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Long-term effect of temperature on bioaccumulation of dietary metals and metallothionein induction in *Sparus aurata*

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ABSTRACT

Previous studies have demonstrated that the commercial feed of aquacultured fish contains trace amounts of toxic and essential metals which can accumulate in tissues and finally be ingested by consumers. Recently rising temperatures, associated to the global warming phenomenon, have been reported as a factor to be taken into consideration in ecotoxicology, since temperature-dependent alterations in bioavailability, toxicokinetics and biotransformation rates can be expected. *Sparus aurata* were kept at 22 °C, 27 °C and 30 °C for 3 months in order to determine the temperature effect on metallothionein induction and metal bioaccumulation from a non-experimentally contaminated commercial feed. A significant temperature-dependent accumulation of cadmium (Cd), copper (Cu), mercury (Hg), zinc (Zn), lead (Pb) and iron (Fe) was found in liver, together with that of manganese (Mn), Fe and Zn in muscle. Hg presented the highest bioaccumulation factor, and essential metal homeostasis was disturbed in both tissues at warm temperatures. An enhancement of hepatic metallothionein induction was found in fish exposed to the highest temperature.

1. Introduction

The aquatic ecosystems are undergoing a warming in their deep and surface waters, a fact which may have significant consequences on the organisms inhabiting them (Noyes et al., 2009). Gilthead sea bream *Sparus aurata* is a cosmopolitan species distributed throughout the Mediterranean and the NE Atlantic, and which is of great economic interest since it is one of the principal species of Mediterranean aquaculture. Being an ectothermic species, it is vulnerable to the effects of temperature variations on its metabolism and physiology. These variations may produce changes in the toxicokinetics, bioavailability, biotransformation, homeostasis, absorption rate and elimination of different compounds (Douben, 1989; Köck et al., 1996; Yang and Chen, 1996). Other key physiological mechanisms such as respiration, feeding rate, growth and reproduction may also be affected (Heugens et al., 2001; Bowen et al., 2006). The thermic induced variations in the toxicokinetics of pollutants, together with the increase in exposure to the same as a consequence of climate warming (Carrie et al., 2010) may present a risk for the development and survival of species of commercial interest, affecting the quality of the end product (Noyes et al., 2009).

In cultured fish, it has been demonstrated that the feed is the principal source of contamination by metals Cadmium (Cd), lead (Pb) and mercury (Hg), among others, are potentially toxic and tend to accumulate in the tissues, which in the end are ingested by the consumers (Maule et al., 2007; Fernandes et al., 2009; Creti et al., 2010). Their accumulation in the organisms depends on the concentration, route of absorption, environmental conditions and other intrinsic factors (Lemus and Chung, 1999; Chowdhury and Blust, 2001; Karakoç and Dinçer, 2003; Bowen et al., 2006; Jezierska and Witeska, 2006).

Due to the growth in the activity of aquaculture in recent decades, it has become of special relevance to learn the influence of the increase in temperature on the possible routes of absorption, accumulation and elimination of metals in these organisms in the context of global warming. Temperature may be a determining factor in the capture, transporting and metabolism of the metals incorporated through the feed, both of the essential metals which may become toxic at high concentrations in the tissues, and of the non-essential metals.

Metallothionein (MT) is a low-molecular-weight metal binding protein and is known to play an important role in protection against heavy metal toxicity. In addition to the detoxification of toxic metals such as Cd and Hg, MT is involved in the maintenance of homeostasis of essential trace elements such as zinc (Zn) and copper (Cu) (Hamilton and Mehrle, 1986; Coyle et al., 2002). Its role in the protection against xenobiotics or in the cellular

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protection against oxidative stress should be underlined (Van Cleef-Toedt et al., 2001; Coyle et al., 2002). Although its synthesis is related to the metal exposure, its levels can be affected by endogenous and exogenous factors such as the reproductive cycle or the temperature (Van Cleef-Toedt et al., 2001). Variations in the water temperature could directly or indirectly modify the behavior of this protein as regards the bioaccumulation of metals, as well as its participation in toxicokinetic processes (Rotchell et al., 2001; Gorbi et al., 2005; Baykan et al., 2007).

Given the lack of information as regards the effect of temperature on the bioaccumulation of metals via dietary sources, and on the synthesis of MT, an understanding of these processes in the light of the problem of global warming and its repercussions on species of commercial interest such as *S. aurata* is required. As such, the aims of this work are:

(1) To determine the effect of temperature on the bioaccumulation of essential (Cu, Fe, Mn and Zn) and non-essential (Cd, Hg and Pb) metals experimentally via a non-contaminated commercial feed.
(2) To discover whether temperature has any influence on the homeostasis of the essential metals.
(3) To elucidate the role of metallothionein in the above mentioned temperature induced changes.

2. Materials and methods

2.1. Animal collection and maintenance

Adult *S. aurata* were distributed and acclimated in 500 L tanks containing seawater (37‰) at a constant temperature of 22 °C, with continuous aeration and natural photoperiod in a closed circuit for 2 months prior to the experiment. Subsequently, the temperature of two of the experimental groups was gradually increased to reach 27 °C and 30 °C respectively. Control groups of fish were maintained at 22 °C throughout the experiments. The animals were fed with commercial pellets (1.5% of body mass per day) and the survival percentage was 99%. Fish were kept under constant conditions for 3 months. After this period, 6–7 fish were removed at random from each experimental group and placed in water containing 30 mg L^{-1} of anesthetic clove oil. Lengths and weights of gilthead sea bream were recorded. Fish were sacrificed; livers and a piece of dorsal muscle tissue were dissected and immediately frozen in liquid nitrogen and stored at −80 °C.

2.2. MT determination by differential pulse polarography

Approximately 0.2 g wet weight portions of frozen liver were homogenized using ultra-turrax in 20 m M Tris-HCl buffer, 1 m M DTT and 0.2 m M PMSF pH 8.6 in an ice bath. The homogenates were centrifuged at 30000 g for 45 min at 4 °C. The supernatant was heated at 80 °C for 10 min in order to denature high molecular weight proteins and subsequently centrifuged at 30000 g for 45 min at 4 °C. The heat-treated supernatant, containing thermally stable MT, was separated from precipitated proteins. MT was measured using differential pulse polarography (Ureña et al., 2007).

An aliquot of the heat-treated supernatant was added to the polarographic cell, containing 20 mL hexamminecobalt chloride buffer (the supporting electrolyte), together with Triton-X (0.025% v/v). The cell was purged for 2 min with purified N_2 prior to analysis. The polarographic response was measured during a potential scan between −1.38 V and −1.7 V (Model 757 VA Computrace Analyser, Methrom, Switzerland) in SMDE mode. Quantification of MT was performed by using the standard addition method with rabbit liver MT I + II (Sigma). Results are expressed as μg g^{-1} wet weight of tissue.

2.3. Metal analysis

Samples of 0.1–0.5 g wet weight of liver and muscle were digested in concentrated nitric acid 65% (Baker) at room temperature overnight, and were heated at 80 °C for 2 h. In order to determine the trace amounts of metal contained in the feed, samples of commercial pellets were also digested ($n = 3$). Within each digestion series, appropriate blanks with ultra-pure water were also subjected to the same procedure in order to take background contamination levels into account. After cooling, solutions were transferred to a standard volume with ultra-pure water. Determination of metals (Cd, Cu, Zn, Fe, Pb, Hg and Mn) was undertaken using an ICP-Mass (Elan DRC-I, Perkin-Elmer Sciex). Samples of similar weight of certified reference material (DOLT-3 and LUTS-1, National Research Council of Canada, Ottawa), were digested and analyzed during each analytical run. The values of all elements found were in good agreement with the certified values, with the recoveries ranging from 91% to 104%.

2.4. Data analysis and calculations

Metal content ratio between muscle and liver was calculated in order to detect changes in the distribution of metals as a consequence of the thermal experimental conditions, and to reveal the proportion of accumulated metal in each tissue.

Bioaccumulation factor (BAF) was calculated for each metal-tissue-temperature combination in order to detect the effects of temperature on the global toxicokinetics among metals. This was calculated as a quotient between the metal concentration in each tissue and the metal content in the commercial pellets as described by Dabrowska et al. (1996).

2.5. Statistical analysis

Statistical analyses were carried out using the software Stata 10 (Stata Corp). Transformations of the data were performed when the assumption of normality of residuals was not met. One-way ANOVA was used in the analysis of data to check the influence of temperature on each variable. The Scheffe test was used as a post hoc test to demonstrate the differences among the three temperature groups. Two-way ANOVA, with temperature and metal as fixed factors, was used in the statistical analysis of BAFs, followed by a post hoc Bonferroni test. The Kruskal Wallis test was used when transformed data did not present homoscedasticity. Pearson correlation coefficients (r) were calculated between MT levels and metal content in liver, in order to measure the strength of association between these variables. Results are presented as means ± SEM and a p value lower than 0.05 was considered as statistically significant.

3. Results

3.1. Metal content in food and biometry

Metal contents of food pellets were 0.38 ± 0.05 μg g^{-1} w.w. for Cd, 11.6 ± 0.7 μg g^{-1} w.w. for Cu, 173 ± 9 μg g^{-1} w.w. for Fe, 40 ± 3 μg g^{-1} w.w. for Mn, 0.05 ± 0.01 μg g^{-1} w.w. for Hg, 0.15 ± 0.09 μg g^{-1} w.w. for Pb and 139 ± 9 μg g^{-1} w.w. for Zn.

The weight and length together with the condition index calculated for gilthead sea bream exposed to three experimental temperatures for 3 months are shown in Table 1. As can be seen, temperature has no effect on the condition index or weight. In contrast, fish kept at 30 °C ($p < 0.05$) were found to be significantly shorter than those kept at 22 °C and 27 °C.