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Influence of food (ciliate and phytoplankton) on the trophic transfer of inorganic and methyl-mercury in the Pacific cupped oyster *Crassostrea gigas*

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Abstract

Diet is an important route of mercury (Hg) uptake in marine organisms. Trophic transfer of Hg throughout the food webs may be influenced by various factors, including diet and Hg speciation. Bivalves such as oysters are widely used as bioindicators of trace element pollution such as Hg. Nevertheless, our current knowledge regarding their ability to accumulate Hg from their diet is mainly based on experiments performed using phytoplankton. In their natural environment, oysters feed on a variety of feeds including ciliates, detritus, in addition to phytoplankton. The present study aimed at examining the influence of diet composition on the trophic transfer of inorganic Hg (iHg) and methylmercury (MeHg) in the Pacific cupped oyster *Crassostrea gigas*. The pulse-chase feeding method was used with two radiolabeled feed items: a heterotrophic protist (*Uronema marina*) and phytoplankton with a diatom model, *Thalassiosira pseudonana*. Depuration of dietary Hg in the oysters was followed for 50 d. Kinetic parameters including Assimilation Efficiency (AE) and efflux rate constant (k_e) were calculated. Our results shown that oysters fed on ciliates assimilated $96 \pm 1\%$ and $31 \pm 2\%$ of the ingested MeHg and iHg respectively while these elements were similarly assimilated in the oysters fed on phytoplankton ($78 \pm 3\%$ and $86 \pm 4\%$ for MeHg and iHg, respectively). Mercury assimilation in oyster is thus diet dependent (significant differences in AE, $p < 0.05$), metal species-dependent and likely resulting from variations in Hg bioavailability in the two feed items tested and a gut passage time-dependent of the ingested matrix.

Keywords: Bivalve, Bioaccumulation, Food pathway, Mercury, Radiotracers

1. Introduction

Mercury (Hg) is a persistent global pollutant released in the coastal environment, mainly released by anthropogenic activities, including combustion of fossil fuels and gold mining (UNEP, 2013; Sundseth et al., 2017). Estimated annual anthropogenic Hg emissions were about 2390 tonnes in 2015 (Streets et al., 2019)). Mercury is a toxic, non-essential element that can be highly bioaccumulated by marine biota and biomagnified along the aquatic food webs, especially in its methylmercury form (MeHg; see e.g., Riisgård and Hansen, 1990; Dijkstra et al., 2013; Eagles-Smith et al., 2018; Harding et al., 2018).

Mercury bioaccumulation processes have been well studied in laboratory conditions in low trophic level aquatic organisms, especially in bivalves, such as Mytilidae (Pan and Wang, 2011; Amachree et al., 2014; Raftopoulou and Dimitriadis, 2011), Ostreidae (Cunningham and Tripp, 1973; Denton and Burdon-Jones, 1981; Pan and Wang, 2011), and Pectinidae (Metian et al., 2008; Pan and Wang, 2011), acting as important vectors for “pumping” Hg to higher trophic levels (Pan and Wang, 2011).

Although bivalves are also exposed to Hg from the water and the sediment (Gagnon and Fisher, 1997; Kidd et al., 2011), previous laboratory studies carried out in the 1970s highlighted that bivalves have substantial capacity to accumulate Hg from their diet especially in MeHg form (e.g., Cunningham and Tripp, 1975; Blackmore and Wang, 2004; Pan and Wang, 2011). Assimilation Efficiency (AE) of inorganic Hg (iHg) in bivalves is variable and ranges from 5 to 60% (Fowler et al., 1978; Riisgård and Hansen, 1990; Blackmore and Wang, 2004; Metian et al., 2008b; Pan and Wang, 2011). Some studies have shown in different bivalves that MeHg is more readily bioavailable than iHg: AE of MeHg is higher than that of iHg, by a factor 1.5 to 4.0 (MeHg AE = 50-95%; e.g., Fowler et al., 1978; Blackmore and Wang, 2004; Pan and Wang, 2011). These observations are valuable for interpreting the trophic transfer of iHg and MeHg from the base of food webs. Nevertheless,

interpreting field data based on laboratory findings has some limitations. Indeed, the experimental characterization of the trophic transfer of Hg in bivalves has mainly been studied using phytoplankton species as diet, which does not fully reflect the diversity of the natural diet of bivalves in the wild.

In their natural environment, bivalves are generally opportunistic, omnivore, filter-feeders feeding on different prey: phytoplankton, resuspended benthic microalgae, detritus or other feed items available in their habitats. Thus, experimental studies highlighted the ability of mussels (*Mytilus edulis* and *Perna viridis*) to assimilate zooplanktonic rotifers in addition to phytoplankton and found that this food source can be an important part of the mussel's energy budget (Wong et al., 2003a, 2003b). Diet of oysters (*Ostrea edulis* and *Crassostrea gigas*) includes phytoplankton, heterotrophic protists, large bacteria, fungi and detritus (Heral, 1990; Dupuy et al., 1999). Oysters retained efficiently particles $> 4 \mu\text{m}$ (Barillé et al., 1993). Thereby, $> 4 \mu\text{m}$ autotrophic protists (primary producers) are directly retained by benthic bivalves. When primary producers are lower than $< 4 \mu\text{m}$ of size, heterotrophic protists, like ciliates, are a trophic link between these small primary producers ($< 4 \mu\text{m}$) and benthic bivalves (Dupuy et al., 1999). Thus, these authors experimentally demonstrated that 94% of ciliates and 86% of flagellates (size between 4 and 72 μm) were retained by the Pacific cupped oyster *Crassostrea gigas*.

Previous experimental works have shown the importance of diet in the trophic transfer of trace elements in bivalves (e.g., Wang and Fisher, 1996; Lee and Luoma, 1998; Metian et al., 2008a). Food quality was shown to have a major influence on trace element assimilation. For example, Wang and Fisher (1996) report that in mussels fed with seven different algal diets, the AEs of Ag varied by a factor 9 (3.8-33.8%) according to the diet. Similar findings were shown for Ag, ^{241}Am , Co, Cd, Cr, Mn, and Zn in several bivalve species including clams, mussels, oysters and scallops (e.g., Lee and Luoma, 1998; Chong and Wang, 2000; Metian et

al., 2008a; Hédouin et al., 2010). Although such effects have not yet been demonstrated for assimilation of Hg, Pan and Wang (2004) suggested that different feed sources might lead to different bioavailability of Hg for bivalves.

The main objective of the present study was to investigate the influence of the diet composition on the trophic transfer of inorganic Hg (iHg) and methyl Hg (MeHg) in the Pacific cupped oyster, *C. gigas*. The depuration kinetics of dietary Hg were determined experimentally in oysters using radiotracer techniques. Thus, AE and efflux rate constant (k_e) of iHg and MeHg were compared in oysters fed on an autotrophic protist model (diatom *Thalassiosira pseudonana*, diameter: 2.5-15 μm) or on a heterotrophic protist model (ciliate protozoan, *Uronema marina*, 20 μm of length). Both protists are components of the oyster natural diet (Le Gall et al., 1997; Dupuy et al., 1999).

2. Materials and Methods

2.1. Acclimation of oysters

Pacific cupped oysters *C. gigas* a cosmopolitan bioindicator of trace element contamination (Rainbow and Philips, 1993; Rainbow, 1995), were purchased from a shellfish farm in La Rochelle, France. Oysters were then transferred to the IAEA Environment Laboratories in the Principality of Monaco. Prior to the experimentation, specimens were acclimated to laboratory conditions (constantly aerated open-circuit aquarium; salinity: 36 ± 1 ; temperature: $19 \pm 1^\circ\text{C}$; pH: 8; light/dark cycle: 12h/12h). During acclimation, bivalves were fed the phytoplanktonic Prymnesiophyceae *Isochrysis galbana* (10^5 cells mL^{-1}). Recorded mortality was lower than 5% over the acclimation period (4 weeks).

2.2. Radiotracer and counting

Depuration kinetics of Hg in oysters were determined using high-specific activity inorganic ^{203}Hg purchased from Isotope Products Laboratories (^{203}Hg as HgCl_2 in 1M HCL, $T_{1/2} = 46.6$ days, specific activity: $4 \cdot 10^9 \text{ Bq g}^{-1}$ at the beginning of the experiment). In order to assess the trophic transfer of methylmercury, MeHg was synthesized from $^{203}\text{HgCl}_2$ according to the method described in Rouleau and Block (1997). Briefly, this method is based on the methylation of inorganic ^{203}Hg by methylcobalamin and isolation of resulting $\text{CH}_3^{203}\text{Hg}$ using a hexane/benzene extraction procedure.

Exposed oysters were counted using a high-resolution γ -spectrometer system composed of three Germanium (N- or P-type) detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner® 4). The radioactivity was determined by comparison with standards of known activity and of appropriate geometry (Cresswell et al., 2017), and corrected for counting efficiency and physical radioactive decay. The counting time was adjusted to obtain a propagated counting error less than 5% (Warnau et al., 1996, 1997).

2.3. Trophic transfer experiment

Trophic transfer of iHg and MeHg in oysters was studied using protozoan and phytoplankton as diets. Ciliate (*Uronema marina*) and diatom (*Thalassiosira pseudonana*, clone 3H) cells were maintained in FAG and F/2 (without EDTA) medium respectively. Cultures were handled aseptically throughout the experimentation and exposed to $1 \text{ kBq } ^{203}\text{HgCl}_2 \text{ L}^{-1}$ or $\text{CH}_3^{203}\text{Hg} \text{ L}^{-1}$ during their exponential growing phase (4-7 d). In terms of stable Hg concentration, this addition corresponded to 12.3 ng L^{-1} . After the radiolabeling period, the diatom culture was centrifuged (2500 g for 25 min). The protozoan culture was filtered (25- μm mesh size; Osmonic® filters) and the filtrate centrifuged (1000 g for 15 min).

Forty oysters (weight: 68.6 ± 3.4 g wet wt, shell length: 89 ± 9 mm) had been randomly placed into four 13-L aquaria 14 days before the start of feeding experiment. Oysters were fed with radiolabeled feeds by resuspension of the centrifuged pellets (same concentration of 10^5 cells mL^{-1} for the two feeds) for 2 h in closed circuit. After the feeding period, all oysters were γ -counted and flow restored in the aquarium. Depuration was followed during 50 d in the same condition as for acclimation (see section 2.1). During the 50-d depuration period, oysters were fed daily with *I. galbana* (10^4 cells mL^{-1}).

Ingestion of food was verified by the γ -counting of the organisms at the end of the 2-h period of feeding. Activity per cell was notably higher in protozoans than diatoms (mean activity of 1150 and 1200 $\mu\text{Bq } ^{203}\text{Hg cell}^{-1}$ for iHg and MeHg in ciliates, and 0.27 and 0.18 $\mu\text{Bq } ^{203}\text{Hg cell}^{-1}$ for iHg and MeHg in diatoms) whereas the quantity ingested was lower (mean quantity of $3.5 \cdot 10^5$ cells for the ciliate and $3.1 \cdot 10^8$ cells for the diatom, for both iHg and MeHg). Bioconcentration capacities of the prey are nevertheless not directly comparable.

2.5. Data analysis

Depuration of 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). The depuration kinetics of the radiotracers were best fitted using either a single-component (Eq. (1)) or a double-component (Eq. (2)) exponential model (decision based on F test and ANOVA tables for two fitted model objects):

$$A_t = A_{0l} e^{-k_{el}t} \quad (1)$$

$$A_t = A_{0s} e^{-k_{es}t} + A_{0l} e^{-k_{el}t} \quad (2)$$

where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively; k_e is the depuration rate constant (d^{-1}); 's' and 'l' are the subscripts for the 'short-lived' and 'long-lived' components, respectively. The short-lived component represents the loss kinetics of the

radiotracer fraction that is weakly associated to the organisms and rapidly eliminated, whereas the long-lived component describes the loss kinetics of the radiotracer fraction that is tightly bound to the organism (Warnau et al., 1996). The long-lived component allows estimating the assimilation efficiency (AE) of the radiotracer ingested with food ($AE = A_{0l}$). For each exponential component (s and l), a biological half-life can be calculated ($T_{b1/2s}$ and $T_{b1/2l}$) from the corresponding depuration rate constant (k_{es} and k_{el} , respectively) according to the relation $T_{b1/2} = \ln 2/k_e$. Kinetic parameters were determined using the R freeware 3.5.2 (R Development Core Team, 2018). They are considered significantly different when 95% their confidence intervals do not overlap (Payton et al., 2003).

3. Results

To evaluate the influence of diet on Hg assimilation in *C. gigas*, depuration kinetics of the iHg and MeHg were followed after a pulse-chase feeding, using radiolabelled food items (diatoms and ciliates). Whole-body depuration kinetics of MeHg in oyster fed with diatoms were described by a two-component exponential model (Fig. 1 and Table 1; R^2 : 0.56) while depuration kinetics from oysters fed with ciliates were described by a one-component exponential model (Fig. 1 and Table 1; R^2 : 0.20). Conversely, depuration kinetics of iHg from oyster fed with diatoms were described by a one-component exponential model (Fig. 1 and Table 1; R^2 : 0.71) while depuration kinetics from oysters fed with ciliates were described by a two-component exponential model (Fig. 1 and Table 1; R^2 : 0.92). A non-negligible fraction (30-96%) of the two radiotracers was strongly to very strongly retained in oysters (average $T_{b1/2l}$ ranging from 28 to 614 d, Table 1) regardless which prey had been ingested.

The assimilation efficiency (AE) of Hg depended both on the food and Hg speciation. Estimated ^{203}Hg AE varied significantly ($p < 0.05$, Fig. 2) according to the prey type. Indeed, AE of ^{203}Hg by oysters when fed with ciliates was $30.7 \pm 2.4\%$, whereas its assimilation was

very high when fed with diatoms ($AE = 85.7 \pm 4.0\%$). MeHg was always better assimilated than iHg ($AE > 60\%$). Its AE was significantly lower ($p < 0.05$, Fig. 2) when oysters were fed with diatoms ($60.7 \pm 4.1\%$) than when fed with ciliates ($96.2 \pm 1.4\%$ Fig. 2, Table 1).

Depuration rate constants (k_{el}) values obtained for MeHg when oysters were fed with diatoms ($k_{el} = 0.012 \pm 0.003 \text{ d}^{-1}$, Table 1) were significantly higher ($p < 0.05$, Fig. 2) than when fed with ciliates ($k_{el} = 0.001 \pm 0.001 \text{ d}^{-1}$, Table 1), indicating that their retention was shorter when fed with diatoms ($T_{b1/2} = 56 \pm 13 \text{ d}$) than when fed with ciliates ($614 \pm 302 \text{ d}$). For iHg, no significant difference between k_{el} was observed (average values ranging from 0.014 to 0.024 d^{-1} , i.e., $T_{b1/2}$ from 28 to 42 days, $p > 0.05$, Fig. 2, Table 1).

4. Discussion

Dietary pathway is now recognized as a major source of Hg bioaccumulation in marine invertebrates (e.g., Blackmore and Wang, 2004; Bustamante et al., 2006; Pan and Wang, 2011). The assimilation efficiency (AE) and efflux rate constant (k_e) or retention time ($T_{b1/2}$) are critical parameters in assessing the dietary uptake of trace elements (Reinfelder et al., 1997) and numerous studies have been dedicated to estimate these parameters in different marine organisms (e.g., Warnau et al., 1996; Wang and Wong, 2003a; Metian et al., 2008b; Lacoue-Labarthe et al., 2009). However, our understanding of the factors influencing the trophic transfer of Hg in bivalves is still limited.

The AE of Hg ingested with marine phytoplankton has been quantified in a few bivalve species, including clams (*Ruditapes philippinarum*), oysters (*Saccostrea cucullata*), mussels (*Mytilus galloprovincialis*, *Perna viridis*, *Septifer virgatus*) and scallops (*Chlamys nobilis*, *C. varia*, *Pecten maximus*) (Fowler et al., 1978; Blackmore and Wang, 2004; Metian et al., 2008b, 2009; Pan and Wang, 2011). In the present study, the AEs of MeHg and iHg in *C. gigas* (61-96% for MeHg and 31-86% for iHg) were quantified for two diets, the diatom

Thalassiosira pseudonana and the ciliate *Uronema* sp. The efficient assimilation of MeHg is presumably due to rapid desorption of the ingested MeHg within the digestive tract, the desorbed MeHg being then rapidly assimilated due to its high lipophilicity (Blackmore and Wang, 2004). The AEs observed in our study for MeHg are corroborating those observed in other bivalves (50-95%) fed with phytoplankton (Fowler et al., 1978; Blackmore and Wang, 2004, Pan and Wang, 2011). Regarding iHg, we found AE of $86 \pm 4.0\%$ in oysters fed with *T. pseudonana*, i.e. a value higher than the one found by Blackmore and Wang (2004) (AE: $31 \pm 2\%$) in the rock oyster *Saccostrea cucullata* fed on the same phytoplankton species. In contrast, the latter value is similar to the AE we observed when *C. gigas* was fed with the ciliate *Uronema* sp (AE: $31 \pm 2\%$). Such contrasting findings suggest that trophic transfer of iHg is both species- and feed-dependent.

Another mechanism partially accounting for difference in AEs between iHg and MeHg in oysters fed on diatoms is how Hg is stored in the phytoplankton cells as shown for other trace elements (e.g., Reinfelder and Fisher, 1991; Wang et al., 1996; Metian et al., 2008a). Indeed, trace elements in algal cytoplasm are assumed to be more bioavailable than the fraction associated with organelles (Wang and Fisher, 1996; Lee and Luoma, 1998). Using subcellular fractioning technique, previous studies have shown that ~40-60% of iHg was found in the bioavailable fraction in the diatoms *Thalassiosira* sp., whereas the proportion of MeHg in the bioavailable fraction was much higher (~70-95%; Wu and Wang, 2011, 2013). Nevertheless, Lee and Fisher (2016) also highlighted that the proportion of MeHg in the cytoplasm of algal cells was dependent on environmental (culture) conditions. Indeed, the percentage of total cellular MeHg in the cytoplasmic fraction varied by a factor of 1.2-2.4 across six phytoplankton species maintained at two different temperatures (4 and 18°C) in the dark or under a 14:10 light-dark cycle. To the best of our knowledge, the effects of culture conditions on the storage of trace elements have never been investigated in ciliates. Further

investigations are needed to assess how the subcellular partitioning (see Wallace and Luoma, 2003) of Hg in diatom and ciliate may affect the trophic transfer of iHg and MeHg in the oysters.

Our study demonstrated that there was a marked difference in the assimilation of Hg (MeHg and iHg) in the oyster *C. gigas* in response to the different diets tested. A number of studies have examined the assimilation of other trace elements from different feeds (i.e., phytoplankton species) in oysters (e.g., Ettajani et al., 2001; Ke and Wang, 2001; Hédouin et al., 2010; Pan and Wang, 2011) and highlighted considerable variability in trace element assimilation according to the diets. As an example, Hédouin et al. (2010) found that Co, Mn and Zn were better assimilated when tree oysters *Isognomon isognomon* was fed with *Isochrysis galbana* than when fed with *Emiliana huxleyi* or *Heterocapsa triquetra*. Similar findings were reported for the oyster *C. gigas* with significantly higher AE of Cd in oysters fed with prasinophyte *Tetraselmis suecica* (AE = 20%) than when fed with the diatom *Skeletonema costatum* (AE = 9%; Ettajani et al., 2001). Observed differences were mainly explained by the subcellular fractioning of the trace elements within the phytoplankton species.

To the best of our knowledge, ciliates were never considered as feed to assess the trophic transfer of trace elements in bivalves. Twining and Fisher (2004) compared the AE of iHg in 3 species of calanoid copepods fed with ciliate (*Uronema* sp.) and phytoplankton (dinoflagellate *Oxyrrhis marina*). They found higher iHg AE (44-53%) in copepods fed with ciliates than in those fed with dinoflagellates (13-14%). For other trace elements (Ag, Cd, Fe, and Zn), AEs in copepods fed with ciliates were higher than when fed with dinoflagellates or diatoms (Fisher et al., 1991; Mason et al., 1996), which was correlated to the higher percentage of trace elements within cytoplasm of the ciliates. In the present study, we found that MeHg was more assimilated in oysters fed with ciliates than when fed with diatoms

whereas the opposite trend was observed for iHg. In addition to interspecific differences in AE of an element from a same diet, the observed differences for the iHg assimilation in the oyster *C. gigas* and the copepods *Acartia tonsa*, *A. hudsonica* and *Temora longicornis*, may be related to the iHg exposure pathways of the ciliates and therefore to the form of storage of iHg. Indeed, Twining and Fisher (2004) exposed ciliates through their diet (i.e., radiolabeled bacteria) while in the present study, ciliates were directly exposed to Hg (iHg and MeHg) from the dissolved pathway. While bioaccumulation of waterborne trace elements is based on transport mechanisms across the cell membrane, ingested matrix enters within digestive vacuoles (where pH 3 was measured; Fok et al., 1982), concentrating the trace elements inside the ciliate cells (Twining and Fisher, 2004) and thus, potentially enhancing their bioavailability for consumers such as bivalves.

Although subcellular partitioning in prey is an important parameter influencing assimilation of trace elements, there is another important process to take into account: the rate at which a trace element passes through the digestive tract (Wang and Wong, 2003b). A long gut passage time (GPT) allows a more efficient assimilation by increasing the absorption time of the ingested trace elements through the intestinal epithelium. In earlier experimental studies, GPT has been shown to be dependent of the ingested diets. In the oyster *C. rivularis* fed with 4 different phytoplankton species and sediment Ke and Wang (2001) found that GPT of Cd, Se and Zn varied by a factor of 10 to 20 with the highest values obtained when oysters fed on *T. pseudonana*. In the present study, the differences in AE of Hg (iHg and MeHg) in the Pacific cupped oyster *C. gigas* are therefore likely to result from (1) a difference in Hg bioavailability in the ciliate (*Uronema* sp.) and the diatom (*T. pseudonana*) and (2) a GPT dependent of the diets. In this study, our results indicated that, for oysters, differences in the bioavailability of Hg or in GPT depending on diets is not the only explanation on the variability of Hg assimilation. Indeed, the comparison of efflux rate constants (k_{el}) reveals

that the retention of ingested Hg is also diet-dependent (especially for MeHg) indicating that excretion processes are also affected by the diet. Overall, the lower k_{el} observed for MeHg indicates a strong retention of this compound in oysters ($T_{b1/2l}$ ranging from 56 to 614 d).

Dietary pathway is an important contributor to the global bioaccumulation of Hg in bivalves (e.g., Blackmore and Wang, 2004, Metian et al., 2009). The present study has shown that the trophic transfer of Hg (iHg and MeHg) in the oyster *C. gigas* is influenced by its diet. As suggested by Hédouin et al. (2010), such experimental data need to be considered to better interpret bioaccumulation data obtained in the framework of biomonitoring programs.

5. Conclusion

Taking into account the major importance of the dietary pathway to the Hg bioaccumulation in bivalves, a special attention should be paid to the factors influencing the trophic transfer of Hg in this group. The present study provides new insights regarding the influence of very different feeds, a heterotrophic protist (the ciliate *Uronema* sp.) and an autotrophic protist (the diatom *T. pseudonana*) on the AE of iHg and MeHg in the Pacific cupped oyster (*C. gigas*). Such information can help refining both Hg bioaccumulation models and interpretation of data from field surveys and biomonitoring programs.

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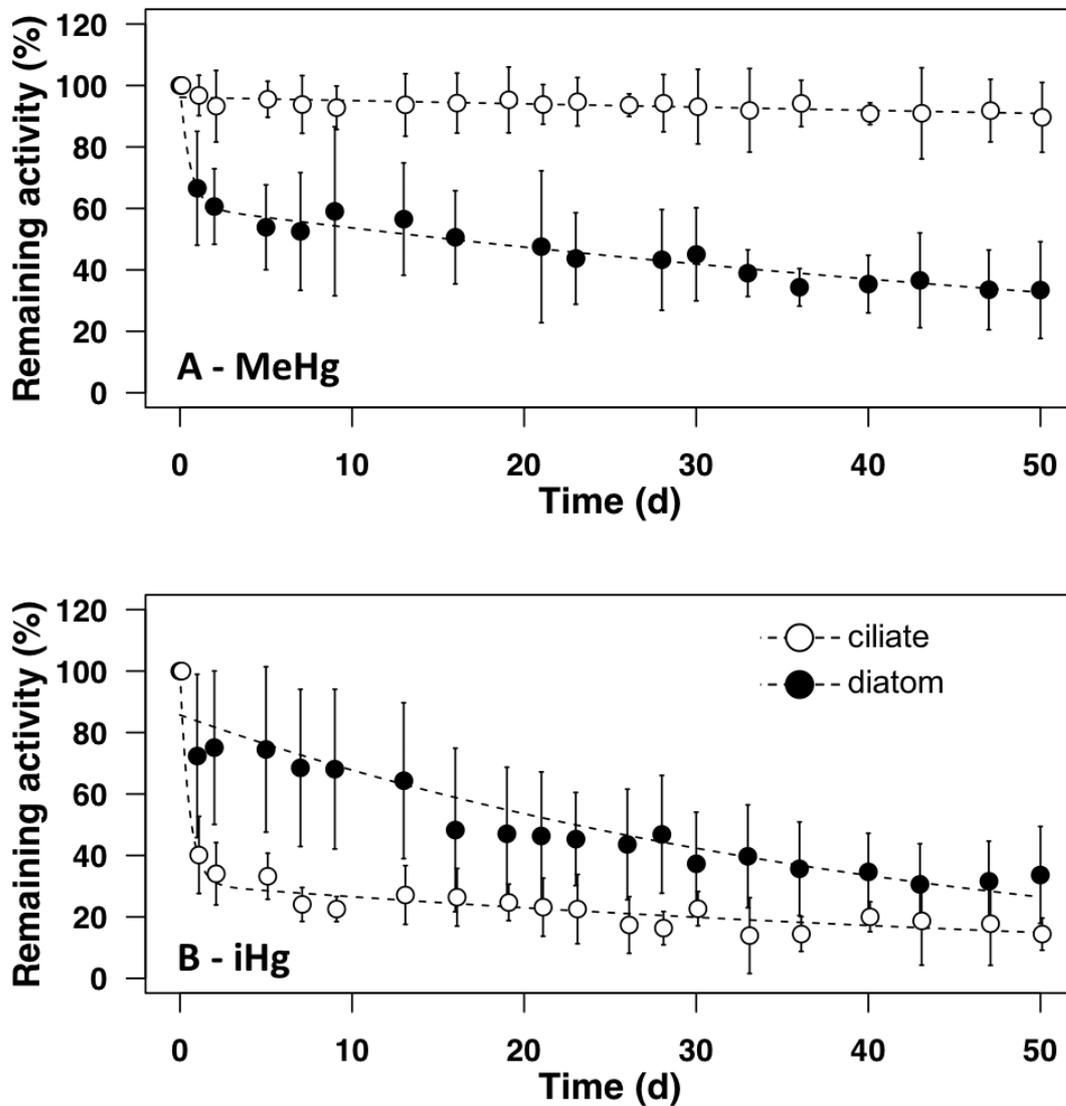


Figure 1. Influence of type of food (ciliate *Uronema* sp. and diatom *Thalassiosira pseudonana*) on whole-body depuration of (A) MeHg and (B) iHg in oysters (n=5, % remaining activities, mean \pm SD). Parameters and statistics of the depuration kinetics are given in Table 1.

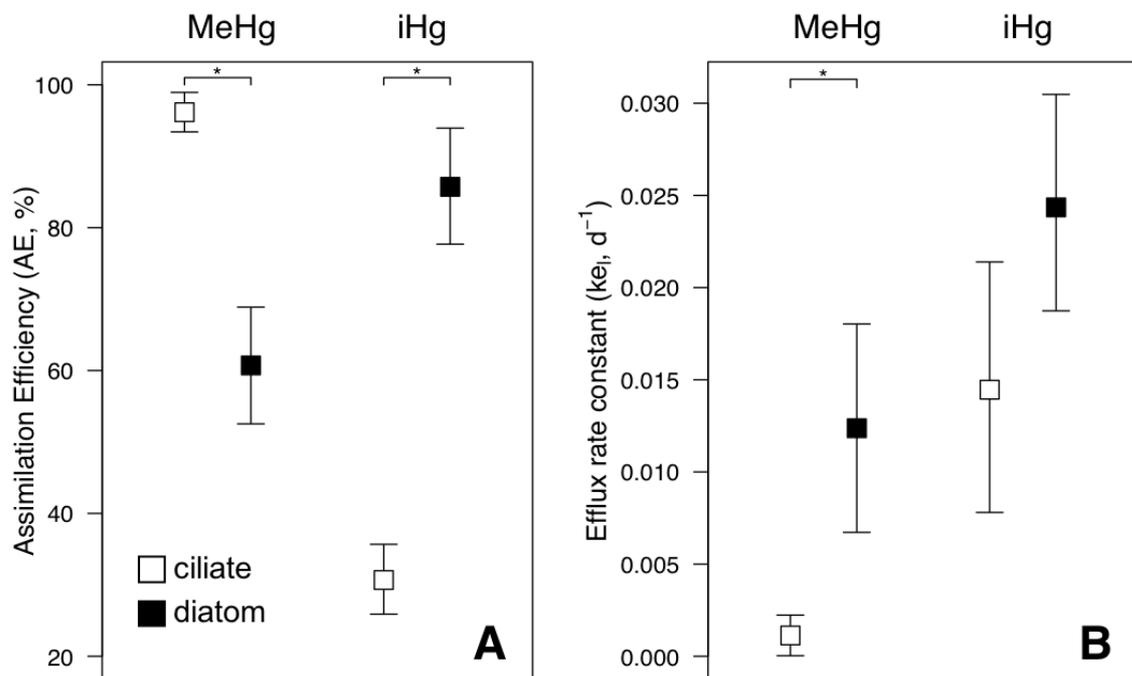


Figure 2. 95% confidence intervals of the main kinetic parameters: (A) Assimilation Efficiency (AE) and (B) Efflux rate constant (k_{e1}) estimated by fitting exponential models in oysters fed radiolabeled prey (ciliate *Uronema* sp. and diatom *Thalassiosira pseudonana*). Symbols denote significant difference.

Table 1. Parameters (mean \pm SE, n = 5) of the whole-body depuration kinetics of iHg and MeHg in the Pacific cupped oyster (*C. gigas*) fed on ciliate (*Uronema* sp.) and diatom (*Thalassiosira pseudonana*). Depuration parameters: A_{0s} and A_{0l} (=AE): activity (%) lost according to the short-and the long-lived exponential component, respectively; $T_{b/2}$: biological half-life (d) [$T_{b/2} = \ln 2/k_e$]; O and T: one-component and two-component exponential model, respectively. R^2 : determination coefficient.

Probability of the model adjustment: ^{NS} $p > 0.05$, * $p < 0.05$, *** $p < 0.001$.

Feed	Element	Model	$A_{0s} \pm SE$	$k_{es} \pm SE$	$T_{b/2s} \pm SE$	$A_{0l} (=AE) \pm SE$	$k_{el} \pm SE$	$T_{b/2l} \pm SE$	R^2
Ciliate	MeHg	O	-	-	-	$96.16 \pm 1.39^{***}$	$0.001 \pm 0.0006^*$	$614 \pm 302^{***}$	0.20
	iHg	T	$69.27 \pm 4.28^{***}$	$1.86 \pm 0.41^{***}$	$0.4 \pm 0.1^{***}$	$30.67 \pm 2.40^{***}$	$0.014 \pm 0.003^{***}$	$48 \pm 11^{***}$	0.92
Diatom	MeHg	T	$39.29 \pm 7.26^{***}$	1.78 ± 1.06^{NS}	0.4 ± 0.2^{NS}	$60.71 \pm 4.11^{***}$	$0.012 \pm 0.003^{***}$	$56 \pm 13^{***}$	0.56
	iHg	O	-	-	-	$85.72 \pm 4.04^{***}$	$0.024 \pm 0.003^{***}$	$28 \pm 3^{***}$	0.71