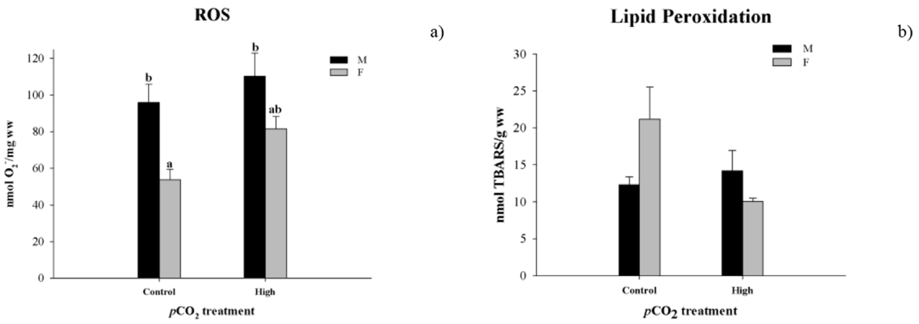

Fig. 2. Oxidative stress related parameters measured in muscle of males and females of *Gobiusculus flavescens* after 4 months of exposure to control (≈600 μatm) and high (≈2300 μatm) CO_2 levels. a) Reactive oxygen species (superoxide anion); b) Lipid peroxidation. Results are expressed as mean ± S.D.; different letters indicate statistical differences between females of different treatments.

~1000 μatm, produced more eggs under elevated pCO$_2$ than control parents (Schade et al., 2014). The tropical damselfish *Amphiprion melanopus* exposed to ~1000 μatm for a 9-month period, which included the breeding season, produced 67% more eggs per clutch compared to fish exposed to control and moderate pCO$_2$ levels (~430 μatm and 580 μatm) (Miller et al., 2013); interestingly, in a posterior study on the same species, no effect of high CO_2 on reproduction was detected (Miller et al., 2015). The authors argued that these conflicting results may be related to differences in the time required to acclimate to captivity, as in the former study fish were studied in their first year of captivity, while in the later reproductive performance was evaluated in the second year of captivity. Supporting the results from the current study is another experimental work on another tropical species - *Amphiprion percula* exposed to ~650 and ~900 μatm for a 9-month period, which also included the breeding season, produced 47–56% more eggs than control breeding pairs (Welch and Munday, 2016). In contrast, in the same study, but on another damselfish, *Acanthochromis polyacanthus*, the authors reported decreased reproductive output with increasing CO_2, with approximately one-third as many clutches produced in high CO_2 compared with controls (Welch and Munday, 2016). In the first study conducted in the wild, Milazzo et al. (2016) found no differences in the number of eggs laid by the ocellated wrasse *Symphodus ocellatus* between nests, along a CO_2 gradient near volcanic seeps. Altogether, these contrasting results suggest a highly variable response of the reproductive performance of different fish species under high CO_2 and highlight the need for further investigation on this matter.

Contrary to the above-mentioned species, the two-spotted goby is a semelparous, short-living species, which typically lives for 1–1.5 years, and has only 1 breeding season, reproducing repeatedly during that season. In face of a stressor, semelparous species have no opportunity to postpone reproduction, or future opportunities to offset low quality progeny, as iteroparous species might have (Love and Williams, 2008). With only a single breeding episode, semelparous species may have evolved strategies to persist and cope with ecological stressors without compromising reproduction and offspring success (Wingfield and Sapolsky, 2003). There are however very few studies addressing effects of parental stress on offspring success in semelparous species and, to our knowledge, our current study is the first addressing the impacts of exposure to high CO_2 on a fish species that breeds only once in their lifetime. Sopinka et al. (2014) showed, for the first time in a wild semelparous fish species, the sockeye salmon, that mothers exposed to a stressor (repeated chase) appear to possess buffering strategies that protect progeny, as egg size and early offspring survival were not affected. Although results of our current study suggest the existence of a trade-off between reproductive output and offspring size at hatch, predictions on offspring survival and success cannot be made. Future studies should aim at addressing how a stressful environment experienced by parents will affect future generations. Transgenerational plasticity (TGP) could act as an effective mechanism to buffer populations against rapid environmental changes (e.g. Salinas and Munch, 2012), and despite the experimental evidence that suggests a high potential for adaptive transgenerational plasticity in fish under ocean warming and acidification conditions (Donelson et al., 2011; Miller et al., 2012; Salinas and Munch, 2012; Murray et al., 2014; Shama and Wegner, 2014), there is still much work to do on this respect.

Increased reproductive output observed in the two-spotted goby was not explained by differences in female size or weight between treatments, as they were evenly distributed at the start and end of the experiment, and it was neither associated with changes in adult body condition (Fulton's K), which is in accordance with other studies reporting changes in reproductive output, but no effect of high CO_2 on adults' body condition (Miller et al., 2013, 2015; Welch and Munday, 2016). This could be explained by the fact that fish were provided with food *ad libitum* throughout the experiment, which allowed them to maintain their body and physiological condition despite an increased reproductive effort, also justifying the observed lack of differences in energy reserves. In the wild, however, it is not likely that food will be fully available, and it is expected that facing different stressors (either abiotic or biotic), reproduction will come at a physiological cost, for both females (egg production) and males (parental care). Moreover, in the wild, organisms require more energy for other activities, such as foraging and predator avoidance, which may involve other energetic trade-offs. Donelson et al. (2010) tested the effects of both water temperature and food supply on the damselfish *Acanthochromis polyacanthus* and found that both affected the reproductive output, with fewer pairs breeding at elevated water temperatures and no pairs reproducing at either of the higher temperatures on the lower quantity diet. However, to our knowledge, all studies to date on effects of simulated ocean acidification have been conducted on fish fed to satiation in laboratory conditions, and future work should investigate whether variation in food supply will influence the effects of exposure to high CO_2 on reproductive performance.

Although there was no effect of high CO_2 on egg size, an apparent trade-off between offspring number and size was observed, as larvae of parental pairs under high CO_2 levels hatched significantly smaller, suggesting differences in parental provisioning which could be related to the fact that these females produce more eggs. Maternal influences are typically more prevalent than paternal ones (Bernardo, 1996). Despite the well-developed acid-base regulatory system in fish, it is expected that compensation of the increased metabolic costs associated to acid-base regulation in a high CO_2 environment (Pörtner et al., 2004; Ishimatsu et al., 2008) would increase physiological costs, particularly, the potential for oxidative stress. However, this was not evident in our results. The present results only revealed a tendency for the decrease of LPO in females from the high CO_2 treatment, which is in accordance to a recent study on the impacts of high CO_2 on oxidative stress of early life stages of another fish species, the sand smelt, *Atherina presbyter* (Silva et al., 2016). It has been hypothesized that the exposure to stressors, such as high CO_2, may change the composition and relative proportion of fatty acids in fish (e.g., Frommel et al., 2011; Díaz-Gil et al., 2015), and in invertebrates (e.g., Timmins-Schiffmann et al., 2014; Valles-Regino et al., 2015), which may contribute to reduce cellular membrane vulnerability to damage and thus, explain lower LPO levels under stressful conditions. In general, the slight (yet not significant) increase of ROS in reproductively active females is not compensated by an increase of the studied antioxidant enzymes. These results seem to sustain the hypothesis of a different energy allocation strategy by females under high CO_2 conditions, likely favoring reproductive success rather than protection against cellular damage, that overall seems to be occurring (decreased LPO) via eventual non-costly metabolically events, such as membrane fatty acid composition changes when faced with stress (e.g., Frommel et al., 2011; Díaz-Gil et al., 2015). In a recent study, Jordão et al. (2016) detected altered lipid storage patterns on *Daphnia magna* and, without any association with differences in energy intake, hypothesized the occurrence of specific mechanisms such as endocrine disruption in stress conditions. However, caution must be taken when interpreting this type of biomarkers' data and its relevance from an ecological perspective once in the present study only the end of the reproductive season was taken into consideration and greater differences could have been observed earlier in time.

Despite the larger influence of maternal provisioning, there is also the potential for male parents to affect offspring phenotype through nest tending in species with paternal care (Green and McCormick, 2005), as the two-spotted goby. Higher energy requirement associated with elevated oxidative stress levels have also been reported in males prior to spawning periods mostly due to parental care (Smith and Wootton, 1995; Gravel et al., 2010; Zolderdo et al., 2016), which is also present in this species. Although time spent in paternal activities (e.g. egg aeration, and removal of dead eggs) was not quantified, there was no apparent difference in activity between treatments, and nest abandonment was never observed. On this matter, a recent study by Sundin et al. (2017) provided evidence of unaltered reproductive behaviours (nest building activities, courtship behaviours, fanning) in the three-spined stickleback *Gasterosteus aculeatus* exposed to 1000 μatm. In the present study, despite no evident effects of the treatment to the exposed males, it can be noticed that males have generally higher ROS levels than females. It is also noted that, under control conditions, LPO levels are lower for males. These difference point to a generally more efficient antioxidant system (reducing lipid damage) which here is seen in higher CAT and SOD activities in males that might explain the higher ETS.

Whether smaller size at hatch will have consequences for larval growth and survival later in the ontogeny was not tested here and would require further investigation. In general, laboratory studies have provided evidence that fast growth during larval life enhances survival, as larvae are better able to capture food, resist starvation, and avoid predators (Miller et al., 1988; Bailey and Houde, 1989). In this sense, it can be hypothesized that the smaller offspring produced by parents under high CO_2 will face higher mortality rates throughout the larval stage. However, other studies have found evidence of compensatory growth (e.g. Gagliano and McCormick, 2007; Donelson et al., 2009), indicating that nongenetic parental effects do not permanently limit offspring's phenotype. It would be valuable for future studies to address the impacts of smaller size at hatching on offspring survival rates, under several scenarios, including food limitation and other stressors.

The mechanisms explaining different reproductive performances under high CO_2 scenarios remains unclear. It is hypothesized that changes in the endocrine pathways associated to reproduction are responsible for altered reproductive output (Pankhurst and Munday, 2011), but it could also be related with GABA-A receptor function, which has been suggested to be implicated in the main behavioural changes observed in fish under ocean acidification scenarios (review by Tresguerres and Hamilton, 2017), but it also plays an important role in mediating secretions of reproduction related hormones (Trudeau et al., 2000; Zohar et al., 2010). Future studies should aim at examining hormonal profiles in fish exposed to high CO_2 and test the involvement of GABA-A receptor.

Overall, this study makes a useful contribution in a research field that has been recognized as a knowledge gap (Heuer and Grosell, 2014; Milazzo et al., 2016). Results reveal clear effects of exposure to high CO_2 on the reproductive success of the two-spotted goby, and support the hypothesis of different energy allocation strategies used by females under high CO_2 conditions.

Authors' contributions

AF conceived the project; AF, AL, CS collected data; AF, CS, SN, ML analyzed data; AF, AL, CS, SN, ML, EG contributed to writing the manuscript. All authors have approved the final article.

Conflicts of interest

Ana Faria, Ana Lopes, Cátia Silva, Sara Novais, Marco Lemos, Emanuel Gonçalves declare that we have no proprietary, financial, professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position presented in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2018.02.027.

References

Alves, L.M.F., Nunes, M., Marchand, P., Le Bizec, B., Mendes, S., Correia, J.P., Lemos, M.F., Novais, S.C., 2016. Blue sharks (Prionace glauca) as bioindicators of pollution and health in the Atlantic Ocean: contamination levels and biochemical stress responses. Sci. Total Environ. 563–564, 282–292. http://dx.doi.org/10.1016/j.scitotenv.2016.04.085.

Bailey, K., Houde, E., 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. Adv. Mar. Biol. 25, 1–83. http://dx.doi.org/10.1016/S0065-2881(08)60187-X.

Bjelvenmark, J., Forsgren, E., 2003. Effects of mate attraction and male-male competition on paternal care in a goby. Behaviour 140, 55–69. http://dx.doi.org/10.1163/156853903763999890.

Bernardo, J., 1996. Maternal effects in animal ecology. Am. Zool. 36, 83–105.

Caldeira, K., Wickett, M.E., 2003. Anthropogenic carbon and ocean pH. Nature 425, 365. http://dx.doi.org/10.1038/425365a.

Cox, R.M., Parker, E.U., Cheney, D.M., Liebl, A.L., Martin, L.B., Calsbeek, R., 2010. Experimental evidence for physiological costs underlying the trade-off between reproduction and survival. Funct. Ecol. 24, 1262–1269.

Depledge, M.H., 1993. The rational basis for the use of biomarkers as ecotoxicological tools. In: Fossi, M.C., Leonzio, C. (Eds.), Nondestructive Biomarkers in Vertebrates, pp. 261–285 Lewis, USA.

Díaz-Gil, C., Catalán, I.A., Palmer, M., Faulk, C.K., Fuiman, L.A., 2015. Ocean acidification increases fatty acids levels of larval fish. Biol. Lett. 11, 0331. http://dx.doi.org/10.1098/rsbl.2015.0331.

Donelson, J.M., Munday, P.L., McCormick, M.I., 2009. Parental effects on offspring life histories: when are they important? Biol. Lett. 5, 262–265. http://dx.doi.org/10.1098/rsbl.2008.0642.

Donelson, J.M., Munday, P.L., McCormick, M.I., Pankhurst, N.W., Pankhurst, P.M., 2010. Effects of elevated water temperature and food availability on the reproductive performance of a coral reef fish. Mar. Ecol. Prog. Ser. 401, 233–245. http://dx.doi.org/10.3354/meps08366.

Donelson, J.M., Munday, P.L., McCormick, M.I., Nilsson, G.E., 2011. Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish. Global Change Biol. 17, 1712–1719. http://dx.doi.org/10.1111/j.1365-2486.2010.02339.x.

Forsgren, E., Amundsen, T., Borg, Å.A., Bjelvenmark, J., 2004. Unusually dynamic sex roles in a fish. Nature 429, 551–554. http://dx.doi.org/10.1038/nature02562.

Forsgren, E., Dupont, S., Jutfelt, F., Amundseen, T., 2013. Elevated CO_2 affects embryonic development and larval phototaxis in a temperate marine fish. Ecol. Evol. 3 (11), 3637–3646. http://dx.doi.org/10.1002/ece3.709.

Frommel, A.Y., Maneja, R., Lowe, D., et al., 2011. Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. Nat. Clim. Change 2, 42–46. http://dx.doi.org/10.1038/nclimate1324.

Gagliano, M., McCormick, M.I., 2007. Compensating in the wild: is flexible growth the key to early juvenile survival? Oikos 116, 111–120. http://dx.doi.org/10.1111/j.2006.0030-1299.15418.x.

Gattuso, J.P., Magnan, A., Billé, R., Cheung, W.W.L., Howes, E.L., Joos, F., Allemand, D., Bopp, L., Cooley, S., Eakin, M., HoeghGuldberg, O., Kelly, R.P., Pörtner, H.O., Rogers, A.D., Baxter, J.M., Laffoley, D., Osborn, D., Rankovic, A., Rochette, J., Sumaila, U.R., Treyer, S., Turley, C., 2015. Contrasting futures for ocean and society from different anthropogenic CO2 emissions scenarios. Science 349, aac4722. http://dx.doi.org/10.1126/science.aac4722.

Gillooly, J.F., Baylis, J.R., 1999. Reproductive success and the energetic cost of parental care in male smallmouth bass. J. Fish. Biol. 54, 573–584. http://dx.doi.org/10.1111/j.1095-8649.1999.tb00636.x.

Gravel, M.A., Couture, P., Cooke, S.J., 2010. Comparative energetics and physiology of parental care in smallmouth bass Micropterus dolomieu across a latitudinal gradient. J. Fish. Biol. 76, 280–300. http://dx.doi.org/10.1111/j.1095-8649.2009.02482.x.

Green, B.S., McCormick, M.I., 2005. Maternal and paternal influences determine size, growth and performance in a tropical reef fish larvae. Mar. Ecol.: Prog. Ser. 289, 263–272. http://dx.doi.org/10.3354/meps289263.

Hartin, C.A., Bond-Lamberty, B., Patel, P., Mundra, A., 2016. Ocean acidification over the next three centuries using a simple global climate carbon-cycle model: projections and sensitivities. Biogeosciences 13, 4329–4342. http://dx.doi.org/10.5194/bg-13-4329-2016.

Heuer, R.M., Grosell, M., 2014. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. Am. J. Physiol. Regul. Integr. Comp. Physiol. 307 (9), R1061–R1084. http://dx.doi.org/10.1152/ajpregu.00064.2014.

Ishimatsu, A., Kikkawa, T., Hayashi, M., Lee, K., Kita, J., 2004. Effects of CO_2 on marine fish: larvae and adults. J. Oceanogr. 60, 731–741. http://dx.doi.org/10.1007/s10872-004-5765-y.

Ishimatsu, A., Hayashi, M., Kikkawa, T., 2008. Fishes in high-CO2, acidified oceans. Mar. Ecol. Prog. Ser. 373, 295–302. http://dx.doi.org/10.3354/meps07823.

Johnsen, S., 1945. Studies on variation in fish in North-European waters. I. Variation in size. Bergens Museums Årbok 1944. Naturvitenskapelig rekke 4, 1–129.

Jordão, R., Garreta, E., Campos, B., Lemos, M.F.L., Soares, A.M.V.M., Tauler, R., Barata, C., 2016. Compounds altering fat storage in Daphnia magna. Sci. Total Environ. 545–546, 127–136. http://dx.doi.org/10.1016/j.scitotenv.2015.12.097.

Kroeker, K.J., Kordas, R.L., Crim, R.N., Singh, G.G., 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol. Lett. 13, 1419–1434. http://dx.doi.org/10.1111/j.1461-0248.2010.01518.x.

Kühnhold, H., Kamyab, E., Novais, S., Indriana, L., Kunzmann, A., Slater, M., Lemos, M., 2017. Thermal stress effects on energy resource allocation and oxygen consumption rate in the juvenile sea cucumber, Holothuria scabra (Jaeger, 1833). Aquaculture 467, 109–117. http://dx.doi.org/10.1016/j.aquaculture.2016.03.018.

Leatherland, J.F., Li, M., Barkataki, S., 2010. Stressors, glucocorticoids and ovarian function in teleosts. J. Fish. Biol. 76, 86–111. http://dx.doi.org/10.1111/j.1095-8649.2009.02514.x.

Love, O.P., Williams, T.D., 2008. The adaptive value of stress-induced phenotypes in the wild: effects of maternally-derived corticosterone on sex allocation, cost of reproduction and maternal fitness. Am. Nat. 172, E135–E149. http://dx.doi.org/10.1086/590959.

Mackereth, R.W., Noakes, D.L.G., Ridgeway, M.S., 1999. Size-based variation in somatic energy reserves and parental expenditure by male smallmouth bass, Micropterus dolomiteu. Environ. Biol. Fish. 56, 263–275. http://dx.doi.org/10.1023/A:1007580323816.

Melzner, F., Gutowska, M.A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M.C., Bleich, M., Pörtner, H.O., 2009. Physiological basis for high CO2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences 6, 2313–2331. http://dx.doi.org/10.5194/bg-6-2313-2009.

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M.A., Bange, H.W., Hansen, H.P., Körtzinger, A., 2013. Future ocean acidification will be amplified by hypoxia in coastal habitats. Mar. Biol. 160, 1875–1888. http://dx.doi.org/10.1007/s00227-012-1954-1.

Metcalfe, N.B., Alonso-Alvarez, C., 2010. Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. Funct. Ecol. 24, 984–996. http://dx.doi.org/10.1111/j.1365-2435.2010.01750.x.

Meylan, S., Miles, D.B., Clobert, J.R., 2012. Hormonally mediated maternal effects, individual strategy and global change. Phil. Trans. R. Soc. B 367, 1647–1664. http://dx.doi.org/10.1098/rstb.2012.0020.

Milazzo, M., Cattano, C., Alonzo, S.H., Foggo, A., Gristina, M., Rodolfo-Metalpa, R., Sinopoli, M., Spatafora, D., Stiver, K.A., et al., 2016. Ocean acidification affects fish spawning but not paternity at CO2 seeps. Proc. R. Soc. Lon. B Biol. 283, 20161021. http://dx.doi.org/10.1098/rspb.2016.1021.

Miller, P.J., 1986. Gobiidae. In: In: Whitehead, P.J.P., Bauchot, M.-L., Hureau, J.-C., Nielsen, J., Tortonese, E. (Eds.), Fishes of the North-eastern Atlantic and the Mediterranean, vol. 3. UNESCO, Paris, pp. 1019–1085 (Ref. 4696).

Miller, T., Crowder, L.B., Rice, J.A., Marschall, E.A., 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework september 1988. Can. J. Fish. Aquat. Sci. 45 (9), 1657–1670. http://dx.doi.org/10.1139/f88-197.

Miller, G.M., Watson, S.A., Donelson, J.M., McCormick, M.I., Munday, P.L., 2012. Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. Nat. Clim. Change 2, 858–861. http://dx.doi.org/10.1038/nclimate1599.

Miller, G.M., Watson, S.A., McCormick, M.I., Munday, P.L., 2013. Increased CO2 stimulates reproduction in a coral reef fish. Global Change Biol. 19, 3037–3045. http://dx.doi.org/10.1111/gcb.12259.

Miller, G.M., Kroon, F.J., Metcalfe, S., Munday, P.L., 2015. Temperature is the evil twin: effects of increased temperature and ocean acidification on reproduction in a reef fish. Ecol. Appl. 25, 603–620. http://dx.doi.org/10.1890/14-0559.1.

Millero, F.J., Graham, T.B., Huang, F., Bustos-Serrano, H., Pierrot, D., 2006. Dissociation constants of carbonic acid in seawater as a function of salinity and temperature. Mar. Chem. 100, 80–94. http://dx.doi.org/10.1016/j.marchem.2005.12.001.

Mobley, K.B., et al., 2009. Multiple mating and a low incidence of cuckoldry for nest-holding males in the two-spotted goby, Gobiusculus flavescens. BMC Evol. Biol. 9, 1–10. http://dx.doi.org/10.1186/1471-2148-9-6.

Murray, C.S., Malvezzi, A., Gobler, C.J., Baumann, H., 2014. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. Mar. Ecol. Prog. Ser. 504, 1–11. https://doi.org/10.3354/meps10791.

Oliveira, A.P., Cabeçadas, G., Pilar-Fonseca, T., 2012. Iberia coastal ocean in the CO2 sink/source context: Portugal case study. J. Coast Res. 28, 184–195. http://dx.doi.org/10.2112/JCOASTRES-D-10-00060.1.

Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R.M., Lindsay, K., Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R.G., Plattner, G.K., Rodgers, K.B., Sabine, C.L., Sarmiento, J.L., Schlitzer, R., Slater, R.D., Totterdell, I.J., Weirig, M.F., Yamanaka, Y., Yool, A., 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437, 681–686. http://dx.doi.org/10.1038/nature04095.

Pélabon, C., Borg, A.A., Bjelvenmark, J., Forsgren, E., Barber, I., Amundsen, T., 2003. Do male two-spotted gobies prefer large fecund females? Behav. Ecol. 14, 787–792. https://doi.org/10.1093/beheco/arg074.

Pörtner, H.O., Langenbuch, M., Reipschläger, A., 2004. Biological impact of elevated ocean CO2 concentrations: lessons from animal physiology and earth history. J. Oceanogr. 60, 705–718. http://dx.doi.org/10.1007/s10872-004-5763-0.

Pankhurst, N.W., Munday, P.L., 2011. Effects of climate change on fish reproduction and early life history stages. Mar. Freshw. Res. 6, 1015–1026. http://dx.doi.org/10.1071/MF10269.

Salinas, S., Munch, S.B., 2012. Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. Ecol. Lett. 15, 159–163. http://dx.doi.org/10.1111/j.1461-0248.2011.01721.x.

Schade, F.M., Clemmesen, C., Wegner, K.M., 2014. Within- and transgenerational effects of ocean acidification on life history of marine three-spined stickleback (Gasterosteus aculeatus). Mar. Biol. 161, 1667–1676. http://dx.doi.org/10.1007/s00227-014-2450-6.

Shama, L.N.S., Wegner, K.M., 2014. Grandparental effects in marine sticklebacks: transgenerational plasticity across multiple generations. J. Evol. Biol. 27, 2297–2307. http://dx.doi.org/10.1111/jeb.12490.

Shaw, E.C., Munday, P.L., McNeil, B.I., 2013. The role of CO2 variability and exposure time for biological impacts of ocean acidification. Geophys. Res. Lett. 40, 4685–4688. http://dx.doi.org/10.1002/grl.50883.

Sih, A., Ferrari, M.C.O., Harris, D.J., 2011. Evolution and behavioural responses to human-induced rapid environmental change. Evol. App 4, 367–387. http://dx.doi.org/10.1111/j.1752-4571.2010.00166.x.

Silva, C.S.E., Novais, S.C., Lemos, M.F.L., Mendes, S., Oliveira, A.P., Gonçalves, E.J., Faria, A.M., 2016. Effects of ocean acidification on the swimming ability, development and biochemical responses of sand smelt larvae. Sci. Total Environ. 563–564, 89–98. http://dx.doi.org/10.1016/j.scitotenv.2016.04.091.

Silva, C.S.E., Novais, S.C., Simões, T., Caramalho, M., Gravato, C., Rodrigues, M.J., Maranhão, P., Lemos, M.F.L., 2017. Using biomarkers to address the impacts of polluted sites on limpets (Patella depressa) and their mechanisms to cope with stress. Ecol. Indicat. http://dx.doi.org/10.1016/j.ecolind.2017.09.046.

Skolbekken, R., Utne-Palm, A.C., 2001. Parental investment of male two-spotted goby, Gobiusculus flavescens (Fabricius). J. Exp. Mar. Biol. Ecol. 261, 137–157. http://dx.doi.org/10.1016/S0022-0981(01)00249-0.

Smith, C., Wootton, R.J., 1995. The costs of parental care in teleost fishes. Rev. Fish Biol. Fish. 5, 7–22. http://dx.doi.org/10.1007/BF01103363.

Sopinka, N.M., Hinch, S.G., Middleton, C.T., Hills, J.A., Patterson, D.A., 2014. Mother knows best, even when stressed? Effects of maternal exposure to a stressor on offspring performance at different life stages in a wild semelparous fish. Oecologia (Berl.) 175, 493–500. http://dx.doi.org/10.1007/s00442-014-2915-9.

Sundin, J., Vossen, L., Nilsson-Sköld, H., Jutfelt, F., 2017. No effect of elevated carbon dioxide on reproductive behaviors in the three-spined stickleback. Behav. Ecol. 28, 1482–1491. http://dx.doi.org/10.1093/beheco/arx112.

Timmins-Schiffmann, E., Coffey, W.D., Hua, W., Nunn, B.L., Dickinson, G.H., Roberts, S.B., 2014. Shotgun proteomics reveals physiological response to ocean acidification in Crassostrea gigas. BMC Genom. 15, 1–59.

Tresguerres, M., Hamilton, T.J., 2017. Acid base physiology, neurobiology and behaviour in relation to CO_2-induced ocean acidification. JJ. Exp.Biol. 220 (12), 2136–2148. http://dx.doi.org/10.1242/jeb.144113.

Trudeau, V.L., Spanswick, D., Fraser, E.J., Lariviere, K., Crump, D., Chiu, S., et al., 2000. The role of amino acid neurotransmitters in the regulation of pituitary gonadotropin release in fish. Biochem. Cell Biol. 78, 241–259. http://dx.doi.org/10.1139/o99-075.

Utne-Palm, A.C., Eduard, K., Jensen, K.H., Mayer, I., Jakobsen, P.J., 2015. Size dependent male reproductive tactic in the two-spotted goby (*Gobiusculus flavescens*). PLoS One 10http://dx.doi.org/10.1371/journal.pone.0143487. e0143487.

Valles-Regino, R., Tate, R., Kelaher, B., Savins, D., Dowell, A., Benkendorff, K., 2015. Ocean warming and CO2-induced acidification impact the lipid content of a marine predatory gastropod. Mar. Drugs 13, 6019–6037. http://dx.doi.org/10.3390/md13106019.

Van der Oost, R., Beyer, J., Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149. http://dx.doi.org/10.1016/S1382-6689(02)00126-6.

Weiss, R.F., 1974. Carbon dioxide in water and seawater: the solubility of a non-ideal gas. Mar. Chem. 2, 203–215.

Welch, M.J., Munday, P.L., 2016. Contrasting effects of ocean acidification on reproduction in reef fishes. Coral Reefs 35, 485–493. http://dx.doi.org/10.1007/s00338-015-1385-9.

Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and resistance to stress: when and how. J. Neuroendocrinol. 15, 711–724. http://dx.doi.org/10.1046/j.1365-2826.2003.01033.x.

Wu, R.S., Siu, W.H., Shin, P.K., 2005. Induction, adaptation and recovery of biological responses: implications for environmental monitoring. Mar. Pollut. Bull. 51 (8), 623–634. http://dx.doi.org/10.1016/j.marpolbul.2005.04.016.

Zohar, Y., Muñoz-Cueto, J.A., Elizur, A., Kah, O., 2010. Neuroendocrinology of reproduction in teleost fish. Gen. Comp. Endocrinol. 165, 438–455. http://dx.doi.org/10.1016/j.ygcen.2009.04.017.

Zolderdo, A.J., Algera, D.A., Lawrence, M.J., Gilmour, K.M., Fast, M.D., Thuswaldner, J., Willmore, W.G., Cooke, S.J., 2016. Stress, nutrition and parental care in a teleost fish: exploring mechanisms with supplemental feeding and cortisol manipulation. J. Exp. Biol. 219, 1237–1248. http://dx.doi.org/10.1242/jeb.135798.