



HAL
open science

The role of marine biotoxins on the trophic transfer of Mn and Zn in fish

Simon Pouil, Rachel J Clausing, Marc Metian, Paco Bustamante,
Marie-Yasmine Dechraoui Bottein

► **To cite this version:**

Simon Pouil, Rachel J Clausing, Marc Metian, Paco Bustamante, Marie-Yasmine Dechraoui Bottein.
The role of marine biotoxins on the trophic transfer of Mn and Zn in fish. *Aquatic Toxicology*, 2018,
198, pp.198-205. 10.1016/j.aquatox.2018.03.004 . hal-02015003

HAL Id: hal-02015003

<https://hal.science/hal-02015003v1>

Submitted on 3 Mar 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The influence of algal toxin ingestion on the transfer of essential nutrients (Mn and Zn) in fish

Simon Pouil^{a,b}, Rachel Clausing^a, Marc Metian^{a*}, Paco Bustamante^b, Marie-Yasmine Dechraoui Bottein^a

^a International Atomic Energy Agency, Environment Laboratories, 4a, Quai Antoine 1er, MC-98000, Principality of Monaco, Monaco

^b Littoral Environnement et Sociétés (LIENSs), UMR 7266, CNRS-Université de La Rochelle, 2 rue Olympe de Gouges, F-17000 La Rochelle, France

* Corresponding author: Marc Metian

Radioecology Laboratory

IAEA Environment Laboratories

4a Quai Antoine 1er

MC-98000 Principality of Monaco

Telephone: +377 97 97 72 17

Abstract

Studying the dietary transfer of essential metals in fish is important to better understand how fish accumulates these metals. Increasing information is available in the literature on the influence of environmental factors such as water temperature, pH and salinity, on the potential trophic transfer of these elements. Algal toxins occurring in the natural environment may also affect metals assimilation through (1) interactions with the metals such as the formation of metal-toxin complexes or (2) by changes to the physiology of the fish (e.g. alteration of ion transport pathways or absorption mechanisms). However, to the best of our knowledge, the potential effects of biotoxins on essential metal trophic transfer have never been investigated. We assessed the influence of dietary exposure to brevetoxins (PbTx_s), biotoxins produced by the dinoflagellate *Karenia brevis*, on trophic transfer of essential metals (Mn and Zn) in fish using highly sensitive radiotracer techniques (i.e. β - and γ -spectrometry). Using radiolabelled mussels *Mytilus edulis* with accumulated PbTx_s under controlled laboratory conditions, juvenile turbot *Scophthalmus maximus* were exposed to both dietary PbTx_s and radiotracers (⁵⁴Mn and ⁶⁵Zn). Treatments included simultaneous exposure in a single-feeding or 3-weeks daily pre-exposure to dietary PbTx_s followed by a single-feeding of radiotracers. After a 21-day depuration period, the assimilation efficiencies (AEs) of Mn and Zn in juvenile turbot were quantified and found not to be affected by the ingestion of PbTx_s ($p > 0.05$). These results can be explained by the rapid turnover and elimination of ingested PbTx_s in the body, likely before distribution to all body compartments, suggesting that dietary PbTx_s have limited effects on Mn and Zn assimilation in fish.

Keywords: Brevetoxins; Teleost; Multiple stressors; Metals; HABs; Nuclear applications

1. Introduction

Some metals found in the marine environment are termed essential metals because marine organisms such as fish require them for physiological processes. In fish, these metals are mainly acquired from their diet (Xu and Wang, 2002; Mathews and Fisher, 2009). For example, these metals are part of the functional groups of various metabolic enzymes, they play a structural role in respiratory pigments and metalloenzymes, and they act as co-factors for various proteins (see e.g. Simkiss, 1979; Watanabe et al., 1997; Williams, 1981). An insufficient or excess quantity of these metals, which are obtained primarily through the diet (e.g. Mathews and Fisher, 2009; Xu and Wang, 2002), can provoke physiological alterations (e.g. Förstner and Wittmann, 1981). Among the essential metals, Mn and Zn are particularly important due to their roles as components of or cofactors in key enzyme systems in lipid, protein, and carbohydrate metabolism (Watanabe et al., 1997; Bury et al., 2003). As a result, these elements are directly involved in growth, bone formation, reproduction, development and immunity in fish (Watanabe et al., 1997). The influence of environmental factors on the trophic transfer of these essential metals in fish is therefore a key focus of research.

In the field, fluctuations in environmental variables such as the temperature, pH or salinity of the surrounding water are known to influence the digestive physiology of fish and thus, potentially, their ability to bioaccumulate dietary essential metals. For example, laboratory experiments on various marine fish species have shown that seawater temperature and pH can strongly affect gut transit time or the activity of enzymes involved in the digestion process (Edwards, 1971; Miegel et al., 2010; Pimentel et al., 2015; Rosa et al., 2016). Salinity also plays a major role in fish physiology, not only in osmoregulation processes, but also in feeding behaviour and food conversion (reviewed by Bœuf and Payan, 2001). Although no significant effect of pH and salinity has yet been found on trophic transfer of essential metals in fish (Pouil et al. 2017, Ni et al. 2005, Jacob et al. 2017), temperature has been shown to exert a positive

effect on Zn trophic transfer in the carp *Cyprinus carpio* (Van Campenhout et al., 2007) and the turbot *Scophthalmus maximus* (Pouil et al., 2017). In addition, naturally produced toxic compounds in the marine environment such as algal toxins can also affect fish physiology.

Although toxic compounds are produced by less than 2% of the described species of marine microalgae (biotoxins; Landsberg, 2002), the occurrence of blooms of these harmful algae (harmful algal blooms: HABs) appear to be increasing over the last decades (Hallegraeff, 1993; Smayda, 1990; Sournia, 1995; Van Dolah, 2000, a trend that is predicted to continue with global changes (Hallegraeff, 2010; Moore et al., 2008; Wells et al., 2015). Depending on the species, bivalves are able to accumulate algal toxins and thus are significant sources for the potential transfer of toxins up the food chain (Landsberg et al., 2009). As consequence, algal biotoxins can impact species interactions, organismal physiology and health, and population dynamics (e.g. Anderson, 2009; Smith and Schindler, 2009; Van Den Bergh et al., 2002), particularly in fishes (reviewed by Landsberg, 2002).

There are strong links between algal toxins and metals. Indeed, it has been reported that the production of algal toxins is affected by change in the environmental conditions resulted from the input of trace metals (Kenefick et al., 1993; Lukac and Aegerter, 1993). The behavior of algal species in responding to metal exposure could be explained by the acquisition of nutritional metals such as Mn and Zn (Sunda, 2012), the detoxification of toxic metals or as the result of responding to the environmental stress caused by metals (Kenefick et al., 1993; Utkilen and Gjølme, 1995; Gong et al., 2009; Li et al. 2009; Zeng et al. 2009). Furthermore, metal ions may be complexed with algal toxins. Since some of the functional groups, such as amino, carboxyl, phenol, sulfhydryl and hydroxyl groups, are generally present in the algal toxin molecules, algal toxins have a potential to complex with metal ions (Sunda, 2012) and thus potentially affect their bioavailability throughout aquatic food chains. Furthermore, some algal toxins, such as brevetoxins (PbTx) cause alteration of membrane potential that occurs with

exposure to several algal toxins (i.e., neurotoxins) impact cellular ion transport (Tian et al., 2011) and consequently has the potential to affect the uptake and depuration of metals.

The aim of the present study was to investigate the influence of single and multiple feedings with brevetoxins (PbTx_s), the biotoxins produced by the red-tide forming dinoflagellate *Karenia brevis* (Landsberg et al., 2009), on the trophic transfer of essential metals in fish. One critical physiological parameter for understanding metal trophic transfer in fish is the assimilation efficiency (AE), defined as the fraction of the ingested metals that is incorporated into biological tissues (Wang and Fisher, 1999). Thus, we experimentally assessed the AEs and tissue incorporation efficiencies of two essential elements (Mn and Zn) in juvenile turbot after one or after multiple feedings of mussels *Mytilus edulis* containing PbTx_s and determined effects in comparison to the AEs of the same metals in turbot not exposed to PbTx. Highly sensitive radiotracer techniques were used (1) to quantify the PbTx_s ingested in juvenile turbot and (2) to determine the AEs of the studied essential metals.

2. Materials and Methods

2.1. Origin, acclimation and maintenance of organisms

Juvenile *S. maximus* were purchased from a fish farm (France Turbot, France). Farmed fish, selected for their very low sensitivity to stress and their ease to handling (Lepage et al. 2000), were acclimated to laboratory conditions for at least 6 months (aerated, open-circuit 700-L tank; flux: 350 L h⁻¹; salinity: 38; temperature: 20 ± 1 °C; pH: 8.0 ± 0.1; light/dark cycle: 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). All experimental fish were individually identified by slits cut into the fins. The daily dietary intake was determined during this period.

Common mussels *M. edulis* (toxin-free as assessed by a receptor binding assay) were purchased from a seafood seller (Les Halles du Midi, Monaco). They were transported to the IAEA-EL laboratory in Monaco and were acclimated in the same conditions as the turbot for 1 month prior to the experiment. During this period, mussels were fed daily with phytoplankton (*Isochrysis galbana*).

Cultures of *K. brevis* (NOAA-1 strain isolated from Charlotte Harbor, FL, USA; 18.5 (Mean) \pm 2.7 (SD) pg PbTx-3 equiv cell⁻¹) were grown in a 20-L plastic container containing aerated seawater (0.45 μ m filtered seawater aged in the dark for 1 month minimum; salinity: 38; temperature: 20 \pm 1°C; pH: 8.0 \pm 0.1; bilateral luminosity 66–80 μ E m⁻² s⁻¹; light/dark: 12h/12h) spiked with an atypical f10k medium (Holmes et al., 1991). The culture was started using 2 L of f10k medium to have an initial cell concentration of approx. 1000 cells mL⁻¹. At regular intervals when the concentration reached 5000 cells mL⁻¹, 2 L of f10k medium was added in order to get 20 L of culture in stationary phase (i.e. approx. 10000 cells mL⁻¹) for experiment. Cell counting was performed in 10% Lugol's solution using a Sedgewick Rafter counting chamber (20 \times 50 μ L) under a light microscope.

2.2. Experimental procedures

To investigate the influence of dietary exposure to PbTxS on the AEs of Mn and Zn in fish, juvenile turbot were fed toxic algae either prior to or concurrent with metals exposure and compared with fish fed with metals alone. The first condition (Treatment 1) simulated a single, concurrent exposure event in which fish consume bivalves long after a *K. brevis* bloom and thus bivalve tissues contain only low concentrations of PbTxS. The second condition (Treatment 2) investigated the effects of repeated dietary exposures to higher concentrations of PbTxS in bivalve tissues as occur shortly after a bloom event. The last condition (Treatment 3) served as the experimental control. One week before the experiment, all the juvenile turbot

were fed daily with non-radiolabelled non-toxic gel food (NRNT, Table 1) to acclimate them to the food matrix. Details of all gel food types used are provided in Table 1.

2.2.1. Preparation of the mussel-based gel foods

To prepare the different PbTx-containing and/or radiolabelled gel foods, *M. edulis* (33.1 ± 5.1 g wet wt) were randomly placed in three aerated, open-circuit 20-L aquaria (n=40; salinity: 38; temperature: 20 ± 1 °C; pH: 8.0 ± 0.1 ; light/dark cycle: 12 h/12 h).

Mussels in two of the 20-L aquaria (aquaria 1&2) were exposed daily for four days to *K. brevis* at an environmentally relevant cell concentration of 980 ± 20 cells mL⁻¹ (Gannon et al., 2009; Stumpf et al., 2003). Each day, *K. brevis* culture was carefully added to the aquaria to preserve *K. brevis* cell integrity and achieve the desired cell concentration. During 1h of feeding, the aquaria were maintained in closed-circuit, after which mussels were fed *I. galbana* for 30 min. At the end of the exposure, a fraction of exposed mussels from each aquarium was used to prepare the NRHT gel food (Table 1). Briefly, the soft tissues of the mussels were collected, homogenized and mixed with food-grade leaf gelatin that had been dissolved in hot seawater (0.1 g mL⁻¹) and cooled to 30°C (ratio of 0.25 mL g⁻¹).

After exposure to *K. brevis*, the remaining mussels from one of the 20-L aquaria (aquarium 1; n=20) were then exposed for 23 days to dissolved radiotracers (⁵⁴Mn and ⁶⁵Zn) of high specific activity (⁵⁴Mn as MnCl₂ in 0.5 M HCl, [T_{1/2}] = 312.2 d and ⁶⁵Zn as ZnCl₂ in 0.1M HCl, [T_{1/2}] = 243.9 d; Isotope Product Lab, USA). Levels of radioactivity in the seawater were kept constant (i.e. 0.40 ± 0.16 kBq ⁵⁴Mn L⁻¹ and 0.80 ± 0.39 kBq ⁶⁵Zn L⁻¹) with regular spiking in closed-circuit, where the frequency was determined by measurements before and after each spike renewal (e.g. Metian et al., 2009). In terms of stable metal equivalent, each spike

corresponded to an addition of 1 ng Mn L⁻¹ and 27 ng Zn L⁻¹ (i.e. concentrations that are substantially lower than the background concentrations of these metals in open sea; Bruland, 1983). After each water renewal (i.e. before each spike renewal), mussels were fed 30 min with *I. galbana*. Mussels were collected at different time intervals and were γ -counted alive and returned to the aquarium. After the 23 d of exposure to the radiotracers, the radiolabelled, PbTx-containing mussels from aquarium 1 were processed to prepare the gel food with radiotracers and low toxin content (RLT; Table 1) using all mussel soft tissues and gelatin as described above.

During the same period, remaining mussels from the second *Karenia*-exposed 20-L aquarium (aquarium 2; n=20) were kept in flow-through seawater and fed daily with *I. galbana*. Twenty-three days after the last exposure to *K. brevis*, mussels were dissected and soft tissues processed for toxin analysis as proxy for toxin content in mussels exposed to radiotracers (aquarium 1) or to prepare a non-radiolabelled-low toxin content gel food (NRLT; Table 1) as previously described.

Mussels from the third 20-L aquarium (aquarium 3, n=20) were exposed to dissolved radiotracers for 23 days as described above without previous exposure to *K. brevis*. Soft tissues were used to prepare the radiolabelled gel food without toxin content (RNT).

All the five experimental gel foods (NRHT, RLT, RNT, NRLT and NRNT; Table 1) were stored at -4°C until use, and radioactivity and PbTx concentrations were measured as described in section 2.3. Furthermore, stable Mn and Zn were measured in gels NRHT and NRNT. Briefly, samples (n = 3 for each gel) of 800 to 1000 mg were digested using 5 ml of 65% HNO₃ and 2 ml of H₂O₂. Acidic digestion was performed overnight at ambient temperature and then heated in a microwave for 40 min, involving a gradual increase to 190°C over 20 min, followed by 20 min at 190°C (1600 W). After the mineralisation process, each sample was diluted to 50 ml

with Milli-Q water, and an extra 1:5 dilution was prepared. Analyses were performed by flame atomic absorption spectrometry (SpectrAA 220, Varian). A certified reference material (fish muscle, IAEA 407) was treated and analysed in the same way as the samples. Results were in good agreement with the certified values (recovery over 80% for Mn and 97% for Zn). For each set of analyses, blanks were included in the analytical batch. These analyses confirmed the homogeneity of the stable metal concentrations in the gels with values of <0.8 and <0.6 ng g⁻¹ wet wt of Mn and 7.8 ± 0.4 and 6.3 ± 0.5 ng g⁻¹ of Zn for the gel NRHT and the NRHT, respectively. All the gels were thawed and cut into small, homogenous pieces (size < 2 mm) a few minutes before being offered to the turbot.

2.2.2. Treatment 1: Influence of a single PbTx ingestion on AE of Mn and Zn in fish

Prior to the experiment, seven juvenile turbot (18.2 ± 2.0 g wet wt) were chosen at random and transferred into an aerated, open circuit 20-L aquarium (water renewal: 60 L h⁻¹; 0.45- μ m filtered seawater; salinity: 38; temperature: 20 ± 1 °C; pH: 8.0 ± 0.1 ; light/dark: 12h/12h). Turbot were fed once with the RLT gel food (see section 2.2.1 and Table 1) for a maximum of 15 min and AEs of Mn and Zn were followed over 21 d. Water flow conditions and feeding duration were selected in order to avoid any risk of dissolved exposure of the fish from the radiotracer leaching from the food. One additional turbot was placed within a net in the same aquarium to restrict access to food and serve as a control for possible radiotracer leaching from the contaminated food or from fish depuration. Two hours after the feeding period, all turbot were whole-body γ -counted alive (section 2.3.2) and replaced in clean, flow-through seawater. Countings were then regularly performed over a 21-d period to follow the depuration kinetics of the radiotracers.

2.2.3. Treatment 2: Influence of multiple PbTx exposures on AE of Mn and Zn in fish

Seven juvenile turbot (22.1 ± 3.7 g wet wt), kept in the same conditions as described in section 2.2.2, were pre-exposed to NRHT gel food for 3 weeks (5 feedings/week) and then given a single-feeding with RNT gel food (Table 1). One additional, non-exposed turbot served as a control (see section 2.2.2). Two hours after the single-feeding, each fish was γ -counted and then replaced in clean, flow-through seawater (parameters previously described). Depuration was followed in each individual by regular, live whole-body γ -counting (as detailed in section 2.3.2) over 21 d as before.

2.2.4. Treatment 3: Mn and Zn AE in fish without dietary PbTx (control condition)

To describe the depuration kinetics of Mn and Zn, juvenile turbot ($n=7$, 21.1 ± 4.3 g wet wt) were given a single-feeding of RNT gel food. Fish were maintained and γ -counted as previously described.

2.2.5. Accumulation of PbTx in fish

To quantify the accumulated PbTxs in fish tissue after both single and multiple exposures, treatments 1 and 2 were repeated in the absence of radiotracers. Quantification of PbTx concentrations cannot be performed on samples with radiotracers due to the interference γ -emitters (here ^{54}Mn and ^{65}Zn) can cause in detection of β -emitters using liquid scintillation counting (here tritiated PbTx: [^3H]PbTx-3; see section 2.3.1). Each time, 8 turbot were single-fed or multi-fed (3 weeks) with NRLT gel food or NRHT gel food, respectively, sacrificed 2 h after feeding, and dissected to collect four body compartments: (1) digestive tract, (2) gall bladder, (3) liver and (4) muscle. Methods of extraction and quantification of toxin concentrations in these tissues are described in section 2.3.

2.3. PbTx₃ and radiotracers quantification

2.3.1. PbTx₃ quantification using β -spectrometry

PbTx₃ in the three mussel-based gel foods (RLT, NRLT and NRHT; Table 1, n=3 per food type) and turbot tissue samples (n=8 for each body compartment) were pooled for homogeneity and to obtain sufficient quantity for measurements, resulting in one pooled sample per body compartment per treatment or per gel food type. Samples were then extracted as previously described (Poli et al., 2000) and tested (recovery: 90-108%, Dickey et al. 1999) with minor modifications. Briefly, samples were homogenized in 3 volumes of acetone, sonicated (min. 1 min in ultrasonic water bath) and centrifuged at 3000 g for 10 min. Each supernatant was collected in a 50-mL Falcon tube, and the process was repeated two more times. Combined supernatants were evaporated under a stream of nitrogen in a water bath at 40 °C. Dried samples were resuspended in 6 mL of 80% methanol to which 6 mL of n-hexane was added. Samples were mixed by inversion and centrifuged at 3000 g for 1 min. The methanol phase was collected in glass tubes and evaporated under a stream of nitrogen. Extracts were resuspended in 100% methanol and stored at -18°C until analysis.

Composite PbTx₃ concentrations in the different tissue extracts were determined using an activity-based radioligand receptor binding assay (RBA; following Dechraoui Bottein and Clausing, 2017). This competitive-inhibition assay measures the binding of a constant low concentration of [³H]PbTx₃ to its specific receptor on voltage gated sodium channels in the presence of extract. The reduction in [³H]PbTx₃ binding is directly proportional to the amounts of PbTx₃ standard or unlabelled toxins present in the sample (Dechraoui, 2014; Poli et al., 1986). In each assay, samples were analysed in triplicate at one (liver, gall bladder and digestive tract) or three (gel foods, muscle) dilutions in a 96-well filterplate format (MSFB N6B 50 MultiscreenHTS), and radioactivity was counted in a liquid scintillation beta counter

(MicroBeta2 Microplate Counter, PerkinElmer) after a 2-h period of incubation. Each sample was run in three independent assays. Toxin quantification of samples was calculated from standard curves of PbTx-3 (4-parameter logistic regressions) determined using GraphPad Prism software version 6.0 (San Diego, USA).

2.3.2. Radiotracer quantification by γ -spectrometry

The radioactivity of the tracers 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 of living organisms and gel-food preparations was determined by comparison with standards of known activity and appropriate geometry (calibration and counting). Measurements were corrected for background and physical radioactive decay (Cresswell et al., 2017). Both radiolabelled experimental gel foods (gel RNT and gel RLT) were weighed (wet wt) and placed in plastic tubes (diameter: 42mm, height: 65mm) in triplicate. Then, 25 mL of 2M HCl was added to each tube to get an appropriate geometry, and samples were stored overnight before radioanalyses. Living organisms were counted in circular plastic boxes (diameter: 160 mm; height: 80 mm) filled with clean seawater. The counting period was adjusted to obtain a propagated counting error less than 5% (e.g. Rodriguez y Baena et al., 2006) while maintaining fish health and normal behaviour, and generally varied between 15 and 60 min.

2.4. Data treatment and statistical analyses

Depuration of the radiotracers was expressed as the percentage of remaining radioactivity (radioactivity at time t divided by the initial radioactivity measured in the organism at the

beginning of the depuration period; following Warnau et al., 1996). The depuration kinetics of Mn and Zn were best fitted using a two-component exponential model Eq. (1):

$$(1) A_t = A_{0s} \cdot e^{-k_{es}t} + A_{0l} \cdot e^{-k_{el}t}$$

where A_t and A_0 are the remaining activities (%) at time t (in days) and time 0, respectively. Here, time 0 refers to the initial γ -counting of the fish performed two hours after the radiolabelled single-feeding. k_e is the depuration rate constant (d^{-1}); and “s” and “l” represent the short- and long-lived radiotracer component during depuration, respectively. The “s” component is the radiotracer fraction that is weakly associated with the organism and rapidly excreted out of the body (i.e. mainly the proportion not absorbed but associated with the faeces; Whicker and Schultz, 1982; Warnau et al., 1996). The “l” component describes the fraction of radiotracer that is actually absorbed by the organism and excreted more slowly. The long-lived component allows estimation of the AE of the radiotracer ingested with food ($AE = A_{0l}$; Fowler and Guary, 1977; Miramand et al., 1982).

A biological half-life can be calculated for both the short- and long-lived components ($T_{b1/2s}$ and $T_{b1/2l}$) from the corresponding depuration rate constants (k_{es} and k_{el}) according to the relation $T_{b1/2} = \ln 2/k_e$. Model constants and their statistics were estimated by iterative adjustment of the model and Hessian matrix computation, respectively, using non-linear curve-fitting routines in Statistica® software 7.0.

Comparison of assimilation of metals among the different experimental conditions was performed using Kruskal-Wallis and Siegel and Castellan non-parametric tests on the AEs calculated for each individual turbot, where the best-fit model obtained for the entire set of turbot was applied to each individual (Zar, 1996). For Mn, two individuals per condition had insufficient initial activity (i.e. <1.5 Bq of ^{54}Mn , activity measured 2 hours after the radiolabelled feeding) and were excluded from statistical analysis. Toxin accumulation data

were not statistically analysed as replicate samples within each treatment/tissue type combination were pooled for to obtain sufficient quantity for rigorous toxin determination. Thus, the given values represent the overall trends between treatments as each sample is a homogenate of 8 individuals. Moreover, each sample was analyzed at multiple dilutions over at least 3 independent assays, giving a high level of confidence to the accuracy of the reported value. The level of significance was $\alpha = 0.05$. All statistical analyses were performed using R software 3.0.1 (R Core Team, 2014).

3. Results

3.1. PbTx concentrations in food and fish

Aliquots taken from the PbTx-containing gel foods without radiotracers (i.e. NRLT and NRHT) confirmed that after 4 exposures to *K. brevis*, the mussels used to prepare the gel food had accumulated PbTxs in their tissues. Low variance among PbTx concentrations among the 3 replicate samples for each food type indicated that the gel foods were homogenous. PbTx concentrations were $188 \text{ (Mean)} \pm 38 \text{ (SD)}$ and $525 \text{ (Mean)} \pm 88 \text{ (SD)}$ ng PbTx-3 equiv g^{-1} food for the NRLT and the NRHT gel foods, respectively (Table 1). Thus, the average quantities of PbTxs given to each turbot during the single (Treatment 1) and the multiple (Treatment 2) dietary exposures were $4 \text{ ng PbTx-3 equiv g}^{-1}$ fish and $222 \text{ ng PbTx-3 equiv g}^{-1}$ fish, respectively (Table 2).

PbTx concentrations in the selected four body compartments of juvenile turbot (the digestive tract, the gall bladder, the liver and the muscles) were found to be highest in the gall bladder (ranging $60\text{-}80 \text{ ng PbTx-3 equiv g}^{-1}$, Fig 2, Table 2). Lower concentrations were found in the digestive tract and the liver ($8\text{-}12 \text{ ng PbTx-3 equiv g}^{-1}$ and $8\text{-}10 \text{ PbTx-3 equiv ng g}^{-1}$, respectively; Fig 2, Table 2). Toxin concentrations in muscle tissue were below the limit of

quantification (i.e. 0.3 ng PbTx-3 equiv g⁻¹; Fig. 2). For all the body compartments measured (pooled for each tissue type within treatment), similar PbTx concentrations were found between the turbot from the different experimental conditions.

3.2. Effects of PbTx on trophic transfer of essential metals in fish

After a single (Treatment 1) and multiple (Treatment 2) dietary exposures to PbTx, the depuration kinetics of ⁵⁴Mn and ⁶⁵Zn were followed in juvenile turbot for 21 d. No growth or mortality were recorded and rates and quantity of food consumption remained constant throughout the experiment. The measured activity levels of each tracer in both radiolabelled gel foods (RLT and RNT) were 48 ± 1 Bq and 37 ± 2 Bq g⁻¹ for ⁵⁴Mn and 1059 ± 30 Bq and 1012 ± 63 Bq g⁻¹ for ⁶⁵Zn, respectively (Table 1). No activity was measured in the control turbot at any time.

Depuration kinetics of ⁵⁴Mn and ⁶⁵Zn were most accurately described by 2-component exponential models (Fig. 3, Table 3; R²: 0.51 - 0.96). For both Mn and Zn, a large proportion (42 - 83%) of the ingested radiotracer was short-lived. The short-lived component was characterized by a very rapid loss with T_{b1/2s} ranged from 0.27 to 0.35 d for Mn and 0.23 to 0.28 d for Zn. Examination of the long-lived component revealed that Zn (AEs: 16-18%) was less assimilated than Mn (AEs: 56-60%). For both metals, AEs calculated from the best fit model for each individual turbot revealed no significant effect of PbTx, whether single or multiple exposures (p > 0.05, Fig. 3). For Mn, T_{b1/2l} average values ranged from 23 to 73 d, suggesting long-term retention of this metal in juvenile turbot. In all experimental conditions, the long-term depuration rate constant (k_{el}) of Zn was not significantly different from 0 (p > 0.05; Table 3), resulting in T_{b1/2l} values tending toward +∞.

4. Discussion

The most common routes of PbTx exposure in aquatic species are by absorption of the toxin from lysed cells across gill epithelium or by direct ingestion of *K. brevis* cells and subsequent toxin absorption across the gastrointestinal epithelia (Kimm-Brinson and Ramsdell, 2001). Although blooms of *K. brevis* are often associated with massive fish mortalities (Landsberg, 2002), PbTx accumulation and toxicological effects in this taxon have not been well characterized. PbTxs bind and activate voltage gated sodium channels (Na_v) that are present in most excitable cells, causing neurotoxic shellfish poisoning (NSP) in mammals exposed through the diet (Flewelling et al 2005; Landsburg et al. 2009). Binding of PbTxs induces cell Na influx, altering cell regulation through activation of ion pumps and/or channels (Baden, 1983). Changes to the state of ion pumps and/or channels with PbTx binding, in turn, may affect the gastrointestinal transport of metals characterized by two ways (1) the active pathway with the occurrence of dedicated transporters and (2) the diffusive pathway (i.e. via channels) (Bury et al., 2003). Although the transport of dietary Mn in fish is not fully elucidated yet, previous works suggested that Mn enters primarily via diffusive routes involved in the uptake of major ions such as Ca channels (Fukuda and Kawa 1977; Anderson 1979; Fasolato et al. 1993). Conversely, Zn absorption seems to be an active process involving specific transporters (Bury et al. 2003) that take the metal into cells through specific channels (ZIP family; Bury et al. 2003; Hogstrand 2011). Since PbTx exposure affects membrane polarization and thus affects other ion transport pathways, the hypothesis of the present study was that this toxin occurring in the food of fish could alter the processes of important essential elements such as reduce the dietary incorporation of Mn and Zn. The comparison of Mn and Zn AEs done in this study allows to characterize potential effects of brevetoxins both on active and passive (i.e. diffusion) mechanisms of metal transportation.

Contrary to expectations, we found that the assimilation of metals in fish from food was not affected by consumption of PbTx. In all treatments, the observed assimilation efficiencies (AEs) of Mn and Zn were in accordance with values previously reported in the literature on turbot (Pouil et al. 2017, 2016, Mathews et al. 2008) and another flatfish species, the European plaice *Pleuronectes platessa* (Pentreath, 1976), with no change exerted by either single or multiple dietary exposures to PbTx while toxin analysis data confirmed the presence of PbTx in the mussel (food), the fish (consumer). Rapid PbTx turnover may explain the absence of differences in the AEs of Mn and Zn in turbot between the experimental treatments. Indeed, we found a relatively limited trophic transfer of PbTx in juvenile turbot that consumed mussels contaminated with PbTx (188 and or 525 ng g⁻¹), where PbTx in fish 2 hours after a single-feeding corresponded to 37% of the given dose (Table 2; percentage estimated based on ratio between radioactivity in food and in whole-body fish after hours). This low transfer could be related to a loss during the feeding process (small particles released in the water were observed during the feeding) for example a portion of the gel food was not ingested). It does nevertheless not affect the difference of ingestion of toxin between the short pre-exposure (single-feeding; RLT) and the pre-exposure (multiple-feeding; NRHT+RNT) experiment.

Despite different toxin concentrations and frequencies of exposure, PbTx concentrations in fish tissues were not significantly different between the single exposed and the 3-weeks exposed fish. These results suggest: (1) Ingested PbTx is rapidly turned over, where the toxin is absorbed in the digestive tract and then quickly eliminated by the hepatobiliary system. This pathway has been recognized as the key route for the metabolization and excretion of brevetoxin in fish (Landsberg, 2002). (2) PbTx are not re-distributed to organs that are not involved in digestion and excretion processes, such as muscle tissue. Although these pathways remain to be confirmed experimentally, Naar et al. (2007) found a similar pattern in field measurements on 42 species of fish exposed to *K. brevis* blooms in the field. PbTx concentrations in these fish

were generally low or below the LOQ in the muscle (11-414 ng g⁻¹), but concentrations reached µg g⁻¹ levels in the liver and digestive tract (levels 10 and 30 times higher than in muscle tissue, respectively).

The current study presents the first findings regarding to the effects of the ingestion of biotoxins on the trophic transfer of selected essential elements in fish. Care needs to be taken in how to interpret and expand on these results. For example, bivalves, which are not natural prey of turbot (Florin and Lavados, 2010; Sparrevohn and Støttrup, 2008), were used for the preparation of the gel food. Therefore, the AEs observed in this study might not reflect actual assimilation of these elements in natural conditions. Furthermore, although in the present study the PbTx concentrations used are relatively low (i.e. < 600 ng g⁻¹), higher concentrations of PbTx can be found in bivalves during *K. brevis* blooms in the natural environment (Landsberg et al., 2009), which could lead to greater physiological effects on their consumers such as fish.

5. Conclusion

This study revealed no statistically significant differences in the metal assimilation efficiencies of juvenile turbot after a single or multiple feedings of gel food containing PbTx in comparison to control fish. Similarities observed indicate that the occurrence of PbTx in the food do not appear to affect the trophic transfer of Mn and Zn essential elements in juvenile fish (integrative process). Nevertheless, since harmful algae can produce a variety of toxins with diverse mode of actions, further investigations are needed to study the influence of (1) higher dietary doses of PbTx, and (2) other biotoxins on metal assimilation in fish.

Acknowledgments

The IAEA is grateful for the support provided to its Environment Laboratories by the Government of the Principality of Monaco. MM is Nippon foundation Nereus program Alumni. The IUF (Institut Universitaire de France) is acknowledged for its support to PB.

References

- Anderson, M., 1979. Mn^{2+} ions pass through Ca^{2+} channels in myoepithelial cells. *J. Exp. Biol.* 82, 227–238.
- Anderson, D.M., 2009. Approaches to monitoring, control and management of harmful algal blooms (HABs). *Ocean Coast. Manag.* 52, 342–347.
- Baden, D.G., 1983. Marine food-borne dinoflagellate toxins. *Int. Rev. Cytol.* 82, 99–150.
- Bœuf, G., Payan, P., 2001. How should salinity influence fish growth? *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* 130, 411–423.
- Bruland, K.W., 1983. Trace elements in seawater, in: Riley, J.P., Chester, R. (Eds), *Chemical oceanography*. Academic Press, New York, USA, pp. 157–220.
- Bury, N.R., Walker, P.A., Glover, C.N., 2003. Nutritive metal uptake in teleost fish. *J. Exp. Biol.* 206, 11–23.
- Cresswell, T., Metian, M., Golding, L.A., Wood, M.D., 2017. Aquatic live animal radiotracing studies for ecotoxicological applications: Addressing fundamental methodological deficiencies. *J. Environ. Radioact.* 178-179, 453-460.
- Dechraoui Bottein, M.-Y.; Clausing, R. J., 2017. Receptor binding assay for the analysis of marine toxins: detection and mode of action, in: Diogène, J., Campàs, M. (Eds), *CAC: Recent Advances in the Analysis of Marine Toxins*, Elsevier, New York, USA.
- Dickey, R., Jester, E., Granade, R., Mowdy, D., Moncreiff, C., Rebarchik, D., Robl, M.,

- Musser, S., Poli, M., 1999. Monitoring brevetoxins during a *Gymnodinium breve* red tide: comparison of sodium channel specific cytotoxicity assay and mouse bioassay for determination of neurotoxic shellfish toxins in shellfish extracts. *Nat. Toxins* 7, 157–165.
- Edwards, D.J., 1971. Effect of temperature on rate of passage of food through the alimentary canal of the plaice *Pleuronectes platessa* L. *J. Fish Biol.* 3, 433–439.
- Fasolato, C., Hoth, M., Matthews, G., Penner, R., 1993. Ca^{2+} and Mn^{2+} influx through receptor-mediated activation of nonspecific cation channels in mast cells. *Proc. Natl. Acad. Sci.* 90, 3068–3072.
- Flewelling, L.J., Naar, J.P., Abbott, J.P., Baden, D.G., Barros, N.B., Bossart, G.D., Bottein, M.-Y.D., Hammond, D.G., Haubold, E.M., Heil, C.A., Henry, M.S., Jacocks, H.M., Leighfield, T.A., Pierce, R.H., Pitchford, T.D., Rommel, S.A., Scott, P.S., Steidinger, K.A., Truby, E.W., Dolah, F.M.V., Landsberg, J.H., 2005. Brevetoxicosis: Red tides and marine mammal mortalities. *Nature* 435, 755–756.
- 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.
- Förstner, U., Wittmann, G.T., 1981. *Metal Pollution in the Aquatic Environment*. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo.
- Fowler, S.W., Guary, J.-C., 1977. High absorption efficiency for ingested plutonium in crabs. *Nature* 266, 827-828.
- Fukuda, J., Kawa, K., 1977. Permeation of manganese, cadmium, zinc, and beryllium through calcium channels of an insect muscle membrane. *Science* 196, 309–311.
- Gannon, D.P., McCabe, E.J.B., Camilleri, S.A., Gannon, J.G., Brueggen, M.K., Barleycorn, A.A., Palubok, V.I., Kirkpatrick, G.J., Wells, R.S., 2009. Effects of *Karenia brevis* harmful algal blooms on nearshore fish communities in southwest Florida. *Mar. Ecol.*

- Prog. Ser. 378, 171–186.
- Gong, Y., Song, L., Wu, X., Xiao, B., Fang, T., Liu, J., 2009. Effects of arsenate on microcystin content and leakage of *Microcystis* strain PCC7806 under various phosphate regimes. *Environ. Toxicol.* 24, 87–94.
- Hallegraeff, G.M., 2010. Ocean climate change, phytoplankton community responses, and harmful algal blooms: A formidable predictive challenge. *J. Phycol.* 46, 220–235.
- Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia* 32, 79–99.
- Hogstrand, C., 2011. 3 - Zinc, in: Chris M. Wood, A.P.F. and C.J.B. (Ed.), *Fish Physiology, Homeostasis and Toxicology of Essential Metals*. Academic Press, New-York, USA, pp. 135–200.
- Holmes, M.J., Lewis, R.J., Poli, M.A., Gillespie, N.C., 1991. Strain dependent production of ciguatoxin precursors (gambiertoxins) by *Gambierdiscus toxicus* (Dinophyceae) in culture. *Toxicon Off. J. Int. Soc. Toxinology* 29, 761–775.
- Kenefick, S.L., Hrudey, S.E., Peterson, H.G., Prepas, E.E., 1993. Toxin release from *Microcystis aeruginosa* after chemical treatment. *Water Sci. Technol.* 27, 433–440.
- Kimm-Brinson, K.L., Ramsdell, J.S., 2001. The red tide toxin, brevetoxin, induces embryo toxicity and developmental abnormalities. *Environ. Health Perspect.* 109, 377–381.
- Landsberg, J.H., 2002. The effects of harmful algal blooms on aquatic organisms. *Rev. Fish. Sci.* 10, 113–390.
- Landsberg, J.H., Flewelling, L.J., Naar, J., 2009. *Karenia brevis* red tides, brevetoxins in the food web, and impacts on natural resources: Decadal advancements. harmful algae, understanding the causes and impacts of the Florida red tide and improving management and response 8, 598–607.
- Lepage, O., Overli, O., Petersson, E., Järvi, T., Winberg, S., 2000. Differential stress coping in

- wild and domesticated sea trout. *Brain. Behav. Evol.* 56, 259–268.
- Li, H., Murphy, T., Guo, J., Parr, T., Nalewajko, C., 2009. Iron-stimulated growth and microcystin production of *Microcystis novacekii* UAM 250. *Limnol. Ecol. Manag. Inland Waters* 39, 255–259.
- Lukac, M., Aegerter, R., 1993. Influence of trace metals on growth and toxin production of *Microcystis aeruginosa*. *Toxicon* 31, 293–305.
- Mathews, T., Fisher, N.S., 2009. Dominance of dietary intake of metals in marine elasmobranch and teleost fish. *Sci. Total Environ.* 407, 5156–5161.
- Mathews, T., Fisher, N.S., Jeffree, R.A., Teysié, J.-L., 2008. Assimilation and retention of metals in teleost and elasmobranch fishes following dietary exposure. *Mar. Ecol. Prog. Ser.* 360, 1–12.
- Metian, M., Warnau, M., Hédouin, L., Bustamante, P., 2009. Bioaccumulation of essential metals (Co, Mn and Zn) in the king scallop *Pecten maximus*: Seawater, food and sediment exposures. *Mar. Biol.* 156, 2063–2075.
- Miegel, R.P., Pain, S.J., van Wettere, W.H.E.J., Howarth, G.S., Stone, D.A.J., 2010. Effect of water temperature on gut transit time, digestive enzyme activity and nutrient digestibility in yellowtail kingfish (*Seriola lalandi*). *Aquaculture* 308, 145–151.
- Miramand, P., Fowler, S.W., Guary, J.C., 1982. Comparative study of vanadium biokinetics in three species of echinoderms. *Mar. Biol.* 67, 127–134.
- Moore, S.K., Trainer, V.L., Mantua, N.J., Parker, M.S., Laws, E.A., Backer, L.C., Fleming, L.E., 2008. Impacts of climate variability and future climate change on harmful algal blooms and human health. *Environ. Health* 7, S4.
- Naar, J.P., Flewelling, L.J., Lenzi, A., Abbott, J.P., Granholm, A., Jacocks, H.M., Gannon, D., Henry, M., Pierce, R., Baden, D.G., Wolny, J., Landsberg, J.H., 2007. Brevetoxins, like ciguatoxins, are potent ichthyotoxic neurotoxins that accumulate in fish. *Toxicon* 50,

707–723.

- 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.
- Pimentel, M.S., Faleiro, F., Diniz, M., Machado, J., Pousão-Ferreira, P., Peck, M.A., Pörtner, H.O., Rosa, R., 2015. Oxidative stress and digestive enzyme activity of flatfish larvae in a changing ocean. PLOS ONE 10, e0134082.
- Poli, M.A., Mende, T.J., Baden, D.G., 1986. Brevetoxins, unique activators of voltage-sensitive sodium channels, bind to specific sites in rat brain synaptosomes. Mol. Pharmacol. 30, 129–135.
- Poli, M.A., Musser, S.M., Dickey, R.W., Eilers, P.P., Hall, S., 2000. Neurotoxic shellfish poisoning and brevetoxin metabolites: A case study from Florida. Toxicol. 38, 981–993.
- Pouil, S., Oberhänsli, F., Bustamante, P., Metian, M., 2017. Investigations of temperature and pH variations on metal trophic transfer in turbot (*Scophthalmus maximus*) fish. Environ. Sci. Pollut. Res. doi: 10.1007/s11356-017-8691-4.
- 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.
- R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rodriguez y Baena, A.M., Metian, M., Teyssié, J.-L., De Broyer, C., Warnau, M., 2006. Experimental evidence for ^{234}Th bioaccumulation in three Antarctic crustaceans: Potential implications for particle flux studies. Mar. Chem. 100, 354–365.
- Rosa, R., Pimentel, M., Galan, J.G., Baptista, M., Lopes, V.M., Couto, A., Guerreiro, M., Sampaio, E., Castro, J., Santos, C., Calado, R., Repolho, T., 2016. Deficit in digestive

- capabilities of bamboo shark early stages under climate change. *Mar. Biol.* 163, 60.
- Simkiss, K., 1979. Metal ions in cells. *Endeavour* 3, 2–6.
- Smayda, T.J., 1990. Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic, in: Graneli, E., Sundström, B., Edler, L., Anderson, D. M. (Eds.), *Toxic Marine Phytoplankton*, Elsevier, New York, USA, pp. 29–40.
- Smith, V.H., Schindler, D.W., 2009. Eutrophication science: Where do we go from here? *Trends Ecol. Evol.* 24, 201–207.
- Sournia, A., 1995. Red tide and toxic marine phytoplankton of the world ocean: an inquiry into biodiversity, in: Lassus, P., Arzul, G., Erard-Le Denn, E., Gentien, P., Marcaillou-Le Baut, C. (Eds.), *Harmful Marine Algal Blooms, Proceedings of the sixth international conference on toxic marine phytoplankton*, Nantes, France, pp. 103–112.
- 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.
- Stumpf, R.P., Culver, M.E., Tester, P.A., Tomlinson, M., Kirkpatrick, G.J., Pederson, B.A., Truby, E., Ransibrahmanakul, V., Soracco, M., 2003. Monitoring *Karenia brevis* blooms in the Gulf of Mexico using satellite ocean color imagery and other data. *Harmful Algae* 2, 147–160.
- Sunda, W.G., 2012. Feedback interactions between trace metal nutrients and phytoplankton in the ocean. *Front. Microbiol.* 3, 204.
- Tian, L., Wang, M., Li, X., Lam, P.K.S., Wang, M., Wang, D., Chou, H.N., Li, Y., Chan, L.L., 2011. Proteomic modification in gills and brains of medaka fish (*Oryzias melastigma*) after exposure to a sodium channel activator neurotoxin, brevetoxin-1. *Aquat. Toxicol.* 104, 211–217.
- Utkilen, H., Gjørlme, N., 1995. Iron-stimulated toxin production in *Microcystis aeruginosa*. *Appl. Environ. Microbiol.* 61, 797–800.

- 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.
- Van Den Bergh, J.C.J.M., Nunes, P.A.L.D., Dotinga, H.M., Kooistra, W.H.C.F., Vrieling, E.G., Peperzak, L., 2002. Exotic harmful algae in marine ecosystems: An integrated biological–economic–legal analysis of impacts and policies. *Mar. Policy* 26, 59–74.
- Van Dolah, F.M., 2000. Marine algal toxins: origins, health effects, and their increased occurrence. *Environ. Health Perspect.* 108, 133.
- Wang, W.-X., Fisher, N.S., 1999. Assimilation efficiencies of chemical contaminants in aquatic invertebrates: A synthesis. *Environ. Toxicol. Chem.* 18, 2034–2045.
- Warnau, M., Teyssié, J.-L., Fowler, S.W., 1996. Biokinetics of selected heavy metals and radionuclides in the common Mediterranean echinoid *Paracentrotus lividus*: Sea water and food exposures. *Mar. Ecol. Prog. Ser.* 141, 83–94.
- Watanabe, T., Kiron, V., Satoh, S., 1997. Trace minerals in fish nutrition. *Aquaculture* 151, 185–207.
- Wells, M.L., Trainer, V.L., Smayda, T.J., Karlson, B.S.O., Trick, C.G., Kudela, R.M., Ishikawa, A., Bernard, S., Wulff, A., Anderson, D.M., Cochlan, W.P., 2015. Harmful algal blooms and climate change: Learning from the past and present to forecast the future. *Harmful Algae* 49, 68–93.
- Whicker, F.W., Schultz, V., 1982. *Radioecology: Nuclear energy and the environment*. CRC press Boca Raton, FL.
- Williams, R.J.P., 1981. The Bakerian Lecture, 1981: Natural selection of the chemical elements. *Proc. R. Soc. Lond. B Biol. Sci.* 213, 361–397.
- 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, 3rd ed. ed. Prentice-Hall, Upper Saddle River, NJ.

Zeng, J., Yang, L., Wang, W.-X., 2009. Cadmium and zinc uptake and toxicity in two strains of *Microcystis aeruginosa* predicted by metal free ion activity and intracellular concentration. *Aquat. Toxicol. Amst. Neth.* 91, 212–220.

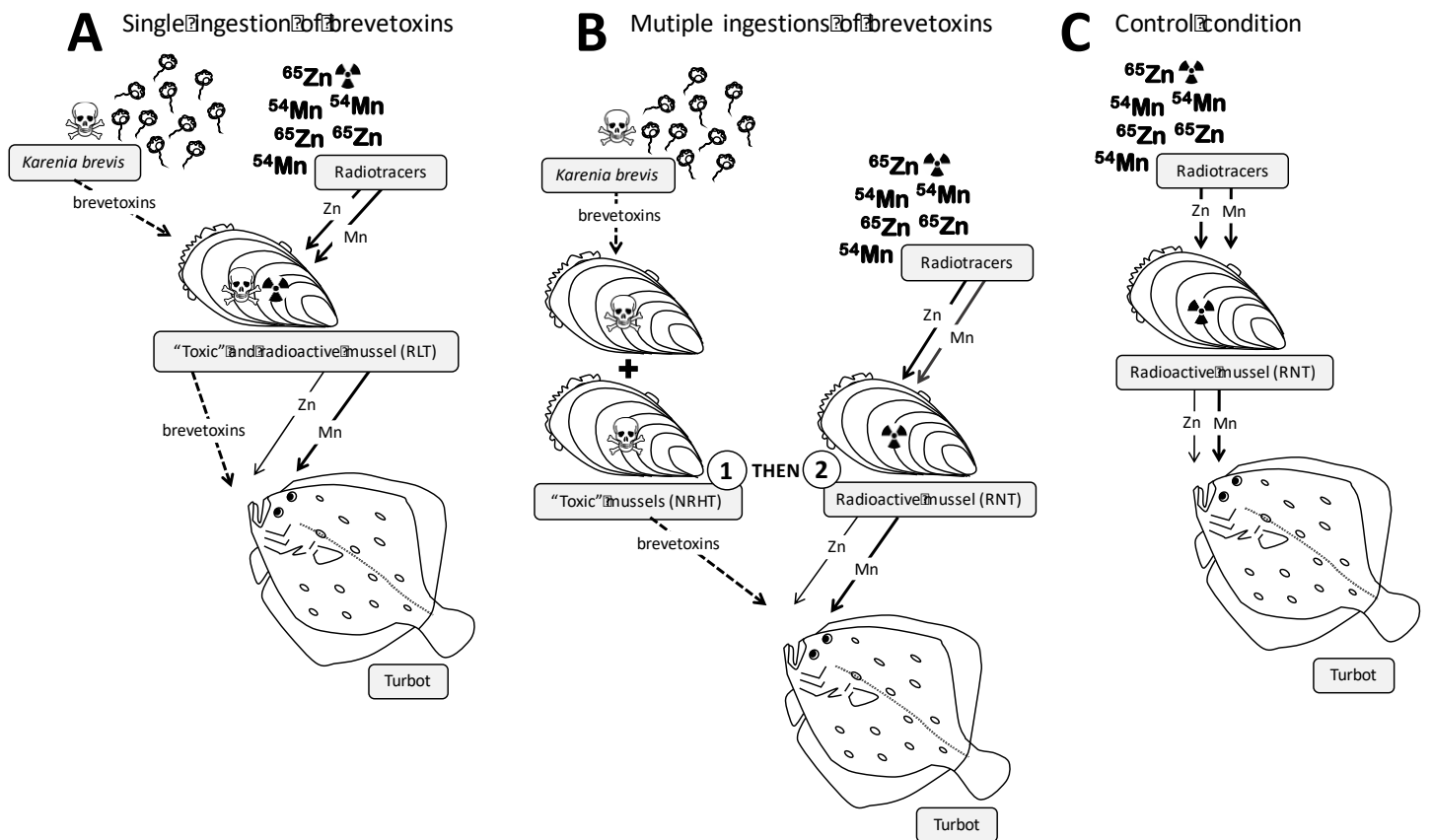


Figure 1. Protocols where (A) turbot were fed a gel food prepared from radiolabelled and low PbTx₃-containing (RLT) a single time (Treatment 1), (B) turbot were given a gel food prepared from high PbTx₃-containing mussels (NRHT) five days per week for three weeks and then single-fed with radiolabelled gel food (RNT; Treatment 2) and (C) turbot were only single-fed a radiolabelled gel food (RNT) and served as the experimental control (Treatment 3).

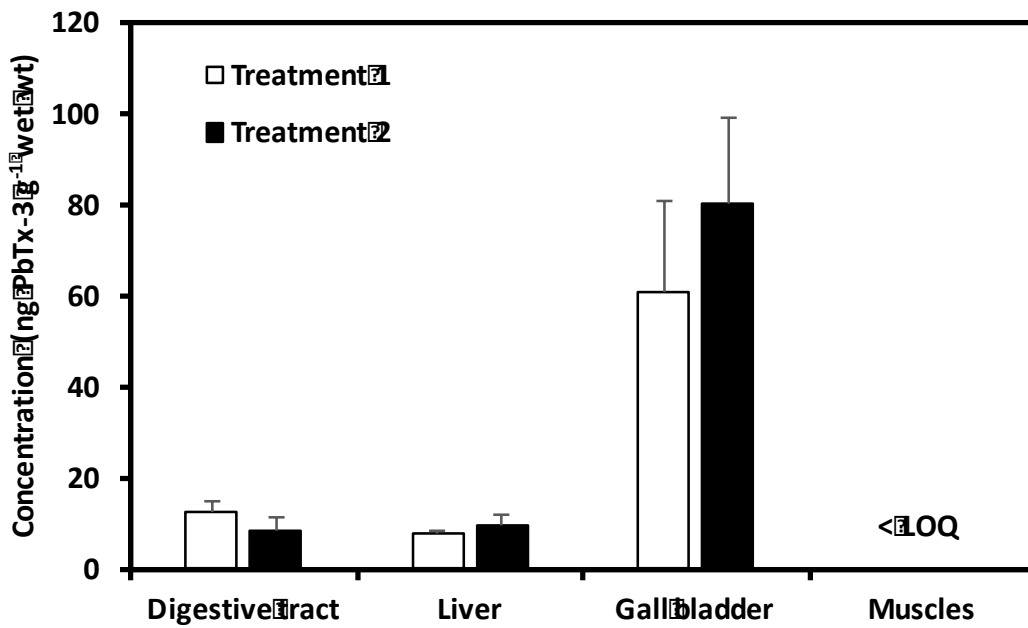


Figure 2. Concentration of PbTx (expressed as PbTx-3 equiv.) in four body compartments (digestive tract, liver, gall bladder and muscles) after single (Treatment 1) or repeated feedings (Treatment 2) of brevetoxic mussel homogenate. Samples were pooled from replicate turbot within each treatment (n = 8) before extraction and analysis as explained in section 2.3.1., Values are Means \pm SD calculated from replicate assays (n=3).

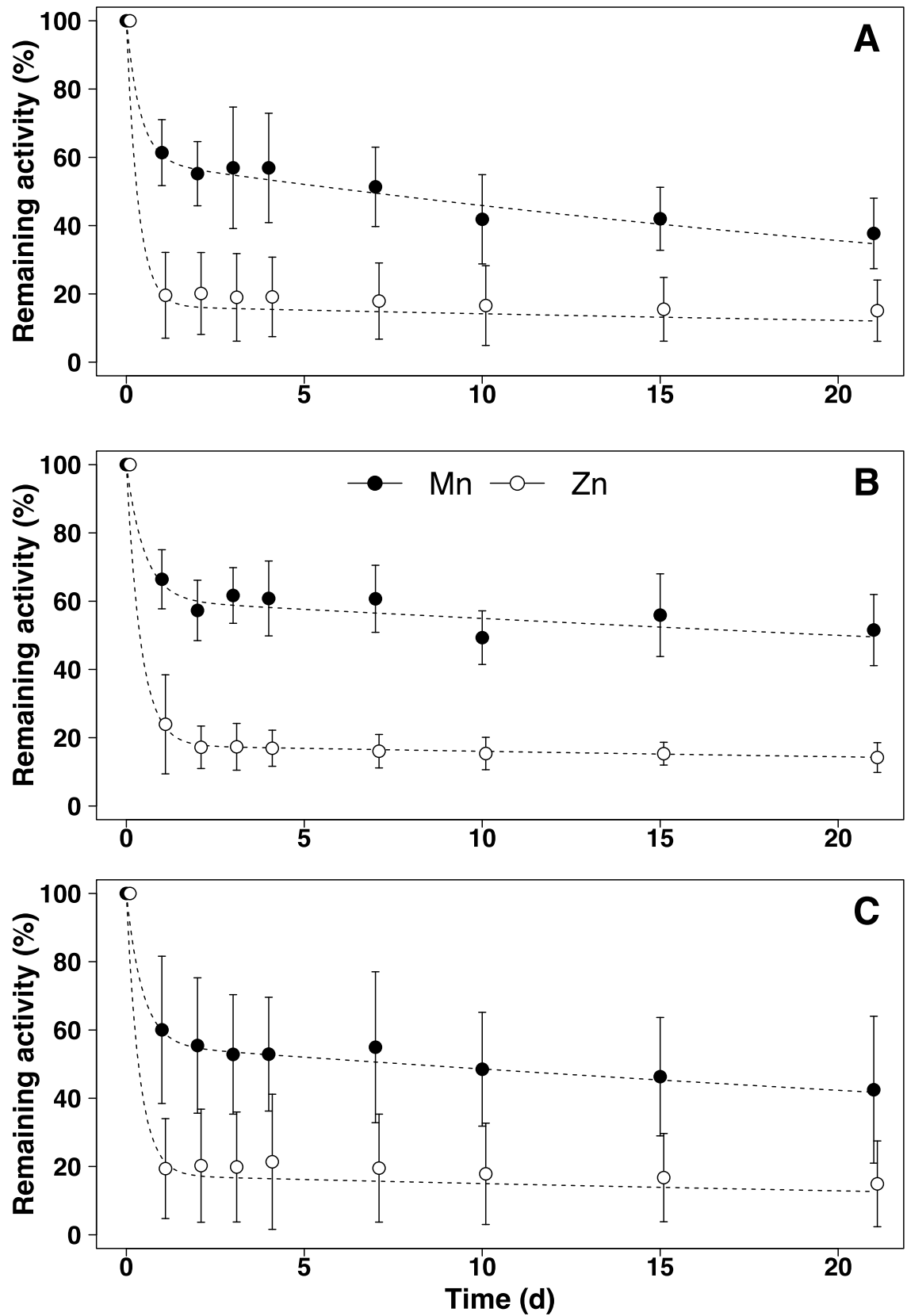


Figure 3. Whole-body depuration, ^{54}Mn and ^{65}Zn in juvenile turbot (A) single-fed with algal toxins, (B) multi-fed with toxins and (C) non-exposed to toxins (n = 5-7). Parameters and statistics of depuration kinetics are given in Table 3.

Table 1. PbTx and radiotracers contents (all values are given relatively to the fresh weight) in the different gel foods prepared from homogenized mussels' soft tissues mixed with gelatin (see details in section 2.2.1). Data are Means \pm SD. For PbTx concentrations, means represent measurements on one homogenized extract over replicate assays (n=3).

Gels	Characteristics	Brevetoxin concentration (ng g ⁻¹)	⁵⁴ Mn concentration (Bq g ⁻¹)	⁶⁵ Zn concentration (Bq g ⁻¹)	Uses
NRNT	Non-radioactive No toxin content	-	-	-	To acclimate all the turbot used in experiment to gel food
RNT	Radioactive No toxin content	-	48 \pm 1	1059 \pm 30	For the single-feeding of turbot from Treatments 2 & 3
NRHT	Non-radioactive High toxin content	525 \pm 88	-	-	For multiple brevetoxin ingestions by turbot (Treatment 2)
RLT	Radioactive Low toxin content	188 \pm 38	37 \pm 2	1012 \pm 6	For single ingestion by turbot (Treatment 1)
NRLT	Non-radioactive Low toxin content	188 \pm 38	-	-	As proxy to estimate the brevetoxin content in the gel RLT

Table 2. Quantities of ingested PbTx for the single (Treatment 1) and multiple (Treatment 2) feeding treatments and the corresponding concentrations subsequently measured in selected body compartments. Data are expressed as averaged concentrations in ng g⁻¹ wet weight (enclosed in square brackets) and total quantities in ng. Body compartment means represent replicate analyses on one pooled extract comprised of 8 replicate samples.

Experimental conditions	PbTx given per feeding and per turbot	Total PbTX given per turbot	Brevetoxin measured in the selected body compartments						
			Digestive tract		Gall bladder		Liver		Muscles
	[ng g ⁻¹] and ng	[ng g ⁻¹] and ng	[ng g ⁻¹] and ng	% of given	[ng g ⁻¹] and ng	% of given	[ng g ⁻¹] and ng	% of given	[ng g ⁻¹] and ng
Single ingestion (Treatment 1)	[4.3] 94.0	[4.3] 94.0	[12.5] 11.1	12	[60.7] 17.6	19	[7.6] 5.6	6	< LOQ
Multiple ingestions (Treatment 2)	[14.8] 262.5	[222] 3937.5	[8.4] 4.9	0.1	[80.1] 20.1	0.5	[9.6] 3.6	<0.1	< LOQ

Table 3. Depuration kinetic parameters of ^{54}Mn and ^{65}Zn in juvenile turbot (n=5-7) (A) single-fed with algal toxins, (B) multi-fed with toxins and (C) non-exposed to toxins (n = 5-7 per treatment) and then maintained for 21d in normal seawater. k_e : depuration rate constant (d^{-1}); $T_{b1/2}$: biological half-life (d), A_0 : remaining activities (%); ASE: asymptotic standard error; R^2 : determination coefficient. Probability of the model adjustment: ^{NS} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Tracer	Experimental conditions	Short-lived			Long-lived			R^2
		$A_{0s} \pm \text{ASE} (\%)$	$k_{es} \pm \text{ASE} (\text{d}^{-1})$	$T_{b1/2s} \pm \text{ASE} (\text{d})$	$A_{0l} (=AE) \pm \text{ASE} (\%)$	$k_{el} \pm \text{ASE} (\text{d}^{-1})$	$T_{b1/2l} \pm \text{ASE} (\text{d})$	
^{54}Mn	A	$40.89 \pm 5.88^{***}$	$2.61 \pm 1.81^*$	0.27 ± 0.19	$59.12 \pm 3.63^{***}$	$0.025 \pm 0.007^{***}$	27 ± 7	0.72
	B	$39.68 \pm 4.80^{***}$	$1.98 \pm 0.81^*$	0.35 ± 0.14	$60.38 \pm 2.87^{***}$	$0.010 \pm 0.004^*$	73 ± 35	0.75
	C	$44.27 \pm 9.36^{***}$	$2.21 \pm 1.18^*$	0.31 ± 0.17	$55.73 \pm 5.66^{***}$	$0.014 \pm 0.010^{\text{NS}}$	50 ± 36	0.51
^{65}Zn	A	$83.60 \pm 3.63^{***}$	$3.06 \pm 0.92^{**}$	0.23 ± 0.07	$16.40 \pm 2.26^{***}$	$0.015 \pm 0.014^{\text{NS}}$	$+\infty$	0.94
	B	$82.22 \pm 3.31^{***}$	$2.48 \pm 0.47^{***}$	0.28 ± 0.05	$17.79 \pm 1.95^{***}$	$0.011 \pm 0.011^{\text{NS}}$	$+\infty$	0.94
	C	$82.52 \pm 3.27^{***}$	$2.74 \pm 0.60^{***}$	0.25 ± 0.06	$17.48 \pm 2.05^{***}$	$0.015 \pm 0.012^{\text{NS}}$	$+\infty$	0.96