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Retention and distribution of methylmercury administered in the food in marine invertebrates:

Effect of dietary selenium

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ABSTRACT

Methylmercury is transported along aquatic food chains from the lower trophic levels and selenium modulates the biokinetics of mercury in organisms in complex ways. We investigated the retention of orally administered methylmercury in various marine invertebrates and the effect of selenium hereon. Shrimps (*Palaemon adpersus* and *P. elegans*), blue mussels (*Mytilus edulis*), shore crabs (*Carcinus maenas*) and sea stars (*Asterias rubens*) eliminated methylmercury slowly ($t_{1/2} = \frac{1}{2}$ to $>1$ year) and the copepod (*Acartia tonsa*) faster ($t_{1/2} \sim 12-24$ h). Orally administered selenite augmented elimination of methylmercury in the copepod (in one of two experiments) and blue mussels, but not in shrimps, crabs and sea stars. Selenium generally alters the distribution of the body burden of mercury, leaving more mercury in muscle and less mercury in digestive glands or rest of the body – also in the species where total body retention is not affected.

Keywords: Methylmercury; Selenium; Invertebrates; Retention

Short title:

Effect of selenium on retention of methylmercury in marine invertebrates

Highlights:

- Retention of orally administered methylmercury was investigated in marine invertebrates
- The copepod *Acartia tonsa* eliminates methylmercury faster than other organisms
- Orally administered selenium augments elimination in some organisms
- Selenium alters the distribution of methylmercury among organs
1. Introduction

Methylmercury is efficiently taken up and assimilated in most organisms and once assimilated, methylmercury is retained very efficiently in aquatic organisms, with biological half lives in various species typically ranging from days in copepods (Lee and Fisher, 2017) over one (Tsui and Wang, 2004a, b) to three (Karimi et al., 2007) weeks in daphnids and weeks to years in some fish (e.g. Amlund et al., 2007; Bjerregaard et al., 2011; Pickhardt et al., 2006; Ruohula and Miettinen, 1975; Tillander et al., 1969; Van Walleghem et al., 2013; Van Walleghem et al., 2007) and decapod crustaceans (Bjerregaard and Christensen, 2012; Evans et al., 2000; Fowler et al., 1978; Headon et al., 1996; Larsen and Bjerregaard, 1995; Rouleau et al., 1999; Tillander et al., 1969).

The major amount of methylmercury enters the aquatic food chains at the lower trophic levels and the level of methylmercury attained by predators at the top of aquatic food chains is generally determined by biomagnification processes up through the food chain (Lavoie et al., 2013; Riget et al., 2007). Hence, the potential to eliminate methylmercury in the organisms along the food chain determines the levels of methylmercury attained at the upper end of the food chain where methylmercury may cause toxic effects in top predators among wildlife (Dietz et al., 2013; Scheuhammer et al., 2015; Scheuhammer et al., 2008; Scheuhammer et al., 2016) and neurological symptoms in children of women with a high fraction of aquatic organisms in their diet (Debes et al., 2016; Grandjean et al., 1997).

Selenium may interact with mercury in aquatic organisms in ways that are not fully understood. Treatment of experimental lakes with selenite resulted in decreased levels of mercury in fish (Paulsson and Lundbergh, 1989, 1991; Turner and Rudd, 1983) and crayfish (Turner and Rudd, 1983), and fish in areas with elevated levels of selenium contain reduced amounts of mercury (Belzile et al., 2006; Peterson et al., 2009; Southworth et al., 2000; Southworth et al., 1994; Yang et al., 2010). Investigations in the laboratory on selenium-mercury interactions in fish and aquatic
invertebrates have shown highly variable results (reviewed by Cuvinaralar and Furness, 1991; Pelletier, 1986); different exposure routes, chemical forms of the elements, timing of the exposure and concentration or dose may lead to different results and different organisms respond differently.

Selenium administered in the food has been shown to reduce the concentrations of methylmercury in liver, kidney and muscle of rainbow trout *Oncorhynchus mykiss* (Bjerregaard et al., 1999) and reduce whole body retention of radiolabelled methylmercury in zebrafish *Danio rerio* and goldfish *Carassius auratus* (Bjerregaard et al., 2011) and the brown shrimp *Crangon crangon* (Bjerregaard and Christensen, 2012).

The purpose of the present investigation was to further elucidate half-lives for mercury and the effect of selenium on methylmercury retention and distribution in various marine invertebrates - the copepod *Acartia tonsa*, the blue mussel *Mytilus edulis*, the sea star *Asterias rubens*, the shore crab *Carcinus maenas* and the shrimps *Paleamon elegans* and *P. adspersus*.

2. Materials and methods

2.1. Experimental principle

The animals were initially exposed to food labelled with $^{203}$HgCH$_3^+$ and subsequently they were exposed to the same type of food, half of them with selenite amended food and the other half without added selenite. The exposure concentrations for selenium were selected with respect to the possibility of identifying effects rather than environmental relevance. The use of the gamma emitter $^{203}$Hg allows repeated counting of the individual animals and the radioactivity of the animals was determined for days or weeks to follow the retention of the mercury. At the termination of the experiments, the distribution of mercury among the organs was determined for the larger organisms.

2.2. Preparation of food
2.2.1 Labelling of the algae with $^{203}$HgCH$_3^+$

The algae were labelled by adding $^{203}$HgCH$_3^+$ to algal cultures with 500000 cells mL$^{-1}$ *Rhodomonas salina* and 100,000 cells mL$^{-1}$ *Thalassiosira weissflogii* and leaving them for 24 hours. The cell cultures were centrifuged at 4500 rpm for 4 min to concentrate the algae and remove them from the $^{203}$HgCH$_3^+$ in the water phase. The radioactivity of the algae was determined and they were re-suspended in medium before they were presented to the mussels and copepods. Assuming a single cell dry weight of 92 pg (Brown et al., 1998), the *R. salina* had an activity of approximately $42 \times 10^6$ and $2.8 \times 10^6$ dpm g$^{-1}$ when presented to the copepods and blue mussels, respectively.

2.2.2 Algal exposure to selenium

Cultures of *R. salina* (500000 cells mL$^{-1}$) were exposed to selenium as selenite for 24 h at concentrations of 500 µg Se-SeO$_3^{2-}$ L$^{-1}$ when fed to the copepods and 1000 µg Se-SeO$_3^{2-}$ L$^{-1}$ when fed to the blue mussels. Light intensity was at 60-90 µE with light intervals of 12 hours light and 12 hours dark; temperature was 15°C. Non-exposed algae were kept under the same conditions as the exposed ones.

Algal cultures were filtered through a glass fiber filter, GC-50 for determination of their selenium content. The control algae contained approximately 1.84 µg Se g$^{-1}$ wet weight (two determinations with 3 replicates each: 1.87±0.23 and 1.81±0.15). The algae exposed to 500 µg Se-SeO$_3^{2-}$ L$^{-1}$ for the copepods and 1000 µg Se-SeO$_3^{2-}$ L$^{-1}$ for the mussels contained 35±7 and 103±9 µg Se g$^{-1}$ wet weight, respectively (3 replicates each).

2.2.3 Food for sea stars, crabs and shrimps

The food for the shrimps, sea stars and shore crabs was cubes of homogenized and solidified blue mussel soft parts with a weight of 6-12 mg wet weight for shrimps and sea stars and 1.6 g wet weight for the crabs. For the sea star and shore crab experiment, the blue mussels (*M. edulis*) were...
collected in Great Belt, Denmark and homogenate and solid cubes with added $^{203}\text{HgCH}_3^+$ or selenite were prepared as described by Bjerregaard and Christensen (2012). For the *Paleamon sp.* experiments, the food was made from commercially obtained, boiled soft parts of *M. chilensis*.

2.3. Experimental organisms

2.3.1. *Rhodomonas salina* culture

The food source for the copepods and blue mussels was the algae *R. salina*. Cultures were obtained from DTU Aqua, Chartottenlund, Denmark and the Marine Biological Research Centre, Kerteminde, Denmark. Semi-continuous cultures of these algae were maintained at 20°C under a 12 hour light (60-90 µE) and 12 hour dark. Water for the experiment was obtained from Great Belt outside Kerteminde, Denmark, and the water was filtered through a glass fiber filter, GC-50. Nutrients added to the algae were based on Walne’s medium for algae cultures (Walne, 1970). The cultures were not grown steriley and between one and two weeks into the experiment with the blue mussels, the culture was contaminated by an unidentified microorganism and a new culture was initiated. One experiment was carried out with the diatom *Thalassiosira weissflogii* (supplied by DTU Aqua and grown in a similar way).

2.3.2. *Acartia tonsa*

The copepod was obtained as eggs from a semi-continuous culture of the copepod obtained from DTU Aqua, Charlottenlund, Denmark. Before starting the experiment, the eggs were stored at 4°C. For a start-up culture eggs were placed in a 10 L container. After hatching, the copepods were fed *ad libitum* with the algae *R. salina* at a cell density of 50000 cells mL$^{-1}$ (Kiorboe et al., 1985; Stottrup and Jensen, 1990).

2.3.3. Field collected animals
Blue mussels *M. edulis*, sea stars *A. rubens*, shore crabs *C. maenas* and shrimps *P. elegans*, *P. adspersus* were collected from the Kerteminde area, Great Belt, Denmark. The salinity in this area typically varies between 15 and 25‰.

2.4. Experiments

2.4.1. *Acartia tonsa*

Three weeks old, adult females were used in this experiment with 10 specimens placed in each 200 mL glass beaker at 20 °C. To be able to move the adults to a new solution, the adults were placed inside mesh-bottomed sieves (8.5 cm height, 4.5 cm diameter, 100 µm mesh size bottom panel). During the whole experiment, the copepods were fed with algae *R. salina* (50000 cells mL⁻¹). At the beginning of the experiment, the copepods were exposed to \(^{203}\)HgCH₃⁺-labelled algae for five hours. Thereafter, the copepods were moved to clean beakers where half of the beakers had selenium exposed *R. salina* added and the other half non-exposed algae. The following days the copepods were moved to new beakers with clean water and algae were added once a day.

Copepods were taken out for determination of radioactivity at 0, 4, 7, 19, 30, 47, 101, 125 and 149 and 0, 4.5, 8, 21, 49, 72, 102 and 120 hours, respectively, after the onset of feeding with selenium amended/control algae in the two experiments carried out. For each treatment and time, three replicate beakers were sampled. We succeeded in retrieving an average of 7.8±0.13 (n=183) out of the 10 added copepods from the beakers.

The influence of temperature on the retention of mercury was investigated in a separate experiment. \(^{203}\)HgCH₃⁺ was presented to the copepods on *R. salina* at 15, 20 and 25 °C and the retention of mercury was monitored at the same temperatures by sampling copepods at 0, 21, 44, 74 and 91 h.

*R. salina* and *T. weissflogii* as the food source for the copepods were compared, also in a separate experiment. \(^{203}\)HgCH₃⁺ was presented to the copepods on *R. salina* and *T. weissflogii* and
the retention of mercury was monitored with the same species as food sources by sampling copepods at 0, 19, 43, 65 and 90 h.

2.4.2. Mytilus edulis

At the beginning of the experiment, 20 blue mussels (shell length 17-21 mm; kept individually in 200 mL glass beakers at 17‰ and 10°C) were fed with $^{203}$HgCH$_3^+$ exposed *R. salina* at 1000 cells mL$^{-1}$ five times at two hours intervals. Thereafter, 10 of the mussels were fed control algae and the other 10 fed selenium exposed algae one to five times a day. Each day the mussels were moved to a new beaker with clean water. For the next 41 days, the radioactivity of the mussels was determined at 1-10 day intervals. Approximately 1 to 2 weeks into the experiment, the *R. salina* culture was contaminated with an unidentified microorganism and a new culture was initiated.

2.4.3. Asterias rubens

Twenty small (wet weight 116-911 mg) sea stars kept individually in 200 mL glass beakers (17‰ and 10°C, aerated) were presented with a cube of mussel homogenate containing $^{203}$HgCH$_3^+$. The experiment started when each sea star had eaten the labelled food. Subsequently, 10 of the sea stars were fed selenium amended food (9.4 µg Se g$^{-1}$) and seven (3 of the 10 control sea stars did not eat the labelled food) were fed control food (0.6 µg Se g$^{-1}$). Once a day the sea stars were checked if they had eaten. Those who had eaten were fed with a new cube of mussel homogenate. Every fourth day the sea stars were moved to a new beaker with clean water. For the next 50 days, the radioactivity of the sea stars was determined at intervals of 3-8 days.

2.4.4. Carcinus maenas

Forty-five shore crabs (carapace width 4.4-5.4 cm) were fed once a day for three days with a cube of mussel homogenate containing $^{203}$HgCH$_3^+$. The following five days the crabs were fed
control food (0.18 µg Se g⁻¹) not amended with selenium. The next 31 days the crabs were divided into three groups and fed either with selenite amended food (53.3 µg Se g⁻¹) or control food (0.18 µg Se g⁻¹ wet weight). One of the groups was fed control food and exposed to 5 mg Se-SeO₃²⁻ L⁻¹ in the water. The crabs were held individually in 1.5 L aerated sea water in plastic beakers at 15 °C. Water was aerated, the light regime was 12:12 and salinity was 20-25 ‰. The water was changed five times a week and the radioactivity of the crabs was determined at 2-4 days intervals.

2.4.5. Palaemon elegans and P. adspersus

Thirty P. elegans or P. adspersus were fed ²⁰³HgCH₃⁺ labelled food every day for 8 days. During the subsequent 3 weeks, half of the shrimps of each species were fed selenite amended food (15 µg Se g⁻¹) and the other half control food (0.21 µg Se g⁻¹). The shrimps were held individually in 1 L sea water in plastic beakers at 15 °C. Water was aerated, the light regime was 12:12 and salinity was 16-26 ‰. Water was changed every 2-3 days and the radioactivity of the shrimps was measured at 4-5 day intervals.

2.5. Dissection

At the end of the experiments, crabs, shrimps, sea stars and blue mussels were dissected to measure ²⁰³Hg contents in the different parts of the body. As the sea stars were small they were only dissected in two parts: pyloric caeca and the rest. The blue mussels were divided in shell, foot, gill, muscle and the remainder, and the shore crabs were divided in muscle, gill, midgut gland, carapace and haemolymph. Shrimps were dissected into tail muscle, midgut gland, green gland and the remainder.

2.6. Chemicals
$^{203}\text{HgCl}$ was obtained from Eckert & Ziegler or Risø. Specific activities ranged from 6.7 to 28.5 GBq/g (1.8 and 0.42 pg Hg/dpm). *P. adspersus* received the highest activity (~35000dpm) corresponding to ~12 ng Hg/g wet weight with the isotope. The methylation to $\text{CH}_3^{203}\text{HgCl}$ followed the procedure of (Toribara, 1985). Selenium(IV)-dioxide (SeO$_2$), purity 98% was from Aldrich. Gelatin from porcine skin, Fluka Analytical, was used for the mussel homogenate.

2.7. Chemical analysis.

Selenium concentrations in the food were determined by hydride generation as after pretreatment of the samples as described by Sørensen and Bjerregaard (1991); a Perkin-Elmer Mercury-Hydride System was used – coupled to PerkinElmer 2380 (shrimp and crab experiments) or FIAS 100 (copepod, mussel, sea star experiments) Atomic Absorption Spectrophotometers. The reliability of the selenium analysis was investigated by including standard material (DORM and TORT); the measured values were within the certified range.

2.8. Determination of radioactivity

The radioactivity of algae, copepods, mussels, shrimps, starfish and dissected tissues was determined in a Wizard 1480 TM3 automatic gamma counter and the radioactivity of the live shore crabs was determined in a well-type Bicron Labtech™ NaI(Tl) crystal gamma counter with a diameter and depth of 7.6 cm. Values were corrected for the physical decay of $^{203}\text{Hg}$. An electrical fault in the output device from the Wizard prevented counting of the sea stars (day 15-23) and mussels (day 16-23) for about a week over the Easter 2012.


For mussels, shrimps, starfish and crabs, the radioactivity of each animal was set to 100%, the day the selenium exposure began. For the copepods, the pooled radioactivity of the animals...
retrieved from each beaker was determined and the activity per animal was calculated; for all
sampling times activity per animal was calculated as percent of the average activity of the animals
at time 0. Repeated measures ANOVA analysis was used to check for difference in elimination of
mercury between the groups of mussels, shrimps, sea stars and crabs. A two-way (+/- Se and time)
ANOVA was used for the copepod results. A significance level: \( \alpha = 0.05 \) was used. The retention of
mercury was fitted to 1\textsuperscript{st} order equations with one (\( C_t = C_0 e^{-kt} \)) or two (\( C_t = A e^{-at} + B e^{-bt} \))
compartments with or without residuals according to Comar (1955). The statistical treatment was
carried out in Systat 13.0 and curve fitting was carried out in FigP. In the text, mean values are
given with \( \pm \)SEM.

3. Results

3.1. Acartia tonsa

The retention of mercury in \( A. \) tonsa fed \( R. \) salina was adequately described by 1\textsuperscript{st} order kinetics
in a two-compartment model (Fig. 1A) or one-compartment models with (Fig. 1B,D) or without
residuals (Fig. 1C). Initial half-lives for mercury in the 4 experiments varied between 12.1 and 23.9
h. The retention of mercury over 6 d in \( A. \) tonsa in the first experiment could be described by 1\textsuperscript{st}
order kinetics in a two-compartment model with half-lives of 12.1 and 55.4 h, respectively, the two
compartments containing 80\% and 20 \% of the body burden (Fig. 1A). For the selenium exposed
group, retention could also be described by a two-compartment model with half-lives of 8.0 and
38.5 h, respectively, in the two compartments which each contained 72\% and 28\% of the body
burden (Fig. 1A). The two-way ANOVA revealed effects of time (\( P<0.001 \)) and selenium treatment
(\( P=0.016 \)) and no interaction between the two (\( P=0.64 \)). In the second experiment, a half-life of
20.9 h was seen in the control group and 16.3 h in the selenium exposed group (Fig. 1B); in this
experiment no statistically significant difference was found between the control and the selenium
exposed group ($P = 0.22$). Temperature (15, 20 or 25 °C) had no effect on the retention of mercury: half-lives were in the range 23.8 to 23.9 h (Fig. 1C). In the experiment where the diatom *T. weissflogii* and *R. salina* were compared as food source (Fig. 1D), the half-life for $^{203}\text{Hg}$ in the copepods fed *R. salina* was 14.0 h while the half-life was longer in copepods fed the diatom (18.6 h).

### 3.2. *Mytilus edulis*

For the initial two weeks no discernible elimination of mercury took place in either of the two groups (Fig. 2A). After d 14, elimination of mercury from the selenium exposed group proceeded faster with a half-life of 94 d and the control group eliminated mercury very slowly with a half-life of 1333 days (Fig. 2A). A statistically significant ($P < 0.001$) difference between the retention in the two groups was seen. The onset of a faster elimination in the selenium exposed group coincided with the contamination of the *R. salina* culture with an unidentified microorganism. At the end of the experiment the selenium exposed group had a higher percentage of the mercury body burden in the gills than the control and less in the adductor muscle (Fig. 2B).

### 3.3. *Asterias rubens*

Retention of mercury in *A. rubens* fitted simple 1st order kinetics (Fig. 3A) with a half-life of 181 d. Retention in the group exposed to 10 µg Se g⁻¹ in the food was similar with a half-life of 129 d. The effect of selenium on the retention of mercury was not statistically significant ($P= 0.32$). At the end of the experiment, mercury was evenly distributed between pyloric caeca and the rest in the control group (Fig. 3B); the group exposed to selenium had less mercury in the pyloric caeca and more in the rest (Fig. 3B).

### 3.4 *Carcinus maenas*
Shore crabs in the three exposure groups eliminated mercury very slowly or not at all during the 31 d exposure period (Fig. 4A). Half-lives in the control group and the group exposed to selenium in the food could be extrapolated to 3394 d and 1195 d, respectively (Fig. 4A); no half-life could be estimated for the group exposed to Se in water: 100.3*e^{-0.00002026*t} (r^2=0.002). Both groups exposed to selenium had higher proportions of mercury in muscle and lower in midgut gland than the control group (Fig. 4B).

3.5. The 2 shrimp species

The control groups of the 2 species (Fig. 5A, C) eliminated no discernible mercury during the exposure period and this was also the case for selenium exposed P. adspersus (Fig. 5C). Selenium exposed P. elegans eliminated mercury very slowly (t_{1/2}=429 d) with no difference from the control group (Fig. 5A). At the end of the experiment, a higher fraction of the mercury was found in the tail muscle of the selenium exposed P. elegans than in the control, and less in the rest (Fig. 5D). A similar – but nonsignificant - trend was seen in P. adspersus (Fig. 5B).

4. Discussion

4.1 Mercury retention

The copepod was the only organism tested where elimination was so fast that the half-life could actually be determined within the time range of the experiment. The half-life for accumulated mercury in A. tonsa varied between 12 and 24 h for the initial elimination in the four experiments which is comparable to the elimination rates reported by Lee and Fisher (2017) although they observed somewhat faster initial and more slow subsequent elimination. Since the gut passage time in A. tonsa feeding at cell densities around 5*10^4 cells mL^{-1} is in the order of 1 h or less (Besiktepe and Dam, 2002; Caparroy and Carlotti, 1996), the loss of ^{203}\text{Hg} from the copepods can be attributed to loss of truly assimilated ^{203}\text{Hg} rather than to a mere emptying of radioactive contents of the gut.
The copepod eliminates mercury faster (Lee and Fisher, 2017) than reported for other small crustaceans such as daphnia where half-lives for methylmercury of 6 d to 15 d in *Daphnia magna* (Tsui and Wang, 2004a, b) and 17 d in juvenile *Daphnia pulex* (Karimi et al., 2007) have been reported. Variation between the half-lives determined in individual experiments have also been noted with *D. magna* exposed to methylmercury in the food; Tsui & Wang (Tsui and Wang, 2004a, b) found half-lives between 6 and 15 days in individual experiments - a relative variability similar to that found for *A. tonsa* in the present experiments. Temperature did not affect the elimination of mercury in the copepods in the present investigation and a similar result was obtained with *D. magna* fed on *Chlamydomonas reinhardtii* at 14, 19 and 24 °C (Tsui and Wang, 2004a). The copepods eliminated mercury more slowly when fed on *T. weissflogii* than on *R. salina* and Lee and Fisher (2017) observed a similar difference when comparing elimination of methylmercury from *A. tonsa* fed the diatom *T. pseudonana* versus *R. salinas* and the chlorophyte *Dunaliella tertiolecta*.

Lee and Fisher (2017) suggest the differences are due to the amount of MeHg incorporated in the cytoplasm of the algae versus the amount adsorbed on the cell wall. Tsui & Wang (2004b) also noted differences in elimination rates in *D. magna* fed on two different food sources (*C. reinhardtii* and *Scenedesmus obliquus*).

For all of the other organisms tested, mercury was retained to a degree where the half-lives could only be estimated by extrapolation of the retention curves beyond the time range of the actual experiment.

Mussels eliminated mercury very slowly in the present experiment which is in accordance with previously reported half-lives between 377 d (Fowler et al., 1978) and 1000 d (Miettinen et al., 1972) for *Mytilus galloprovincialis* in laboratory experiments - depending on the route of administration.
The shrimps and crab of the present investigation also showed no or very slow elimination of ingested methylmercury. Most previous investigations draw comparable conclusions with reported half-lives of 529 d in the shrimp *Lysmata seticaudata* (Fowler et al., 1978), 630-770 d in *C. crangon* (Bjerregaard and Christensen, 2012), 520-650 d in the snow crab *Chionoecetes opilio* (Rouleau et al., 1999), 3-5 y in crayfish *Oronectes virilis* (Headon et al., 1996), 144-297 d in the crayfish *Astacus astacus* (Tillander et al., 1969), 400 d (Miettinen et al., 1972) and 753 d (Larsen and Bjerregaard, 1995) in *C. maenas* and no loss in blue crabs *Callinectes sapidus* (Evans et al., 2000); pink shrimp *Penaeus duorarum* eliminated ingested methylmercury somewhat faster with a half-life of 58 d (Evans et al., 2000).

There is no information in the scientific literature on biokinetics of mercury in echinoderms but in the present investigation, sea stars appear to eliminate ingested methylmercury somewhat faster than the mussels and decapod crustaceans.

4.2. Effect of selenium on distribution of mercury

Exposure to selenium alters the distribution of accumulated mercury in most of the organisms tested, and also when no effect on half-lives for mercury was observed. In *P. elegans* and *C. maenas*, exposure to selenium results in a higher proportion of the whole body mercury being accumulated in the muscle tissue and less in the midgut gland or rest. For *C. maenas* this is in accordance with previous observations (Bjerregaard and Christensen, 1993; Larsen and Bjerregaard, 1995) and it is also in accordance with observations in the brown shrimp *C. crangon* (Bjerregaard and Christensen, 2012). *A. rubens* exposed to 10 µg L\(^{-1}\) inorganic mercury in the water accumulated much lower concentrations of mercury in the pyloric caeca when simultaneously exposed to 75 µg Se-SeO\(_3^{2-}\) L\(^{-1}\) and more in body wall, tube feet and coelomic fluid (Sorensen and Bjerregaard, 1991), comparable to the effect of orally administered selenium on the distribution of methylmercury in the present investigation.
4.3. Effect of selenium on the retention of mercury

Exposure to selenium marginally affected the retention of mercury in one out of two experiments with *A. tonsa*, clear effects were observed in blue mussels, weak trends were observed in *P. elegans* and *A. rubens* and no effects were seen in *P. adspersus* and *C. maenas*.

The principle and technique used to obtain the results on the effects of selenium on the retention of ingested methylmercury are fairly simple and robust. Thus, the likelihood that the different results for the various organisms can be explained by experimental error is considered to be very low.

Between 44% and 75% of the mercury eliminated from the brown shrimp after selenium supplementation in Bjerregaard and Christensen (2012) were accounted for by the content in the faeces of the shrimps. It was suggested that the interaction between selenium and methylmercury takes place in the gut and also that the microflora of the gut might play a role in this interaction also seen in fish (Bjerregaard et al., 2011). It is noteworthy that the onset of the faster elimination of mercury in the selenium exposed group of the mussels coincided with the contamination of the algal culture with an unidentified microorganism which may be speculated to have played a role in the selenium mercury interaction. That microbial activity in the gut may affect the fate of methylmercury was shown already in 1977 by Rowland et al. who observed a higher mercury accumulation in the tissues and a lower faecal elimination in rats (Rowland et al., 1980a, b; Rowland et al., 1977a, 1978) and mice (Rowland et al., 1984) treated with antibiotics. They also demonstrated volatilization of methylmercury in *in vitro* studies with gut contents (Rowland et al., 1977b, 1978). Formation of inert Hg-Se compounds have been demonstrated in cultures of both *Pseudomonas fluorescens* (Yang et al., 2011) and *Desulfovibrio desulfuricans* (Truong et al., 2013; Truong et al., 2014) upon addition of selenium and mercury. Demethylation of methylmercury to inorganic mercury – in fish also believed to take place in the gut (e.g. Wang et al., 2017) – might
also increase the mercury elimination rate since inorganic mercury is generally retained less efficiently than methylmercury (e.g. Bjerregaard et al., 2011; Pentreath, 1976). It has been suggested that selenium plays a role in the demethylation of methylmercury in fish (Palmisano et al., 1995), rats (Iwata et al., 1982; Masukawa et al., 1982) and marine mammals (Nigro, 1994; Nigro and Leonzio, 1996).

The clear effects of dietary selenium on the retention of methylmercury observed in rainbow trout (Bjerregaard et al. 1999), zebrafish and goldfish (Bjerregaard et al. 2011) and brown shrimps (Bjerregaard and Christensen, 2012) were only seen in the blue mussel in the present investigation and this emphasizes that we still do not understand the mechanisms involved in the selenium-mercury interactions. It is especially noteworthy that the two *Palaemon* species in the present investigation showed such a markedly different response from that of the brown shrimp (Bjerregaard and Christensen, 2012) and this discrepancy is under further investigation in our laboratory.

**Acknowledgement**

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**Figure legends**

Fig. 1. *Acartia tonsa*. A and B: Retention of $^{203}$Hg in copepods fed $^{203}$HgCH$_3^+$ exposed algae *Rhodomonas salina*. ○: Control copepods. ■: Copepods fed *R. salina* exposed to 500 µg Se-SeO$_3^{2-}$ for 1 day. C: Retention of $^{203}$Hg in copepods fed $^{203}$HgCH$_3^+$ exposed algae *R. salina* maintained at 15 (∆), 20 (○) or 25 (□) °C. D: Retention of $^{203}$Hg in copepods fed $^{203}$HgCH$_3^+$ exposed algae *R. salina* (○) or *T. weissflogii* (▼). Mean±SEM for three replicates, each consisting of an average of 7.8±0.13 (SEM) animals.

Fig. 2. *Mytilus edulis*. A: Retention of $^{203}$Hg in mussels fed $^{203}$HgCH$_3^+$ exposed algae *Rhodomonas salina*. ○: Control mussels. ■: Mussels fed *R. salina* exposed to 1000 µg Se-SeO$_3^{2-}$ L$^{-1}$ for 1 day. Mean±SEM; n=10 in each group. Equations show retention between day 14 and 39. B: Distribution of the $^{203}$Hg body burden. * and ** indicates statistically significant difference between control and Se-exposed group at $P < 0.05$ and $P < 0.010$, respectively.

Fig. 3. *Asterias rubens*. A: Retention of $^{203}$Hg in sea stars fed $^{203}$HgCH$_3^+$ containing food. ○: Sea stars fed control food (n=7). ■: Sea stars fed Se amended food (10 µg Se-SeO$_3^{2-}$ g$^{-1}$; n=10). Mean±SEM. B: Distribution of the $^{203}$Hg body burden. * and *** indicates statistically significant difference between control and Se-exposed group at $P < 0.05$ and $P < 0.001$, respectively.

Fig. 4. *Carcinus maenas*. A: Retention of $^{203}$Hg in shore crabs fed $^{203}$HgCH$_3^+$ containing food. ○: Shore crabs fed control food (n=14). ■: Shore crabs fed Se amended food (53 µg Se-SeO$_3^{2-}$ g$^{-1}$; n=15). ∆: Shore crabs fed control food and exposed to 5000 µg Se-SeO$_3^{2-}$ L$^{-1}$ in the water; n=15. Mean±SEM. B: Distribution of the $^{203}$Hg body burden. *** indicates statistically significant difference between control and Se-exposed groups at $P < 0.001$.

Fig. 5. *Palaemon sp*. A, C: Retention of $^{203}$Hg in shrimps fed $^{203}$HgCH$_3^+$ containing food. ○: Shrimps fed control food (n=13-14). ■: Shrimps fed Se amended food (15 µg Se-SeO$_3^{2-}$ g$^{-1}$; n=14-15). B, D: Distribution of the $^{203}$Hg body burden. * and *** indicate statistically significant difference between control and Se-exposed group at $P < 0.05$ and $P < 0.001$, respectively. Mean±SEM.
Reference List


Pickhardt, P.C., Stepanova, M., Fisher, N.S., 2006. Contrasting uptake routes and tissue distributions of inorganic and methylmercury in mosquitofish (Gambusia affinis) and redear sunfish (Lepomis microlophus). Environmental Toxicology and Chemistry 25, 2132-2142.


Environmental Pollution 210, 361-370.


Fig. 1: The graphs show the percentage of 203 Hg retained over time for different conditions. The equations for the control and selenium (Se) treatments are as follows:

- **Control**: 
  \[\% \text{ retained} = 80e^{-0.0574t} + 20e^{-0.0125t}\]
  \(r^2=0.997\)

- **Se**: 
  \[\% \text{ retained} = 72e^{-0.0867t} + 28e^{-0.018t}\]
  \(r^2=0.998\)

For Thalassiosira weissflogii:

- \(15\ C\): 
  \[\% \text{ retained} = 82e^{-0.0321t} + 12\]
  \(r^2=0.983\)

- \(20\ C\): 
  \[\% \text{ retained} = 77e^{-0.0373t} + 23\]
  \(r^2=0.989\)

- \(25\ C\): 
  \[\% \text{ retained} = 92e^{-0.0495t} + 8\]
  \(r^2=0.996\)

For Rhodomonas salina:

- \(15\ C\): 
  \[\% \text{ retained} = 91e^{-0.0434t} + 7\]
  \(r^2=0.989\)

- \(20\ C\): 
  \[\% \text{ retained} = 91e^{-0.0397t} + 8\]
  \(r^2=0.995\)

- \(25\ C\): 
  \[\% \text{ retained} = 100e^{-0.0289t}\]
  \(r^2=0.987\)

These equations describe the exponential decay of Hg retention over time in different conditions.
Fig. 2

% $^{203}$Hg retained = 99.3\(e^{-0.00052t}\)

% retained = 100\(e^{-0.00738t}\)

\(r^2 = 0.96\)

\(r^2 = 0.19\)
Fig. 3

**A**

- **O: Control**
  - % retained = 97.9\(e^{-0.0038t}\)
  - \(r^2 = 0.92\)

- **+ Se**
  - % retained = 97.6\(e^{-0.0054t}\)
  - \(r^2 = 0.95\)

**B**

- Dissection water
- Rest
- Pyloric caeca

Elimination time (days)

Hg retained

% of total Hg

% retained = 97.9\(e^{-0.0038t}\)

% retained = 97.6\(e^{-0.0054t}\)
Fig. 4

**A**
- Control
- Se in food
- Se in water

**B**
- % Hg retained
- Elimination time (days)

<table>
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<th>Elimination time (days)</th>
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<tbody>
<tr>
<td>0</td>
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<td>97%</td>
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<tr>
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### Equations
- Control:
  \[ %\text{Hg retained} = 101.7e^{-0.0004761t} \]
  \[ r^2 = 0.56 \]

- Se in food:
  \[ %\text{Hg retained} = 100.7e^{-0.0002042t} \]
  \[ r^2 = 0.15 \]
**Palaemon elegans**

- **A**: Graph showing the percentage of mercury retained over time. The equation for mercury retention is given as
  \[ \% \text{ Hg retained} = 102e^{-0.00162t} \]
  with \( r^2 = 0.55 \).

- **B**: Bar graph comparing the percentage of total mercury in different parts of the shrimp (Tail muscle, Midgut gland, Green gland, Rest) between Control (+Se) and treated (+Se) conditions. The y-axis represents the percentage of total mercury. There is a significant difference (***). A Tukey HSD post hoc test was used.

**Palaemon adspersus**

- **C**: Graph showing the percentage of mercury retained over time. The y-axis represents the percentage of mercury retained.

- **D**: Bar graph comparing the percentage of total mercury in different parts of the shrimp (Tail muscle, Midgut gland, Green gland, Rest) between Control (+Se) and treated (+Se) conditions. The y-axis represents the percentage of total mercury. A Tukey HSD post hoc test was used.