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Ocean warming alters cellular metabolism and induces mortality in fish early life stages: A proteomic approach

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ABSTRACT

Climate change has pervasive effects on marine ecosystems, altering biodiversity patterns, abundance and distribution of species, biological interactions, phenology, and organisms' physiology, performance and fitness. Fish early life stages have narrow thermal windows and are thus more vulnerable to further changes in water temperature. The aim of this study was to address the sensitivity and underlying molecular changes of larvae of a key fisheries species, the sea bream *Sparus aurata*, towards ocean warming. Larvae were exposed to three temperatures: 18 °C (control), 24 °C (warm) and 30 °C (heat wave) for seven days. At the end of the assay, i) survival curves were plotted for each temperature treatment and ii) entire larvae were collected for proteomic analysis via 2D gel electrophoresis, image analysis and mass spectrometry. Survival decreased with increasing temperature, with no larvae surviving at 30 °C. Therefore, proteomic analysis was only carried out for 18 °C and 24 °C. Larvae up-regulated protein folding and degradation, cytoskeletal re-organization, transcriptional regulation and the growth hormone while mostly down-regulating cargo transporting and porphyrin metabolism upon exposure to heat stress. No changes were detected in proteins related to energetic metabolism suggesting that larval fish may not have the energetic plasticity needed to sustain cellular protection in the long-term. These results indicate that despite proteome modulation, *S. aurata* larvae do not seem able to fully acclimate to higher temperatures as shown by the low survival rates. Consequently, elevated temperatures seem to have bottleneck effects during fish early life stages, and future ocean warming can potentially compromise recruitment's success of key fisheries species.

1. Introduction

The Earth's climate is dynamic; it has changed many times according to historical records. However, while past changes can be attributed to natural causes, more recent changes (and rate of change) have been associated with an increase in greenhouse gas emissions by direct anthropogenic activities (Brierley and Kingsford, 2009; National Research Council USA, 2010; Doney et al., 2012; Godbold and Calosi, 2013). This phenomenon has led to an increase in global temperature (ranging from +1.8 to +4.0 °C), a change in weather patterns and hydrodynamics, sea level rise, stratification of ocean waters and ocean acidification (Solomon et al., 2007; Brierley and Kingsford, 2009; Okey et al., 2012; Storch et al., 2014; Bradley et al., 2015). Growing evidence is showing that climate change has pervasive impacts on terrestrial and marine ecosystems, altering biodiversity patterns, abundance and distribution of species, biological interactions, phenology, and organisms' physiology, performance and fitness (Walther et al., 2002; Perry et al., 2005; Doney et al. 2012, Okey et al., 2012). However, the extent of these effects may differ regionally and is dependent upon several factors such as regional rate of warming, as well as organisms' tolerance limits, phenotypic plasticity, adaptive capacity, generation time, dispersal, and reproductive output (e.g. Pörtner and Farrell, 2008). Moreover, tolerance limits and performance are dependent upon ontogenetic stage, because throughout development organisms have different physiological requirements. Several studies have approached this issue by studying thermal window widths and physiological performance across life stages (i.e. Pörtner and Farrell, 2008; Pörtner and Peck, 2010). According to these studies, thermal windows widths are narrower for egg and larval stages, as well as for spawners. Broader thermal windows are characteristic of juveniles and growing adults (Pörtner and Farrell, 2008). As such, early life

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stages are highly vulnerable to ocean warming (e.g. Storch et al., 2001; Pörtner and Farrell, 2008). Therefore, exposure of early life stages to stress can induce harmful downstream effects, in a process called 'developmental domino effect' (Pechenik , 2006; Byrne 2012; Byrne et al., 2013).

In the context of climate change, an overview of the current scientific literature shows that warming has greater physiological effects than ocean acidification in many species (see Byrne et al., 2009; Findlay et al., 2010; Rodolfo-Metalpa et al., 2010; Pansch et al., 2012; Moya et al., 2015). However, Byrne (2012) states that research concerning the vulnerability of early developmental stages to climate change has mainly focused on ocean acidification. Nevertheless, some studies have shown that temperature induces both sublethal and lethal effects on larvae. Among sublethal effects, changes in metabolism, disease resistance, growth and development, and increased incidence of malformations were reported by several authors (e.g. Polo et al., 1991; Werner et al., 2007; Georgakopoulou et al., 2010; Byrne, 2011; Vinagre et al., 2013). Moreover, increasing temperatures can induce low performance and increased mortality, creating a bottleneck effect at early life stages, impairing recruitment and population sustainability (Houde, 1989, 2008; Faria et al., 2011; Landsman et al., 2011; Bartolini et al., 2013). This may have severe effects in several species including commercial ones, compromising fish stocks and ecosystem services.

A major limitation in climate change research and associated effects on marine larvae is the lack of information concerning the molecular basis of responses to stress. Even though this has been addressed by several authors, studies are usually focused on a few specific biomarkers. Some improvement was possible with the expansion of genomic and transcriptomic tools; however, these are focused on analyzing DNA sequences or transcripts and not the final product of gene expression (i.e. proteins). Therefore, direct links with phenotype and fitness are hard to establish especially due to the poor correlation between transcript and protein levels (Vogel and Marcotte, 2012; Diz et al., 2012). Proteomics overcame this issue by enabling the study of a vast set of proteins within the cell, which can be linked to the cell's phenotype and can thus be related to fitness and adaptation (Dupont et al., 2007; Feder and Walser, 2005; Diz et al., 2012). Surprisingly, proteomics has not been widely applied in marine ecology, possibly due to the lack of sequencing data for marine organisms. Indeed, Tomanek (2014) already stated that proteomic studies are still scarce and restricted to few species and habitat types. Therefore, proteomic research applied to ecology is still in its infancy and may unravel new pathways that play a role in adaptation processes (Karr, 2008; Dalziel and Schulte, 2012). The few proteomic studies performed on marine organisms have shown that several pathways are affected by temperature including cytoskeletal dynamics, energetic metabolism, oxidative stress metabolism, chaperoning activity, immune response, transcriptional regulation, protein turnover and signal transduction (e.g. López et al., 2002; Gardeström et al., 2007; Tomanek and Zuzow, 2010; Tomanek, 2011; Fields et al., 2012; Garland et al., 2015).

The aims of this study were to assess the sensitivity of larval fish to ocean warming and extreme events in a highly commercial species, the sea bream *Sparus aurata* (*Linnaeus,* 1758) (IUCN Red List – Least Concern). *S. aurata* has a distributional area ranging from the Mediterranean and Black Seas to Eastern Atlantic and from the British Isles to Cape Verde (Froese and Pauly 2006, Sola et al. 2007). Spawning takes place between October and April in the open sea (Kissil et al., 2001; Dimitriou et al., 2007; Mylonas et al., 2011; Ibarra-Zatarain and Duncan, 2015). Early life stages are planktonic and the larval phase lasts about 50–60 days at 18 °C (Andrades et al., 1996; Sola et al., 2007). Larvae migrate across several environments during spring and summer to settle in shallow water habitats such as estuaries and coastal lagoons (Suau and Lopez, 1976; Arabaci, 2010; Verdiell-Cubedo et al., 2013), where they will grow until the adult stage. We used an integrative approach connecting proteome changes with organism-level indicators to unravel both molecular and fitness alterations providing a mechanistic insight into stress tolerance pathways and consequent fitness outcomes. We hypothesize that for larvae to survive heat stress, they must regulate at least two crucial processes, i) pro-survival pathways by up-regulating proteins with cytoprotective functions and ii) adjusting the energetic metabolism to cope with higher energetic demands of warm water, promoting homeostasis and sustaining performance. Proteomic approaches allow us to explore stress response networks and their regulatory steps, identify new candidate proteins (see Diz et al., 2012) and predict the vulnerability of fish larvae to climate change and environmental extremes, improving our understanding of ecological processes.

2. Methods

2.1. Assessment of S. aurata thermal environments

Both Portuguese coastal water temperatures and estuarine temperatures were assessed. Coastal water temperatures were retrieved from the sea temperature database (satellite data available from http://seatemperature.info/portugal-water-temperature.html), which has monthly sea surface temperatures for the main coastal cities of Portugal (data from the past five years – 2011–2015 for January until October and data from 2010 to 2014 for November and December). Maximum ± sd and minimum ± sd temperatures were calculated from this time-series considering all locations. Water temperatures in the Tagus estuary were obtained from the Marine and Environmental Sciences Center (MARE) database (data obtained from measurements carried out with YSI loggers) considering a time-series from 1978 to 2006. Moreover, future thermal environments were projected taking into account that Portuguese waters will undergo 2–3 °C increase by 2100 (Miranda et al., 2002).

2.2. Housing and husbandry of larvae

Larvae (n = 75; 35 days post-hatch – dph – larvae from a brood stock of 50 males and 25 females – breeding scheme in Fig. SM1a; total length range of 10–15 mm) were obtained from a fish farm (MARESA, Ayamonte, Huelva, Spain) and transported to the laboratory in 10 L opaque plastic containers with constant aeration and stable temperature conditions (18 ± 0.5 °C). The sample size was calculated considering that natural daily mortalities expected for larvae at 18 °C are around 20% at 30 dph, 9.9% at 40 dph and 4.9% at 50 dph (Andrades et al., 1996).

Distant and recent thermal history of the larvae were assessed considering i) the origin of parental fish, and ii) culture conditions at the fish farm. The first parental fish of the hatchery (collected in the late '90s) were wild fish caught in the nearby coastal lagoon mixed with adults obtained from an aquaculture in Almería region (Spain). In the fish farm, larvae are reared under tightly controlled conditions in indoor tanks (20 °C, high water quality) until they reach 0.1 g (approximately at 60 days post-hatch). Afterwards, they are placed in other less controlled indoor tanks but keep being reared at 20 °C. When they reach 1 g (approximately 90 days post-hatch), they are moved into land-based outdoor ponds (with water from the nearby coastal lagoon) and subjected to a natural temperature regime (temperate climate with seasonal variation: colder during winter and warmer during summer). According to data (from 1984 to 2010) obtained from the Spanish Agencia