ARTICLE IN PRESS

Marine Environmental Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

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

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ARTICLE INFO

Keywords:
Biomarker
Energy metabolism
Fish
Gobiusculus flavescens
Ocean acidification
Reproductive success

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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https://doi.org/10.1016/j.marenvres.2018.02.027
Received 3 January 2018; Received in revised form 23 February 2018; Accepted 23 February 2018

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