Exposure to methylmercury and inorganic mercury in the food does not lead to trophic magnification in the sea star Asterias rubens

Bjerregaard, Poul; Møller, Lise Marianne

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Exposure to methylmercury and inorganic mercury in the food does not lead to trophic magnification in the sea star *Asterias rubens*

Poul Bjerregaard and Lise Marianne Møller*

Department of Biology

University of Southern Denmark

Campusvej 55, DK-5230

Odense

Denmark

*Present address:

DHI-Group, Water Environment Health, Agern Allé 5, 2970 Hørsholm, Denmark

Abstract. Methylmercury accumulated at the top of aquatic food chains constitutes a toxicological risk to humans and other top predators. Biomagnification of methylmercury takes place among vertebrates at the higher trophic levels, but this process is less elucidated in benthic invertebrates at the lower trophic levels. Therefore, we investigated the accumulation from food and elimination of methylmercury and inorganic mercury in the benthic sea star *Asterias rubens* (L.) – a representative of trophic level ~ 3 - in laboratory experiments. Sea stars fed over 49 days with contaminated mussels (*Mytilus edulis*) accumulate methylmercury and inorganic mercury to the highest concentrations in the digestive glands, the pyloric caeca, less in stomach, gonad, tube feet, aboral body wall and not to detectable levels in the coelomic fluid. Concerning whole body contents, steady states were reached for both methylmercury and inorganic mercury during the 7-week feeding period and the sea stars reached approximately ½ and ¼ of the concentrations in the mussel food for the two mercury forms, respectively. Half-lives for the elimination of the two mercury forms varied between 45 and 173 days in a 140-d elimination period following the feeding period; inorganic mercury was eliminated faster than methylmercury. Examination of total mercury concentrations in field-collected sea stars confirmed this lack of trophic magnification in relation to the major food items, soft parts of molluscs. We suggest that mercury is not trophically magnified in sea stars 1) because they eliminate methylmercury faster than larger fish and decapod crustaceans and 2) maybe more importantly, because inorganic mercury with its faster elimination constitutes a larger fraction of the total mercury in the food at the lower trophic levels - as opposed to methylmercury which dominates at the higher trophic levels.

Key words: Methylmercury; Inorganic mercury; Sea star; Oral exposure; Trophic magnification

Capsule:

Neither inorganic mercury nor methylmercury is magnified in the sea stars relative to the concentrations in their food.

1. **Introduction**

Anthropogenic releases of mercury to the environment have led to elevated concentrations at both local and global scales (UNEP, 2019). Mercury is generally biomagnified along aquatic food chains and mercury concentrations at the higher trophic levels in aquatically-based food chains may reach levels that challenge the health of top predators such as humans (Grandjean et al., 1997) and fish-eating birds (Scheuhammer et al., 2008).

Although the extent of direct discharges of mercury to the coastal environments have decreased during the latest decades, the discharge of mercury from various sources to the atmosphere such as burning of coal, artisanal goldmining, cement production etc. (UNEP, 2019) still has the potential to add to the biomagnification of mercury in aquatically-based food chains.

Whereas biomagnification [defined as increase of concentration over 2 or more trophic levels (Nordberg et al., 2009)] of mercury has been clearly demonstrated along aquatic food chains (reviewed by Lavoie et al., 2013; Wu et al., 2019; Wu et al., 2020), a relatively high number of the investigations concern fish and aquatic mammals and birds at the higher levels of the food chain (i.e. Bowles et al., 2001; Dietz et al., 2009; Kidd et al., 1995; Riget et al., 2007; Ruus et al., 2015). The situation appears less clear at the lower levels of the food chains: Daphnia (Mathews and Fisher, 2008) and copepods (Lee and Fisher, 2017) concentrate methylmercury from phytoplankton at the transition from trophic level one to two and consistent biomagnification has been shown at trophic levels 1 to 4 planktonic and pelagic food webs in field investigations (Di Beneditto et al., 2012; Kehrig et al., 2010). However, increases in mercury concentrations do not necessarily take place from trophic level ~ 2 to ~ 3 in benthic, coastal organisms and food webs. In laboratory investigations, neither brown shrimps *Crangon crangon* (Riisgard and Famme, 1986), Nordic shrimp *Pandalus borealis* (Rouleau et al., 1992)

76 nor the shore crab *Carcinus maenas* (Bjerregaard and Christensen, 1993; Larsen and
77 Bjerregaard, 1995) fed food contaminated with methylmercury and/or inorganic mercury
78 accumulated any of the two mercury forms to higher concentrations than found in the food.
79 Also in the freshwater crayfish *Astacus astacus* concentrations did not exceed prey
80 concentrations - although a steady-state was not reached within the 30-d exposure period
81 (Simon and Boudou, 2001). In field investigations, the shore crab *C. maenas* does not
82 concentrate mercury to higher levels than potential food items [the clam *Scrobicularia plana*
83 and the polychaete *Hediste diversicolor* (Coelho et al., 2013; Fonseca et al., 2019) or
84 periwinkles *Littorina littorea* or blue mussels *M. edulis* (Bjerregaard et al., 2020)]. Likewise,
85 American lobsters (*Homarus americanus*) and rock crabs (*Cancer irroratus*) do not appear to
86 concentrate mercury compared to *M. edulis* (Chaudhary et al., 2020).
87 Sea stars are important predators in many benthic ecosystems and the common starfish *A.*
88 *rubens* may occur in high numbers in populations of blue mussels *Mytilus edulis/trossulus*
89 (e.g. Khaitov et al., 2018). *A. rubens* itself is preyed upon by fish and birds in coastal areas;
90 e.g. by the herring gull *Larus argentatus* (Bukacinska et al., 1996). Contaminants in sea stars
91 may also find their way into human food items because sea stars are harvested locally to be
92 used as a protein source in the breeding of pigs (Skovborg, 2018) and poultry (Afrose et al.,
93 2016).
94 *A. rubens* accumulates mercury from both water (Rouleau et al., 1993; Sorensen and
95 Bjerregaard, 1991) and from food in short-term experimental investigations (Bjerregaard et al.,
96 2018) and so does the starfish *Leptasterias polaris* (Maheu and Pelletier, 1994; Pelletier and
97 Larocque, 1987; Rouleau et al., 1995a, b) in different experimental settings. It is not known if
98 prolonged exposure to methylmercury and inorganic mercury in the food will lead to trophic

magnification [here defined as increase in concentration from one step in the food chain to the next] of mercury in *A. rubens*.

The present study was initiated to investigate accumulation of methylmercury and inorganic mercury from food into the tissues of *A. rubens* with the aim of elucidating if exposure leads to mercury concentrations in the starfish exceeding the concentrations in the food.

2. Materials and methods

2.1. Experimental specimens

Sea stars *Asterias rubens* (body weight 44 ± 16 g) and blue mussels *Mytilus edulis* (shell length 4.8 ± 1.8 cm) for the exposure experiment were collected in Lille Bælt at Middelfart, Funen, Denmark, in late September.

For the investigation of the background concentrations of total mercury, ten specimens (body weight 8.7±1.6 g) of *A. rubens* were collected from Kerteminde, Funen, Denmark in January.

None of the two sites have any known history with regard to mercury contamination.

2.2. Animal husbandry

The experiments were carried out at Bøgebjerg Marine Biological Station situated next to Great Belt at the north-eastern coast of the island of Funen, Denmark. Great Belt water was pumped into the station to allow a high throughput of running water to the tanks. Salinity in the Great Belt water varies between approximately 12 and 28 ‰, depending on prevailing wind and stream. The locations at which the animals were collected show the same variability in salinity.

2.3. Exposure experiment

122 *2.3.1. Preparation of food*

123 Mussels *M. edulis* were exposed to 16 µg Hg-CH_3HgCl L^{-1} (purity not given) and 104 µg
124 Hg-$HgCl_2$ L^{-1} (purity ≥ 99.5%; both from Sigma-Aldrich, Denmark) for 7 days. The mussels
125 were held in glass aquaria, each containing 6 L sea water. Water in the aquaria was replaced
126 every day. After the exposure, the mussels were frozen immediately for further preparation.

127 To obtain a homogenous source of food, soft parts of the Hg-exposed mussels were
128 homogenized in a food processor (Waring Commercial Blender). Forty g of commercial gelatin
129 were dissolved to 100 mL with tap water and added to 500 mL mussel homogenate (heated and
130 stirred). The mixture was cooled in a grid, yielding solid cubes of 1.55±0.12 g with 430±185
131 ng Hg-CH_3HgCl and 4913±760 ng Hg-$HgCl_2$ g^{-1} wet wt (mean±SD). The food blocks were
132 frozen at -18 ºC and stored at this temperature until use. Before the food blocks were fed to the
133 sea stars, they were thawed for one hour. The food blocks were ingested by the sea stars without
134 visible loss of material to the seawater.

135

136 *2.3.2. Exposure procedure*

137 Ninety sea stars were selected and kept individually in separate 1.8 L polystyrene aquaria or
138 2.0 L PVC aquaria. Running sea water and aeration were provided in each aquarium. Water
139 temperature decreased from 11°C to 6.8°C during the exposure period (October 10 to December
140 19). Salinity was 12 ± 7‰.

141 The sea stars were starved for 34 days prior to the experiment. Then each sea star received
142 one contaminated food block three times a week for 7 weeks. Six specimens were dissected
143 after 0 (not exposed), 7, 14, 21, 28, 35, 42 and 49 days of exposure. Coelomic fluid and samples

144 of soft tissue (pyloric caeca, stomach, gonads, tube feet and aboral body wall) were taken from
145 each specimen; the endoskeleton was not included. The tissue samples were frozen at -18°C.

146 *2.3.3. Elimination procedure*

147 The elimination of mercury was studied during the following 5 months (December to May)
148 in the remaining 42 sea stars. During the elimination period, live blue mussels (5.6±0.4 cm)
149 were placed in the aquaria to allow the sea stars to eat *ad libitum.* Six specimens were collected
150 after 2, 5, 9, 21, 41, 71 and 141 days in the elimination period, respectively, and treated as the
151 animals in the exposure experiment. Each specimen was weighed at the initial day in the
152 elimination period and at the day of dissection. Water temperature in the elimination period
153 varied between 4°C and 8°C.

154 *2.4. Determination of natural background concentrations of total mercury*

155 The animals were dissected into stomach, pyloric caeca, aboral body wall, tube feet, gonads
156 and rest. The dissection was carried out on pre-weighed paper towel and the amount of coelomic
157 fluid lost during the dissection was estimated from the weight increase of the paper. The rest
158 was constituted mainly by the calcareous ambulacral plates (endoskeleton). The tissues were
159 weighed, frozen and freeze dried for subsequent determination of concentrations of total
160 mercury.

161 *2.5. Chemical analyses*

162 *2.5.1. Exposure experiment*

163 Methylmercury and inorganic mercury were determined by means of the stepwise and
164 selective reduction Cold Vapour Atomic Absorption Spectrometry method described by Oda
165 and Ingle (1981) and used by Riisgard and Hansen (1990) and Bjerregaard et al. (1999) at our

166 department. We used the same analytical equipment as Riisgard and Hansen and tests of the
167 reliability of the method are thoroughly described by Riisgard and Hansen (1990). The limit of
168 detection was approximately 15 ng Hg g^{-1} ww for both inorganic mercury and methylmercury
169 – depending on the amount of tissue available.

170 *2.5.2. Natural background concentrations*

171 Total mercury was determined by means of a Milestone DMA-80 Direct Mercury Analyser
172 as recommended in product instructions. Up to 50 mg freeze dried tissue were weighed and
173 placed in the sample boats. Samples were heated to 650ºC and the evaporated mercury was
174 amalgamated on a gold filter, which was subsequently heated to 800ºC to release the
175 amalgamated mercury. The absorption at 253.65 nm of the released mercury was determined
176 in a quartz cuvette to quantify mercury (related to standards with known amounts on mercury).
177 The quality of the determinations was validated using TORT-standards (lobster
178 hepatopancreas) with certified mercury contents from the National Research Council of Canada
179 (Institute for Environmental Chemistry, Ottawa, Canada); values were within ±10% of the
180 certified values. The detection limit (<0.2 ng Hg) was not challenged in any of the
181 determinations.

182 *2.6. Data handling and statistical treatment*

183 During the uptake phase, data for concentrations in the tissues and whole animal soft parts
184 were fitted to either linear ($y=a*x+b$) or polynomial ($y=a*x^2+b*x+c$) equations with y = [Hg]
185 and x = time. During the elimination phase, data for concentrations in the tissues and whole
186 animals were fitted to either exponential ($y=P*e^{a*x}$), biexponential ($y=P*e^{a*x} + Q*e^{b*x}$) [with or
187 without residuals] or polynomial ($y=a*x^2+b*x+c$) equations. Half-lives for mercury were
188 calculated from the elimination coefficient ($T_{½} = \ln 2/a$). Curve fitting was carried out in FigP.

189 In the feeding experiment, the total body mercury content was calculated as the sum of the
190 contents in the soft tissues (thus omitting the calcareous endoskeleton); retention in the
191 exposure period was calculated from the ingested amount of mercury and the amount remaining
192 in the soft tissues. Regression analyses were used in the statistical treatment of the data
193 (SYSTAT, version 13).

194 **3. Results**

195 *3.1. Exposure experiment*

196 During the experimental period (October 31st to May 1st) all the *Asterias rubens* specimens
197 appeared to be in good condition and there was no mortality during the experiments. During
198 the elimination phase, the sea stars dissected during day 5 to 21 in the elimination phase had
199 lost between 4 and 11 % of their body weight while the specimens dissected at day 41, 71 and
200 141 had gained between 15 and 23% of their body weight; since the variability in mercury
201 concentrations in the tissues was generally higher than these values, loss or gain in weight of
202 the entire animal were not considered in the presentation of the results.

203 *3.1.1. Tissue concentrations*

204 *3.1.1.1. Methylmercury*

205 Sea stars exposed to contaminated food accumulated methylmercury to detectable levels in
206 body wall, tube feet, stomach, pyloric caeca and gonads but not in the coelomic fluid (Fig. 1).
207 Sea stars accumulated methylmercury at initial rates of 65 and 19 ng Hg g^{-1} wet wt d^{-1} in pyloric
208 caeca and stomach, respectively; after 20-30 days of exposure the accumulation rate levelled
209 off in both tissues (Fig. 1A, B). Methylmercury only reached detectable levels (> 15 ng Hg g^{-1}
210 wet wt) in gonad and tube feet after 2- and 3-weeks exposure, respectively. Thereafter, gonads
211 and tube feet accumulated mercury at 7 and 0.6 ng Hg g^{-1} wet wt d^{-1} (Fig. 1C, D). The aboral

212 body wall accumulated mercury at a rate of 1.5 ng Hg g^{-1} wet wt d^{-1} (Fig. 1E). The analysis of
213 organic mercury for the 42d gonad sample was defective and therefore omitted.

214 Rather than losing methylmercury when the exposure via the food stopped, gonads, tube
215 feet and aboral body wall kept on accumulating methylmercury during the depuration phase
216 with uptake rates of 1.2, 1.6 and 0.2 ng Hg g^{-1} wet wt d^{-1} in body wall, tube feet and gonad,
217 respectively (Fig 1H, J, I). The pyloric caeca and stomach eliminated methylmercury during
218 the initial part of the depuration period with half-lives of 2.4 and 1.7 days, respectively (Fig.
219 1F, G.); later in the elimination phase, elimination took place more slowly ($T_{½}$: 8-22 d).

220 *3.1.1.2 Inorganic mercury*

221 Sea stars exposed to contaminated food accumulated inorganic mercury linearly with time
222 over 49 days in stomach and body wall at rates of 12 and 14 ng Hg g^{-1} wet wt d^{-1}, respectively
223 (Fig. 2A, E). The pyloric caeca accumulated inorganic mercury at an initial rate of 277 ng Hg
224 g^{-1} wet wt d^{-1}; after 21 days the accumulation rate levelled off (Fig 2B). The gonad and tube
225 feet accumulated inorganic mercury at an initial rate of 55 and 14 ng Hg g^{-1} wet wt d^{-1}; after 28
226 days the accumulation rate increased further (Fig. 2C, D).

227 The sea stars continued uptake of inorganic mercury in stomach, body wall and tube feet
228 during the elimination period with uptake rate of 6, 7 and 6 ng Hg g^{-1} wet wt d^{-1}, respectively;
229 after 60 days the mercury concentration attained a plateau and remained more stable (Fig. 2F,
230 I, J). After an initial rapid loss over the first few days, the gonad eliminated inorganic mercury
231 with a rate of 3 ng Hg g^{-1} wet wt d^{-1} and a half-life of 1300 days (Fig. 2H). The pyloric caeca
232 eliminated inorganic mercury immediately after the beginning of the elimination period (Fig.
233 2G) and elimination can be described by a two-compartment model with half-lives of 18 and
234 224 days, respectively (Fig. 2G).

235 *3.1.2. Tissue:food ratios during uptake phase*

236 The pyloric caeca accumulated both methylmercury (Fig. 3A) and inorganic (Fig. 3B) to
237 concentrations exceeding the concentrations in the food. Since the other tissues accumulated
238 both mercury forms to lower levels than did the pyloric caeca (Fig. 3A, B), the concentrations
239 in the aggregated soft tissues did not exceed those in the food (Fig. 3C). At the end of the
240 exposure period, concentrations of inorganic mercury in the aggregated soft tissues were
241 approximately ¼ of the concentration in the food while the concentration of methylmercury
242 reached approximately ½ of the concentration in the food (Fig. 3C).

243 *3.1.3. Assimilation and retention of mercury*

244 Assimilation efficiency for ingested inorganic mercury could be estimated to 77% (Fig. 4A)
245 while all of the ingested methylmercury was assimilated (Fig. 4B). During the exposure period,
246 inorganic mercury (Fig. 4A) and methylmercury (Fig. 4B) were retained with half-lives of 45
247 and 107 days, respectively. During the elimination phase, inorganic mercury was eliminated
248 with a half-life of 81 days (Fig. 4C). During the elimination phase, the concentrations of
249 methylmercury showed a fairly high variability; elimination could be described by a two-
250 compartment model with half-lives of 8.4 and 173 days, respectively (Fig. 4D).

251 *3.1.4. Hg tissue distribution*

252 During the uptake phase, 80-90% of both the accumulated methylmercury (Fig. 5A) and
253 inorganic (Fig. 5B) mercury were located in the pyloric caeca. During the elimination phase,
254 most of the body burden of both forms was still present in the pyloric caeca, but the percentage
255 decreased – especially for inorganic mercury. The percentage of the body burden present in the
256 aboral body wall increased during the elimination period for both mercury forms.

257 *3.2. Background levels of total mercury*

258 The concentrations of total mercury in sea stars collected from a site with no known history
259 of mercury contamination showed a high variability (Fig. 6A). Average concentrations were
260 approximately 60 ng Hg g^{-1} wet wt in stomach, pyloric caeca and gonad, 30 ng Hg g^{-1} wet wt
261 in tube feet and 20 ng Hg g^{-1} wet wt in aboral body wall and rest. Rest (mainly endoskeleton),
262 pyloric caeca and aboral body wall contained most of the body burden of total mercury (Fig.
263 6B).

264 **4. Discussion**

265 The main conclusion from this investigation is that no trophic magnification of mercury
266 takes place at the transition from mussels at trophic level ~ 2 to sea stars at trophic level ~ 3.

267 Individual tissues (pyloric caeca and partly stomach) concentrate inorganic mercury as well
268 as methylmercury to concentrations higher than the levels in the food but from the point of view
269 of the entire organism, neither of the mercury forms are magnified relative to the food. Although
270 the term biomagnification is normally used to describe increases between trophic levels for
271 entire organisms (Nordberg et al., 2009) some authors also use the term when they consider
272 concentrations in individual tissues (e.g. cadmium in kidneys of Arctic mammals (Dehn et al.,
273 2006) and several metal(loid)s in ringed seal (*Phoca hispada*)/polar bear (*Ursus maritimus*)
274 liver, kidney or muscle (Woshner et al., 2001)). The latter use may be relevant if predators prey
275 on selected tissues rather than entire organisms; in most cases entire organisms are selected for
276 prey by their predators. Starfish prey on the soft parts of mussels and other molluscs so the
277 relevant consideration regarding magnification is to compare the concentrations in the sea stars
278 with the concentrations in the soft parts of the molluscs, thus excluding the shells.

279 Ingestion of mussels in *Asterias rubens* is normally initiated with a protrusion of the stomach
280 between the shells of the bivalve where digestive enzymes are secreted to partly digest the soft

281 parts of the mussels. Extracellularly digested particles are transported by ciliary activity from
282 the stomach into the pyloric caeca where digestion continues intracellularly in the diverticula
283 (Jangoux, 1982). Nutrients are transported from the pyloric caeca to the growing gonads via the
284 haemal system of the coelomic cavity (Plas et al., 1983). *A. rubens* in Northern Europe spawn
285 in June-July and the gonads gradually increase in size during the period from August until May
286 concomitantly with a decrease in the size of the pyloric caeca (Plas et al., 1983). It is consistent
287 with this knowledge on food handling processes in *A. rubens* that the major body burden of
288 both methylmercury and inorganic mercury after uptake from the food for seven weeks is found
289 in the pyloric caeca. Since the experiment was carried out in the period in which the gonads
290 grow due to transfer of nutrients from the pyloric caeca it also makes sense that concentrations
291 of both inorganic mercury and methylmercury increase slowly in the gonads during the
292 exposure phase and for methylmercury continues to do so also in the elimination phase. Both
293 mercury forms are also transported from the pyloric caeca to the tube feet and the aboral wall -
294 during the elimination phase especially to the aboral body wall. The stone canal system may be
295 involved in the transport of mercury to the tube feet but the precise function of the various parts
296 of the sea stars' complicated circulatory system is not fully elucidated (Ezhova et al., 2013). It
297 is apparent that the amount of accumulated mercury is not at equilibrium between the tissues at
298 the end of the elimination (Fig. 4A-B) period and the distribution between organs appears to
299 approach the distribution of total mercury in the background sea stars. Only the soft parts of the
300 sea stars were analysed in the laboratory experiment on the assumption that mercury would not
301 be incorporated in the endoskeleton in the – compared to the life span of the sea stars – relatively
302 short exposure period. Judged from the fact that the content in the soft parts accounted for
303 approximately 100% of the ingested mercury, this assumption appears to be true. However,
304 mercury seems to be incorporated into the endoskeleton under natural conditions, since the

305 'rest' mainly consisting of the ambulacral calcareous plates contained detectable amounts of
306 mercury. Likewise, Pelletier and Larocque (1987) demonstrated uptake of mercury in the
307 calcareous skeleton of the sea star *Leptasterias polaris* after exposure to methylmercury
308 exposed mussels; uptake rates were lower in the calcareous skeleton than in pyloric caeca and
309 stomach but since the calcareous skeleton constituted 58% of the body weight, the skeleton
310 contained a considerable amount of the mercury body burden (Pelletier and Larocque, 1987).
311 The accumulation of methylmercury to higher levels relative to the food levels than
312 inorganic mercury in starfish is consistent with information from other groups of animals
313 (Riisgard and Famme, 1986; Riisgard and Hansen, 1990) and so is the more rapid elimination
314 of inorganic mercury than methylmercury (Bjerregaard et al., 2011; Larsen and Bjerregaard,
315 1995; Pentreath, 1976a, c, d). The half-life for methylmercury in *A. rubens* found in the present
316 experiment is considerably shorter than found in most fish species (Pentreath, 1976b, c, d;
317 Ruohtula and Miettinen, 1975; Tillander et al., 1969). Elimination of the two mercury forms
318 from the sea stars was examined through winter and spring conditions in the present experiment.
319 The rate of biochemical and other biological processes generally increases with temperature in
320 poikilothermic animals (Schmidt-Nielsen, 1975) and the possibility exists that elimination
321 would occur faster at higher temperatures during summer. The feeding rates for *A. rubens* on
322 mussels increased with temperature at temperatures between 1ºC and 12ºC (Aguera et al., 2012)
323 and St-Pierre and Gagnon (2015) demonstrated approximately identical feeding rates (also on
324 mussels) at 11ºC and 15ºC but only 64% and 38% of this feeding rate at than at 5ºC and 2ºC,
325 respectively. Although both accumulation and elimination rates might be hypothesized to be
326 higher during summer than shown in the present autumn-winter-spring experiments, the half-
327 life of 181 d for methylmercury determined at 10ºC (Bjerregaard et al., 2018) was similar to the
328 half-life (173 d) in the slowly exchanging compartment at 4ºC to 8ºC in the present experiment.

329 Whereas benthic predators in field investigations can be assumed to be in a steady state
330 regarding bioaccumulation of methylmercury from their food, this may or may not be the case
331 in laboratory experiments and the extent of trophic transfer can only be predicted if steady state
332 has been obtained. The shrimp *Pandalus borealis* appears to enter a steady state (approximately
333 1.5 μg Hg g^{-1} wet weight) regarding the mercury content in the food (mussels with 6 μg Hg g^{-1}
334 wet weight) within 10 days (Rouleau et al., 1992). It is not quite clear from the data if
335 equilibrium has been reached after 4 week's feeding of the brown shrimp *Crangon crangon*
336 with soft parts of mussels collected at a mercury contaminated site (Riisgard and Famme, 1986;
337 NOTE! the two figures in the paper have been switched around). Results for the shore crab
338 *Carcinus maenas* during 1 month's exposure to methylmercury in their food vary as to whether
339 or not steady states are obtained – but all with methylmercury concentrations in the predator
340 considerably lower than in the food items (Bjerregaard and Christensen, 1993; Larsen and
341 Bjerregaard, 1995). In the present investigation on sea stars, steady state was reached for both
342 methylmercury and inorganic mercury after approximately 1 month's exposure.
343 The lack of trophic magnification of mercury in *A. rubens* demonstrated in the present
344 laboratory experiments was corroborated by the findings in the background sea stars. Brügmann
345 and Lange (1988) reported mercury concentrations in *A. rubens* from the Western Baltic in the
346 range 17-163 (mean 60±22) ng Hg g^{-1} dry weight (dw), comparable to the results of the present
347 investigation (79±24) ng Hg g^{-1} dw. Soft parts of the potential prey items blue mussels *Mytilus*
348 *edulis* and periwinkles *Littorina littorea* collected at the same location as the background sea
349 stars of the present investigation contained 12.2±1.04 (n=28) and 13.3±0.5 (n=28) ng Hg g^{-1}
350 ww, respectively (Bjerregaard et al., 2020) – also indicating lack of trophic magnification in
351 the sea stars with 15±5 ng Hg g^{-1} ww. Thus, sea stars apparently do not concentrate mercury to
352 higher concentrations than found in the food and this also appears to be the case for another

353 benthic invertebrate predator, the shore crab *C. maenas* which in field investigations did not
354 show highly elevated mercury concentrations compared to potential prey items: Coelho et al.
355 (2013) found 1330 ng Hg/g dw in the muscle of the shore crab and 900 and 2080/670 ng Hg,
356 respectively, in the potential prey items the polychaete *Hediste diversicolor* and the clam
357 *Scrobicularia plana*. Bjerregaard et al. (2020) also found lower mercury concentrations in the
358 soft parts of *C. maenas* than in their potential prey items the periwinkle *L. littorea* and the blue
359 mussel *M. edulis,* both at contaminated sites and sites with no history of mercury contamination.
360 If the lack of trophic magnification at the lower – as opposed to the higher - trophic levels is
361 a phenomenon that extends beyond sea stars and decapod crustaceans, the interesting question
362 is of course which mechanism is underlying this. To try to answer this question, it is relevant
363 to look at differences and similarities in key processes [1) assimilation efficiency for the metal
364 content in the food, 2) uptake directly from the water phase and 3) retention efficiency for the
365 accumulated contaminant - often described by the half-life in the organism] between sea
366 stars/decapod crustaceans on the one hand side and fish on the other.
367 Ad 1) Assimilation efficiencies for ingested methylmercury in fish are generally reported to be
368 high [e.g 96.6% in thornback ray *Raja clavata* (Pentreath, 1976b), 89% in plaice,
369 *Pleuronectes platessa* (Pentreath, 1976c) 84% in rainbow trout *Salmo gairdneri* (Boudou
370 and Ribeyre, 1985) and 77% in lake trout, *Salvelinus namaycush* (Madenjian et al., 2012)]
371 which are similar to assimilation efficiencies in most decapod crustaceans [72% and 76%
372 in pink shrimp, *Penaeus duorarum* and blue crabs, *Callinectes sapidus*, respectively
373 (Evans et al., 2000), 76% in brown shrimps *C. crangon* (Riisgard and Famme, 1986), >
374 89% in snow crabs, *Chionoecetes opilio* (Rouleau et al., 1999); 42% in Nordic shrimps,
375 *Pandalus borealis* (Rouleau et al., 1992), 50-80% in shore crabs *C. maenas* (Bjerregaard
376 and Christensen, 1993; Larsen and Bjerregaard, 1995)] and sea stars (50±10% in *L.*

377 *polaris* (Pelletier and Larocque, 1987); ~ 100% in the present study). Reported values for
378 assimilation efficiencies for inorganic mercury in fish appear to be more variable with
379 values of 11% in plaice, *P. platessa* (Pentreath, 1976c), 14.3% in thornback ray, *R. clavata*
380 (Pentreath, 1976b), 23% in rainbow trout, *S. gairdneri* (Boudou and Ribeyre, 1985) and
381 63.5% in lake trout, *S. namaycush* (Madenjian et al., 2012); Trudel and Rasmussen (1997)
382 conclude that assimilation efficiency from food in fish is 5 to 10 times higher for
383 methylmercury than for inorganic mercury. Among decapods some variability is reported
384 [4% in brown shrimp, *C. crangon* (Riisgard and Famme, 1986), 60-90% in snow crabs, *C.*
385 *opilio* (Rouleau et al., 1999), 50-70% in shore crabs, *C. maenas* (Bjerregaard and
386 Christensen, 1993)]. Sea stars assimilate 60-90% (Pelletier and Larocque, 1987) and 77%
387 (present study) of inorganic mercury from their food.
388 Ad 2) Accumulation in predators of trace metals directly from the water phase in
389 uncontaminated areas with only background concentrations (typically in the low ng L^{-1}
390 range) may be insignificant compared to accumulation from the content in the prey; this
391 has been shown for methylmercury in a freshwater fish, fine scale dace (*Phoxinus*
392 *neogaeus*) (Hall et al., 1997). Information on this for mercury in decapods or sea stars is
393 not available, but for another trace metal, cadmium, uptake directly from water in shore
394 crabs, *C. maenas* is insignificant compared to the uptake from food (Bjerregaard et al.,
395 2005).
396 Ad 3) Laboratory experiments show that methylmercury may be retained very efficiently in fish
397 – especially in larger fish – with half-lives of more than one year (summarized by Trudel
398 and Rasmussen, 1997) and Madenjian et al. (2021) suggest that results obtained in the
399 laboratory actually may underestimate real retention times in the field. Most
400 investigations of retention of methylmercury in decapod crustaceans also show half-lives

401 in the range of 1 to 2 years (Bjerregaard and Christensen, 2012; Bjerregaard et al., 2018;
402 Evans et al., 2000; Fowler et al., 1978; Headon et al., 1996; Larsen and Bjerregaard, 1995;
403 Miettinen et al., 1972; Rouleau et al., 1999; Tillander et al., 1969). Sea stars eliminate
404 methylmercury faster [$T_{½}$ = 173 to 181 d present study and (Bjerregaard et al., 2018)]
405 than both fish and decapods. Inorganic mercury is generally retained less efficiently in
406 fish than methylmercury with 3-fold higher excretion rates (Trudel and Rasmussen, 1997)
407 and decapod crustaceans also show lower half-lives for inorganic mercury than for
408 methylmercury [$T_{½}$ = 112 d in the shrimp *Lysmata seticaudata* (Fowler et al., 1978) and
409 56 days in the shore crab *C. maenas* (Larsen and Bjerregaard, 1995)].
410
411 Obvious differences in these key processes between fish and decapods/sea stars could not
412 be identified and one potential – and probably the most plausible – explanation for the lack of
413 trophic magnification in the latter could be that inorganic mercury with its shorter retention
414 times plays a more important role at the lower trophic levels than methylmercury.
415 Biomagnification in marine and freshwater food chains is often seen to begin in fish and
416 typically the ratio of methylmercury to inorganic mercury increases with trophic level, ending
417 at 80-100% methylmercury at the highest levels (Bernhard, 1985; Bowles et al., 2001). The
418 biomagnification in fish is normally explained by the high assimilation efficiency and long
419 retention time for methylmercury; inorganic mercury ingested with the food is assimilated in
420 fish with lower efficiency and the retention time is much shorter. Whereas methylmercury
421 constitutes the major mercury form in the food of fish high in the food chain, this is not the case
422 in the food for sea stars and shore crabs: Italian (Di Leo et al., 2010) and French (Briant et al.,
423 2017) investigations show that inorganic mercury generally constitutes more than two thirds of
424 the total mercury in blue mussels (49-87%). Although this inorganic mercury is assimilated

425 fairly efficiently from food in sea stars (77%) and shore crabs (60-70%), it is also lost relatively
426 quickly with half-lives in sea stars and shore crabs of 45-81 d and 56 d (Larsen and Bjerregaard,
427 1995), respectively.

428 With assimilation efficiencies for methylmercury between 50 and 70% and half-life > 2
429 years, magnification patterns for total mercury in the shore crab *C. maenas* might be
430 hypothesized to be similar to those in predatory fish higher in the food chain. However,
431 although benthic invertebrates are the preferred food items for the shore crab, it also has an
432 opportunistic, omnivorous feeding behaviour including food items at trophic level 1 such as
433 macroalgae (Baeta et al., 2006) and aquatic plants, e.g. eelgrass (Malyshev and Quijon, 2011).
434 Methylmercury's proportion of the total mercury is generally much lower in macroalgae (1.6%
435 in *Fucus*; Coquery et al., 2000) and eelgrass (6.5%; Morrison and Weber, 1997) than in
436 invertebrates which further emphasizes the role of inorganic mercury in the mercury
437 accumulation pattern in the shore crab.

438 The sea star *A. rubens* eliminates methylmercury considerably faster than both larger fish
439 and decapod crustaceans which will make trophic magnification less likely – even if
440 methylmercury is assimilated from the food.

441 **5. Conclusion**

442 Both laboratory experiments and field investigations indicate that concentrations of both
443 methylmercury and inorganic mercury in the sea stars *A. rubens* do not exceed the
444 concentrations in the food items. This lack of trophic magnification is suggested to be the result
445 of the relatively fast (compared to decapod crustaceans and larger fish) elimination of
446 methylmercury by the sea stars and, maybe more importantly, because inorganic mercury with
447 its faster elimination constitutes a larger fraction of the total mercury in the food at the lower
448 trophic levels - as opposed to methylmercury which dominates at the higher trophic levels.

Declaration of competing interests

The authors declare no conflict of interests.

CRediT authorship contribution statement

Poul Bjerregaard: Conceptualization, Writing – original draft, Writing review & editing, Formal analysis. **Lise M. Møller:** Investigation, Formal analysis.

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657

658

659 **Figure legends**

660 Figure 1. Concentrations of methylmercury in the tissues of starfish fed mussel homogenate
661 containing 430±185 ng Hg-CH_3HgCl and 4913±760 ng Hg-$HgCl_2$ g^{-1} wet wt for 7 weeks (A-
662 E) and subsequently fed uncontaminated food for 20 weeks (F-J). Mean±SEM for six animals.
663 The dotted horizontal line indicates the detection limit.

664

665 Figure 2. Concentrations of inorganic mercury in the tissues of starfish fed mussel homogenate
666 containing 430±185 ng Hg-CH_3HgCl and 4913±760 ng Hg-$HgCl_2$ g^{-1} wet wt for 7 weeks (A-
667 E) and subsequently fed uncontaminated food for 20 weeks (F-J). Mean±SEM for six animals.

668

669 Figure 3. Ratio for mercury in starfish relative to food. A: Methylmercury in the tissues. B:
670 inorganic mercury in the tissues. Curve fits from Fig. 1 and 2 are shown. C: Methylmercury (○)
671 and inorganic mercury (□) in aggregated soft tissues. Mean±SEM for six animals in C.

672

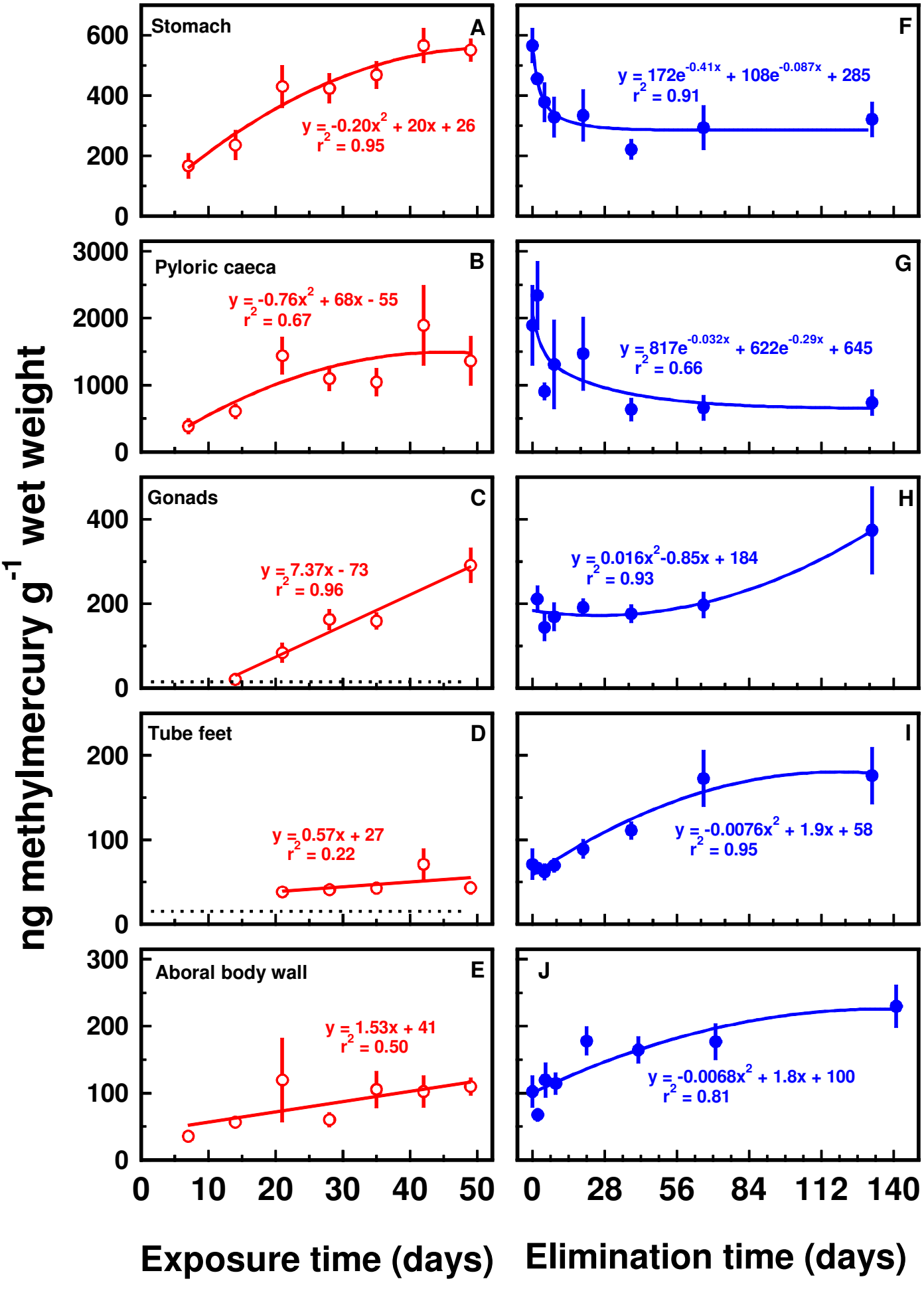
673 Figure 4. Retention of inorganic mercury (□) and methylmercury (○) during uptake (A & B)
674 and elimination (C & D) phase. Mean±SEM for six animals.

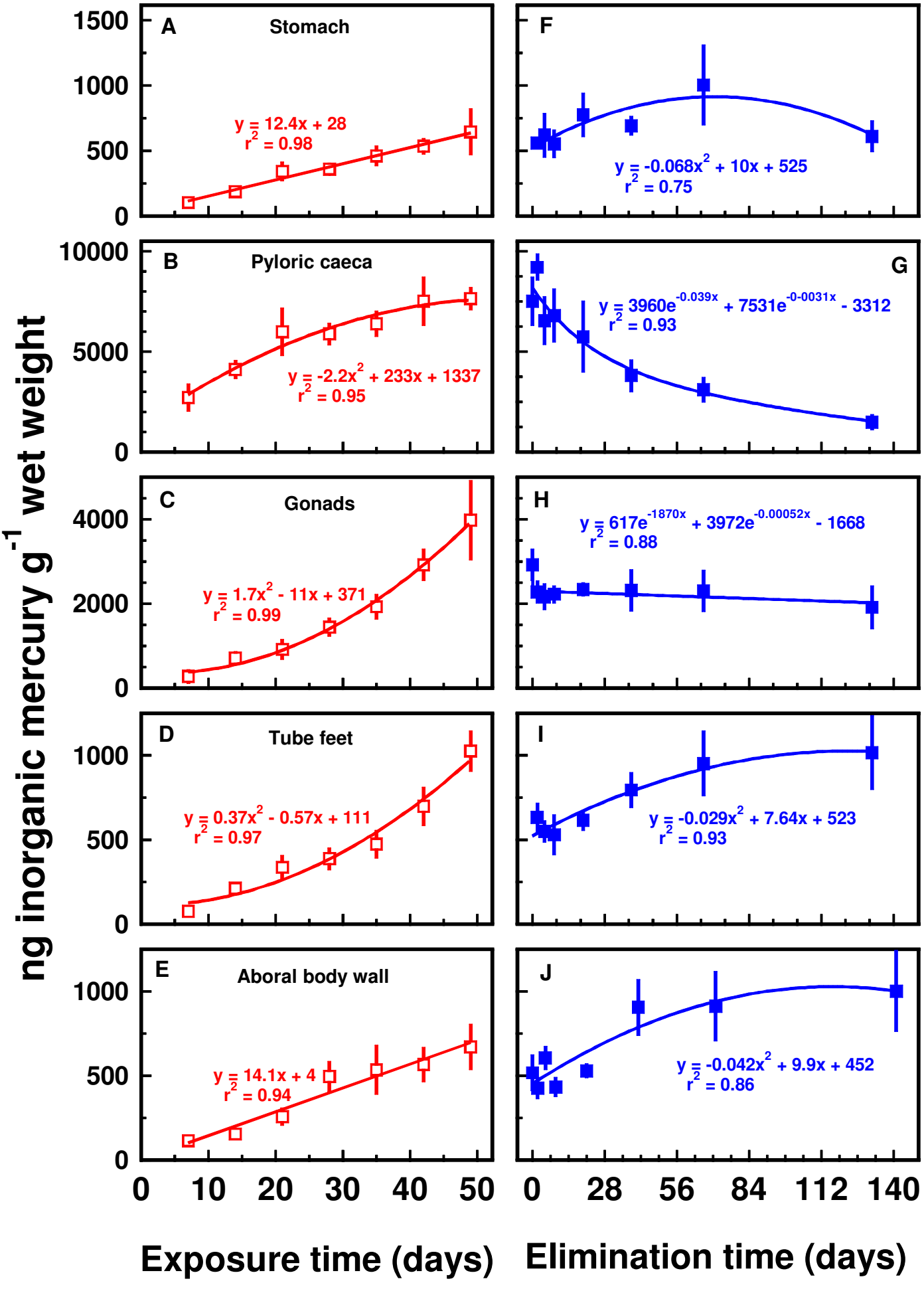
675

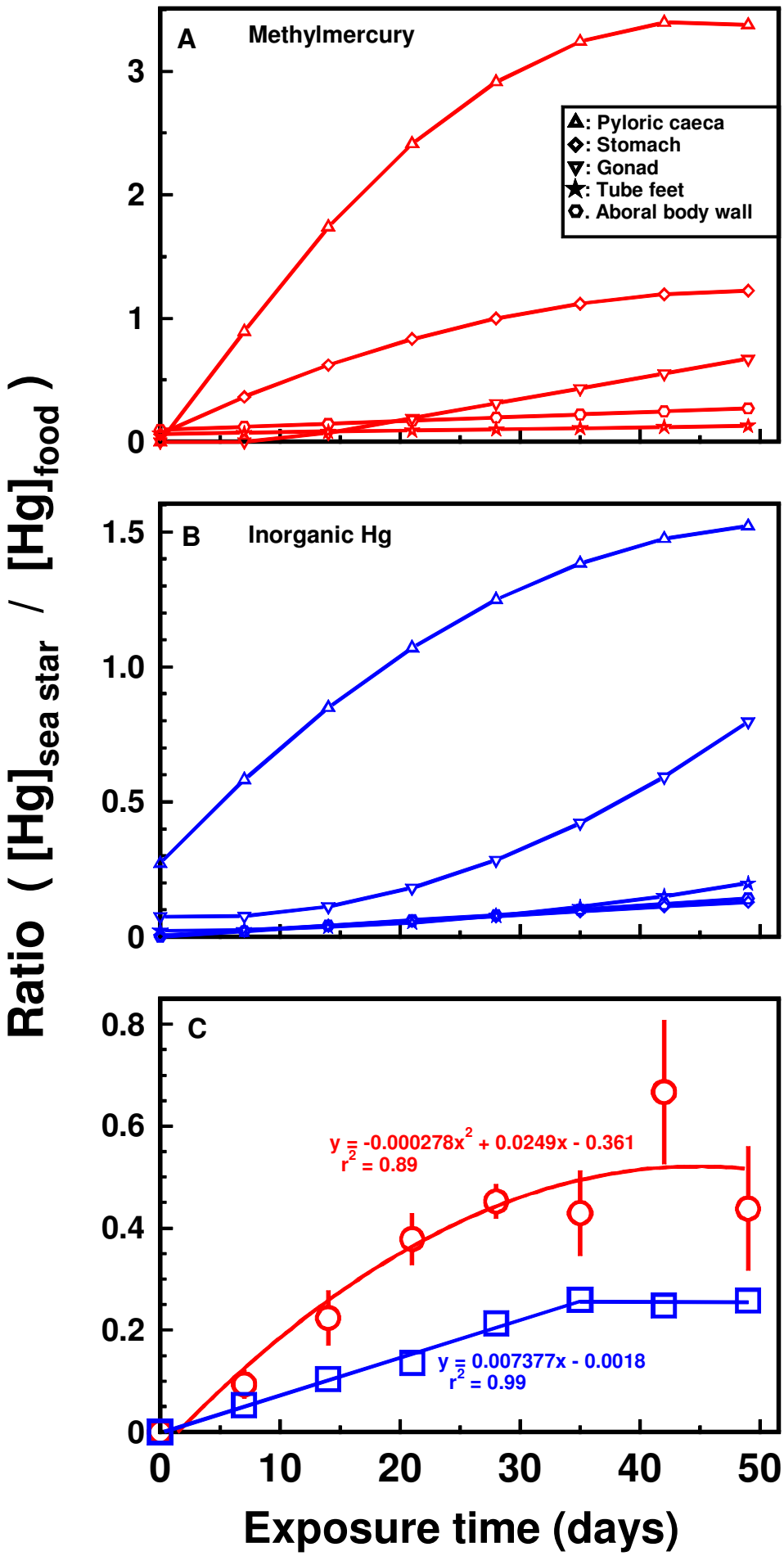
676 Figure 5. Distribution of methylmercury (A) and inorganic mercury (B) among the tissues
677 during uptake and elimination phase. C: Concentrations of inorganic mercury (□) and methyl
678 mercury (○) in the aggregated soft tissues.

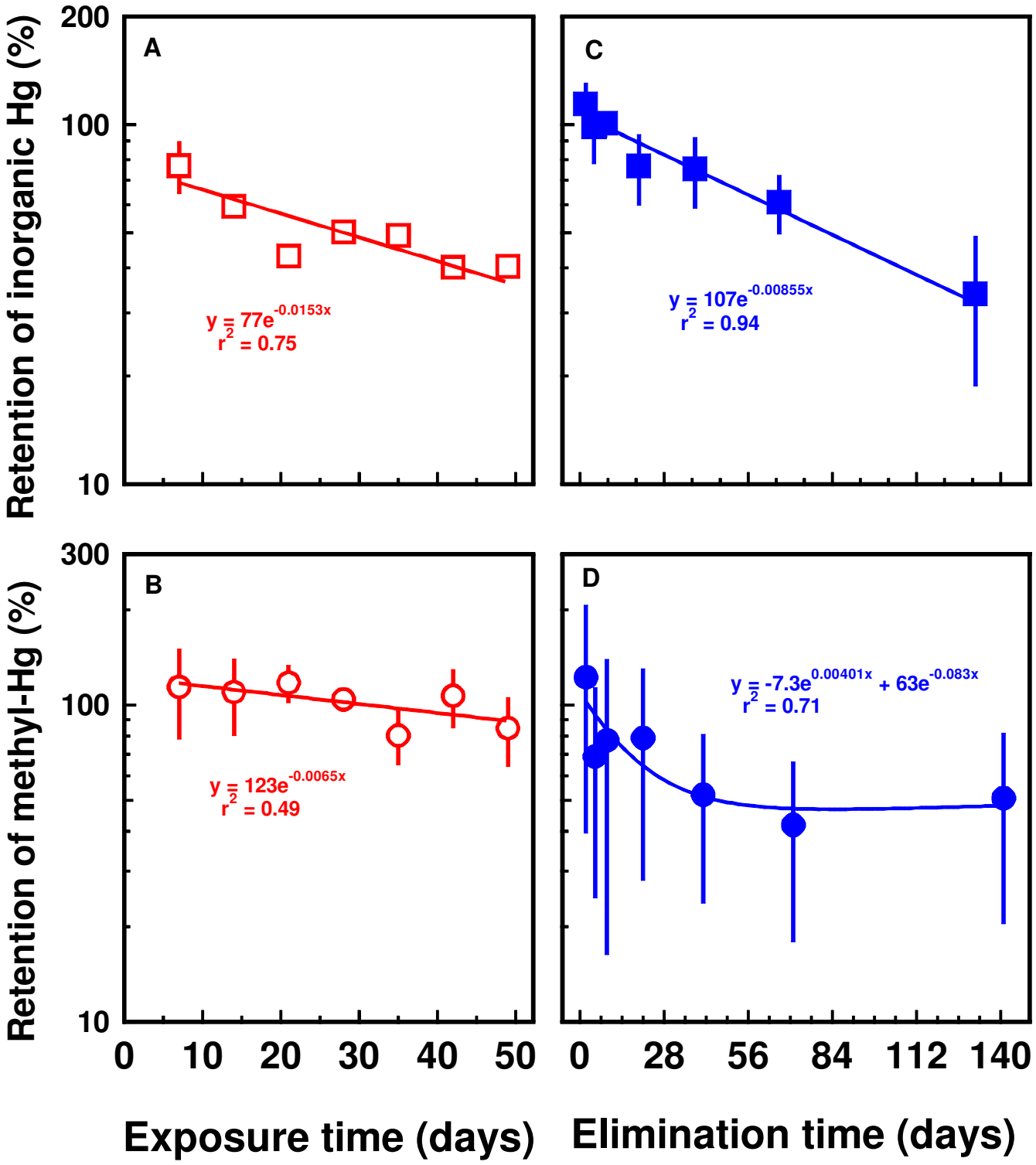
679

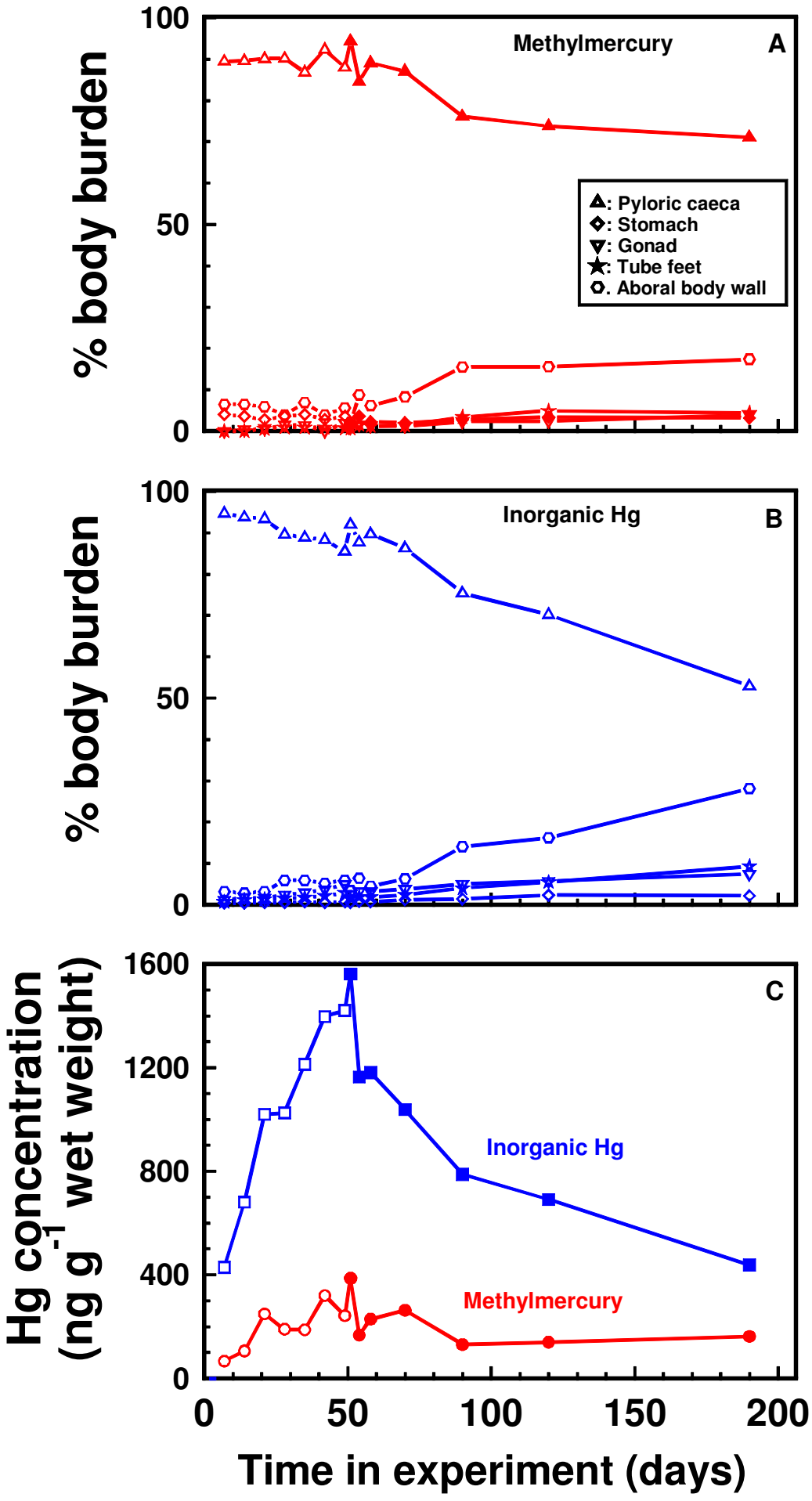
680 Figure 6. Concentrations (A) and distribution of total body burden (B) of total mercury in the
681 tissues of sea stars collected at an uncontaminated site.

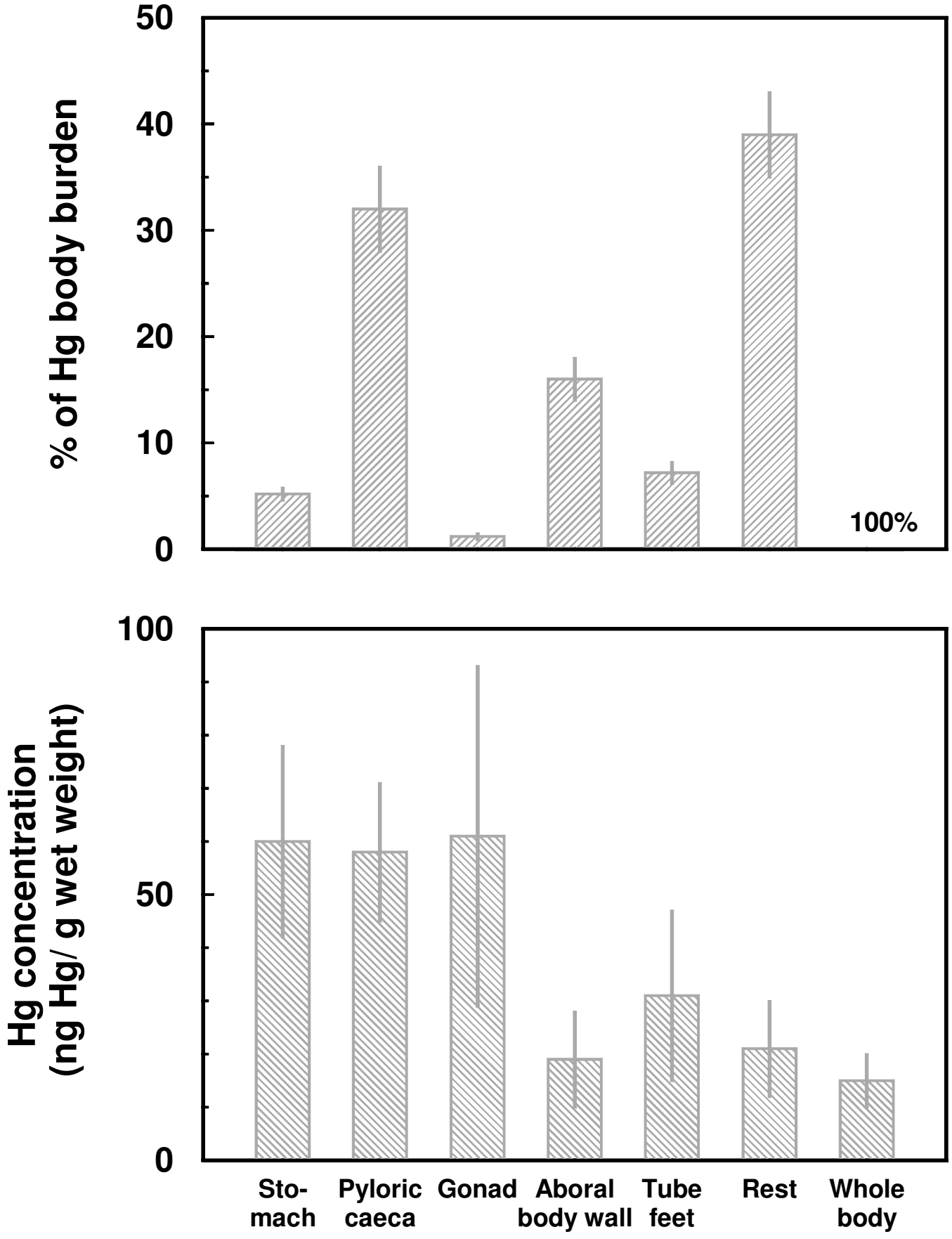

Stomach
A
y = -0.20x2 + 20x + 26
r2 = 0.95
F
y = 172e-0.41x + 108e-0.087x + 285
r2 = 0.91
Pyloric caeca
B
y = -0.76x2 + 68x - 55
r2 = 0.67
G
y = 817e-0.032x + 622e-0.29x + 645
r2 = 0.66
Gonads
C
y = 7.37x - 73
r2 = 0.96
H
y = 0.016x2-0.85x + 184
r2 = 0.93
Tube feet
D
y = 0.57x + 27
r2 = 0.22
I
y = -0.0076x2 + 1.9x + 58
r2 = 0.95
Aboral body wall
E
y = 1.53x + 41
r2 = 0.50
J
y = -0.0068x2 + 1.8x + 100
r2 = 0.81
ng methylmercury g-1 wet weight
Exposure time (days)
Elimination time (days)

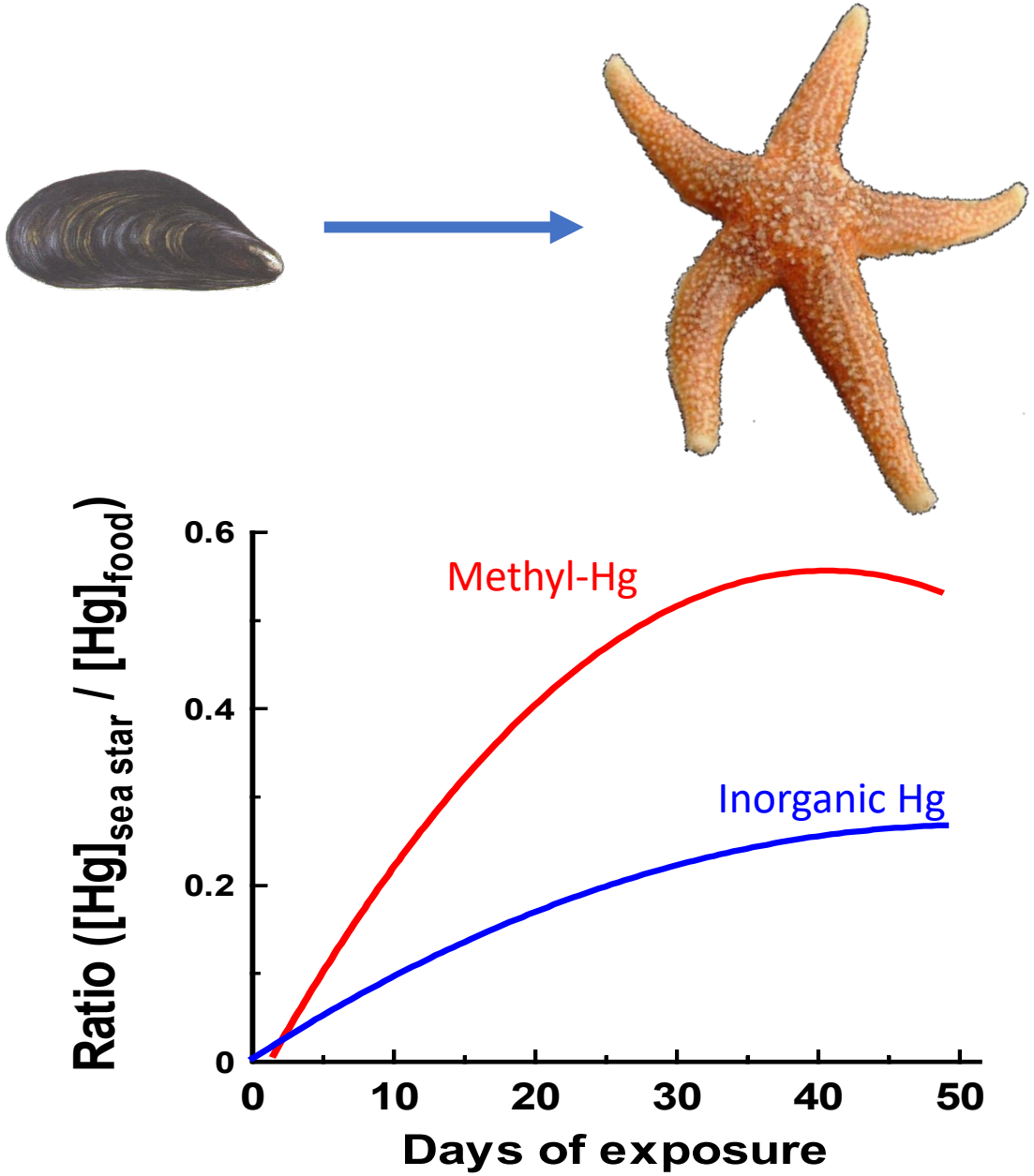

A
Stomach
y = 12.4x + 28
r2 = 0.98
F
y = -0.068x2 + 10x + 525
r2 = 0.75
B
Pyloric caeca
y = -2.2x2 + 233x + 1337
r2 = 0.95
G
y = 3960e-0.039x + 7531e-0-0031x - 3312
r2 = 0.93
C
Gonads
y = 1.7x2 - 11x + 371
r2 = 0.99
H
y = 617e-1870x + 3972e-0.00052x - 1668
r2 = 0.88
D
Tube feet
y = 0.37x2 - 0.57x + 111
r2 = 0.97
I
y = -0.029x2 + 7.64x + 523
r2 = 0.93
E
Aboral body wall
y = 14.1x + 4
r2 = 0.94
J
y = -0.042x2 + 9.9x + 452
r2 = 0.86
1500
1000
500
0
10000
5000
4000
2000
0 10 20 30 40 50
0 28 56 84 112 140
ng inorganic mercury g-1 wet weight
Exposure time (days)
Elimination time (days)


A
Methylmercury
▲: Pyloric caeca
◇: Stomach
▽: Gonad
★: Tube feet
○. Aboral body wall
B
Inorganic Hg
C
y = -0.000278x2 + 0.0249x - 0.361
r2 = 0.89
y = 0.007377x - 0.0018
r2 = 0.99
Ratio ([Hg]sea star / [Hg]food)
Exposure time (days)


Retention of inorganic Hg (%)
Retention of methyl-Hg (%)
200
100
10
300
100
10
A
B
C
D
y = 77e^-0.0153x
r^2 = 0.75
y = 123e^-0.0065x
r^2 = 0.49
y = 107e^-0.00855x
r^2 = 0.94
y = -7.3e^0.00401x + 63e^-0.083x
r^2 = 0.71
0
10
20
30
40
50
0
28
56
84
112
140
Exposure time (days)
Elimination time (days)


Methylmercury
A
% body burden
100
50
0
: Pyloric caeca
: Stomach
: Gonad
: Tube feet
. Aboral body wall
Inorganic Hg
B
% body burden
100
50
0
C
Hg concentration (ng g-1 wet weight)
1600
1200
800
400
0
Inorganic Hg
Methylmercury
0
50
100
150
200
Time in experiment (days)


% of Hg body burden
50
40
30
20
10
0
100%
Hg concentration
(ng Hg/ g wet weight)
100
50
0
Sto-
mach
Pyloric
caeca
Gonad
Aboral
body wall
Tube
feet
Rest
Whole
body


Methyl-Hg
Inorganic Hg
Ratio ([Hg]sea star / [Hg]food)
0.6
0.4
0.2
0
0
10
20
30
40
50
Days of exposure