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Effects of ocean acidification on the swimming ability, development and biochemical responses of sand smelt larvae

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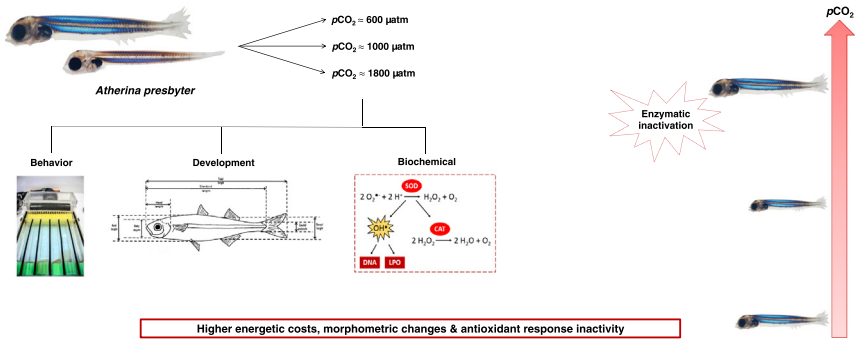
HIGHLIGHTS

- Exposure to high pCO_2 has several impacts on fish early life stages.
- Behavioural, morphometric and biochemical approaches were used to assess impacts.
- Critical swimming speed was unaffected by high pCO_2, contrary to larval development
- High pCO_2 leads to increased oxidative stress and differential energy allocation.

GRAPHICAL ABSTRACT



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ABSTRACT

Ocean acidification, recognized as a major threat to marine ecosystems, has developed into one of the fastest growing fields of research in marine sciences. Several studies on fish larval stages point to abnormal behaviours, malformations and increased mortality rates as a result of exposure to increased levels of CO_2. However, other studies fail to recognize any consequence, suggesting species-specific sensitivity to increased levels of CO_2, highlighting the need of further research. In this study we investigated the effects of exposure to elevated pCO_2 on behaviour, development, oxidative stress and energy metabolism of sand smelt larvae, *Atherina presbyter*. Larvae were caught at Arrábida Marine Park (Portugal) and exposed to different pCO_2 levels (control: ~600 µatm, pH = 8.03; medium: ~1000 µatm, pH = 7.85; high: ~1800 µatm, pH = 7.64) up to 15 days, after which critical swimming speed (U_{crit}), morphometric traits and biochemical biomarkers were determined. Measured biomarkers were related with: 1) oxidative stress — superoxide dismutase and catalase enzyme activities, levels of lipid peroxidation and DNA damage, and levels of superoxide anion production; 2) energy metabolism — total carbohydrate levels, electron transport system activity, lactate dehydrogenase and isocitrate dehydrogenase enzyme activities. Swimming speed was not affected by treatment, but exposure to increasing levels of pCO_2 leads to higher energetic costs and morphometric changes, with larger larvae in high pCO_2 treatment and smaller larvae in medium pCO_2 treatment. The efficient antioxidant response capacity and increase in energetic metabolism only registered at the medium pCO_2 treatment may indicate that at higher pCO_2 levels the capacity of larvae to restore their internal balance can be impaired. Our findings illustrate the need of using multiple approaches to explore the consequences of future pCO_2 levels on organisms.

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1. Introduction

Ocean acidification is recognized as an important driver of change in biological systems (Hoegh-Guldberg and Bruno, 2010; Doney et al., 2012), and has developed into one of the fastest growing fields of research in marine sciences (Rudd, 2014). The continuous uptake of additional atmospheric CO_2 is expected to cause severe changes in seawater chemistry, and depending on the emission trajectory, CO_2 partial pressure (pCO_2) levels are projected to exceed 900 ppm by 2100 (pH decreases of approximately 0.4 units) in an open ocean scenario (IPCC, 2013). In coastal environments, the effects of ocean acidification may be even greater and more variable due to eutrophication (Borges and Gypens, 2010; Cai et al., 2011) and upwelling events (Feely et al., 2008; Lachkar, 2014).

Most studies on the impacts of ocean acidification have focused on marine calcifying invertebrates, particularly molluscs and *Cnidaria* (Heuer and Grosell, 2014) due to their calcium carbonate dependence (Orr et al., 2005). Less attention has traditionally been given to fish, but in the past few years an impressive body of studies on the effects of ocean acidification has been published, covering a wide range of species. The early life stages have been particularly studied as they are most likely to be affected by increasing pCO_2, due to poorly developed mechanisms of physiological regulation (Brauner, 2008). Results point towards disturbances across a wide range of sensory systems and neurological functions, like olfaction (Dixson et al., 2010), vision (Chung et al., 2014), lateralization (Domenici et al., 2012), hearing (Simpson et al., 2011), learning (Ferrari et al., 2012), and activity levels (Munday et al., 2010). These disturbances will presumably have substantial impacts on predator-prey interactions (Domenici et al., 2012; Ferrari et al., 2012) and orientation abilities (Siebeck et al., 2015), and may ultimately lead to increased mortality rates (Munday et al., 2010). Recent studies have also detected impacts on survival, growth (Baumann et al., 2012) and development (Munday et al., 2009a), as well as on physiological and biochemical responses (Pimentel et al., 2015) of fish early life stages.

Despite these significant impacts, other studies fail to find any dramatic consequence of high pCO_2 on larval development and survival (e.g. Munday et al., 2011) or behaviour (e.g. Jutfelt and Hedgarde, 2013), suggesting species-specific levels of sensitivity to a changing environment. It is still unclear what justifies different species sensitivities to ocean acidification, but it might be related to the spawning mode of fishes (Munday et al., 2009a) or metabolic rates (Pane and Barry, 2007). Additional experimentation across a wide range of fish species with contrasting life histories and habitats will help identifying the factors associated with relative sensitivity to ocean acidification, as this will be critical for assessing the impacts on marine biodiversity and ecosystem function (Fabry et al., 2008).

In this study, we investigated the impact of ocean acidification on the early life stages of sand smelt (*Atherina presbyter*). Sand smelt is one of the two species of *Atherinidae* in the north-eastern Atlantic Ocean (Whitehead et al., 1986). It lives inshore, occasionally entering coastal lagoons and estuaries, and is an important prey species for some commercially valuable coastal predators. Adults spawn on filamentous algae, and larvae hatch after approximately 15 days of embryonic development (Bamber et al., 1985), as competent larvae capable of swimming (Faria et al., 2014), and form shoals (Bamber et al., 1985). We were particularly interested in evaluating treatment effects on critical swimming speed (U_{crit}), development and biochemical responses. U_{crit} is one of the most common methods to assess swimming abilities in fish larvae, enabling standardized comparisons of different taxa and developmental stages (Plaut, 2001; Leis, 2006). Swimming abilities have the potential to influence survival as they are critical to find food, avoid predators and influence dispersal (Fisher, 2005a; Leis, 2006). Survival of larval fishes may also be deeply impacted by changes in growth and development (Houde, 1997), which in turn may be related to altered metabolic demand or reallocation of metabolic resources under stressful conditions (Matson et al., 2012). While organisms normally allocate their energy for growth, reproduction and maintenance of their basal metabolism, the disruption in acid-base balance due to exposure to environmental stressors can result in the reallocation of metabolic resources to deal with stress and maintain homeostasis. This may, ultimately, impact the energy balance and disrupt the development and somatic growth of the organism (Matson et al., 2012). Exposure to environmental stressors can dramatically increase levels of reactive oxygen species (ROS) (Lesser, 2011), potentially compromising the organism's ability to reestablish balance. The failure of defense mechanisms to respond to these threats can lead to oxidative stress and, consequently, damage at cellular level, including proteins, DNA, and lipids (Young and Woodside, 2001; De Jesus and De Carvalho, 2008). The antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), are part of the defense mechanisms of the organisms, playing a major role protecting or delaying the oxidative DNA damage, lipid peroxidation (LPO) and enzymatic inactivation (Halliwell and Gutteridge, 1999; Novais et al., 2014). Other enzymes, related to energy metabolism, such as lactate dehydrogenase (LDH) (associated with anaerobic metabolism, Ribeiro et al., 1999; Diamantino et al., 2001), and isocitrate dehydrogenase (IDH) (associated with aerobic metabolism, Moreira et al., 2006) also play an important role in the production of the necessary energy for the maintenance of organisms' physiological homeostasis. The reallocation of metabolic resources to avoid/repair internal stress and damages may be challenging for larvae (Cunha et al., 2007), with severe consequences for development, performance and survival, which will eventually impact the population and communities, and ultimately the ecosystem.

Here we tested the hypothesis that increased pCO_2 and decreased pH conditions would damage the organism at the cellular and metabolic level while also negatively affecting swimming and larval development.

2. Materials and methods

The current study was undertaken under the supervision of an accredited expert in laboratory animal science by the Portuguese Veterinary Authority (DGV-Portugal, following FELASA category C recommendations), according to the guidelines on the protection of animals used for scientific purposes from the European directive 2010/63/UE.

2.1. pCO_2 treatments

Sand smelt larvae were exposed to local ambient conditions, pH 8.0, ~600 μatm, and to 2 elevated pCO_2 treatments: medium, pH 7.8, ~1000 μatm; and high, pH 7.6, ~1800 μatm. The medium pCO_2 treatment was chosen based on values already reported in the coastal waters where sand smelt inhabits, where pCO_2 values up to 1170 μatm have been recorded under upwelling conditions (Cabeçadas and Oliveira, 2005). The high pCO_2 treatment was chosen as an extreme condition, but with the amplifying effects of anthropogenic ocean acidification (Melzner et al., 2013), future pCO_2 during upwelling events could easily exceed 1800 μatm.

Artificial seawater adjusted to a salinity of 34.5‰ was used in the experiments by blending a commercial salt mixture (Tropic Marin®) with filtered freshwater. Seawater was diffused with ambient air (control) or CO_2 in a 200-L sump to achieve the required pH, controlled by two pH controllers (Tunze Aquarientechnik, Germany) attached to the sump of each treatment. Each sump was equipped with mechanical, biological, chemical and ultraviolet filtration and delivered equilibrated seawater into 35-L aquariums at a flow-rate of ~600 mL min^{-1}. A number of 30–50 fish were randomly assigned to 2-replicate aquariums per treatment.

Temperature, salinity and pH_{NBS} (National Bureau of Standards Scale) were daily measured using a portable meter (SevenGo DuoPro, SG23). Diffusion pumps on each sump kept oxygen levels above 90% saturation in all treatments. Samples for total alkalinity