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Fish energy budget under ocean warming and flame retardant exposure

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ABSTRACT

Climate change and chemical contamination are global environmental threats of growing concern for the scientific community and regulatory authorities. Yet, the impacts and interactions of both stressors (particularly ocean warming and emerging chemical contaminants) on physiological responses of marine organisms remain unclear and still require further understanding. Within this context, the main goal of this study was to assess, for the first time, the effects of warming (+ 5 °C) and accumulation of a polybrominated diphenyl ether congener (BDE-209, brominated flame retardant) through dietary exposure on energy budget of the juvenile white seabream (*Diplodus sargus*). Specifically, growth (G), routine metabolism (R), excretion (faecal, F and nitrogenous losses, U) and food consumption (C) were calculated to obtain the energy budget. The results demonstrated that the energy proportion spent for G dominated the mode of the energy allocation of juvenile white seabream (56.0–67.8%), especially under the combined effect of warming plus BDE-209 exposure. Under all treatments, the energy channelled for R varied around 26% and a much smaller percentage was channelled for excretion (F: 4.3–16.0% and U: 2.3–3.3%). An opposite trend to G was observed to F, where the highest percentage (16.0 ± 0.9%) was found under control temperature and BDE-209 exposure via diet. In general, the parameters were significantly affected by increased temperature and flame retardant exposure, where higher levels occurred for: i) wet weight, relative growth rate, protein and ash contents under warming conditions, ii) only for O:N ratio under BDE-209 exposure via diet, and iii) for feed efficiency, ammonia excretion rate, routine metabolic rate and assimilation efficiency under the combination of both stressors. On the other hand, decreased viscerosomatic index was observed under warming and lower fat content was observed under the combined effect of both stressors. Overall, under future warming and chemical contamination conditions, fish energy budget was greatly affected, which may dictate negative cascading impacts at population and community levels. Further research combining other climate change stressors (e.g. acidification and hypoxia) and emerging chemical contaminants are needed to better understand and forecast such biological effects in a changing ocean.

1. Introduction

Climate change is one of the greatest environmental challenges that cause profound and diverse impacts on marine organisms and ecosystems (IPCC, 2014). Anthropogenic activities are strongly influencing climate, primarily through fossil fuels, industrial, agricultural, and other land use emissions that alter atmospheric carbon dioxide (CO_2). Hence, one direct consequence of cumulative and continuing emissions of CO_2 since the industrial revolution is the increase of surface ocean temperatures (Doney et al., 2012). According to the latest report of the Intergovernmental Panel on Climate Change (IPCC), the average temperatures on the Earth's surface are expected to rise between 0.3 °C and 4.8 °C by the end of the 21st century with strong local variations. Additionally, it is predicted that extreme high temperature events (heat

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waves) will become more extreme and frequent (IPCC, 2014). Ocean warming represents a major threat to many marine organisms deeply affecting their physiological responses, namely growth, feeding, acid–base balance, metabolism and behaviour in ways that often compromise species fitness and survival (Pörtner and Peck, 2010; Rosa et al., 2012a). Thus, temperature is one of the main abiotic factors that can lead to significant changes on the energy budget of an organism (Yurista, 1999). The energy budget describes the energy intake and expenditure within the whole organism, leading to the partitioning of ingested energy into the major physiological components of the energy budget equation: $C = G + R + F + U$, where energy consumed in form of food (C) is the sum of energy retained in materials deposited during growth (G), energy spent for respiration (R), energy losses through faeces (F) and energy losses via nitrogenous excretory products (U; non-faecal) (Jobling, 1994). At optimal temperature conditions, an organism allocates a great part of its energy input into vital functions, as well as in the build-up of energy storage. In contrast, under stress (sub-optimal high temperatures), fish cannot consume enough food to meet, for example, the increasing metabolic costs, whereby energy is often limited and can only cover costs of essential functions, such as standard metabolism (Clarke and Johnston, 1999). Indeed, warming greatly affect metabolic rate of fish, since there is a dependent relation between the variables (temperature-oxygen) and oxygen supply becomes limited at high temperature borders (Pörtner and Peck, 2010; Rosa et al., 2014). Consequently, all of biological processes are directly affected by temperature, namely food intake, nutritional efficiency and whole-body composition (Burel et al., 1996; Fang et al., 2010). Zheng et al. (2008) also reported that growth rate and faecal production increased in fish species at higher temperatures, while nitrogen excretion decreased, leading to a higher O:N ratio (ratio of oxygen consumed to nitrogen excreted). On the other hand, ocean warming impacts are also expected to directly influence the availability and toxicological effects of marine pollutants (Marques et al., 2010). Recent studies, evidenced that marine fish species tend to accumulate emerging chemical contaminants in warmer conditions (Maulvault et al., 2016, 2017). Such trend was demonstrated in our recent work with seabass (*Dicentrarchus labrax*) juveniles exposed to methylmercury (MeHg) via diet, with specimens exposed to warmer temperatures (22 °C) showing higher levels of MeHg in different tissues (muscle, liver and brain; Maulvault et al., 2016). The contaminants of emerging concern are a subject of growing interest for the scientific community and regulatory authorities, since constitute a risk for human health and biota, and for which there is still insufficient scientific knowledge (Cruz et al., 2015). Among these contaminants, polybrominated diphenyl ethers (PBDEs) are an important class of flame retardants (FRs) consisting of 209 congeners, which have been commercialized as penta-, octa-, and decabrominated mixtures. PBDEs are additive flame retardants, i.e. they are not bound to the polymer, but have been widely used in a variety of products (e.g. plastics, furniture, vehicles) to make them less combustible and to retard flames (Alaee et al., 2003). These FRs are persistent in the environment, accumulate in food chains and show toxic effects on hormonal regulation and affect neuronal, thyroid and liver-related activities (Costa and Giordano, 2011). Hence, these substances are the subject of a wide range of toxicologic and ecotoxicologic studies. Due to these concerns of environmental persistence, bioaccumulation and toxicity, lower brominated PBDEs have been banned in several countries, but the congener decabromodiphenyl ether (BDE-209), primary constituent of deca-BDE, is still used. Nonetheless, it is worth noting that some European countries (e.g. Norway) or states in the U.S.A. (e.g. Maine) have recently banned their use (Costa and Giordano, 2011). Despite the lower levels of BDE-209 found in water, seafood has been identified as the major dietary source of this compound for human uptake, since it can be biotransformed to lower and more toxic congeners via the food web (Kierkegaard et al., 1999; Elliott et al., 2005; Stapleton et al., 2006). Several studies have addressed the effects of water temperature on energy budget of some fish species (e.g. Xie et al., 2011), but the conclusions were not consistent among them, and others only focused on the effects of chemical contaminants under climate change (e.g. Maulvault et al., 2016, 2017; Sampaio et al., 2018) without ever reporting the effects on energy budgets. Yet, a small number of studies investigated the effects of BDE-209 dietary exposure only on growth of marine species (Kuo et al., 2010; Zhang et al., 2013; Sha et al., 2015) and, to our knowledge, there are no studies that investigated the interactive effects of both stressors (ocean warming and the presence of emerging chemical contaminants) in the perspective of fish bioenergetics. In this context, the main goal of this study was to investigate, for the first time, the effects of ocean warming (+ 5 °C) and the dietary exposure to the emerging BDE-209 (60 ng g^{-1} dry weight, dw) on the energy budget of juvenile white seabream (*Diplodus sargus*), by measuring growth, routine metabolism, excretion and food consumption. This species was selected as a suitable biological model to evaluate these impacts since it generally inhabits coastal areas particularly susceptible to hydrographic changes and where climate change effects may certainly pose greater ecological and toxicological challenges (Marques et al., 2010; Rosa et al., 2012b). Additionally, this seabream species plays an important role in the coastal food web, as they link top predators with species occupying low trophic levels.

2. Materials and methods

2.1. Experimental design and sampling

Juvenile white seabream (*Diplodus sargus*) specimens from the same batch and with similar biometric characteristics (6.2 ± 0.6 cm total length; 3.9 ± 1.2 g total weight; mean ± standard deviation) were reared at the aquaculture pilot station of the Portuguese Institute for the Sea and Atmosphere (EPPO-IPMA, Olhão, Portugal) and transported in thermal isolated containers, with constant aeration, to Guia Marine Laboratory (MARE-FCUL, Cascais, Portugal). Upon arrival, fish were randomly distributed in 12 rectangular incubating glass tanks (98 × 33 × 24.7 cm; 100 L total capacity each), each with independent recirculating aquaculture systems (RAS). Each RAS was equipped with biological filtration (model FSBF 1500, TMC Iberia, Portugal), physical filtration (protein skimmer; ReefSkimPro, TMC-Iberia, Portugal), UV disinfection (Vecton 300, TMC Iberia, Portugal), independent and automatic temperature (Frimar, Fernando Ribeiro Lda, Portugal) and pH control (model Profilux 3.1 N, GHL, Germany) via solenoid valves system. Each incubation system ($n = 12$) had independent pH measurements, by means of pH electrodes ($n = 24$ in total), connected to the Profilux system apparatus. pH values were monitored every 2 s and lowered by injection of a certified CO_2 gas mixture (Air Liquide, Portugal) via air stones or increased by tank aeration with CO_2-filtered (using soda lime, Sigma-Aldrich) air. Seawater used in the RAS was filtered (0.35 µm) and UV sterilized (Vecton 600, TMC Iberia, Portugal). Ammonia (NH_3/NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) levels were monitored regularly, by means of colorimetric tests kits (Tropic Marin, USA) and kept below 0.05 mg L^{-1}, 0.20 mg L^{-1} and 2.0 mg L^{-1}, respectively. In order to avoid physiological stress related to high animal density, fish density was kept below 5 g body weight L^{-1} in each incubation tank. Daily, mortality was registered in each treatment and seawater was partially replaced (around 20%).

Fish were acclimated to laboratory conditions during two weeks before the start of the experiment at the following abiotic conditions: dissolved oxygen (DO) above 5 mg L^{-1}; temperature = 19 ± 0.4 °C; pH = 8.06 ± 0.10; salinity = 35 ± 1‰ (WTW handheld Meter Multi 350i, Germany) and photoperiod of 12 h light and 12 h dark (12 L:12D). Five days before initiating the climate change exposure scenarios, seawater temperature was slowly raised (1 °C per day) until reaching 24 °C in tanks simulating warming conditions, in order to allow specimens to acclimate at this temperature before the beginning of the trial. No mortality occurred during the acclimation period.

Fish were exposed to four scenarios during 56 days to understand