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Ecophysiological responses of juvenile seabass (*Dicentrarchus labrax*) exposed to increased temperature and dietary methylmercury

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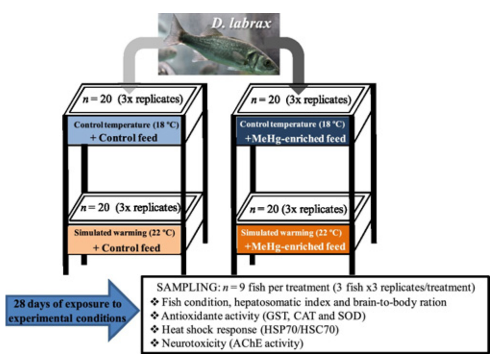
HIGHLIGHTS

- MeHg exposure decreased K and BB-ratio, but increased HSI
- Muscle GST was induced by warming and MeHg, but not by their combination
- Brain CAT was induced by warming and MeHg, but not by their combination
- Liver GST and CAT were induced by MeHg, regardless of temperature exposure
- MeHg exposure did not enhance HSP70/HSC70 induction caused by warming
- Increased temperature did not enhance AChE inhibition due to MeHg exposure

GRAPHICAL ABSTRACT



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ABSTRACT

The ecotoxicological effects of methylmercury (MeHg) exposure have been intensively described in literature. Yet, it is still unclear how marine biota will respond to the presence of MeHg under climate change, namely ocean warming. The present study aimed to investigate, for the first time, fish condition [Fulton's K index (K), hepatosomatic index (HIS) and brain-to-body mass ratio (BB-ratio)] and several stress-related responses in an ecologically and commercially important fish species (*Dicentrachus labrax*) exposed for 28 days to dietary MeHg (8.0 mg kg-1 dw) and temperature increase (+4 °C). Results showed significant impairments on fish condition, i.e. up to 34% decrease on K, >100% increase on HIS and 44% decrease on BB-ratio, compared to control conditions. Significant changes on tissue biochemical responses were observed in fish exposed to both stressors, acting alone or combined, evidencing the relevance of assessing possible interactions between different environmental stressors in ecotoxicological studies. For instance, muscle showed to be the least affected tissue, only revealing significant alterations in GST activity of MeHg-enriched fish. On the other hand, liver exhibited a significant induction of GST (>100%) and CAT (up to 74%) in MeHg-enriched fish, regardless of temperature exposure, as well as decreased SOD activity (19%) and increased HSP70/HSC70 content (87%) in fish exposed to warming alone. Brain showed to be affected by temperature (69% of GST inhibition and >100% of increased

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CAT activity), MeHg (>100% of increased CAT activity, 47% of SOD inhibition and 55% of AChE inhibition), as well as by the combination of both (GST, SOD and AChE inhibition, 17%, 48% and 53%, respectively). Hence, our data provides evidences that the toxicological aspects of MeHg ca be potentiated by warmer temperatures, thus, evidencing the need for further research combining contaminants exposure and climate change effects, to better forecast ecological impacts in the ocean of tomorrow.

1. Introduction

Anthropogenic derived impacts, such as the cumulative emissions of greenhouse gases and the introduction of pollutants in the environment, have resulted in remarkable contamination and unequivocally contributed to warming at a global scale (IPCC, 2014). Coastal environments are particularly vulnerable to climate change and anthropogenic pollution, once they are naturally and frequently exposed to a wide range of environmental stressors (e.g. tidal changes, salinity and temperature fluctuations), as well as subjected to constant inputs of industrial, agricultural and domestic chemical wastes (Boldt et al., 2014).

Over the last 30 years, an increase of the sea surface temperature (SST) has been globally observed, with particular emphasis in the Northern hemisphere, and the most up-to-date projections indicating an average SST increase of 3.7 °C by the year 2100 (scenario RCP8.5, IPCC, 2014). Increased temperatures may lead to deleterious effects over marine organisms, for instance, promoting metabolic depression (Aurélio et al., 2013), as well as, notorious changes in tissues' heat shock response (e.g. HSP70/HSC70) and antioxidant machinery (e.g. CAT, SOD, GST), which play a key role in cell defence against the formation of reactive oxygen species (ROS) induced by stress (Madeira et al., 2013). Thus, the ability of marine species to acclimate or even adapt (or not) to rising temperatures will pose major challenges at a species, community and population level (Schiedek et al., 2007; Pörtner and Knust, 2007).

On the other hand, environmental warming may also result in higher availability and toxicity of marine pollutants, not only by facilitating their release from sediments and altering their chemistry, but also by affecting contaminants uptake/detoxification rates, as well as the metabolic rates and enzymatic activity of marine organisms (e.g. Schiedek et al., 2007; Noyes et al., 2009; Marques et al., 2010). Although literature is still limited, recent studies provide empirical proof to sustain that marine species' propensity to accumulate chemical contaminants might be exacerbated in a warmer environment (Dijkstra et al., 2013; Siscar et al., 2014; Maulvault et al., 2016). Such trend was evidenced in our recent work performed with *Dicentrarchus labrax* juveniles exposed to methylmercury (MeHg) through dietary sources, with specimens exposed to warmer temperatures exhibiting higher contents of MeHg in muscle, liver and brain, along with diminished ability to eliminate this element at the liver level (Maulvault et al., 2016). Despite being a contaminant of priority concern, up until now, little attention has been paid to the toxicological aspects and ecological implications of dietary MeHg exposure. Furthermore, the way marine vertebrate species will cope with its presence while dealing also with other environmental stressors, such as climate change effects, is still unclear. Therefore, it is of paramount importance to undertake research in this innovative and poorly-understood field.

European seabass (*D. labrax*) is as top-predatory species, thus, being susceptible to accumulate high levels of biomagnifying pollutants, such MeHg (e.g. Miniero et al., 2013). Additionally, by inhabiting coastal areas (wild specimens) or often being reared in offshore aquaculture systems (farmed specimens), which are particularly vulnerable to hydrographic alterations, it is expected that climate change effects may pose great ecological and toxicological challenges to this fish species (Marques et al., 2010; Rosa et al., 2012). These ecological features along with the great economical value of *D. labrax*, both wild or farmed (FEAP, 2014; FAO, 2014), make this species a suitable biological model to assess the possible impacts of climate change and chemical contamination in marine ecosystems.

In this context, our work aimed to assess the effects of increased temperature (+4 °C) and MeHg dietary exposure (8.0 $mg \cdot kg^{-1}$), when acting alone or in combination, on the ecophysiological responses (fish condition, hepatosomatic index and brain-to-body mass ratio, antioxidant enzymes activities – GST, CAT and SOD, heat shock protein concentration and AChE activity) of juvenile seabass *D. labrax* (brain, muscle and liver).

2. Materials and methods

2.1. Control and MeHg-enriched diets

Non-contaminated (control) and MeHg-enriched diets with the same nutritional composition were manufactured by a specialized feed producing company (SPAROS Lda, Olhão, Portugal), considering the nutritional requirements of juvenile seabass (detailed feed composition can be consulted in Appendix A.1.; adapted from Maulvault et al., 2016). Feed preparation, as well as the methodologies used to determine its proximate chemical composition were performed as previously described in detail in Maulvault et al. (2016). For the MeHg-enriched diet, MeHg(II) chloride (CH_3ClHg, 99.8%, Sigma-Aldrich) was dissolved in a small volume of ethanol (<6 mL, 100% *v*/*v*) and then mixed with the oils before pellets extrusion. Diets were maintained at 4 °C, and MeHg stability in the diets was evaluated throughout the experiment, by quantification of total and MeHg contents in the two feeds, using an automatic Hg analyser (AMA 254, LECO, USA), as described in detail in Maulvault et al. (2016). Methylmercury concentration in control and MeHg-enriched diets were, in dry weight (dw), 0.60 ± 0.01 $mg \cdot kg^{-1}$ and 8.02 ± 0.14 $mg \cdot kg^{-1}$ (mean ± standard deviation, $n = 3$; see also Appendix A.2., adapted from Maulvault et al., 2016), respectively, with the control diet representing a low Hg level [~0.12 $mg \cdot kg^{-1}$ wet weight (ww)] and the MeHg-enriched diet representing a high Hg level (~1.6 $mg \cdot kg^{-1}$ ww), commonly found in species inhabiting contaminated coastal areas, susceptible to accumulate Hg, and that are natural preys of juvenile seabass (e.g. Cardoso et al., 2014).

2.2. Experimental design and biological sampling

D. labrax specimens with similar biometric characteristics were reared until juvenile stage (12.8 ± 0.7 cm total length; 19.2 ± 4.0 g total weight; Table 1) at the aquaculture pilot station of the Portuguese Institute for the Sea and Atmosphere (EPPO-IPMA, Olhão, Portugal) using routine hatchery conditions. Subsequently, fish were transported to the aquatic facilities of "Laboratório Maritimo da Guia (MARE-FCUL, Cascais, Portugal)", where they were randomly and equitably distributed in 12 rectangular shaped incubating glass tanks (100 L each, total volume), within independent recirculating aquaculture systems. As previously described in Maulvault et al. (2016), each system was equipped with independent and automatic temperature (Frimar, Fernando Ribeiro Lda, Portugal) and pH control (model Profilux 3.1 N, GHL, Germany), protein skimmer (Reef SkimPro, TMC Iberia, Portugal), UV disinfection (Vecton 300, TMC Iberia, Portugal), biological filtration (model FSBF 1500, TMC Iberia, Portugal) and chemical filtration (activated carbon, Fernando Ribeiro Lda, Portugal). In order to avoid