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Sand smelt ability to cope and recover from ocean's elevated CO_2 levels

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ABSTRACT

Considered a major environmental concern, ocean acidification has induced a recent research boost into effects on marine biodiversity and possible ecological, physiological, and behavioural impacts. Although the majority of literature indicate negative effects of future acidification scenarios, most studies are conducted for just a few days or weeks, which may be insufficient to detect the capacity of an organism to adjust to environmental changes through phenotypic plasticity. Here, the effects and the capacity of sand smelt larvae *Atherina presbyter* to cope and recover (through a treatment combination strategy) from short (15 days) and long-term exposure (45 days) to increasing pCO_2 levels (control: ~515 µatm, pH = 8.07; medium: ~940 µatm, pH = 7.84; high: ~1500 µatm, pH = 7.66) were measured, addressing larval development traits, behavioural lateralization, and biochemical biomarkers related with oxidative stress and damage, and energy metabolism and reserves. Although behavioural lateralization was not affected by high pCO_2 exposure, morphometric changes, energetic costs, and oxidative stress damage were impacted differently through different exposures periods. Generally, short-time exposures led to different responses to either medium or high pCO_2 levels (e.g. development, cellular metabolism, or damage), while on the long-term the response patterns tend to become similar between them, with both acidification scenarios inducing DNA damage and tending to lower growth rates. Additionally, when organisms were transferred to lower acidified condition, they were not able to recover from the mentioned DNA damage impacts.

Overall, results suggest that exposure to future ocean acidification scenarios can induce sublethal effects on early life-stages of fish, but effects are dependent on duration of exposure, and are likely not reversible. Furthermore, to improve our understanding on species sensitivity and adaptation strategies, results reinforce the need to use multiple biological endpoints when assessing the effects of ocean acidification on marine organisms.

1. Introduction

The unprecedented amount of carbon dioxide being released by anthropogenic sources (Kerr, 2010) has surpassed the oceans' capacity to absorb it, leading to changes in its chemistry in a process known as ocean acidification. With current rates of CO_2 emissions, pH is expected to drop 0.3–0.4 units until the end of this century, from 8.1 to 7.8 or 7.7 (IPCC, 2014). These ocean pH changes, which are occurring at an unprecedented rate compared with similar events in the geological past, pose serious threats to marine life, which evolved over millions of years in a stable pH environment (Kerr, 2010).

Initially focused on corals and other calcified organisms due to its carbonate dependence (Orr et al., 2005; Hoegh-Guldberg et al., 2007), research into the effects of elevated CO_2 has spread to other non-calcified marine organisms, such as fish. Early life stages might be particularly vulnerable (Melzner et al., 2009) given that physiological regulation mechanisms are poorly developed (Brauner, 2008). The available scientific findings report detrimental effects on larval growth (e.g. Baumann et al., 2012; Chambers et al., 2014; Silva et al., 2016; Rato et al., 2017), metabolism (e.g. Pimentel et al., 2015; Silva et al., 2016), sensorial perception (e.g. Dixson et al., 2010; Castro et al., 2017; Chung et al., 2014), and behaviour (e.g. Devine et al., 2012; Lopes et al., 2016). Moreover, exposure to increased CO_2 levels may contribute to divert energy towards internal balance mechanisms, with increased metabolic costs (Silva et al., 2016), resulting in less energy available for other important tasks. As a result, these trade-offs may have major implications for survival, recruitment, and ultimately affect population replenishment and sustainability (Stiasny et al., 2016; Le

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Quesne and Pinnegar, 2012). Despite these evidences, most studies to date, on fish, involve short-term exposure periods to elevated pCO_2 (usually less than 3 weeks), which may be insufficient to capture species' ability to acclimate to the environmental changes through phenotypic plasticity.

In a previous work with larvae of sand smelt, *Atherina presbyter* – a temperate, commercial, pelagic fish species from the Atlantic Ocean coasts – evidence of increased size and an ineffective antioxidant response capacity of larvae exposed to elevated pCO_2 levels (~1800 µatm, pH = 7.64) for 15-days was found, while larvae from intermediate pCO_2 levels (~1000 µatm, pH = 7.85) presented smaller sizes, associated to increased energetic metabolism and an active antioxidant response system (Silva et al., 2016). These results suggest a fight-back cellular response at intermediate pCO_2 levels, but the consequences of a longer-term exposure period are still unclear – the increased energetic costs could either result in higher mortality, later on when energy depots lower the minimum needed for regular organism homeostasis, or fish could potentially acclimate to increased pCO_2 levels. Here the consequences of a longer-term exposure period (45 days) of sand smelt larvae to increasing pCO_2 levels (control: ~515 µatm, pH = 8.07; medium: ~940 µatm, pH = 7.84; high: ~1500 µatm, pH = 7.66), at biochemical, developmental, and behavioural level are addressed. Behavioural lateralization was chosen as the behavioural endpoint as a previous work with sand smelt larvae reported changes in lateralization under elevated pCO_2 levels (Lopes et al., 2016). As the early assessment of climate change effects at the community and ecosystem level is particularly difficult, measurements at lower levels of ecological relevance have been extensively used to detect, and possibly extrapolate changes occurring at individual level (Lemos et al., 2010; Pan et al., 2015; Schunter et al., 2016). Biochemical biomarkers, mainly related with energy metabolism and oxidative stress, have been widely used as efficient tools to detect and monitor environmental variations and fitness costs (Huggett et al., 1992; Tonn et al., 2016; Silva et al., 2016; Kamyab et al., 2017). The exposure to environmental stressors may induce the formation of free radicals, contributing to the cellular vulnerability of organisms through lipid peroxidation (LPO) and/or DNA damage (Aguilera and Rautenberger, 2010; Lesser, 2011). To maintain cellular integrity, a set of antioxidant enzymes, including catalase (CAT) and superoxide dismutase (SOD), prevent the formation and/or remove these reactive metabolites (Abele and Puntarulo, 2004), while isocitrate and lactate dehydrogenase are associated with shifts on energetic metabolism, from aerobic to anaerobic, respectively (Huggett et al., 1992).

Together with the biomarkers approach, lateralization was chosen to assess the biochemical and morphological impacts in *Atherina presbyter* larvae induced by exposure to high levels of pCO_2, while gaining insight on the adaptation and recovery to ocean acidification, through a treatment combination strategy and extended exposure period.

2. Materials and methods

2.1. Ethics statement

The current study was performed under the guidelines of the Portuguese Veterinary Authority (DGV-Portugal, following FELASA category C recommendations) and the European directive 2010/63/UE for the protection of animals used for scientific purposes.

2.2. pCO_2 treatments

Sand smelt were exposed to control pCO_2 levels (pH 8.07, ~515 µatm), and two elevated pCO_2 treatments: medium (pH 7.84, ~940 µatm) and high (pH 7.66, ~1500 µatm). Control treatment was selected based on previous pH measures at the sampling site (pH 8.05–8.07); the medium treatment was chosen based on pCO_2 levels reported in the coastal areas where larvae of this species occur, where concentrations up to 1170 µatm have been reported under upwelling events (Cabeçadas and Oliveira, 2005); the highest pCO_2 treatment was chosen as an extreme condition, which may be reached in upwelling systems if the worst IPCC scenarios are met by 2100.

The experiments were performed with artificial seawater resulting from the mixing of filtered freshwater with a salt mixture (TropicMarin®) and adjusted to a salinity of 34.5‰. Except for the control treatment, where pH was directly influenced by ambient air, the two pCO_2 treatments were achieved by CO_2 injection. pH in the two pCO_2 treatments was regulated by a pH computer (Tunze Aquarientechnik, Germany) connected to a pH probe connected to a 200-L sump. The pH_{NBS} (National Bureau of Standards Scale), in the three treatments, was daily cross-checked using a portable meter (SevenGo DuoPro, SG23), which was also used for daily measures of temperature and salinity. Due to diffusion pumps on each sump, oxygen levels were always kept above 90% saturation. Each sump was equipped with diverse filtration sets (ultraviolet, chemical, biological, and mechanical), and delivered a continuous supply of recirculated seawater into five 35-L aquariums at a flow-rate of ~600 mL min^{-1}. Aquariums were sealed on top with a clear glass lid to limit CO_2 exchange with the atmosphere. Ammonia, nitrates, and nitrites were monitored weekly and kept below critical levels.

Total alkalinity (TA) was determined on a weekly basis, using automated Gran titrations, with certified reference material supplied by A. Dickson (Scripps Institutions of Oceanography, San Diego). pCO_2 was calculated in CO_2SYS (Pierrot et al., 2006) using *in situ* temperature, TA, and pH, the carbonic acid dissociation constants given by Millero et al. (2006) and the CO_2 solubility coefficient of Weiss (1974) for each of the experimental treatments (Table 1).

2.3. Test organisms and experimental design

Sand smelt larvae were collected at the surface, in the very nearshore, at Portinho da Arrábida, Portugal (38°28′48″ N | 8°58′59″ W), using a 1 mm mesh hand net. Larvae were immediately transported to the laboratory and allowed to recover from handling effects for 2 days in 35-L tanks with recirculating seawater. Except for the two-days recovery period, larvae were daily fed *ad libitum* with *Artemia* nauplii and maintained under controlled temperature and salinity, and a summer light cycle of 14 h light:10 h dark. Individuals were then randomly assigned to a control (C), medium (M), or high (H) pCO_2 treatment, and maintained in five replicate 35-L tanks, per treatment, throughout the experiment. Initial larval density was 12–15 larvae per tank (N = 73 in control treatment, N = 70 in mid pCO_2 treatment, and N = 72 in high pCO_2 treatment). After 30 days in treatment, larvae were either transferred between treatments or left in the original treatment for another 15 days, thus totalling 45 days in treatment (CC, CM, CH; MM, MC, MH; HH, HC, HM). No mortality was observed during the experiment, in any of the treatments. Larvae were randomly sampled at 15, 30, and 45

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

pCO_2 condition	pH_{NBS}	T(°C)	S (psu)	TA (µmol kg^{-1})	pCO_2 (µatm)
Control	8.07 ± 0.03	16.0 ± 0.3	34.7 ± 0.6	2253 ± 5	516 ± 43
Medium	7.84 ± 0.01	16.4 ± 0.3	34.9 ± 0.2	2245 ± 3	943 ± 29
High	7.66 ± 0.02	16.6 ± 0.2	34.6 ± 0.8	2247 ± 5	1504 ± 71