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Short Communication

Dietary Zn and the subsequent organotropism in fish: No influence of food quality, frequency of feeding and environmental conditions (pH and temperature)

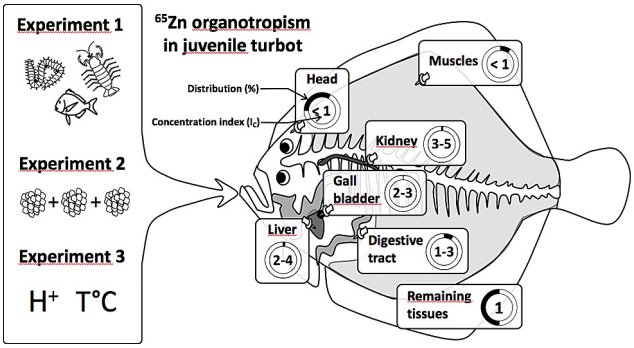
Simon Pouil [a, b], François Oberhänsli [a], Paco Bustamante [b], Marc Metian [a, *]

[a] International Atomic Energy Agency - Environment Laboratories (IAEA-EL), 4 Quai Antoine 1er, MC-98000, Monaco
[b] Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS - Université de La Rochelle, 2 rue Olympe de Gouges, F-17000 La Rochelle, France

HIGHLIGHTS

- Distribution and concentration of dietary Zn was experimentally assessed in turbot.
- Zn organotropism is not affected by food or pH and temperature.
- Consistency in the Zn organotropism can be explained by its homeostasis in fish.

GRAPHICAL ABSTRACT



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ABSTRACT

Trophic transfer of Zn in fish is affected by the type of food and environmental variables such as temperature. However, there is still a lack of knowledge regarding the effects of such factors on Zn organotropism. For this reason, a series of experimental studies have investigated how the distribution and the concentration of Zn is affected by some environmentally-relevant factors (food quality, food availability, water pH, and temperature) in turbot *Scophthalmus maximus* using radiotracer techniques. In three different experiments, Zn distribution in seven body compartments of juvenile turbot and the calculation of Zn concentration index (I_C) for each compartment were compared. Its distribution as well as its concentration in the body compartments of juvenile turbots were not affected by the experimental conditions tested. This apparent consistency in the Zn organotropism can be explained by the ability of the fish to maintain Zn homeostasis at non-toxic Zn concentrations in their diet. These results are important to better understand the trophic transfer of Zn in fish under realistic environmental conditions.

1. Introduction

Zinc (Zn) is a crucial microelement for living organisms, including fish (Watanabe et al., 1997). Indeed, it is an essential element for fish playing a vital role in lipid, protein, and

* Corresponding author. Radioecology Laboratory, IAEA Environment Laboratories, 4a Quai Antoine 1er, MC-98000 Monaco, Monaco.
E-mail address: m.metian@iaea.org (M. Metian).

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carbohydrate metabolism but it also can be potentially toxic at high concentrations in the environment (Spry and Wood, 1985; Eisler, 2009; Hogstrand, 2011). Due to these two opposite aspects, the accumulation of Zn by fish has been extensively studied (*e.g.* Spry et al., 1988; Clearwater et al., 2002; Van Campenhout et al., 2007). Fish have mainly two sources of Zn uptake: the surrounded water and their diet (Bury et al., 2003) and it is now well-identified that food is the major pathway of Zn intake in fish especially at low ambient Zn concentrations (Spry et al., 1988). Indeed, as early as the 1980s, Willis and Sunda (1984) have highlighted that food ingestion represented up to 82% of total Zn accumulation for two species of fish *Gambusia affinis* and *Leiostomus xanthurus* fed with radiolabelled brine shrimp. More recently, Xu and Wang (2002) and Mathews and Fisher (2009) have confirmed using biokinetic models that Zn in teleosts and elasmobranchs was predominantly bioaccumulated from the dietary source. Numerous experimental studies have focused on the study of Zn trophic transfer in fish (*e.g.* Pentreath, 1976; Milner, 1982; Zhang and Wang, 2007; Pouil et al., 2016) but the understanding of the physiological mechanisms governing the assimilation of this element is still very limited. It is known that Zn trophic transfer may, in some cases, be affected by the type of dietary supply or by the environmental conditions (*e.g.* Van Campenhout et al., 2007). The observed effects are usually related to the differential Zn subcellular fractionation in the food items or the chemical forms of this element that can affect the bioavailability of dietary Zn and, by extension the ways of its storage in the organisms (*e.g.* Zhang and Wang, 2007; Pouil et al., 2016). At present, however, there is little known about how Zn is stored in fish.

The organotropism of Zn (*viz.* whole-body distribution of Zn) can provide a better understanding of the variability observed in Zn trophic transfer under different conditions. Measurements carried out in the field highlighted that a series of factors (e.g., trophic habits, gender, season) can affect Zn body burden and its distribution among organs and tissues (Andres et al., 2000; Kojadinovic et al., 2007; Dural et al., 2007). It is, however, always complex with an *in-situ* approach to establish unambiguous trends because origins of Zn intakes cannot be properly identified and the life-history traits of the organisms that may play a role in its body distribution are usually unknown (Gray, 2002). The experimental approach, in controlled conditions, is a relevant option to unequivocally assess Zn organotropism, especially using radiotracer techniques (Warnau and Bustamante, 2007).

In the present work, we experimentally assessed the dietary Zn organotropism in juvenile turbots *Scophthalmus maximus* single-fed with ^{65}Zn radiolabelled food and exposed to different experimental conditions. Thus, after a 21-d depuration period, the fish from the different experimental conditions were dissected in order to (1) understand the long-term storage of dietary Zn in fish through the measurement of the body concentration and distribution of this element and (2) investigate potential effects of some environmentally relevant factors: food quality, feeding frequency, seawater pH and temperature on Zn organotropism. These factors were selected for their previously-shown potential to affect the trophic transfer of Zn in fish and/or their physiology (*e.g.* Van Campenhout et al., 2007; Pouil et al., 2016, in press). The food items used were natural prey of the juvenile turbot (*i.e.* crustaceans, fish and polychaetes; Florin and Lavados, 2010; Sparrevohn and Støttrup, 2008) and compounded pellets used in aquaculture for this species. The values of pH (7.5 and 8.0) and temperature (17 °C and 20 °C) were chosen based on the optimal temperature for food conversion efficiency in juvenile turbots (Imsland et al., 2001) and the current projections of these factors provided by the literature for the next two centuries ($\Delta T°C$: +3 °C and ΔpH: −0.5; IPCC, 2013; Orr et al., 2005).

2. Materials and methods

2.1. Origin and acclimation of organisms

Juvenile turbots *Scophthalmus maximus* were purchased from a fish farm (France Turbot, France) and shipped to the International Atomic Energy Agency premises in the Principality of Monaco. Fish were acclimated to laboratory conditions for a minimum of 3 weeks (open circuit; 700-L tank, water renewal: 300 L h^{-1}; 0.45 μm filtered seawater; salinity: 38; temperature: 17 ± 2 °C; pH: 8.0 ± 0.1; light/dark: 12 h/12 h). During the acclimation period, the fish were fed a daily ration of 2% of their biomass with 1.1-mm pellets (proteins: 55% and lipids: 12%; Le Gouessant, France).

Natural prey of juvenile turbots *i.e.*, crustaceans (common prawn *Palaemon serratus*), fish (seabream *Sparus aurata*), and ragworms (estuary ragworm *Hediste diversicolor*), were respectively purchased from French suppliers (Poissons du Soleil, Poissons Vivants and Normandie Appâts). All prey were acclimated to the same laboratory conditions as the turbots for a minimum of 2 weeks prior to experiments.

2.2. Radiolabelling and counting

For Zn radiolabelling, radiotracer of high specific activity was purchased from Polatom, Poland (^{65}Zn as $ZnCl_2$ in 0.1 M HCl, $t_{1/2}$ = 244 d). The use of gamma-emitting isotope allowed accurate measurements using environmentally realistic zinc concentrations. All the natural prey (crustaceans, fish and polychaetes) were exposed to dissolved ^{65}Zn for 1–3 weeks following the protocol described by Pouil et al. (2016). For radiolabelling, pellets were dipped in ^{65}Zn radiolabelled seawater for 1 h (ratio: 0.32 g dry wt mL^{-1}). Then, radioactive pellets were dried and stored in dry conditions (for more details see Pouil et al., 2017b).

The radioactivity in prey, fish and dissected samples was measured using a high-resolution γ-spectrometer system composed of 4 Germanium - N or P type - detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multi-channel analyser and a computer equipped with a spectra analysis software (Interwinner 6, Intertechnique®). The radioactivity in living turbots and samples from dissections was determined by comparison with standard of known activity and of appropriate geometry (calibration and counting; see Pouil et al., in press; Pouil et al., 2016). The counting time was adjusted to obtain a propagated counting error less than 5% (*e.g.* Rodriguez y Baena et al., 2006).

2.3. Zn organotropism in juvenile turbots

2.3.1. General experimental approach

The following conditions were applied to all the Zn organotropism experiments, unless stated otherwise. The experiments were performed in 20-L or 70-L aquaria (open-circuit, same conditions that during acclimation, see section 2.1). Two weeks before the start of the experiments, juvenile turbots were randomly transferred from the acclimation tank to the aquaria used for experiments and kept 2 d without food until the beginning of the experiment. Slits cut into the fins were used to facilitate individual recognition. Two hours after the unique (Experiments 1 and 3) and the last (Experiment 2) radiolabelled feeding, all the turbots from the different experiments were counted to determine the ingested ^{65}Zn activity, estimate the ingestion rate for each individual and the stable Zn quantity eaten (see Pouil et al., 2016 for Zn stable analysis methodology for the different food items). After a 21-d depuration period, when the whole-body Zn activity as reached a stable level in fish (*i.e.*, when Zn distribution among tissues has been achieved and depuration is low, see Pouil et al., 2016), fish were sampled, were

anaesthetized, euthanized by exposure to high concentrations of anaesthetic (Eugenol) and dissected: (1) muscles (the 4 fillets without dorsal skin), (2) the kidney, (3) the liver, (4) the gall bladder, (5) the digestive tract, (6) the head (including gills) and (7) the remaining tissues (including remaining skin, skeleton, fins, heart and muscle residues) were separated, weighed (wet wt) and placed in plastic tubes (diameter: 42 mm, height: 65 mm) for further radioactivity counting. Then, 20 mL of 2 M HCl were added in each tube to digest the tissues in order to get an appropriate geometry and samples were stored overnight before radioanalyses. During all the experiments, no mortality was recorded.

2.3.2. Experiment 1: influence of food items

For this experiment, natural prey of the juvenile turbots (common prawns, seabreams and ragworms) were exposed to dissolved ^{65}Zn as described in section 2.3.1. The average activities in the prey at the end of the exposure period were 144 Bq g^{-1} wet wt, 67 Bq g^{-1} wet wt and 250 Bq g^{-1} wet wt, respectively for common prawn, seabream and ragworm. The stable Zn concentrations were 56, 110 and 127 μg g^{-1} dry wt, respectively for common prawn, seabream and ragworm. Then, three batch of turbots (n = 5, 12.1 ± 4.9 g wet wt) were fed *ad libitum* with the three different freshly killed radiolabelled prey. After the depuration period following the single feeding, fish were dissected as described in section 2.3.1.

2.3.3. Experiment 2: influence of feeding frequency

In order to understand the effect of feeding frequency on Zn organotropism in fish, 5 juvenile turbots were single-fed (23.9 ± 6.0 g wet wt) using radiolabelled pellets (2350 Bq g^{-1} in average) and another batch of turbots (n = 5, 23.7 ± 3.1 g wet wt) were pre-exposed to the radiolabelled pellets during a 12-day period (one-labelled pellet feeding every 4 d; Pouil et al., 2017b). Between each labelled-pellet feeding, turbots were fed daily with non-labelled pellets. The stable Zn concentrations in the pellets was 148 μg g^{-1} dry wt (see Pouil et al., 2016 for Zn stable analysis methodology). After the last radiolabelled feeding, depuration of the single-fed and multi-fed turbot was followed for 21 d and all the fish were dissected (see details in section 2.3.1).

2.3.4. Experiment 3: influence of pH and temperature

In order to study the effects of pH and temperature on Zn organotropism, 4 batches of juvenile turbots (n = 4, 23.7 ± 5.3 g wet wt) were acclimated for 2 months to the target experimental conditions (pH 8.0 at 17 °C, pH 8.0 at 20 °C, pH 7.5 at 17 °C and pH 7.5 at 20 °C) controlled using an IKS system (Pouil et al., in press). Then, all the turbot were fed with radiolabelled common prawn (213 Bq g^{-1} wet wt in average). After the 21-d depuration period, fish were dissected as detailed in section 2.3.1.

2.4. Data analysis

Data obtained by radioanalyses of dissected samples (*viz.* fish compartments) were used to calculate the distribution (expressed in %) of Zn in fish whole body. The concentration index (I_C) was also calculated as defined by Rouleau et al. (2000) using the following equation (Eq. (1)):

$$I_C = \frac{[^{65}Zn]\ \ in\ tissue}{[^{65}Zn]\ \ in\ whole\ body} \quad (1)$$

Values of $I_C > 1$ indicate that the considered tissue is enriched in Zn compared to the whole-body average Zn concentration.

Distribution and concentration (i.e. I_C values) of Zn in the body compartments of fish exposed to the different experimental conditions (type of food, frequency of feeding, pH and temperature), were compared using the Kruskal-Wallis non-parametric test, followed by a multiple-comparison test of Siegel and Castellan (Zar, 1996). The level of significance for all statistical analyses was always set at $\alpha = 0.05$. All the statistical analyses were performed using either the Statistica® software 7.0 or R freeware 3.0.1 (R Development Core Team, 2014).

3. Results and discussion

It is already known that the digestive physiology of fish and especially their ability to assimilate dietary Zn can be influenced both by biotic factors such as the food items ingested (*e.g.* Ni et al., 2000; Pouil et al., 2016) or abiotic factors such as the water temperature (*e.g.* Pouil et al., in press). Information related on the long-term storage of dietary Zn is nevertheless still limited. To fill this gap, dietary Zn distribution in the body compartments of juvenile turbots exposed to several environmentally relevant factors (food quality, feeding frequency, seawater pH and temperature) was investigated after a 21-d depuration period.

Our results indicated that the proportion of Zn in the remaining tissues (including remaining skin, skeleton, fins, heart and muscle residues), in each of the tested conditions, was always the highest with approx. 50% of the Zn total body burden (Fig. 1), which can be related to the high weight of this compartment (always over 45% of the total body weight). The high proportion of dietary Zn found in the remaining tissues indicates that there is no specialized storage organ for Zn presumably because of it essentiality for the normal physiological functions of the fish (Hogstrand, 2011). Nevertheless, the Zn distribution among the other body compartments (*viz.* excluding remaining tissues) systematically ranked according to the following decreasing order (Fig. 1, Table 1): Head (28–36%) ≫ Muscles (8–11%) > Digestive tract (7–10%) ≫ Liver (1–2%) > Kidney (1–1.5%) > Gall bladder (<0.5%). Although the weight of the head and the muscles are comparable (20–30% of the total body weight), a much higher proportion of Zn is stored in the head and Zn concentration I_C is always > 1 in the head (Table 1). We assume two reasons to understand this observation. Indeed, field investigations have already shown that the highest concentration of Zn can be found in the eyes of fish (Bowness and Morton, 1952; Eckhert, 1983) although the exact function(s) of Zn in the eyes remain(s) unclear. Furthermore, Pouil et al. (2017a; supplementary material) have shown, experimentally, a high concentration index, I_C (up to 2), of Zn in the eyes of silver moony *Monodactylus argenteus* and spotted scat *Scatophagus argus*. Another reason to explain the high Zn concentration in the head is the presence of the gills in this compartment. Indeed, branchial excretion has been suggested as the major Zn excretory route in euryhaline fish exposed through dietary Zn (Hardy et al., 1987). The high concentration index, I_C (1–4) found by Pouil et al. (2017a) in the gills of *M. argenteus* and *S. argus*, indicates that this organ plays an important role in Zn metabolism in fish. Although branchial excretion dominates, Zn can be also excreted, in a lesser extent, via the bile (Hardy et al., 1987; produced by the gall bladder) and urine (Spry and Wood, 1985). It is thus not surprising to observe the highest I_C values in gall bladder and kidney, ranking from 2 to 5 for these organs (Table 1). In the same time, it is important to note that these organs separately only represent less than 1% of the total body weight which also can explain a higher I_C. Some field investigations have shown that, in some species, Zn concentration in the kidney can be incredibly high (e.g. 23 500 μg g^{-1} in the yellowfin tuna *Thunnus albacares*, Kojadinovic et al., 2007) suggesting an important role of this organ in Zn storage in fish. In our study, we found that a non-negligible part of Zn is distributed in the digestive tract (7–10%, Fig. 1 and Table 1). Furthermore, we calculated relatively high Ic values observed (up to 3) for this body compartment.

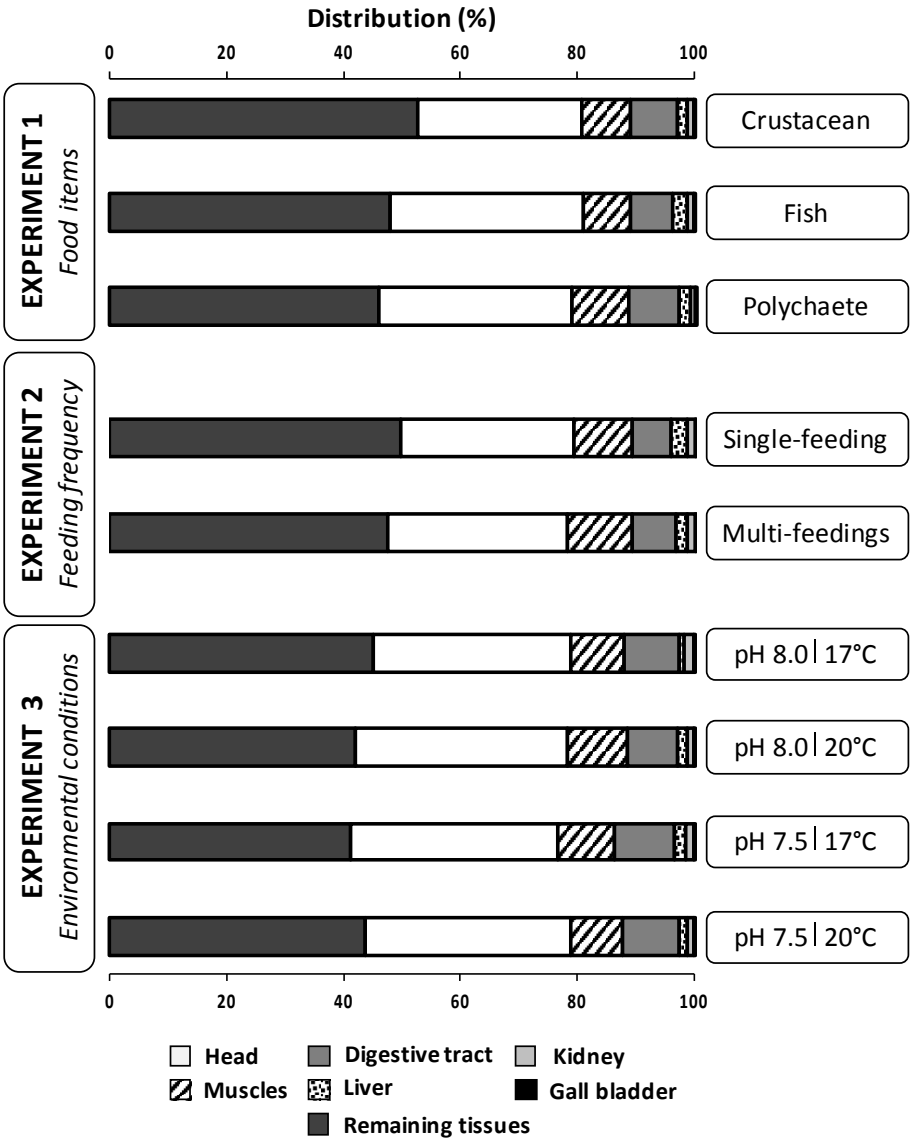

Fig. 1. Distribution patterns of Zn (%) in the 7 body compartments of turbots exposed single time to different food items (Experiment 1), fed one and multiple times with compounded pellets (Experiment 2) or maintained under different pH and temperature conditions and single fed with shrimp (Experiment 3). Values are means (n = 4–5). Details are provided in Table 1. The remaining tissues included remaining skin, skeleton, fins, heart and muscle residues.

These findings are in accordance with the literature. Indeed, very high Zn concentrations (up to 500 μg Zn g^{-1} wet wt) have been measured in the digestive tract of common carp *Cyprinus carpio* (Sun and Jeng, 1999; Reynders et al., 2006). Thus, the digestive tract is not only involved in the absorption of the dietary Zn but is also an important sink for long-term storage of this element. This fact could be due to the presence in high quantity of specific low molecular weight Zn-binding membrane proteins (Jeng et al., 1999).

In this study, several biotic factors were examined to understand Zn organotropism in juvenile turbots: the food quality (different prey items with different Zn bioavailability) and the feeding frequency. Our results did not show any significant effect of these parameters in Zn distribution and concentration in body compartments of juvenile turbots after a 21-d depuration period ($p > 0.05$; Fig. 1, Table 1). The absence of changes in distribution and concentration of this element in the body compartments could be related to the fact that the experimental context is reflecting non-polluted conditions (*i.e.*, no excess of Zn in the diet) and rather reflects normal physiological processes. The stable Zn concentrations in the different types of food used were ranging between 56 and 148 μg g^{-1} dry wt for all the food items used which represent concentration of prey living in non-polluted environments (Eisler, 2009). In addition, during the depuration period, we estimated an average dietary Zn input of approx. 15–20 μg g^{-1} dry wt per fish based on the stable Zn concentration in pellet and daily food eaten by each fish. These values presumably satisfied the daily Zn requirements for fish (Antony Jesu Prabhu et al., 2016). Since the Zn intake is not in excess, we therefore assumed that it is stored in fish tissues without activation of any abnormal excretion mechanisms. This assumption is supported by the results of Experiment 2 where fish were single-fed or multi-fed with ^{65}Zn radiolabeled pellets without any effect on Zn long-term storage in fish. Furthermore, a preliminary assessment of the relationship between stable Zn ingested dose and the ^{65}Zn stored in the different body compartments was done for single-fed turbots from the Experiment 2 (Fig. 2) to determine if we reached a tipping point from a physiological perspective. The linear relation observed indicates that, even if the ingested dose of Zn can be multiplied by 3 for

Table 1
Distribution (%) and concentration index (I_C) of Zn calculated from the 7 body compartments of turbot exposed single time to different food items (Experiment 1) or fed one and multiple times with compounded pellets (Experiment 2) or maintained under different pH and temperature conditions and single fed with shrimp (Experiment 3). Values are means ± SD (n = 4–5).

Compartments	Experiment 1 (n = 5 for each condition)			Experiment 2 (n = 5 for each condition)		Experiment 3 (n = 4 for each condition)			
	Crustacean	Fish	Polychaete	Single-fed	Multi-fed	pH 7.5 at 17 °C	pH 7.5 at 20 °C	pH 8.0 at 17 °C	pH 8.0 at 20 °C
Distribution (%)									
Digestive tract	7.97 ± 0.78	6.98 ± 0.71	8.78 ± 1.68	7.34 ± 1.20	6.67 ± 1.41	10.16 ± 2.02	9.62 ± 1.42	9.22 ± 1.23	8.80 ± 0.46
Head	28.12 ± 0.99	32.74 ± 3.94	32.95 ± 1.50	30.64 ± 2.59	29.70 ± 1.10	35.34 ± 2.48	35.11 ± 2.09	33.78 ± 5.83	36.23 ± 2.06
Gall bladder	0.40 ± 0.24	0.34 ± 0.13	0.10 ± 0.05	0.13 ± 0.07	0.82 ± 0.28	0.35 ± 0.24	0.21 ± 0.07	0.36 ± 0.24	0.11 ± 0.00
Liver	1.70 ± 0.73	2.51 ± 0.71	1.75 ± 0.67	2.01 ± 0.41	2.76 ± 0.87	2.03 ± 0.24	1.33 ± 0.86	0.95 ± 0.28	1.55 ± 0.75
Kidney	1.14 ± 0.44	1.27 ± 0.65	0.96 ± 0.36	1.26 ± 0.15	1.31 ± 0.12	1.31 ± 0.58	1.26 ± 0.65	1.56 ± 0.24	1.26 ± 0.69
Muscles	8.19 ± 1.33	8.16 ± 0.97	9.54 ± 0.88	11.24 ± 1.90	9.87 ± 1.29	9.75 ± 0.59	8.94 ± 2.08	9.22 ± 1.30	10.13 ± 0.95
Remaining tissues[a]	52.48 ± 1.22	48.00 ± 3.65	45.92 ± 3.39	47.44 ± 4.37	49.62 ± 2.22	41.15 ± 3.47	43.59 ± 1.99	45 ± 6.27	41.96 ± 1.80
Concentration index (I_C)									
Digestive tract	1.35 ± 0.22	1.75 ± 0.16	1.67 ± 0.38	2.34 ± 0.62	2.36 ± 0.42	2.87 ± 0.66	2.76 ± 0.23	2.82 ± 0.56	2.82 ± 0.09
Head	1.40 ± 0.06	1.39 ± 0.08	1.25 ± 0.05	1.27 ± 0.04	1.30 ± 0.08	1.26 ± 0.06	1.27 ± 0.12	1.15 ± 0.20	1.19 ± 0.07
Gall bladder	3.01 ± 2.20	2.82 ± 1.43	2.03 ± 0.44	1.70 ± 0.37	1.82 ± 0.28	1.93 ± 1.15	1.87 ± 0.22	2.22 ± 2.03	1.90 ± 0.10
Liver	1.79 ± 0.78	2.13 ± 0.56	2.80 ± 0.27	2.59 ± 0.33	2.44 ± 0.33	3.48 ± 1.05	2.18 ± 0.86	2.66 ± 1.46	3.62 ± 1.11
Kidney	3.70 ± 1.02	3.7 ± 1.44	3.78 ± 0.67	2.98 ± 0.52	2.87 ± 0.47	4.64 ± 1.04	3.96 ± 1.69	4.26 ± 0.63	3.61 ± 1.06
Muscles	0.39 ± 0.06	0.44 ± 0.06	0.42 ± 0.03	0.39 ± 0.04	0.48 ± 0.04	0.49 ± 0.02	0.45 ± 0.09	0.44 ± 0.04	0.51 ± 0.04
Remaining tissues[a]	1.02 ± 0.03	1.04 ± 0.05	1.04 ± 0.06	1.08 ± 0.04	0.98 ± 0.05	0.87 ± 0.06	0.91 ± 0.05	1.00 ± 0.20	0.92 ± 0.05

[a] The remaining tissues included remaining skin, skeleton, fins, heart and muscle residues.

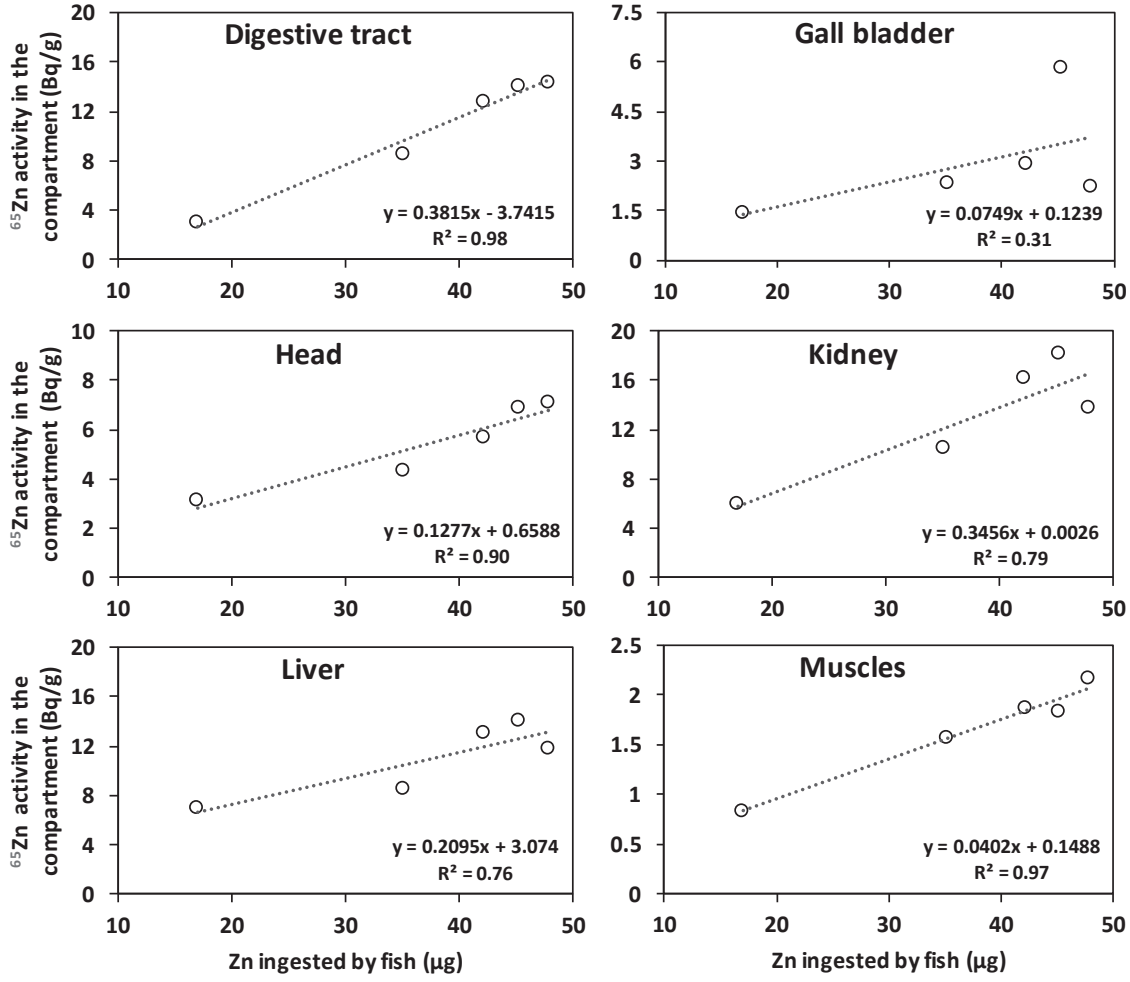

Fig. 2. Relationship between the estimated Zn ingested by fish (µg) and ^{65}Zn stored in each body compartments (Bq g^{-1} wet wt) for the five single-fed turbot from the Experiment 2.

juvenile turbots, there is no visible saturation of the ^{65}Zn burden in the body compartments.

It is however important to keep in mind that, at higher Zn concentrations in food, the uptake and excretion pathways of Zn may be impacted (Bury et al., 2003). Furthermore, at high Zn concentrations, an increase in synthesis of metallothioneins, active

metal transporters, may be observed (Zhang and Wang, 2005). These physiological changes may affect the distribution and concentration of Zn in body compartments and further investigations are needed to clarify this point.

Concerning the potential effects of abiotic factors on Zn trophic transfer in fish, very few studies have been done to date with salinity (Ni et al., 2005), pH (Jacob et al., 2017), temperature (Van Campenhout et al., 2007), pH and temperature (Pouil et al., in press) as influencing factors. Although some of these studies have highlighted significant effects of these parameters on Zn trophic transfer (Van Campenhout et al., 2007; Pouil et al., in press), to the best of our knowledge, only one study considered the potential effects of such parameters (i.e. salinity, Ni et al., 2005) on dietary Zn organotropism. These authors showed that, when the intertidal mudskipper *Periophthalmus cantonensis* was exposed to dietary Zn through a single-feeding at different salinities, Zn body distribution after a 2-d depuration period was not affected. In the present study, we showed that water pH and temperature, either considered separately or in combination, have no significant effect on Zn organotropism (Table 1). The body distribution of this essential element is governed in fish by homeostatic regulation (Hogstrand, 2011). The ability of fish to regulate themselves their acid-base balance and their osmolality (Brauner et al., 2004; Kültz, 2015) can explained that, in the range of pH and temperature values presently used, there is no disturbance of Zn homeostasis, and by extension, no change in the Zn organotropism. Nevertheless, in the context of global change, local important variations of such abiotic stressors can occur, especially in coastal areas and further investigations are needed to explore the dietary trace element organotropism in fish under a larger range of pH and temperature values.

In summary, this study revealed no statistically significant effect of food quality, feeding frequency, and water pH and temperature in Zn organotropism of juvenile turbot. The concentration index (I_C) values, indicate that, when Zn was provided by food, digestive tract played a major role in the long-term storage of this element as well as kidney and gall bladder. We also suspected a non-negligible role of gills for Zn excretion as shown by others (Hardy et al., 1987) but we were not able to prove it. Because metal concentrations in surrounded environment are likely to affect their body concentration (Le Pabic et al., 2015), further investigations are needed to study the influence of this parameter on Zn organotropism.

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References

Andres, S., Ribeyre, F., Tourencq, J.-N., Boudou, A., 2000. Interspecific comparison of cadmium and zinc contamination in the organs of four fish species along a polymetallic pollution gradient (Lot River, France). Sci. Total Environ. 248, 11–25.

Antony Jesu Prabhu, P., Schrama, J.W., Kaushik, S.J., 2016. Mineral requirements of fish: a systematic review. Rev. Aquac. 8, 172–219.

Bowness, J.M., Morton, R.A., 1952. Distribution of copper and zinc in the eyes of fresh-water fishes and frogs. Occurrence of metals in melanin fractions from eye tissues. Biochem. J. 51, 530–535.

Brauner, C.J., Wang, T., Wang, Y., Richards, J.G., Gonzalez, R.J., Bernier, N.J., Xi, W., Patrick, M., Val, A.L., 2004. Limited extracellular but complete intracellular acid-base regulation during short-term environmental hypercapnia in the armoured catfish, *Liposarcus pardalis*. J. Exp. Biol. 207, 3381–3390.

Bury, N.R., Walker, P.A., Glover, C.N., 2003. Nutritive metal uptake in teleost fish. J. Exp. Biol. 206, 11–23.

Clearwater, S.J., Farag, A.M., Meyer, J.S., 2002. Bioavailability and toxicity of dietborne copper and zinc to fish. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 132, 269–313.

Dural, M., Göksu, M.Z.L., Özak, A.A., 2007. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. Food. Chem. 102, 415–421.

Eckhert, C.D., 1983. Elemental concentrations in ocular tissues of various species. Exp. Eye Res. 37, 639–647.

Eisler, R., 2009. Compendium of Trace Metals and Marine Biota. In: Plants and Invertebrates, vol. 1. Elsevier.

Florin, A.-B., Lavados, G., 2010. Feeding habits of juvenile flatfish in relation to habitat characteristics in the Baltic Sea. Estuar. Coast. Shelf. Sci. 86, 607–612.

Gray, J.S., 2002. Biomagnification in marine systems: the perspective of an ecologist. Mar. Pollut. Bull. 45, 46–52.

Hardy, R.W., Sullivan, C.V., Koziol, A.M., 1987. Absorption, body distribution, and excretion of dietary zinc by rainbow trout (*Salmo gairdneri*). Fish. Physiol. Biochem. 3, 133–143.

Hogstrand, C., 2011. Zinc. In: Chris, M., Wood, A.P.F., Brauner, C.J. (Eds.), Fish Physiology. Academic Press, pp. 135–200.

Imsland, A.K., Foss, A., Gunnarsson, S., Berntssen, M.H.G., FitzGerald, R., Bonja, S.W., Ham, E., Naevdal, G., Stefansson, S.O., 2001. The interaction of temperature and salinity on growth and food conversion in juvenile turbot (*Scophthalmus maximus*). Aquaculture 198, 353–367.

IPCC, 2013. In: Climate Change 2013: The Physical Sciences Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. ed. Intergovernmental Panel on Climate Change (IPCC), Cambridge, United Kingdom and New York, NY, USA.

Jacob, H., Pouil, S., Lecchini, D., Oberhänsli, F., Swarzenski, P., Metian, M., 2017. Trophic transfer of essential elements in the clownfish *Amphiprion ocellaris* in the context of ocean acidification. PLoS One e0174344.

Jeng, S.-S., Wang, J.-T., Sun, L.-T., 1999. Zinc and zinc binding substances in the tissues of common carp. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 122, 461–468.

Kojadinovic, J., Potier, M., Le Corre, M., Cosson, R.P., Bustamante, P., 2007. Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean. Environ. Pollut. 146, 548–566.

Kültz, D., 2015. Physiological mechanisms used by fish to cope with salinity stress. J. Exp. Biol. 218, 1907–1914.

Le Pabic, C., Caplat, C., Lehodey, J.P., Milinkovitch, T., Koueta, N., Cosson, R.P., Bustamante, P., 2015. Trace metal concentrations in post-hatching cuttlefish *Sepia officinalis* and consequences of dissolved zinc exposure. Aquat. Toxicol. 159, 23–35.

Mathews, T., Fisher, N.S., 2009. Dominance of dietary intake of metals in marine elasmobranch and teleost fish. Sci. Total Environ. 407, 5156–5161.

Milner, N.J., 1982. The accumulation of zinc by O-group plaice, *Pleuronectes platessa* (L.), from high concentrations in sea water and food. J. Fish. Biol. 21, 325–336.

Ni, I.-H., Chan, S.M., Wang, W.-X., 2005. Influences of salinity on the biokinetics of Cd, Se, and Zn in the intertidal mudskipper *Periophthalmus cantonensis*. Chemosphere 61, 1607–1617.

Ni, I.-H., Wang, W.-X., Yin, K.T., 2000. Transfer of Cd, Cr and Zn from zooplankton prey to mudskipper *Periophthalmus cantonensis* and glassy *Ambassis urotaenia* fishes. Mar. Ecol. Prog. Ser. 194, 203–210.

Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R.M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R.G., Plattner, G.-K., Rodgers, K.B., Sabine, C.L., Sarmiento, J.L., Schlitzer, R., Slater, R.D., Totterdell, I.J., Weirig, M.-F., Yamanaka, Y., Yool, A., 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437, 681–686.

Pentreath, R.J., 1976. Some further studies on the accumulation and retention of ^{65}Zn and ^{54}Mn by the plaice, *Pleuronectes platessa* L. J. Exp. Mar. Biol. Ecol. 21, 179–189.

Pouil, S., Oberhaënsli, F., Bustamante, P., Metian, M., 2017c. Investigations of temperature and pH variations on metal trophic transfer in turbot (*Scophthalmus maximus*) fish. Environ. Sci. Pollut. Res. http://dx.doi.org/10.1007/s11356-017-8691-4 (in press).

Pouil, S., Warnau, M., Oberhänsli, F., Teyssié, J.-L., Bustamante, P., Metian, M., 2016. Influence of food on the assimilation of essential elements (Co, Mn, and Zn) by turbot *Scophthalmus maximus*. Mar. Ecol. Prog. Ser. 550, 207–218.

Pouil, S., Teyssié, J.-L., Rouleau, C., Fowler, S.W., Metian, M., Bustamante, P., Warnau, M., 2017a. Comparative study of trophic transfer of the essential metals Co and Zn in two tropical fish: a radiotracer approach. J. Exp. Mar. Biol. Ecol. 486, 42–51.

Pouil, S., Warnau, M., Oberhänsli, F., Teyssié, J.-L., Bustamante, P., Metian, M., 2017b. Comparing single-feeding and multi-feeding approaches for experimentally assessing trophic transfer of metals in fish. Environ. Toxicol. Chem. 36, 1227–1234.

R Development Core Team, 2014. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reynders, H., Van Campenhout, K., Bervoets, L., De Coen, W.M., Blust, R., 2006. Dynamics of cadmium accumulation and effects in common carp (*Cyprinus carpio*) during simultaneous exposure to water and food (*Tubifex tubifex*). Environ. Toxicol. Chem. 25, 1558–1567.

Rodriguez y Baena, A.M., Miquel, J.C., Masqué, P., Povinec, P.P., La Rosa, J., 2006. A single vs. double spike approach to improve the accuracy of ^{234}Th measurements in small-volume seawater samples. Mar. Chem. 100, 269–281.

Rouleau, C., Gobeil, C., Tjälve, H., 2000. Accumulation of silver from the diet in two marine benthic predators: the snow crab (*Chionoecetes opilio*) and American plaice (*Hippoglossoides platessoides*). Environ. Toxicol. Chem. 19, 631–637.

Sparrevohn, C.R., Støttrup, J., 2008. Diet, abundance and distribution as indices of turbot (*Psetta maxima* L.) release habitat suitability. Rev. Fish. Sci. 16, 338–347.
Spry, D.J., Hodson, P.V., Wood, C.M., 1988. Relative contributions of dietary and waterborne zinc in the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 45, 32–41.
Spry, D.J., Wood, C.M., 1985. Ion flux rates, acid–base status, and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water. Can. J. Fish. Aquat. Sci. 42, 1332–1341.
Sun, L.-T., Jeng, S.-S., 1999. Accumulation of zinc from diet and its release in common carp. Fish. Physiol. Biochem. 20, 313–324.
Van Campenhout, K., Bervoets, L., Blust, R., 2007. Assimilation efficiencies of Cd and Zn in the common carp (Cyprinus carpio): effects of metal concentration, temperature and prey type. Environ. Pollut. 145, 905–914.
Warnau, M., Bustamante, P., 2007. Radiotracer techniques: a unique tool in marine ecotoxicological studies. Environ. Bioindic. 2, 217–218.
Watanabe, T., Kiron, V., Satoh, S., 1997. Trace minerals in fish nutrition. Aquaculture 151, 185–207.
Willis, J.N., Sunda, W.G., 1984. Relative contributions of food and water in the accumulation of zinc by two species of marine fish. Mar. Biol. 80, 273–279.
Xu, Y., Wang, W.-X., 2002. Exposure and potential food chain transfer factor of Cd, Se and Zn in marine fish *Lutjanus argentimaculatus*. Mar. Ecol. Prog. Ser. 238, 173–186.
Zar, J.H., 1996. Biostatistical Analysis, third ed. Prentice-Hall, Upper Saddle River, NJ.
Zhang, L., Wang, W.-X., 2007. Gastrointestinal uptake of cadmium and zinc by a marine teleost *Acanthopagrus schlegeli*. Aquat. Toxicol. 85, 143–153.
Zhang, L., Wang, W.-X., 2005. Effects of Zn pre-exposure on Cd and Zn bioaccumulation and metallothionein levels in two species of marine fish. Aquat. Toxicol. 73, 353–369.