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Negative synergistic impacts of ocean warming and acidification on the survival and proteome of the commercial sea bream, *Sparus aurata*

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ABSTRACT

Global change is impacting aquatic ecosystems, with high risks for food production. However, the molecular underpinnings of organismal tolerance to both ocean warming and acidification are largely unknown. Here we tested the effect of warming and acidification in a 42-day experiment on a commercial temperate fish, the gilthead seabream *Sparus aurata*. Juvenile fish were exposed to *control* (C 18 °C pH 8), *ocean warming* (OW 22 °C pH 8), *ocean acidification* (OA 18 °C pH 7.5) and *ocean warming and acidification* (OWA 22 °C pH 7.5). Proxies of fitness (mortality; condition index) and muscle proteome changes were assessed; bioinformatics tools (Cytoscape, STRAP, STRING) were used for functional analyses. While there was no mortality in fish under OW, fish exposed to OA and both OWA showed 17% and 50% mortality, respectively. Condition index remained constant in all treatments. OW alone induced small proteome adjustments (up-regulation of 2 proteins) related to epigenetic gene regulation and cytoskeletal remodeling. OA and both OWA induced greater proteome changes (12 and 8 regulated proteins, respectively) when compared to OW alone, suggesting that pH is central to proteome modulation. OA exposure led to increased glycogen degradation, glycolysis, lipid metabolism, anion homeostasis, cytoskeletal remodeling, immune processes and redox based signaling while decreasing ADP metabolic process. OWA led to increased lipid metabolism, glycogen degradation, glycolysis, cytoskeleton remodeling and decreased muscle filament sliding and intermediate filament organization. Moreover, as rates of change in temperature and acidification depend on region we tested as proof of concept an (i) acidification effect in a hot ocean (22 °C pH 8 *vs* 22 °C pH 7.5) which led to the regulation of 7 proteins, the novelty being in a boost of anaerobic metabolism and impairment of proteasomal degradation; and (ii) warming effect in an acidified ocean (18 °C pH 7.5 *vs* 22 °C pH 7.5) which led to the regulation of 5 proteins, with an emphasis on anaerobic metabolism and transcriptional regulation. The negative synergistic effects of ocean warming and acidification on fish survival coupled to the mobilization of storage compounds, enhancement in anaerobic pathways and impaired proteasomal degradation could pose a serious threat to the viability of sea bream populations.

Abbreviation list: ACT, actin isoform; ACT2, actin, muscle-type/alpha cardiac muscle 2; ACTS, actin alpha skeletal muscle; ACTSB, actin alpha skeletal muscle B; ADP, adenosine diphosphate; Ambic, ammonium bicarbonate; APOA1, apolipoprotein A-I; ATP, adenosine triphosphate; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; Da, dalton; DOC/TCA, Na-deoxycholate/trichloroacetic acid; DTT, dithiothreitol; G3P, glyceraldehyde-3-phosphate dehydrogenase; GDE, glycogen debranching enzyme; GO, gene ontology; Hsp, heat shock proteins; IF2A, eukaryotic translation initiation factor 2 subunit 1; IPG, immobilized pH gradient; KAD1, adenylate kinase isoenzyme 1; KDM3A, lysine-specific demethylase 3A; LDHBA, L-lactate dehydrogenase B-A chain; MALDI TOF-TOF, Matrix-assisted laser desorption/ionization time-of-flight; MAPK, mitogen activated protein kinase; NADH, nicotinamide adenine dinucleotide reduced form; NADPH, nicotinamide adenine dinucleotide phosphate reduced form; NEBU, nebulin; OA, ocean acidification; OW, ocean warming; OWA, ocean warming and acidification; PERI, peripherin; PMF, peptide mass fingerprints; PSA4, proteasome subunit alpha type-4; PSA6, proteasome subunit alpha type-6; SDS, sodium dodecyl sulphate; TPISB, triose phosphate isomerase B; ZFP69, zinc finger protein ZFP69

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1. Introduction

Global change forcing owing to greenhouse gas emissions (*e.g.* global warming and ocean acidification) is imposing biodiversity changes across terrestrial, coastal and oceanic habitats, with high risks for food production (IPCC, 2014; Walther et al., 2002). Sea surface temperature has risen 0.8 °C over the past century concomitantly with a decrease of 0.1 in ocean pH, which corresponds to a 26% increase in water acidity (IPCC, 2014). Model projections further indicate that oceans will warm up by 3 to 4 °C and will undergo additional acidification (Δ pH -0.3 to −0.5) until 2100, depending on region, habitat and emission scenario (Mora et al., 2013). Such changes are expected to decrease the fitness of marine biota (Kroeker et al., 2010; Mora et al., 2013) even though the sensitivity to environmental change may depend on taxonomic group. Organisms in high trophic levels such as carnivore fish will be highly impacted *via* elevated metabolic costs due to a rise in temperature coupled to a decrease in secondary production due to acidification (Nagelkerken and Connell, 2015). Some species (*e.g.* temperate killifish and tropical damselfish) are expected to show phenotypic plasticity over short (one generation) and long (transgenerational) time-scales allowing them to acclimate to new environmental conditions (Donelson et al., 2014; Donelson et al., 2011; Fangue et al., 2006). However, negative effects may be exacerbated in exploited fish populations as their genetic diversity is highly reduced, further decreasing their potential for adaptation facing the changing world (Ottersen et al., 2006).

Physiological mechanisms of acclimation may not be specific for each stressor and overall they can be related to changes in gene expression (and protein levels), metabolism, behavior, life history traits, growth and reproductive tactics. For instance, organisms adjust metabolic rates (oxygen consumption) and modulate cellular pathways related to cytoskeleton dynamics, protein quality control system, antioxidant response, metabolic reprogramming, immune system, transcriptional regulation and signal transduction in response to elevated water temperature and/or acidification, in order to promote survival (Bresolin de Souza et al., 2014; Carter et al., 2013; Garland et al., 2015; Gunnarsson, 2010; Jayasundara et al., 2015; Madeira et al., 2016a; Pörtner, 2010; Stillman and Tagmount, 2009; Timmins-Schiffman et al., 2014; Tomanek, 2014). However, elevated mortality rates and changes in the distributional range of species associated with global change and extreme climatic events have already been observed in the marine environment (Pearce and Feng, 2013; Walther et al., 2002; Wernberg et al., 2013; Wernberg et al., 2011), suggesting that physiological limits can be exceeded. Demersal sea breams may be particularly vulnerable to global change as their Critical Thermal Maxima values are not far from mean coastal and estuarine water temperatures and these could be surpassed by maximum temperatures reached during heat waves (Madeira et al., 2014; Madeira et al., 2012). Additionally, thermal stress has been shown to induce tissue damage and mortality in the commercial gilthead seabream, *Sparus aurata* (*Linnaeus* 1758), paralleled to an increase in mitogen activated protein kinase (MAPK) signaling, glycolytic potential and markers of protein denaturation and oxidative stress (Feidantsis et al., 2009; Madeira et al., 2016c; Madeira et al., 2014). Warming is expected to have greater effects in physiology than acidification, as marine biota seem to be quite tolerant to a pH decrease (Byrne et al., 2009; Fabry et al., 2008; Findlay et al., 2010; Perry et al., 2015), although acidification may especially affect early-life stages (metabolic suppression, lower condition and impaired olfactory discrimination), particularly when combined with elevated temperature (Byrne, 2011; Fabry et al., 2008; Flynn et al., 2015; Munday et al., 2009a; Rosa et al., 2014a,b). Nevertheless, there seems to be no agreement on the combined effects of temperature and acidification. Some authors report antagonistic effects (Davis et al., 2013; Ferrari et al., 2015; Pistevos et al., 2016) while others report additive (Anlauf et al., 2011; Talmage and Gobler, 2011) and synergistic effects (Ferrari et al., 2015; Flynn et al., 2015), depending on species, developmental stage and parameters analyzed (reviewed by Byrne and Przeslawski, 2013). Thus, knowledge on the interactive effects of global change drivers in marine biota is still limited (Byrne, 2011; Ferrari et al., 2015).

Sea breams are ecologically and economically relevant species in Southern Europe and North Africa. As predators, they exert top-down control of coastal ecosystem functioning and are a highly relevant group for the fishing (6703 t in 2014) and aquaculture industries (158,389 t in 2014) (EUMOFA, 2015; FAO, 2015). Thus, the aim of this study was to investigate the long-term combined effects of ocean warming and acidification on a relevant sea bream, *Sparus aurata*. We hypothesized that (i) temperature and the combination of temperature and acidification induce greater physiological effects on fish than acidification alone; (ii) fish alter their proteome in response to single and both stressors, inducing proteins with cytoprotective functions and enhancing glycolytic potential to try to sustain the cellular stress response. To test these hypotheses we subjected fish to a 42-day experiment simulating global change conditions for 2100 (+4 °C and -0.5 in pH) and calculated mortality and Fulton's K condition index concomitantly with the use of proteomics tools to assess protein changes in the muscle of fish. Such tools allow the establishment of direct links between molecular responses and phenotypes/fitness and the unravelling of pathways that characterize acclimation and adaptation processes (Dalziel and Schulte, 2012; Diz et al., 2012; Dupont et al., 2007; Karr, 2008), providing a mechanistic insight into the impacts of global change drivers on exploited high trophic level fish.

2. Material and methods

2.1. Ethical statement

This study was approved by *Direcção Geral de Alimentação e Veterinária* and followed EU legislation for animal experimentation (Directive2010/63/EU). Two authors have a level C (persons responsible for directing animal experiments) certification by the Federation of European Laboratory Animal Science Associations.

2.2. Sparus aurata housing and husbandry

Fish (n = 48, mean ± sd; total lenght of 11 ± 3 cm and 38 ± 8 g of weight) were obtained from a fish farm (MARESA, Spain) and transported to the laboratory in 100 L opaque plastic boxes with constant aeration and stable temperature conditions (100% survival during transport). Sample sizes were calculated following previous omics studies (Jayasundara et al., 2015; Logan and Somero, 2011). Fish were placed in a re-circulating system consisting of two 400 L glass tanks (57 × 100 × 70 cm) with 24 individuals *per* tank. The tanks were filled with clean natural aerated sea water (95–100% air saturation), with a constant temperature of 18 °C, salinity 35‰ and pH 8.0 (conditions of the fish farm). The fish were acclimated for 2 weeks and their welfare was assessed (*e.g.* presence/absence of wounds, external parasites, spots, ragged fins, lack of appetite). During the acclimation and experimental trials, a regime of period feeding was carried out (twice a day) with commercial food pellets (Gemma Diamond 1.8, Skettring).

2.3. Experimental design

Fish were randomly divided into four 227 L tanks (37 × 98 × 62.5 cm) (1) *control* (C) 18 °C, pH 8 similar to natural water conditions to wild fish; (2) simulating conditions of *ocean warming* (OW, +4 °C) 22 °C, pH 8; (3) *ocean acidification* (OA, -0.5 in pH) 18 °C, pH 7.5; (4) *ocean warming and acidification* (OWA) 22 °C, pH 7.5 (according to IPCC, 2014) and maintained at these conditions for 42 days (n = 12 fish *per* tank). Temperature was maintained using thermostats and pH levels were adjusted with CO_2 gas mixture injection. Water parameters (ammonia, nitrites, nitrates, O_2, temperature and pH) were monitored