Title: Mercury distribution with size between the tissues of the northern quahog (= hard clam) (*Mercenaria mercenaria*)

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Introduction

Elevation of toxic metal mercury (Hg) in coastal waters is influenced by anthropogenic activities, with detrimental impacts on both wildlife and human health (Mason and Gill 2005, Chen et al. 2012). There are multiple chemical forms of Hg present in the environment, including both inorganic mercury (iHg) and methylmercury (MeHg). While both forms are toxic, MeHg is cause for greater concern due to its efficient accumulation and transfer to higher trophic levels (Bryan and Darracott 1979, Fitzgerald et al. 2007, Mason et al. 2012). When dissolved in seawater iHg and MeHg are efficiently taken up by unicellular phytoplankton (Mason et al. 1995, Lee and Fisher 2016), and transferred to their consumers, i.e., planktivorous organisms, both sessile suspension feeders such as bivalve molluscs, and zooplankton (Blackmore and Wang 2004, Hammerschmidt and Fitzgerald 2006, Pan and Wang 2011). MeHg is transferred efficiently in the food web, while iHg is not. This efficient transfer of MeHg from seawater to phytoplankton and then from lower to higher trophic levels results in the highest MeHg concentrations being found in fish and other marine predators, while iHg represents a very small fraction in fish tissues. Additionally, %MeHg in muscle tissue is high (>90%; Bloom 1992, Baumann et al. 2017, Anatone et al. 2020) and this might be caused by the binding between MeHg and thiol groups in proteins (Bradley et al. 2017, Man et al. 2019). Since the analysis of THg is much simpler, compared to the more tedious analysis of MeHg, many studies report only THg for fish, and often as well for bivalve molluscs (e.g. Paulson et al. 2003; Sarkar et al. 2008; Kim et al. 2017; Costa et al. 2020). Yet, the variability in %MeHg values can be very high in bivalve molluscan tissues, as demonstrated by Hansen et al. (2024), and analysis of both THg and MeHg is needed to gain more insight into the cycling of iHg and MeHg in tissues.

Among suspension-feeding bivalves in New England waters, the northern quahog (= hard clam) (*Mercenaria mercenaria*) is an important species from both ecological and economic

perspectives. Quahogs, as other suspension feeders, influence nutrient cycles and contaminant cycling and often serve as food for predators of a broad range of taxa, including species of crabs, sea stars, gastropods, finfish, birds, and humans (Mackenzie Jr 1977, Dame and Libes 1993, Smaal and Prins 1993, Prins et al. 1997, Kraeuter 2001, Lonsdale et al. 2009).

Data on concentrations of iHg, MeHg, and THg for northern quahogs is virtually absent with the exception of one study (Hansen et al. 2024) and thus, there remain major knowledge gaps concerning patterns of bioaccumulation in these organisms. Prior studies that investigated Hg bioaccumulation patterns in suspension-feeding bivalve molluscs showed conflicting results for THg and did not report MeHg concentrations. For example, THg and size showed a positive relationship for *Anomalocardia brasiliana, Mytilus trossulus, Mytilus galloprovincialis,* and *Ostrea edulis* (Najdek and Sapunar 1987, da Silveira Fiori et al. 2018, Jędruch et al. 2019), and negative relationships for *Ruditapes philippinarum* and *Senilia senilis* (Otchere et al. 2002, Giani et al. 2012). Still, others have found no correlation between THg concentration and animal size for *Mytilus edulis* and *Corbicula fluminea* (Cossa and Rondeau 1985, Neufeld 2010). Hansen et al. (2024) showed an inverse relationship between MeHg concentrations and animal size, but such a relationship was not observed for iHg or THg, and the mechanism underpinning these patterns is not understood.

The distribution of MeHg, iHg, and THg between tissue types in different bivalve mollusc species is not well understood, but some prior research has addressed changes in concentrations for different tissues in a number of bivalve molluscan species. Several studies exposed bivalve molluscs to iHg and MeHg and, following short-term depurations, tracked concentrations in dissected tissues. For example, Denton and Burden-Jones (1981) exposed *Saccostrea echinata* to aqueous iHg for up to 30 days, while Kopfler (1974) exposed

48 *Crassostrea virginica* to both aqueous iHg and MeHg for up to 42 days. Both studies reported
49 the highest concentrations of iHg and MeHg in the gill and mantle, while the lowest
50 concentrations were found in the adductor muscles. Gagnon and Fisher (1997) reported higher
51 iHg and MeHg concentrations in mantle and digestive gland compared to the gill and foot of *M.*
52 *edulis* exposed for 20 minutes via radioactively labeled particles. Other studies followed iHg and
53 MeHg concentrations after both short- and long-term depuration periods following both aqueous
54 and dietary exposures. Cunnigham and Tripp (1975a; *C. virginica*) and Fowler (1978; *M.*
55 *galloprovincialis*) reported that iHg and MeHg concentrations were highest in the gill, mantle,
56 and digestive gland after short-term depuration (<5 days), but further depuration for 35 and 45
57 days, showed the highest MeHg concentrations in the adductor muscles. Conversely, Sarkar et al.
58 (2008) performed a field-based study in which any short- vs. long-term depuration process would
59 be masked, reported typically higher THg concentrations (MeHg was not measured) in the gills
60 and mantle compared to the foot in three clam species, i.e., *Sanguinolaria acuminata*, *Meretrix*
61 *meretrix*, and *Pelecyora trigona*.

62 the objective of the present study was to investigate the distribution of MeHg, iHg, and
63 THg in terms of concentrations, and total mass allocation within four soft tissues in northern
64 quahogs: mantle, foot, adductor, and viscera. This study was motivated by the overall hypothesis
65 that concentration and MeHg allocation change as animals grow. It was hypothesized (H1) that
66 MeHg concentrations for all tissues combined would be inversely related to shell heights, and the
67 same pattern would be evident for all the dissected tissues. It was also hypothesized (H2) that in
68 northern quahogs, the muscular tissues - specifically the adductor and foot – would exhibit the
69 highest concentrations of MeHg, contain the largest mass of MeHg, and have the highest
70 percentages of MeHg relative to THg (%MeHg).

Materials and Methods

Sample Collection and Preparation

Northern quahogs (*Mercenaria mercenaria*) spanning the available size range (35.6 − 98.3 mm) were collected from Mumford Cove in Groton, CT (Lat, Long: 41° 19' 36.86" N, 72° 1' 13.60" W) on July 26, 2016 (n = 29), and August 17, 2018 (n = 24) from the subtidal zone as described in Hansen et al. 2024. Upon collection, animals were transported in a cooler to the laboratory for further processing. Each quahog was measured for shell height (H; 35.6–98.3 mm) and tissue wet weight (W; 36.4–276.8 g). Tissues were separated from the shell and placed in clean conical polyethylene vials. For the quahogs sampled in 2016, tissues were kept intact, while those collected in 2018 were dissected to isolate the adductor muscle (A), mantle (M), and foot (F) tissue. The remainder of the soft tissues were labelled as "viscera" (V). All tissue samples were kept frozen (-18 °C) until they were freeze dried, after which, they were reweighed and homogenized into a fine powder using a mortar and pestle.

Mercury Analyses

The concentrations of MeHg in tissue samples were measured using a procedure based on Hammerschmidt and Fitzgerald (2005), which was modified from EPA method 1630. A subsample of soft tissue was weighed (40–60 mg) and digested overnight in 5 mL of 4.5 N nitric acid (HNO_3) at 60 °C. A 100-µL aliquot of the digest was then diluted to 30 mL in MilliQ water and pH adjusted to 4.7–4.9 using 2 M acetate buffer and 8 N potassium hydroxide (KOH). Sodium tetraethyl borate ($NaBET_4$) was added to the samples before they were measured for MeHg by the Tekran 2700™ system. Every 10^{th} sample was triplicated with a fourth sample

being spiked with a known amount of MeHg to assess sample recovery. Total mercury (THg) concentrations in the tissue samples were measured by the Nippon Mercury Analyzer 3000™ using EPA method 7473. Approximately every 10^{th} sample was analyzed in triplicate.

To assess accuracy and recoveries, the standard reference material (SRM) used for both THg and MeHg was TORT-2 (lobster hepatopancreas, National Research Council of Canada). The average measured concentrations were 0.253 ± 0.011 μg g^{-1} for THg (n =10) and 0.151 ± 0.010 μg g^{-1} for MeHg (n = 16). Both values were within the accepted ranges for the SRM. Analytical triplicate MeHg sample analyses produced a relative standard deviation (RSD) of 3.7% while the THg RSD was 2.0%. The MeHg spike recoveries were 108.1 ± 9.8%.

Mercury Calculations

The reported THg and MeHg concentrations (μg g^{-1} dry weight) for each individual tissue type were measured directly, while the iHg concentrations were calculated by finding the difference between the THg and MeHg values (Equation 1).

$$C_{THg} - C_{MeHg} = C_{iHg} \quad \text{(eq. 1)}$$

Further, the accumulated masses (μg) of THg, MeHg, and iHg were calculated by using both the concentrations and the measured dried mass (g) of each tissue type (W_T; where T is A, F, M or V; Equation 2).

$$C_{Hg} \times W_T = Hg_T \quad \text{(eq. 2)}$$

The bulk mass (μg) of Hg is the mass of all soft tissues combined (Hg_B; Equation 3).

$$H_A + Hg_F + Hg_M + Hg_V = Hg_B \quad \text{(eq. 3)}$$

The bulk tissue concentrations were calculated by dividing Hg_B by the sum of specific tissue dry masses (Equation 4).

$$\frac{Hg_B}{\sum_{T:A,F,M,V} W} = C_{Hg,B} \quad \text{(eq. 4)}$$

The percent allocation (Al) of these Hg forms in specific tissues was calculated based on their tissue specific masses (Hg_T) in relation to Hg_B (Equation 5).

$$\frac{Hg_T}{Hg_B} \times 100 = Al_T \quad \text{(eq. 5)}$$

%MeHg describes the relative amount of MeHg to THg (Equation 6).

$$\frac{\text{MeHg } (\mu g\ g^{-1})}{\text{THg } (\mu g\ g^{-1})} \times 100 = \%\text{MeHg} \quad \text{(eq. 6)}$$

Statistical Analyses

A t-test was used to compare the THg, MeHg, and iHg concentrations in all of the tissue types combined, defined here as bulk tissue concentrations (eq. 3), between the 2016 and 2018 quahogs. To compare the growth curves of the two populations, the analysis of covariance

(ANCOVA) was used. Prior to the ANCOVA, dry weight values were $\log_{10}$ transformed to satisfy the assumption of linearity of the data (Gotelli and Ellison 2018).

When comparing the THg, MeHg, and iHg concentrations in the four tissue types, the data were transformed using power functions to achieve homoscedasticity (Gotelli and Ellison 2018). The iHg data was normalized through this transformation, thus an ANOVA and Tukey post-hoc tests were utilized; however, the tissue THg and MeHg concentration data could not be transformed to fit the assumption of normality, thus nonparametric Kruskal-Wallace test with a Dunn post-hoc test was used.

Linear regressions were used to test for significance of Hg concentrations or allocation against shell heights. The assumptions of homoscedasticity and normality were met by observing the residual and q-q plots respectively and no transformations were needed for this analysis. All statistical analyses were performed in R version 3.5.1.

Results

Biometrics and Tissue Mass

When comparing quahogs collected in 2016 and 2018, dry weights of the soft tissues for quahogs collected in 2016 ranged from 0.34 to 7.37 g, and their shell heights ranged from 35.6 to 98.3 mm, while for quahogs collected in 2018, dry weights ranged from 0.61 to 4.91 g, and shell heights ranged from 44.7 to 87.6 mm (Hansen et al. 2025). Based on ANCOVA, the 2018 quahogs were significantly heavier than those collected in 2016 ($p < 0.002$), yet the relationship between shell height (H) and tissue dry weight (W) was not statistically different between the two collection years (ANCOVA, $p = 0.053$). The equation 7 describes this relationship for data derived from both the 2016 and 2018-collections.

$$W = 2 \times 10^{-5} \times H^{2.8} \quad \text{(eq. 7)}$$

The allocation of biomass in soft tissues from the 2018 samples revealed that the viscera accounted for 56.5 ± 6.7% (mean ± standard deviation) of the total mass, while the adductor muscles accounted for 21.1 ± 4.2% of the total mass. The foot and mantle accounted for 10.2 ± 1.7% and 12.2 ± 2.5% of the total mass respectively (Figure S1). Further, the mass allocation of each of the tissue types did not vary with shell height (Linear Regression, $p > 0.05$).

Mercury Concentrations and Allocation in Tissues

The comparison of concentrations of THg, MeHg, and iHg for all tissues combined, i.e., bulk tissues, between the quahogs collected in summers of 2016 and 2018 (Hansen et al. 2025), revealed some similarities (t-test, $p > 0.05$); however, concentrations of THg, MeHg, and iHg in the bulk tissues of quahogs collected in 2016 and 2018 showed differences in relation to the animal size. Quahogs collected in July 2016 showed a significant inverse relationship between MeHg and shell height ($p = 0.001$), but these relationships were not significant for either the THg or iHg ($p > 0.05$; Figure 1). In contrast to samples from 2016, the observed significant inverse relationship between MeHg and shell height was not found in the 2018-collected quahogs ($p > 0.05$), while THg and iHg concentrations in the 2018-collected quahogs showed positive relationships with shell heights ($p = 0.002$, $p < 0.001$; Figure 1).

Concentrations of THg, MeHg, and iHg for dissected tissues of quahogs collected in 2018 (Hansen et al. 2025) revealed no significant differences for THg (Kruskal-Wallace, $p > 0.05$; Figure 2); however, concentrations of MeHg in the adductor muscles (0.182 ± 0.051 μg g^{-1}) and foot (0.213 ± 0.036 μg g^{-1}), which were comparable to each other (Dunn, $p > 0.05$), were

184 significantly higher compared to the mantle (0.121 ± 0.023 μg g^{-1}) and viscera (0.122 ± 0.023 μg
185 g^{-1}; Dunn, $p < 0.001$). Further, MeHg concentrations in the mantle and viscera were also similar
186 to each other (Dunn, $p > 0.05$). The iHg concentrations showed the opposite pattern to what was
187 observed for MeHg. The adductor and foot muscles which had similar concentrations (0.020 ±
188 0.024 μg g^{-1}, 0.030 ± 0.020 μg g^{-1}; Tukey, $p > 0.05$), were, however, significantly lower
189 compared to the mantle (0.114 ± 0.086 μg g^{-1}) and viscera (0.124 ± 0.121 μg g^{-1}; Tukey, $p <$
190 0.001; Figure 2). Concentrations of iHg in the mantle and viscera were similar (Tukey, $p > 0.05$).
191 Further reflecting the differences in iHg and MeHg between the tissue types, the values
192 of %MeHg also differed between the tissues. On average, %MeHg values were higher in the
193 muscular than other tissues. They were higher in the adductor muscles (90 ± 15%) and foot (88 ±
194 8%) and lower in the mantle (56 ± 16%) and viscera (58 ± 20%; Dunn, $p<0.01$; Figure 2).
195 Further, the masses of THg, MeHg and iHg were all allocated largely within the viscera
196 (THg: 56.8 ± 8.5%, MeHg: 48.2 ± 7.5%, iHg: 72.0 ± 9.0%; Figure 2). Despite the highest MeHg
197 concentrations in the adductor muscles, the relatively small mass of this tissue accounted for
198 only 26.4 ± 6.6% of the accumulated MeHg. Even smaller percentages of all accumulated THg
199 and iHg were allocated to the adductor muscles (THg: 19.5 ± 5.7%, iHg: 5.8 ± 5.1%). The
200 mantle and foot each contained between 10 and 15% of the accumulated THg, MeHg, and iHg
201 (Figure 2).
202 The investigation into the relationship between the tissue-specific THg, MeHg, and iHg
203 concentrations and the quahog shell heights revealed an inverse relationship for THg
204 concentration in the adductor muscles (Linear Regression, $p = 0.006$), and positive relationship
205 in the mantle and viscera (Linear Regression, $p \leq 0.001$; Figure 3). Shell heights and THg
206 concentrations in the foot were not significantly related (Linear Regression, $p > 0.05$). For MeHg

concentrations in the four separated tissues, the only significant relationship with shell height was observed for the adductor, where it was inverse (Linear Regression, $p < 0.001$; Figure 3). Both the mantle and viscera had positive relationships between iHg concentrations and shell heights (Linear Regression, $p \leq 0.002$; Figure 3). The iHg concentrations and shell heights were not related in either the adductor or foot (Linear Regression, $p > 0.05$). Overall, the changes in THg in the viscera and mantle were driven by the changes in iHg, mostly while the negative relationship for THg in the adductor muscle was driven by changes in the MeHg content with size.

In addition, the possible change of THg, MeHg, and iHg allocation with animal size was also investigated given the differences in the mass allocations for different tissues. The results presented in Figure 4 and Table 1 show an inverse relationship between the accumulated THg mass and shell heights for the adductor muscles (Linear Regression, slope: -0.443, $p < 0.001$), while there was a positive relationship for the viscera (Linear Regression, slope: 0.631, $p < 0.001$). THg allocation in the foot declined significantly as the shell heights increased (Linear Regression, slope: -0.126, $p = 0.004$), and remained unchanged in the mantle ($p > 0.05$). Similarly to the THg, the relative amount of MeHg stored in the adductor muscles was inversely related to shell heights (Linear Regression, slope: -0.365, $p < 0.001$), but positively correlated in the viscera (Linear Regression, slope: 0.356, $p = 0.002$). The MeHg allocation in the foot and mantle did not vary with shell heights (Linear Regression, $p > 0.05$). Figure 4 also shows the iHg allocation to the viscera increases with shell height (Linear Regression, slope: 0.468, $p = 0.001$). Further, both the foot (Linear Regression, slope: -0.166, $p = 0.001$) and mantle (slope: -0.243, $p = 0.004$) showed an inverse relationship in the relative iHg accumulation with shell height. The

amount of iHg allocated to the adductor muscle remained similar across animal sizes (Linear Regression, $p > 0.05$).

Discussion

Mercury Concentration and Quahog Size

The quahogs collected in 2018 demonstrated a lack of an inverse relationship between shell height and the MeHg concentrations for all tissues combined, but also for specific tissues i.e., foot, mantle, and viscera; however, this relationship held for the adductor muscle, thus partially supporting the first hypothesis. The results for MeHg in combined tissues for the 2018-collected quahogs differed from those found in the 2016-collected quahogs, where the inverse relationship was found (Hansen et al. 2024). Further, this study supported the hypothesis that MeHg in the muscular tissue of the adductor and foot was at higher concentrations and higher %MeHg relative to THg compared to the mantle and viscera.,The muscular tissues of foot and adductor did not contain most of the MeHg accumulated by the quahogs.

Interannual variability in the size-related concentration patterns of iHg and MeHg, and thus, THg concentrations was clearly demonstrated in this study. While the quahogs collected in 2016 and 2018 had similar average THg, MeHg, and iHg concentrations, the correlations between the bulk tissue concentrations and shell height differed between the two years. This variability is likely not the result of potential interannual differences in environmental Hg concentration, as a change in environmental Hg concentration would cause a change in the overall Hg concentration in the quahogs, which was not observed. A possible explanation, however, for these interannual differences may be related to the exact timing of sampling. The quahogs in 2016 were collected at the end of July, while those collected in 2018 were collected in the middle of August. Previous work (Hansen et al. 2024) showed that, while overall Hg

concentrations remain relatively constant, the slope of the relationship between Hg concentrations and quahog shell heights can fluctuate throughout the growing season. One potential cause of the observed differences is the timing of sampling relative to the major spawning period. Previous work by Cunningham and Tripp (1973 and 1975b) on eastern oysters, *Crassostrea virginica* and Najdek and Sapunar (1987) and Zorita et al. (2007) on Mediterranean mussels, *Mytilus galloprovincialis,* suggested spawning as a potential mechanism for Hg depuration in their respective study species. Furthermore, Wilman et al. (2023) reported up to 30% decrease in accumulated Hg concentrations in the zebra mussel, *Dreissena polymorpha*, after spawning. Thus, spawning would not only reduce the tissue mass, but also could potentially alter the tissue Hg concentration overall, and potentially the relative distribution of iHg and MeHg between tissues. Further, the spawning season for quahogs in Long Island Sound typically occurs from early July to late August (Eversole 2001, Doall et al. 2008), overlapping with the 2016 and 2018 sampling events. Since sampling occurred during different stages of the spawning season, this may have influenced the proportion of quahogs having already spawned, affecting the relationship between Hg concentrations and shell height in these quahogs. This, however, could not be confirmed in this study as the reproductive stages were not observed in either sampling event.

According to Hofmann et al. (2006), spawning occurs when the reproductive tissue mass reaches a specific relative mass compared to the overall tissue mass (0.2 for clams of <3 g dry wt but a decreasing relative mass for larger clams). The significant trend with size for the quahogs collected in 2016 suggests that they potentially had not yet spawned. The concentrations in 2018 are more consistent across the size range suggesting that this could be a result of spawning as it would reduce the MeHg concentration in the smaller clam more than the larger clams if there is

preferential removal of MeHg during spawning, as suggested in the studies discussed above. Thus, it is possible that the differences across years could be related to spawning. While this conclusion fits the data, it cannot be confirmed by the results of this study as, again, the reproductive stages were not observed in either sampling event.

Additionally, while there have been few studies, it is possible that differences in food type influence the assimilation efficiency. Metian et al. (2020) showed that there were differences in the assimilation of iHg and MeHg by oysters fed ciliates, so differences in years may be related to differences in food in the months prior to sampling. These differences could impact the relative amount of iHg relative to MeHg, as iHg is relatively rapidly depurated from bivalve tissue, while the deputation rate of MeHg is much slower (Pan and Wang 2011, Cardoso et al. 2015). These factors could account to some degree for the shorter time scale and inter-annual variability in the overall tissue concentration.

Mercury Distribution between Different Tissues

In the present study, it was shown that in the northern quahogs MeHg accumulated preferentially in the muscular tissues, specifically the adductor muscle and foot, at concentrations that were 30-40% higher than in the viscera and mantle. Additionally, MeHg was the dominant form of Hg in the muscular tissues, reaching values that ranged from 79 to 100%. This higher percentage of MeHg in muscular tissues versus the viscera or mantle may be due to the higher protein content in the adductor muscles and foot, which are 60−70% protein on a dry weight basis, compared to other tissues, which are 40−50% protein (Ansell et al. 1964, Eble 2001, Chantler 2006). Thus, the relative difference in the MeHg content is consistent with the differences in the amount of protein between tissue types. Higher concentrations of MeHg in the

muscle compared to other tissues have been documented in finfish, where the protein rich dorsal tissue contains more MeHg than other organs and the %MeHg in the muscle tissue generally exceeds 90% (Bloom 1992, Dutton and Fisher 2010). The biochemical make-up of the tissue thus strongly influences the partitioning of Hg forms. For example, in intracellular environments, MeHg predominately forms complexes with thiol-containing molecules, particularly the amino acid cysteine present in proteins (Harris et al. 2003, Lemes and Wang 2009, Man et al. 2019). For example, cellular fractionation studies show that iHg is more widely distributed within the cells of invertebrates, such that they are bound to membranes, organelles, and proteins, than MeHg, which is mostly found in the heat-stable protein fraction for bivalves (Dang and Wang 2010). The findings from this study add to the prior supporting evidence, which suggests that the preferential accumulation of MeHg in muscular tissues may be a conserved trait across different groups of organisms, both vertebrate and invertebrate, although more research is required to confirm such a conclusion.

This conclusion is consistent with prior studies of the short- and long-term storage of Hg in soft tissues of several bivalve molluscan species. Previous laboratory studies followed distribution of Hg forms among the soft tissues following an initial exposure to iHg and MeHg (Kopfler 1974, Cunningham and Tripp 1975a, Gagnon and Fisher 1997). In a short-term experiment by Kopfler (1974), *C. virginica* was exposed to dissolved iHg and MeHg and found to have the highest iHg and MeHg concentrations in the gill and mantle, and lowest in the adductor muscles. An additional short-term experiment the blue mussel *Mytilus edulis*, performed by Gagnon and Fisher (1997) reported higher iHg and MeHg concentrations in the mantle than in the foot. In an experiment performed by Denton and Burden-Jones (1981), the black-lip oysters, *Saccostrea echinata* were exposed to iHg for a maximum of 30 days. During

this exposure, Denton and Burden-Jones (1981) found that the uptake rate of iHg was greater in the gills and mantle compared to the adductor muscles, which would result in higher initial concentrations in these tissues. Denton and Burden-Jones (1981) reported a slower loss of Hg from the gill than from the adductor muscles after a depuration period.

Longer-term studies have found that the distribution of iHg and MeHg in tissues can change over time. For example, based on experiments using *C. virginica*, Cunningham and Tripp (1975a) found that iHg and MeHg concentration were higher in the gills, digestive gland, and mantle compared to the muscular tissues initially following exposure; however, after a 45-day depuration period, iHg and MeHg concentrations rapidly declined in the gills and digestive tissue, while concentrations remained constant in the mantle and increased in the muscular tissue. Fowler et al. (1978), who experimented with *M. galloprovincialis*, reported similar results with a 5-fold decrease in MeHg concentrations in the gills with a 4-fold increase in the muscle tissue over a 21-day depuration period. Additionally, Inza et al. (1998) also reported a 45% decrease in MeHg concentrations in the gills of the Asiatic clam, *Corbicula fluminea*, within 30 days. At the end of the depuration period, the adductor muscles and foot had more than double the MeHg concentration of the other isolated tissues. Further, Cardosa et al. (2015) observed THg concentrations in various tissues in transplanted peppery furrows, *Scrobicularia plana*, and then modelled the uptake and depuration of THg from these tissues. They reported that while the *S. plana* were deployed at the contaminated sites, the highest concentrations were in the digestive gland. After being transferred to a site with a lower level of contamination, it was observed that the different tissues had different depuration rates with the digestive gland having the highest depuration rate. The results from these exposure studies suggest that the MeHg and iHg initially accumulate in the gills and mantle and then MeHg is transferred for longer storage

345 the adductor and foot - the muscular tissue. Preferential allocation of MeHg in muscular tissues
346 is consistent with the findings of the present study.

347 The results showing MeHg preference for the muscular tissue in bivalves are also
348 consistent with the results of studies that employed fish. For example, Leaner and Mason (2004)
349 showed that after a single feeding of MeHg-spiked food, the concentrations in the intestine, gills,
350 liver and blood of the minnow peaked within a few days and after that, the MeHg was mostly
351 transferred to the muscle tissue with the muscle tissues increasing in concentration, while the
352 concentrations in the other organs decreased. After two weeks, more than 80% of the assimilated
353 MeHg was in the muscle tissue, as seen in other similar exposure studies (Pentreath 1976,
354 Rouleau et al. 1998, Oliveira Ribeiro et al. 1999).

355 Despite MeHg preferentially accumulating in the adductor muscles and foot given
356 continual exposure, these tissues do not control the patterns of MeHg bioaccumulation on the
357 bulk tissue scale because of their relatively small fraction of the total tissue mass. While the
358 muscular tissues have the highest MeHg concentrations, the foot and adductor only account for
359 approximately 30% of the total soft tissue dry weight (combined) in quahogs. This results in
360 these tissues only containing 40% of the accumulated MeHg. Conversely, the viscera account for
361 over 50% of the tissue mass and consequently contain approximately 50% of the accumulated
362 MeHg (present study). Further, while the adductor muscle MeHg concentrations and shell height
363 were inversely correlated, the MeHg concentrations in the bulk tissue did not vary with size.
364 Again, this discrepancy was likely the result of the adductor muscles being a relatively small
365 fraction of the tissue and only containing 25% of the accumulated MeHg. This is strongly in
366 contrast to what is observed in finfish species as the dorsal muscle has the highest MeHg
367 concentrations when compared to other tissue types and, in most species, the muscle tissue

368 comprises over 60% of the fish total body mass (Dutton and Fisher 2010, Johnston et al. 2011).
369 Thus, the dorsal muscle is where the vast majority of the MeHg is accumulated and is the tissue
370 type that drives the MeHg concentration at the organismal level. Overall, despite multiple studies
371 observing the concentration of MeHg in different tissue types in various bivalve molluscan
372 species, no study, including this study, has shown a specific tissue type controlling the MeHg
373 concentrations in bivalve soft tissue as a whole (Cunningham and Tripp 1975a, Fowler et al.
374 1978, Denton and Burdon-Jones 1981, Gagnon and Fisher 1997, Inza et al. 1998).

375 The results of the present study also demonstrated that larger proportions of THg, MeHg,
376 and iHg are accumulated in the viscera as quahogs increase in size, primarily driven by changes
377 in Hg concentration with size as tissue mass allocation remained unchanged with size. For the
378 MeHg specifically, concentrations in the adductor muscles and shell heights were negatively
379 correlated, while all other tissues showed no correlation between size and MeHg concentration.
380 Since the MeHg concentrations in the adductor muscles decreased in the larger animals, the
381 relative amount of MeHg stored in this tissue type also decreased, and MeHg allocation shifted
382 to the other tissues, specifically the viscera. For iHg, both the viscera and mantle had positive
383 correlations between shell height and concentration, but only the viscera had an increase in iHg
384 allocation with shell height. Thus, the increase of iHg concentration in the viscera translated to
385 the increased iHg mass there. The results of this study, however, cannot be used to determine
386 which specific tissue type preferentially accumulated iHg because the viscera tissue class in this
387 study is a collection of different tissues, including the gills, digestive gland, and gonads. As
388 noted above, these tissues may initially be the sites of MeHg bioaccumulation upon ingestion,
389 but over time the MeHg could be transferred to the muscle tissue and stored there for a much
390 longer period.

391 Further, a possible explanation for the observed simultaneous decrease of MeHg
392 concentration in the adductor muscles with increasing size, along with the increase of iHg
393 concentration in the viscera, is that MeHg is being demethylated into iHg. This notion is partially
394 supported by previous studies suggesting that different Hg species can move between tissue
395 types (Cunningham and Tripp 1975a, Fowler et al. 1978, Inza et al. 1998). Conversely, as MeHg
396 demethylation process was not investigated here nor in previous studies in bivalve mollusc
397 tissues, it thus remains as a hypothetical mechanism and requires further research, especially as
398 MeHg demethylation in the liver has been found for marine vertebrates (Palmisano et al. 1995,
399 Wagemann et al. 1998, Wang et al. 2013, Wang et al. 2017). Future studies should consider if
400 demethylation or any other MeHg excretion processes are present in bivalve molluscs and are
401 sufficient to alter *in situ* MeHg concentration in various tissues.
402 In summary, this study demonstrated that MeHg preferentially accumulates in the
403 adductor muscles and foot of northern quahogs with these tissues containing the highest MeHg
404 concentrations and highest %MeHg. Despite the elevated MeHg relative to iHg in these tissues,
405 however, the MeHg concentrations in these muscular tissues are insufficient to serve as its major
406 reservoir, or to drive concentration patterns in the whole organism. Further, this study
407 demonstrated that the MeHg, iHg, and THg allocation among different tissues changes according
408 to the quahog size but future studies are required to build on these results and investigate
409 mechanisms behind these changes, including possibly MeHg demethylation, inter-tissue transfer
410 of iHg and MeHg forms, spawning and other processes of depuration. Finally, the size-related
411 changes in concentrations of MeHg and iHg have implications for exposure to predators.
412
413
414

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423

Tables

Table 1: Results of linear regression (alpha = 95%) testing the relationships between Hg allocation within each tissue type and shell height (mm) from quahogs collected in 2018; n.s. denotes non-significance.

	THg	MeHg	iHg
Adductor Muscle	$y = -0.443x + 47.4$ $R^2 = 0.693$ $p < 0.001$	$y = -0.365x + 49.3$ $R^2 = 0.355$ $p < 0.001$	n.s.
Foot	$y = -0.126x + 19.1$ $R^2 = 0.296$ $p = 0.004$	n.s.	$y = -0.166x + 15.1$ $R^2 = 0.318$ $p = 0.001$
Mantle	n.s.	n.s.	$y = -0.243x + 33.0$ $R^2 = 0.262$ $p = 0.004$
Viscera	$y = 0.631x + 17.1$ $R^2 = 0.665$ $p < 0.001$	$y = 0.356x + 25.8$ $R^2 = 0.292$ $p = 0.002$	$y = 0.468x + 42.5$ $R^2 = 0.309$ $p = 0.001$

Figure captions

Figure 1: Relationships for bulk THg, MeHg, and iHg concentrations with shell height for quahogs collected in 2016 and 2018. n.s. denotes non-significance.

Figure 2: The concentrations and allocation of THg, MeHg, and iHg in the adductor muscle, foot, mantle, and viscera dissected from quahogs collected in 2018 (n = 29). Error bars denote standard deviation. Lettering signifies statistically significant differences ($p < 0.05$).

Figure 3: Correlations between size and Hg concentrations of individual tissue types from quahogs collected in 2018. n.s. denotes non-significance.

Figure 4: Linear regressions between size with THg, MeHg, and iHg allocation for each tissue type.

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Figure 1

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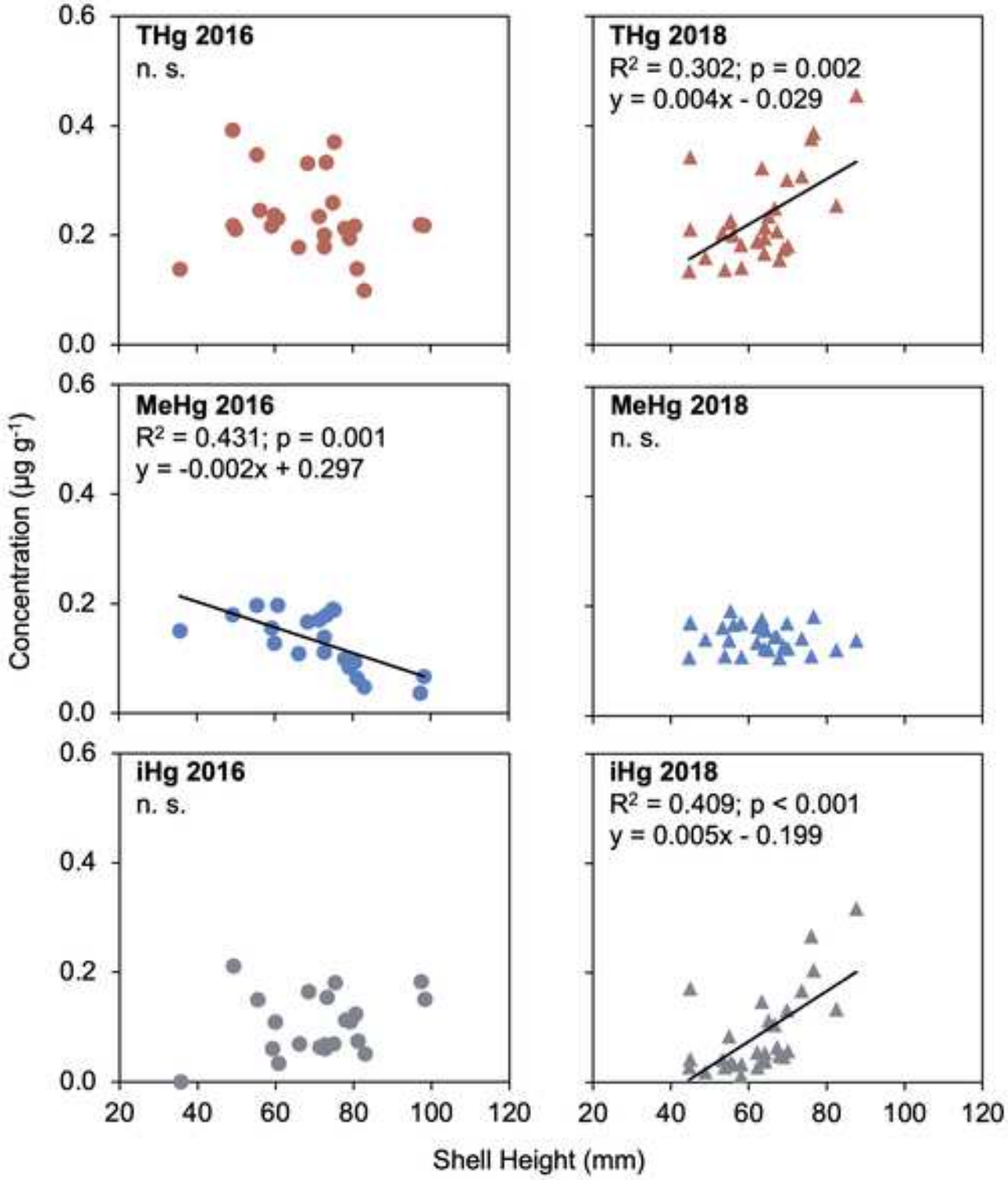

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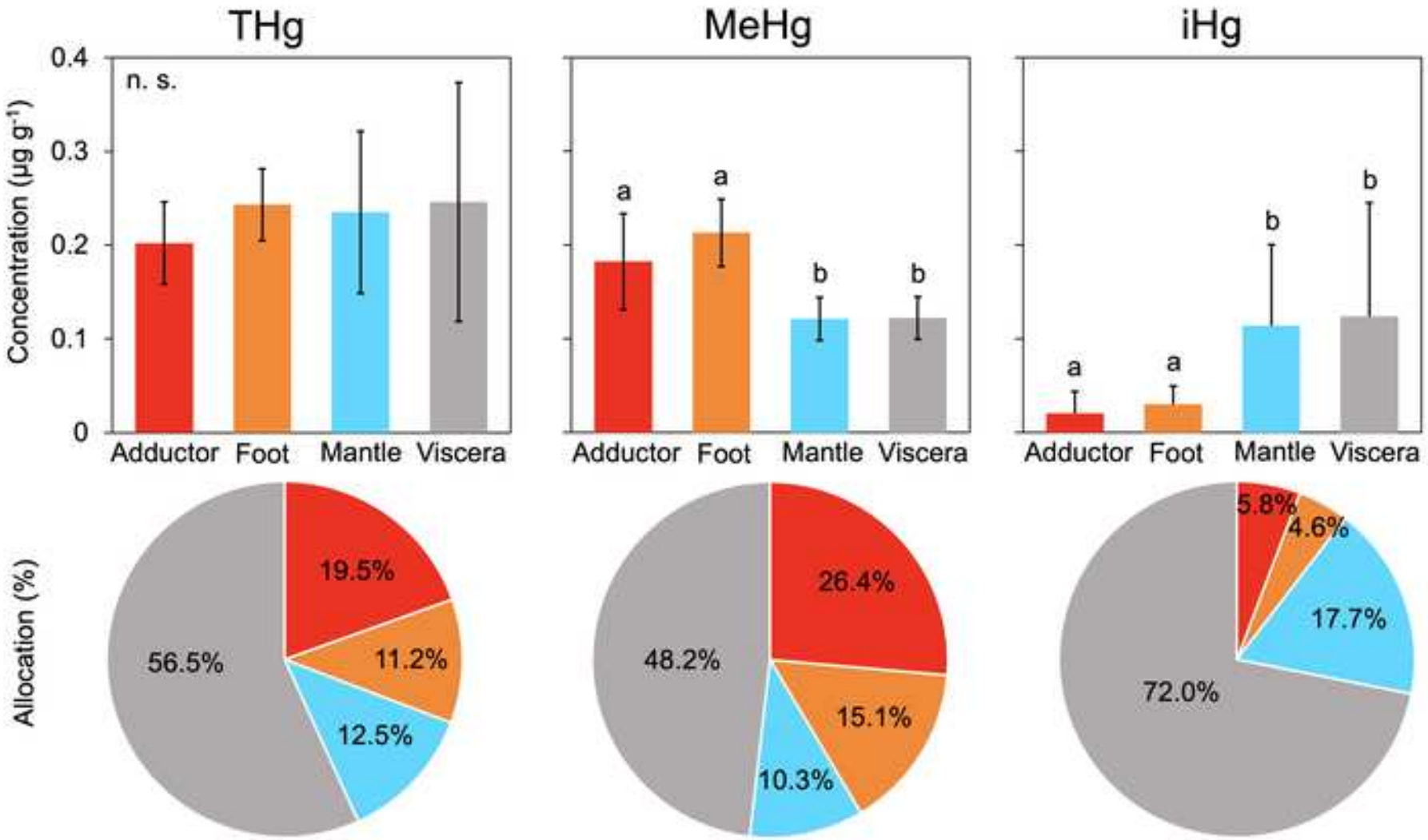

Figure 3

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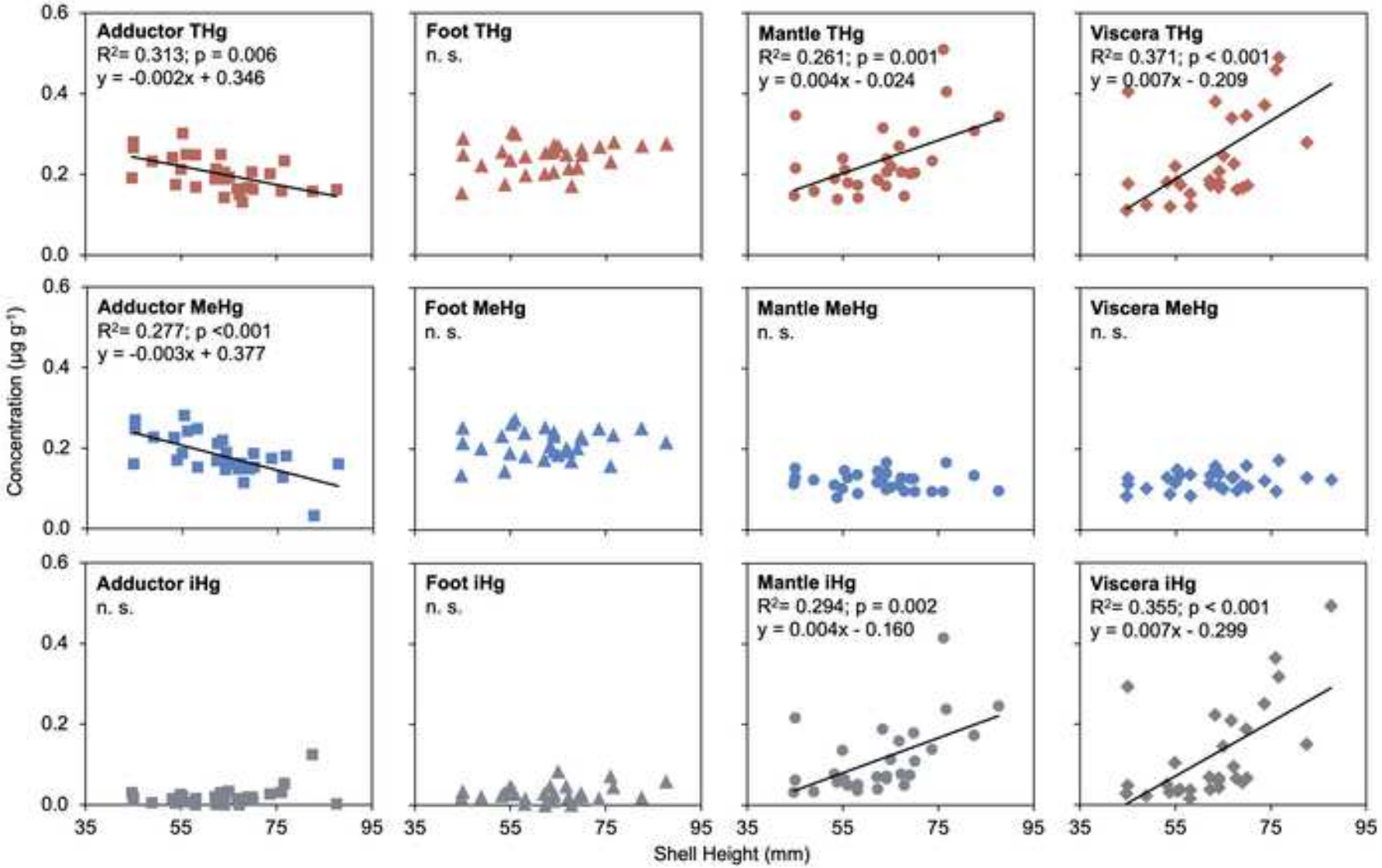

Figure 4

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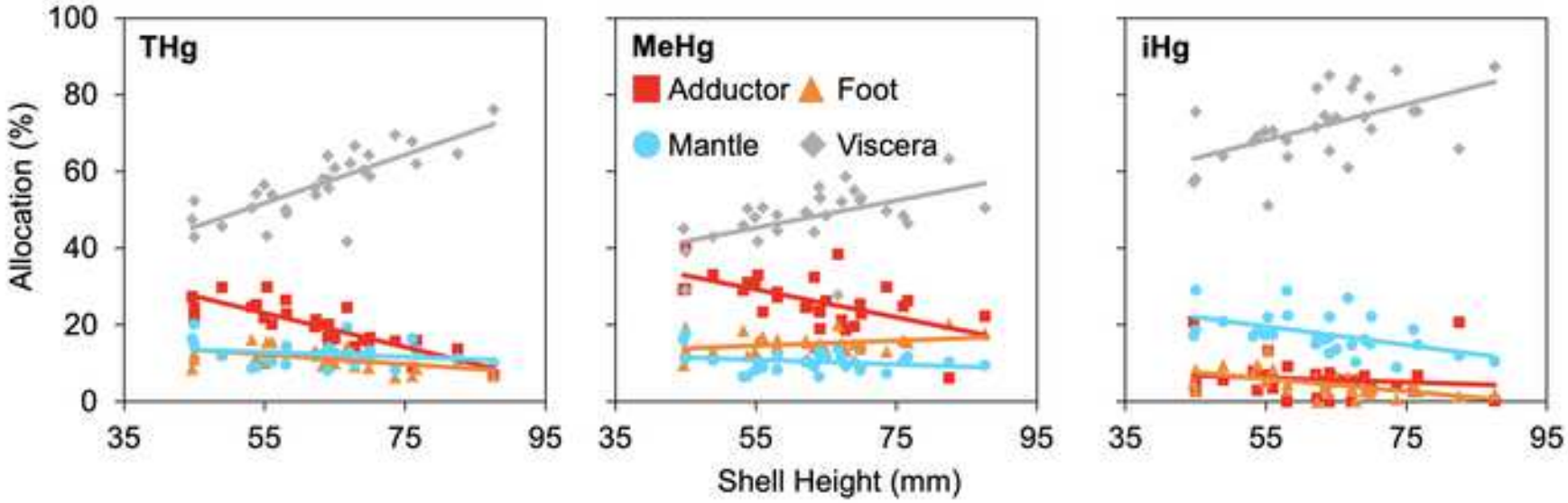

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Gunnar Hansen: Investigation, Data curation, analysis, and visualization, Writing - Original Draft, Reviewing and Editing, Preparation; **Robert P. Mason:** Conceptualization, Methodology, Resources, Writing - Original Draft, Reviewing and Editing, Funding acquisition; **Sandra Shumway:** Writing - Original Draft, Reviewing and Editing; **Zofia Baumann:** Conceptualization, Investigation, Methodology, Resources, Data Curation, Writing - Original Draft, Reviewing and Editing, Funding acquisition.