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Examining molluscs as bioindicators of shrimp aquaculture effluent contamination in a southeast Asian mangrove

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ABSTRACT

This study evaluates the presence of metal(loid) contamination from shrimp aquaculture effluent in a nearby mangrove ecosystem in Khung Krabaen Bay (KKB), Thailand. Our objectives were to: 1) examine how sediment metal(loid) concentrations change spatially in KKB relative to the aquaculture ponds; (2) compare mollusc trophic dynamics of elements associated with shrimp aquaculture; and (3) determine if certain mangrove mollusc species present better ecological indicators of aquaculture elemental contamination. We analyzed targeted elemental concentrations (As, Mn, Cu, Zn, Cr, Pb) in sediments sampled at increasing distance from intensive shrimp aquaculture and within the tissues of five species of molluscs. Differences in mollusc diet were studied using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes, and biomagnification and biodilution of metal(loid)s were examined using $\delta^{15}N$ values to infer trophic position. Elemental concentrations were low in farmed shrimp and pellet food relative to mangrove molluscs, but high in the sediments of drainage ponds filtering pond effluent. Declining elemental concentrations from the aquaculture drainage ponds to the mangrove sediments closest to shrimp farms suggested that the management settling ponds are effective at attenuating metal(loid) contamination. Large differences in mollusc mean $\delta^{13}C$ (–25.8 to –20.1‰) and $\delta^{15}N$ (1.9 to 8.3‰) values indicate variable diets across species, and trophically-elevated molluscs had greater marine-based diets resulting in a significant, positive correlation between mollusc $\delta^{13}C$ and $\delta^{15}N$ values. KKB molluscs biomagnify As, Zn, and Se, whereas declines in Mn and Cu with increasing trophic position indicate biodilution. The metals Pb, Fe, Al, Cr were elevated in the shrimp drainage pond sediments and in the tissues of the gastropods *Ellobium aurisjudae, Cerithidea obtusa* (both mud grazing), and *Nerita lineata* (algal grazing at low tide), identifying these gastropod species as probable bioindicators of aquaculture contamination in southeast Asian mangroves. Although we find biomagnification of some elements, mangrove mollusc metal(loid) concentrations are not likely to be of toxicological concern, and in fact, were lower than many mangroves elsewhere impacted by industrial and sewage contamination.

1. Introduction

The deforestation of mangroves for aquaculture raises concern over the loss of ecosystem services, coastal contamination and pollution, and the introduction of nonnative aquaculture species (Goldburg and Naylor, 2005). In particular, common components of shrimp aquaculture effluent contamination are: solid matter (e.g., eroded pond soils), organic matter (e.g., shrimp feed, dead shrimp, shrimp feces), and dissolved metabolites (e.g., ammonia, urea) (Gradlund and Bengtsson, 2001). Most shrimp farms dispose of their effluent into nearby waterways, which run through mangroves and towards the sea (Molnar et al., 2013). Because of the proximity of remaining mangroves to present shrimp culture ponds, aquaculture effluent and its solid component may accumulate within the mangrove environment and in the food web.

Many elements (Zn, Mn, Cu, Fe, etc.) are essential for culturing marine organisms, while others, such as As, Cd, Pb and Hg, are generally non-essential and particularly toxic at high concentrations (Marín-Guirao et al., 2008; Nascimento et al., 2017). Manganese, found within the soybean and wheat ingredients used to manufacture shrimp feed and commonly added to shrimp aquaculture as a pond disinfectant (as potassium permanganate), can accumulate within ponds and be

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discharged in pond effluent. While Mn is an important mineral element for shrimp cellular and protein metabolism and formation of skeletal structures, chronic exposure to high Mn concentrations may cause an organism stress and can lead to mortality (Hernroth et al., 2004; Bordean et al., 2014). Furthermore, with water column hypoxia from the degradation of settling organic matter, reduced Mn (Mn^{2+}) can accumulate to toxic concentrations at the bottom of shrimp ponds (Gibson et al., 2006), which ultimately is rapidly discharged to the environment at the end of shrimp rearing cycles. In addition to Mn, other elements often associated with shrimp-aquaculture include: As, Cu, Cr, Zn, Cd, and Pb (Nemati et al., 2009; Tu et al., 2011; Cheng et al., 2013).

Sediment element concentrations provide time-integrated signals of metal(loid) contamination. However, sediment elemental concentrations are also affected by sediment characteristics that vary across the land-to-sea transition within mangrove forests, including particle size, organic carbon content, and redox conditions (Marchand et al., 2011). Measuring metal(loid) bioaccumulation can help establish the bioavailability of metal(loid)s in the environment, offering time-integrated estimates on the proportion of total elemental load that is of ecological relevance (Rainbow, 1995). Mangrove molluscs represent ideal biomonitors of environmental metal(loid)s because of their large size (i.e. enough tissue for analyses), long lifespan, limited movement, and are abundant in mangroves (Elder and Collins, 1991; Zhou et al., 2008). In molluscs, metal(loid) bioaccumulation is influenced by exposure routes (diet and solution) and geochemical effects on bioavailability (Rainbow, 1995). Therefore, examination of metal(loid) bioaccumulation across a range of mollusc species recognizes different sources of metal(loid) contamination, as well as physiological differences in metal regulation (Rainbow, 1995; Zhou et al., 2008).

Carbon and nitrogen stable isotope ($\delta^{13}C$, $\delta^{15}N$) analyses are important in the study of elemental trophic dynamics (Quinn et al., 2003; Ikemoto et al., 2008; Revenga et al., 2012). $\delta^{15}N$ values fractionate isotopically between a consumer and its prey (approximately + 3‰), and thus is an useful tool to track an organism's trophic position in a food web, reflecting long-term feeding behavior and ingested prey (Matthews and Mazumder, 2008). Regressing the concentrations of an element against $\delta^{15}N$ values allows for the quantification and modeling of metal and metalloid trophic transfer in food webs (Croteau et al., 2005). By contrast, $\delta^{13}C$ values demonstrate lower trophic fractionation between a consumer and its diet (+0.8-2‰), and as such, are useful for evaluating sources of primary production within food webs (Vander Zanden and Rasmussen, 2001).

This study examines the combined use of sediments and mangrove molluscs as environmental indicators of metal(loid) contamination in an ecosystem adjacent to intensive shrimp aquaculture. Given a wide variety in mangrove physical habitat (prop roots, downed woody debris, forest floor) and chemical gradients (e.g. tidal flushing and salinity), we hypothesize that mangrove mollusc niches yield differences in trophic position. This difference in trophic position (mean $\delta^{15}N$ value) amongst mangrove molluscs in KKB may amplify the concentrations of some elements in the tissues of high-trophic molluscs. Thus, the study objectives were to: 1) examine whether sediment elemental concentrations change spatially in KKB relative to the aquaculture ponds; 2) compare the trophic dynamics of elements associated with shrimp aquaculture (e.g., Mn, As, Cu, Zn); and 3) determine if certain mangrove mollusc species present better ecological indicators of aquaculture elemental contamination in mangroves. The trophic dynamics of aquaculture-associated metal(loid)s among molluscs are evaluated using linear regressions between log-transformed concentrations of elements and $\delta^{15}N$ values. Here, a statistically significant and positive slope indicates biomagnification of trace elements in the food web, whereas a statistically significant and negative slope indicates trophic dilution of an element. Using multivariate analyses, we compare the elemental concentrations in the sediments of ponds filtering shrimp aquaculture effluent, canal, mangrove and bay sediments to mollusc elemental concentrations to ascertain whether tissue elemental concentrations of some molluscs more closely resemble that of the shrimp farm sediments.

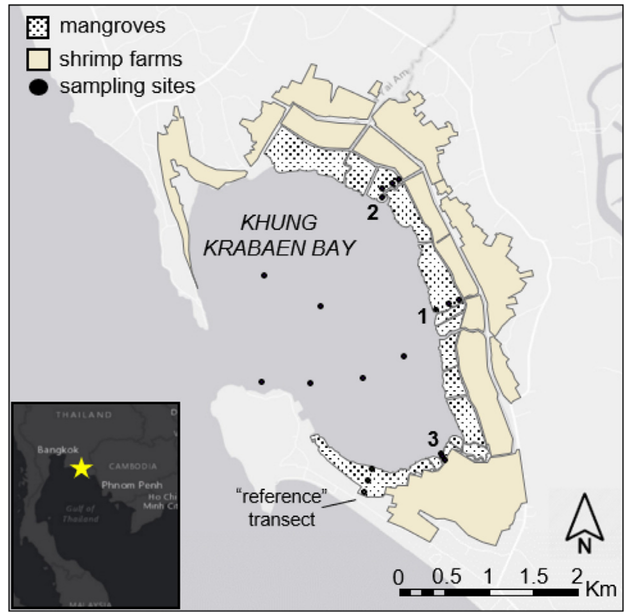

Fig. 1. The study system, Khung Krabaen Bay (KKB), located on the north-eastern Gulf of Thailand (shown in the inset map), in the province of Chanthaburi, and the sampling locations along each mangrove transect and within KKB.

2. Materials and methods

2.1. Study site description

The study was conducted in the mangrove fringing Khung Krabaen Bay (KKB) (12°33 – 12°36, 101°53′– 101°55′E, Fig. 1), located in the Khlong Khut sub-district, Tha Mai district, of the Chanthaburi province in Thailand (Fig. 1). Approximately 260 km southeast from Bangkok, KKB is a rural area of Thailand, with many of its inhabitants fishing or farming for a living. The mangrove is part of the Khung Krabaen Bay Royal Development Study Centre, established in 1981 by the late Majesty King Bhumibol Adulyadej of Thailand. Approximately 264 ha encompasses the mangrove forest, which is about 200–500 m wide from landward to seaward edge, and shrimp farms are locations around the bay encompassing ~ 160 ha (Thimdee et al., 2003; Google Earth Pro measurement tool, Fig. 1). Shrimp aquaculture in KKB yields ~ 2080 tons/year (mean from 2006 to 2013) in shrimp and shrimp products. During our sampling we observed that *Ceriops tagal* were more abundant towards the landward edge of the mangrove although still found throughout the mangrove; *Rhizophora apiculata* and *R. mucronata* were most abundant in the intermediate zone of the mangrove; and *Avicennia* species became more common towards the seaward edge. *Lumnitzera littorea* and *L. racemosa* were also common throughout the KKB mangrove, although less so towards the seaward edge. KKB is a semi-enclosed, oval-shaped bay about 4.6 km long, 2.6 km wide, and between 0.7 and 3.0 m deep. A diurnal tide in KKB replaces ~ 86% of the bay water during each tidal cycle (Thimdee et al., 2003).

2.2. Sampling design and sample collection

Samples were collected from three "impact" transects that receive effluent from nearby shrimp ponds and one "reference" transect which receives no effluent from shrimp ponds, but is located within the same bay as the impact transects (Fig. 1). Sediment and mollusc samples were collected during the dry season in March 2018 and at the start of the wet season in May 2018. The mangrove forest along the impact transects were accessed by canals that direct the shrimp pond effluent through the mangrove to KKB. No canal spans the reference transect, and interior mangroves along this transect were accessed by an educational trail. Samples were collected along each transect approximately every 50 m in order to account for the differences that may arise in metal(loid) levels for samples closer to the ocean compared to those closer to shrimp farms. At every 50 m interval along each transect, a circular (14 m diameter) sampling plot was established. One surface sediment sample (top ~ 1 cm) was collected close to the center of the plot and molluscs were collected within the circumference of the plot. Molluscs were identified to the lowest taxa when possible (see Supplementary material for a general description of each species, a list of identification resources, and mollusc photos) and include: *Nerita lineata* (common nerite), *Littoraria* sp. (periwinkle), *Ellobium aurisjudae*, *Thais gradata* (drill), *Cerithidea obtusa* (mud creeper), and *Isognomon ephippium* (tree/leaf oyster). The number of molluscs collected differed for each species due to their size differences and the requirement that enough tissue was obtained for both elemental and stable isotope analyses. In each plot, 1–2 individuals of each *T. graduata*, *E. aurisjudae*, and *I. ephippium*, and 4–5 individuals of each *N. lineata*, *Littoraria* sp., and *C. obtusa* were collected when present.

At the time of shrimp harvest in May 2018, *Penaeus monodon* (Asian tiger shrimp), shrimp feed, and shrimp pond drainage sediments were collected from three farms that discharge their effluent into canals that run into the adjacent mangrove (Fig. 1).

2.3. Sample preparation

At Burapha University Marine Technology Lab, mollusc and farmed shrimp samples were placed in a laboratory freezer overnight at −20 °C. Samples were then thawed, and the soft tissues removed with tweezers from the mollusc shells, and shrimp carapace. In cases where the gastropods had a deep shell whorl, shells were crushed with a mallet and the soft tissue separated from the shell with tweezers. All mollusc soft tissue was utilized and individuals combined to form one sample per species per plot. Mollusc and shrimp tissues and sediment samples were dried in a lab oven at 70 °C for ~ 12 h.

2.4. Elemental analysis

To prepare samples for acid digestion, the shrimp, snail, and oyster tissues were finely ground with a mortar and pestle. Sediment samples were ground and homogenized with a mortar and pestle and sifted with a stainless-steel sieve with a mesh size of 35-μm. All elemental analyses were performed by SGS North American Inc. (an accredited National Environmental Laboratory) in Dayton, New Jersey. The Environmental Protection Agency (EPA) method 3050B for acid digestion of sediments, sludges, and soils (U.S. EPA, 1996) was used to prepare the samples for metal analyses by inductively coupled plasma-mass spectrometry (ICP-MS). To summarize, ~0.5 g (dry weight) of sample was digested with repeated additions of reagent-grade nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). The resultant digestate was reduced in volume while heating and then diluted to a final volume of 100 mL. The EPA 6020B (SW-846) method (U.S. EPA, 2014) was used to determine the concentration of 22 elements on the ICP-MS (limits of detection in μg/g): Al (1 3 0), Sb (5.0), As (1.3), Ba (2.5), Be (1.3), Cd (1.3), Ca (6 3 0), Cr (5.0), Co (1.3), Cu (5.0), Fe (1 1 0), Pb (1.3), Mg (6 3 0), Mn (10), Ni (5.0), K (6 3 0), Se (1.3), Ag (1.3), Na (6 3 0), Tl (1.3), V (5.0), Zn (25). Sample preparation for Hg analyses and quantification of sample Hg concentration (limit of detection = 0.064 μg/g) followed the EPA method 7471B (W-846) (U.S. EPA, 2007) for a cold-vapor atomic absorption procedure. Quality control was ensured by running internal standards, blanks, and certified reference material.

2.5. Stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$)

Approximately 1.0 mg of molluscs was utilized for stable isotope analysis. The stable isotope methods for particulate organic matter, sediment organic matter, and mangrove leaves are published in Hargan et al. (2020). Isotopic ratios were analyzed using an Isotope Ratio Mass Spectrometer (IRMS) (ThemoFisher Delta V Plus) at The David W. and Claire B. Oxtoby Environmental Isotope Lab (Pomona College, Claremont, CA). Acetanilide (C_8H_9NO) was used as an internal standard to determine the analytical precision for $\delta^{13}C$ (–29.53‰) and $\delta^{15}N$ (1.18‰) (Indiana University, ID). The standard deviation on 10 aliquots of the same sample was lower than 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$. Samples were calibrated to national and international standards: USGS40 and USGS24 for ^{13}C, and USGS40 and IAEA N-2 for ^{15}N. Results of stable isotope analyses are reported in δ units where $\delta = [(R_{sample}/R_{standard}) - 1] \times 1{,}000$. R_{sample} are the ratios of the isotopes (i.e. $^{13}C/^{12}C$, $^{15}N/^{14}N$) in samples, and $R_{standard}$ are the ratios of isotopes in the international standards, unique for each element. $\delta^{15}N$ is reported relative to atmospheric nitrogen and $\delta^{13}C$ relative to Vienna Pee Dee Belemnite, and isotopes are expressed in units of per mill (‰).

2.6. Data analyses

To visualize differences in mollusc trophic position, $\delta^{13}C$ values were plotted against $\delta^{15}N$ values with isotope signatures for mangrove leaves, particulate organic matter (POM), and sediment organic matter (SOM) collected along the land-to-sea transects (Hargan et al., 2020). One-way analysis of variance (ANOVA) with pairwise Tukey tests were used to test whether the metal concentrations in the sediments from different habitats (i.e. shrimp farm, canal, mangrove, marine) significantly differed. Tests for normality (Shapiro-Wilk's test) rendered significantly non-normal distributions ($p > 0.05$) for all elements and normal distributions for isotopes. Elements were log10 transferred prior to one-way ANOVAs and only for Cr concentration was a Kruskal-Wallis ANOVA on ranks used as this transformed element maintained a non-normal distribution. To test whether mollusc elemental concentrations were significantly different between impact transects and reference transect, we first examined the differences in the mean elemental concentrations of mollusc species between impact transects using an ANOVA. There was no significant difference in species elemental concentrations amongst impact transects. Therefore, to avoid pseudo-replication, we averaged the species elemental concentrations across the three impact transects and compared to mean species concentrations on the reference transect using a paired *t*-test.

Trends in element concentrations with changing trophic level (as measured by $\delta^{15}N$) in molluscs were assessed using linear regression analysis, with element concentration ([element]) log10 transformed. Stable nitrogen isotopes for molluscs were not corrected using basal, primary producer $\delta^{15}N$ values. The sole primary producers with measured $\delta^{15}N$ in our study are mangrove tree leaves (e.g., *Rhizophora apiculata*). Many of the snails are known opportunistic grazers, likely consuming algal sources and detritus when available (Christensen et al., 2001; Lee et al., 2001).

Mangrove elemental concentrations were summarized through a principal component analyses (PCA) including all sediment, aquaculture, and mollusc samples ($n = 68$). Samples were log-transformed prior to PCA analysis to equalize the variance among the different elements. This unconstrained ordination procedure was used to examine how the shrimp pond, sediment, and molluscs samples varied with respect to elemental composition. Among the 23 elements, only those that registered concentrations above detection limit (DL) in at least half of the samples were included in the PCA. If < 50% of measured variables fell below DL, an element was included and samples with elemental measurements below the DL were approximated using the DL of variable divided by the square root of 2 (Hornung and Reed, 1990). Cd, Co, Hg, Ni, Se, V were removed due to a large amount of below detection values. Skewed distributions occurred for some of the elements, where the concentrations are high in sediments and low in molluscs (e.g., Al) or high in molluscs (e.g., Zn) and low in sediments; and thus even with log transformations, some of the elements still failed the normality test. However, with large sample sizes (> 30 samples), the violation of the normality assumption should not cause major problems implying that parametric procedures can be used even when the data are not normally distributed (Ghasemi and Zahediasl, 2012). Therefore, redundant variables were identified with a Pearson correlation matrix ($p \le 0.05$) with Bonferroni-adjusted probabilities (Supplementary File, Table S1). Pb, Al, and Fe were significantly correlated (see Table S1), and grouped using Al, which explained the largest amount of variation. Mn was also correlated to Pb and Fe, but not Al, so it was left in the PCA, leaving 10 elements included in the PCA ordination.

3. Results

3.1. Sediments

Mangrove sediment elemental concentrations did not differ between the reference and impact transects ($p > 0.05$). Mean [As] significantly differed among the sediment groups (One-way ANOVA, df = 3, F = 3.2, p = 0.038); although, this difference is largely driven by higher [As] in the shrimp farm sediments (10.3 ± 5.2 μg/g) than in the mangroves (4.9 ± 2.3 μg/g) (q = 4.3, p = 0.027), with more similar [As] between canal (4.8 ± 1.3 μg/g) and KKB sediments (5.8 ± 1.3 μg/g) (Table 1). Sediment [As] beyond the shrimp ponds gradually increased from land-to-sea (Fig. 2). Mean [Cu] were highest in the drainage ponds (69.2 ± 63.6 μg/g), yet [Cu] were still nine times higher in canal (18.4 ± 22 μg/g) compared to KKB sediments (2.1 ± 1.2 μg/g) (Table 1). This yielded significant differences in mean sediment [Cu] between all KKB habitats (One-way ANOVA, df = 3, F = 15.5, $p < 0.001$), although differences between shrimp pond [Cu] and canal [Cu] (Tukey test, q = 3.7, p = 0.07) and the canal and mangrove [Cu] (Tukey test, q = 1.8, p = 0.57) were insignificant. Manganese concentrations ranged from 51 ± 30 μg/g in mangrove sediments to 221 ± 67 μg/g in KKB sediments, with significantly higher [Mn] measured in KKB sediments relative to the mangrove (Tukey test, q = 7.9, $p < 0.001$) and canal (q = 4.4, p = 0.022) (Table 1). Overall, this resulted in a gradient of increasing sediment [Mn] from land-to-sea (Fig. 2). Average [Pb] in sediments were highest in the shrimp drainage ponds (mean = 9.2 μg/g), but were otherwise consistent and did not significantly differ across habitats in KKB (means = 4.9–5.5 μg/g) (One-way ANOVA, df = 3, F = 1.6, p = 0.212) (Table 1, Fig. 2). Sediment [Cr] significantly differed among habitats (Kruskal-Wallis ANOVA on ranks, df = 3, H = 9.5, p = 0.023) with significantly higher [Cr] in shrimp pond sediments than mangrove (Dunn's Method, Q = 2.9, p = 0.024) and marine KKB (Dunn's Method, Q = 2.7, p = 0.039) (Fig. 2).

3.2. Farmed shrimp and shrimp feed

Shrimp feed mean [Mn] (39.2 μg/g) was lower than the average [Mn] of all mollusc species (Table 1); however, shrimp feed average [Mn] was higher than the average [Mn] in *Penaeus monodon* (2.8 ± 0.4 μg/g) (Table 1). Shrimp feed also had a slightly higher average [Zn] (66.0 μg/g) compared with mean *P. monodon* [Zn] (62.0 ± 9.5 μg/g) (Table 1). The average [Cu] was similar between shrimp feed (31.9 μg/g) and *P. monodon* samples (31.0 ± 9.3 μg/g) (Table 1). Arsenic concentrations were lowest in the shrimp feed (< 1.3 μg/g) compared with average [As] in *P. monodon* (2.7 ± 0.5 μg/g) (Table 1) and higher concentrations in mangrove mollusc species.

3.3. Mangrove mollusc stable carbon and nitrogen isotopes ($\delta^{13}C$, $\delta^{15}N$)

The $\delta^{13}C$ and $\delta^{15}N$ values significantly positively correlated among the molluscs (r^2 = 0.411, $p < 0.001$, Table 2, Fig. 3A). Thus, the significant relationships present between $\delta^{15}N$ values and an element are also often true for $\delta^{13}C$ values and the same element (Table 2). Mollusc samples captured a broad range in $\delta^{13}C$ and $\delta^{15}N$ values from –27.2 to –17.3‰ and 0.1 to 10.3‰, respectively. *Thais gradata* had the highest mean $\delta^{15}N$ value (8.3‰, n = 4) and mean $\delta^{13}C$ value (–20.1 ± 2.1‰), while *C. obtusa* had the lowest mean $\delta^{15}N$ value (1.9‰, n = 5) (Table 1). Mean mollusc tissue $\delta^{13}C$ values were enriched relative to mean mangrove leave $\delta^{13}C$ values (+5.4–13.1‰), mean mangrove SOM $\delta^{13}C$ values (+2.9–8.6‰), and mean shrimp farm POM $\delta^{13}C$ values (+2.3–8.0‰) (Fig. 3B).

3.4. Trophic relationships to elements

Across mollusc samples, elements that were routinely above instrumental detection limit were: Cu, As, Mn, Zn, Cr, Fe, Al, Pb, and Se; and therefore, we focus on trends between these metal(loid)s, mollusc diet (inferred using known diet preferences and $\delta^{13}C$ values), and trophic position (inferred with $\delta^{15}N$ values). Of these elements, we found no significant difference of transect type (comparing the mean species elemental concentrations across the impact transects to the

Table 1
Mean stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values and elemental concentrations (± standard deviation) in mangrove, canal, marine Khung Krabaen Bay (KKB), and shrimp pond sediments, shrimp feed, farmed shrimp tissue, and mollusc tissue. All samples from the reference and impact transects are combined. For sediment samples, if > 50% of the samples fell above the element detection limit, the element concentration was estimated using the same methods outlined for the principal components analyses. Below detection (b.d.) noted only if all samples measured fell below the instrumental method detection limit.

	n	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	**As** (μg/g)	**Cu** (μg/g)	**Zn** (μg/g)	**Mn** (μg/g)	**Fe** (mg/g)	**Cr** (μg/g)	**Pb** (μg/g)
Sediments & aquaculture										
Mangrove	12	3.1 ± 0.9	−28.7 ± 0.4	4.9 ± 2.3	10.8 ± 15	19.7 ± 7	51 ± 30	6.9 ± 4.3	8.6 ± 4.5	5.5 ± 3.1
Marine KKB	8	3.8 ± 0.6	−24.5 ± 0.6	5.8 ± 1.3	2.1 ± 1.2	8.1 ± 3	221 ± 67	10.1 ± 2.3	7.4 ± 2.4	5.3 ± 2.4
Canal	5	4.1 ± 0.2	−27.1 ± 0.7	4.8 ± 1.3	18.4 ± 22	23.5 ± 9	77 ± 49	7.5 ± 3.4	8.5 ± 3.6	4.9 ± 2.3
Shrimp pond	5	4.4 ± 0.7	−25.1 ± 1.4	10.3 ± 5.2	69.2 ± 64	72.1 ± 24	185 ± 129	22.6 ± 10.9	22.1 ± 10.1	9.2 ± 4.1
Shrimp feed	3	2.5 ± 0.1	−25.4 ± 0.1	< 1.3	31.9	66.0	40	0.42 ± 0	b.d.	b.d.
Peneaus monodon	3	5.0 ± 0.2	–23.1 ± 0.1	2.7 ± 0.5	31.0 ± 9	62.0 ± 9	2.8 ± 0.4	b.d.	b.d.	b.d.
Molluscs										
Cerithidea obtusa	5	1.9 ± 1.2	−24.8 ± 1.2	5.1 ± 1.6	100.8 ± 52	66.0 ± 32	192 ± 149	0.8 ± 0.3	9.3 ± 4.9	b.d.
Littoraria sp.	5	3.3 ± 1.8	–23.7 ± 2.2	7.4 ± 2.1	87.4 ± 31	118.2 ± 66	86 ± 20	1.1 ± 0.5	7.1 ± 3.5	1.3 ± 0.5
Nerita lineata	7	3.5 ± 2.6	−25.5 ± 1.9	6.1 ± 1.2	47.4 ± 25	69.5 ± 35	59 ± 22	0.8 ± 0.3	13.5 ± 6.7	1.0 ± 0.3
Ellobium aurisjudae	6	4.9 ± 0.8	−25.8 ± 1.3	8.8 ± 1.4	90.3 ± 21	150.2 ± 65	51 ± 21	2.5 ± 0.8	9.6 ± 9.9	2.1 ± 0.9
Isognomon ephippium	6	7.0 ± 0.6	–22.5 ± 1.2	13.4 ± 2.3	11.7 ± 5	1938.5 ± 835	211 ± 304	1.3 ± 1.4	4.9 ± 2.9	1.7 ± 1.5
Thais gradata	4	8.3 ± 2.0	−20.1 ± 2.1	20.1 ± 15	197.4 ± 228	657.8 ± 536	18 ± 15	0.5 ± 0.4	20.5 ± 24.2	b.d.

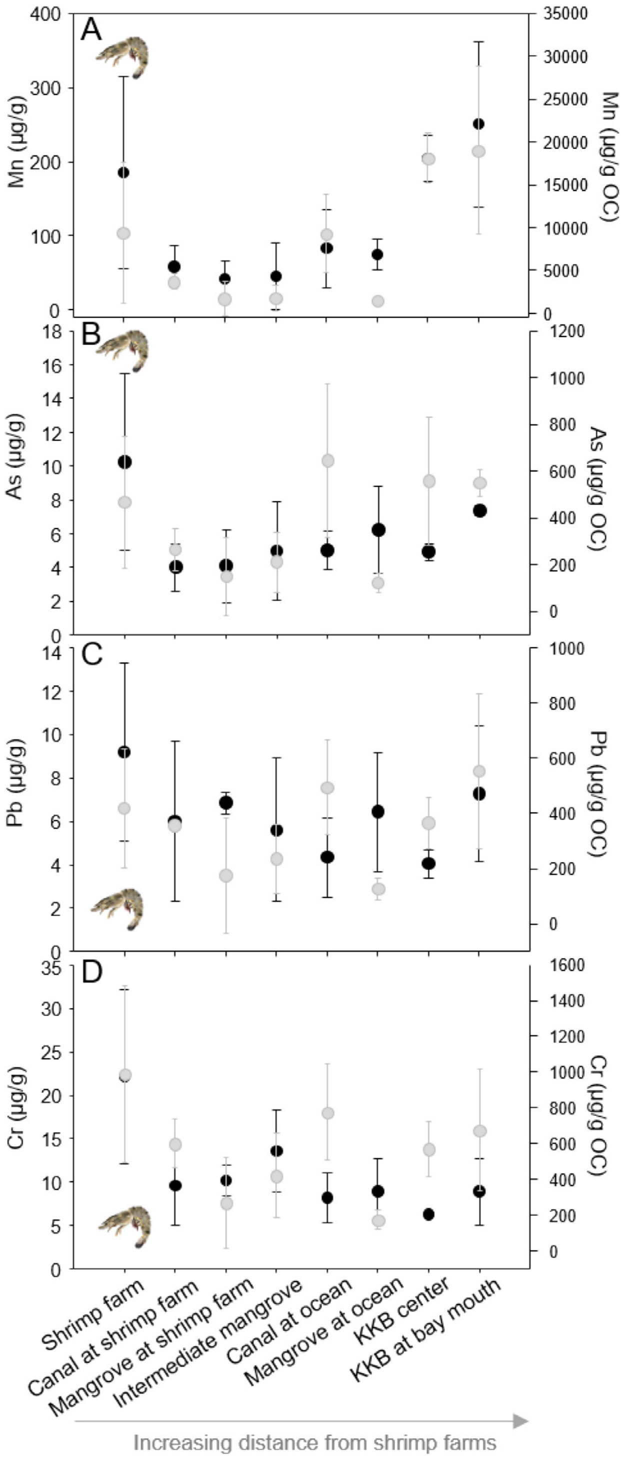

Fig. 2. Mean (± standard deviations) sediment element concentrations (µg/g d.w.) for A) manganese (Mn), B) arsenic (As), C) lead (Pb), and D) chromium (Cr) along a gradient of increasing distance from the shrimp farms, beginning with the shrimp farm drainage ponds, canal and mangrove samples closest to the farm, and progressing out into the Khung Krabaen Bay (KKB) marine system. Grey circles and standard deviation bars correspond to the element concentrations plotted in µg/g of organic carbon (OC) on the right y-axis.

reference transect) on mollusc species body concentration. Mercury concentrations were above instrumental detection limits in all *E. aurisjudae* samples (0.10 ± 0.03 µg/g) and 5 of 7 *Nerita lineata* samples (0.09 ± 0.03 µg/g); otherwise [Hg] was below detection for more than half of the other mollusc species as well as all sediment samples.

Table 2
Linear regression analysis of mangrove mollusc elemental concentration (µg/g d.w.) versus tissue $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios (‰). Relationships with both a *p*-value < 0.05 and r^2 > 0.3 are bolded.

Element	*n*	Slope	Intercept	r^2	p-value
$\delta^{13}C$ *vs.* $\delta^{15}N$	33	**0.697**	**21.409**	**0.411**	< **0.001**
$\delta^{15}N$ vs.					
$log_{10}As$	33	**0.0723**	**0.584**	**0.756**	< **0.001**
$log_{10}Mn$	33	−0.0894	2.176	0.253	0.003
$log_{10}Cu$	33	−0.0511	1.960	0.0939	0.083
$log_{10}Zn$	33	**0.164**	**1.502**	**0.547**	< **0.001**
$log_{10}Cr$	33	−0.0210	0.993	0.0312	0.325
$log_{10}Se$	33	0.0372	0.0661	0.126	0.046
$log_{10}Pb$	33	−0.00764	0.0866	0.00673	0.650
$log_{10}Al$	33	−0.0373	2.805	0.0417	0.255
$\delta^{13}C$ vs.					
$log_{10}As$	33	**0.0552**	**2.246**	**0.374**	< **0.001**
$log_{10}Mn$	33	−0.0335	0.953	0.0302	0.334
$log_{10}Cu$	33	−0.0433	0.683	0.0570	0.181
$log_{10}Zn$	33	**0.132**	**5.445**	**0.303**	< **0.001**
$log_{10}Cr$	33	−0.00404	0.789	0.000983	0.862
$log_{10}Se$	33	−0.00516	0.105	0.00197	0.806

Minimum and maximum [As] ranged from 2.2 to 41.3 µg/g for individual molluscs. The highest mean [As] was measured in *T. gradata* (30.2 ± 15.7 µg/g, $n = 4$). For each individual species of mollusc sampled, [As] increased with increasing $\delta^{15}N$ values. The strongest relationship measured between an element and both $\delta^{15}N$ and $\delta^{13}C$ values was for [As] (Table 2), with a significant, positive correlation between log[As] and $\delta^{15}N$ values ($n = 33$, $r^2 = 0.756$, $p < 0.001$), as well as $\delta^{13}C$ values ($r^2 = 0.374$, $p < 0.001$) (Table 2, Fig. 4A, Fig. S2).

Average mollusc [Zn] ranged from 42.9 to 2960 µg/g (Table 1). Within species, *C. obtusa*, *N. lineata*, and *Littoraria* sp. [Zn] demonstrated little change within increasing $\delta^{15}N$. However, [Zn] did increase with increasing $\delta^{15}N$ values for *Isognomon ephippium* and *T. gradata* (Fig. 4B). There was an overall positive, significant linear relationship between log[Zn], $\delta^{15}N$ ($r^2 = 0.547$, $p < 0.001$), and $\delta^{13}C$ values ($r^2 = 0.303$, $p < 0.001$) (Table 2, Fig. 4B).

Mean Mn values were lowest in *N. lineata*, *Littoraria* sp., and *E. aurisjudae* (18–86 µg/g), while [Mn] were highest in *I. ephippium* (211 ± 304 µg/g) and *C. obtusa* (192 ± 149 µg/g) (Table 1, Fig. 4C). Standard deviation in [Mn] was highest for *I. ephippium*, as two oyster samples (of the three collected along the reference transect) had [Mn] several orders of magnitude higher (453 and 722 µg/g) than oyster samples collected along the impact transect. Therefore, [Mn] decreased as $\delta^{15}N$ value increased for each snail species except *I. ephippium* tree oysters (Fig. 4C). Mollusc log[Mn] significantly decreased with increasing $\delta^{15}N$ values ($r^2 = 0.253$, $p = 0.003$) (Table 2, Fig. 4C). In contrast, a significant negative regression between $\delta^{13}C$ values and log [Mn] was not observed (Table 2, Fig. S2).

Average mollusc [Cu] ranged from 8.0 to 537 µg/g. Cu concentrations increased with increasing $\delta^{15}N$ value in *E. aurisjudae* and *C. obtusa*. However, [Cu] decreased with increasing $\delta^{15}N$ value in *I. ephippium* and *T. gradata* (Fig. 4D). *Isognomon ephippium* had low [Cu] relative to all other molluscs. Two *N. lineata* samples with high $\delta^{15}N$ values also had low tissue [Cu]. An insignificant, negative slope represents the relationship between $\delta^{15}N$ values and log[Cu] (Table 2, Fig. 4D).

A negative slope between mollusc $\delta^{15}N$ values and log[Cr] was observed ($p = 0.325$) (Table 2, Fig. 4E). Additionally, we found a positive relationship between mollusc $\delta^{15}N$ and log[Se] ($p = 0.05$) (Fig. 4F). No relationships were observed between mollusc $\delta^{15}N$ and $\delta^{13}C$ values, and log[Pb] and log[Al] (Table 2).

3.5. Comparisons between sediment and molluscs using multivariate analysis

Axis 1 and 2 of the PCA explained 71% of variation in elemental

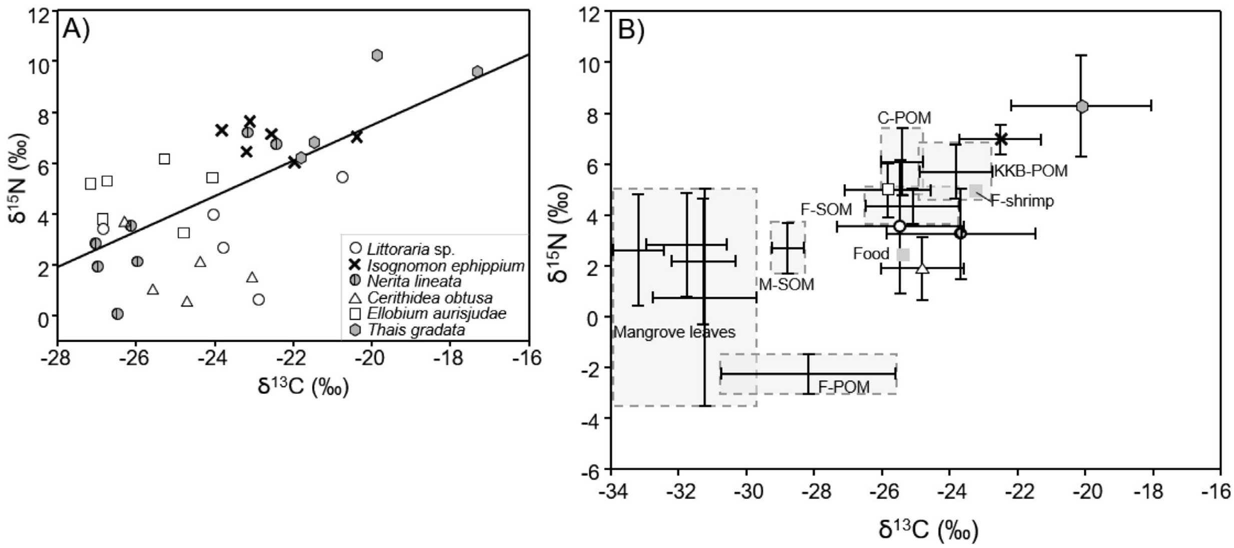

Fig. 3. A) Mollusc stable carbon isotopes ($\delta^{13}C$, ‰) *vs* stable nitrogen isotopes ($\delta^{15}N$, ‰) with a significant, positive linear regression shown (Table 2). B) Mean mollusc, farmed shrimp ('F-shrimp') and shrimp pellet food ('Food') $\delta^{13}C$ and $\delta^{15}N$ values plotted with mean stable isotope values of mangrove leaves, and particulate and sediment organic matter (POM, SOM) sources for the shrimp farms ('F-POM', 'F-SOM'), canals ('C-POM'), mangrove ('M−SOM'), and marine KKB ('KKB-POM') (from Hargan et al., 2020). The organic matter pools are captured in grey boxes outlined with dashes and the molluscs are delineated using symbols from the legend in Fig. 3A.

concentrations among canal, mangrove, shrimp drainage pond, and bay sediments, molluscs, and farmed shrimp samples ($n = 68$) (Fig. 5). The PCA distinguished marine sediments with high [Al] and shrimp pond drainage sediments by high [Mn]. Trophically-elevated *T. gradata* and *I. ephippium* are represented by high [Zn] and [As] (Fig. 5). *Cerithidea obtusa* and *N. lineata* samples are clustered closest to the shrimp drainage pond sediments (Fig. 5). In general, primary consumers (*Littoraria* sp., *E. aurisjudae, N. lineata*) are distinguished by high [Cu], [Ca], and [Mg] (Fig. 5).

4. Discussion

4.1. Sediment elemental concentrations

Sediment elemental concentrations were significantly higher in the shrimp drainage ponds than all other sediments analyzed in KKB (Table 1). Since metal(loid) concentrations were low in the shrimp and shrimp food (Table 1), this suggests that collectively the dense number of shrimp, shrimp feed, and shrimp feces contributes to elevated elemental concentrations that accumulate over time in the drainage ponds. In general, the prawn *P. monodon* accumulates some metals under particular aquaculture pond conditions and does not accumulate other metals regardless of the pond conditions (Hashmi et al., 2002). This means that often there can be a decoupling effect between concentrations in shrimp tissue and the water and sediments, where although low concentrations are measured in shrimp (i.e. little bioaccumulation) they can be high in the water and sediments. Thus, even in relatively pristine landscapes with regards to heavy metal contamination, significant increases in sediment metals (Zn, Cu, Pb) close to aquaculture ponds occur suggesting that trace metal enrichment in the sediments may be attributed to the aquaculture pond effluents (Mendiguchía et al., 2006). In mixed-species aquaculture in natural wetland tidal pools of Deep Bay, Hong Kong, the sediments collected from sites closest to the aquaculture also contained higher Zn, Cr, Cu, Ni and Cd concentrations than those sediments collected from the seaward sites (Cheung and Wong, 2006); also suggesting that the large abundance of animals in coastal areas produce trace metal contamination.

In our study system, the abundances of elements in the shrimp drainage ponds and mangroves were very similar, with concentrations in sediments of the ponds and mangroves following the distribution from Fe > Mn > Zn > Cu > Cr > Pb > As (Table 1). In contrast, abundances of elemental concentrations in marine KKB sediments were distinct with elemental abundances following the pattern of Fe > Mn > Zn > Cr > Pb > As > Cu (Table 1). These differences were likely due to separate sources of elements to each system, but also changes in abiotic conditions including salinity and redox potential, which contribute to the settling and resuspension of elements (Marchand et al., 2011). For example, the release of effluent within an *Avicennia* mangrove stand modified the duration of waterlogging and thus modified the redox conditions, which influenced the carrier phase of heavy metals, becoming largely associated with sulfides during effluent release (Marchand et al., 2011). Metals were thus less mobile, and consequently when the mangrove received effluents, they acted as a sink for trace metals (Marchand et al., 2011). In KKB, shrimp drainage ponds first receive effluent followed by the canals and mangroves, indicating that despite their landward location with less inundation at high tide, these areas of the bay would be water logged for a longer time, attenuating some of the highest elemental contamination in the drainage ponds and canals closest to the farms. However, in KKB elemental concentrations still rapidly declined from the drainage pond sediments to the mangrove and canal sediments closest to shrimp farms (Fig. 2). This implies that the drainage ponds are effective at attenuating elemental contamination and/or that metal(loid)s are flushed beyond the mangrove into the marine ecosystem.

In KKB, canals likely direct shrimp aquaculture effluent beyond the mangroves to the marine environment (Hargan et al., 2020). Here, Mn, As, Cr, and Pb concentrations are high in shrimp pond sediments, low in the mangrove sediments, and increase again in the marine KKB sediments (Fig. 2). However, with our methodology we cannot ascertain the source of these elements in the marine sediments. Overall, we find that KKB mangrove sediment elemental concentrations are similar to unimpacted mangroves studied in Hong Kong (Tam and Wong, 2000; MacFarlane et al., 2007), except that in some cases KKB mangrove sediments have higher [Cr] and [Cu]. Cu and Cr can increase in sediments adjacent to aquaculture (Mendiguchía et al., 2006; Cheung and Wong, 2006) and are associated with ecosystem contamination from untreated sewage (Montgomery and Price, 1979; Revenga et al., 2012). In general, our KKB sediment concentrations are lower than those measured in tropical bays receiving industrial and sewage pollution (Ong Che, 1999; Tam and Wong, 1995, 2000; Defew et al., 2005). KKB elemental

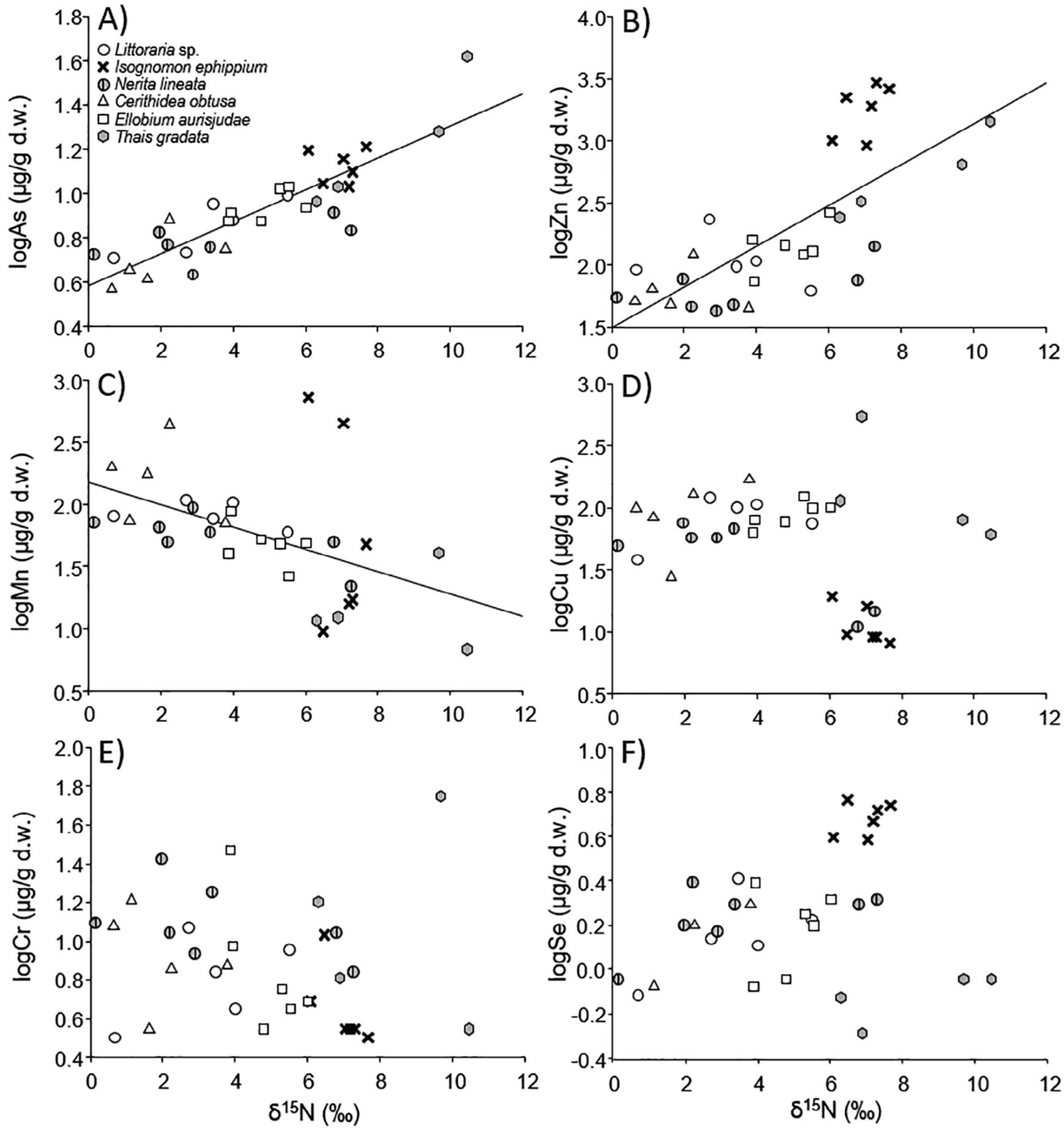

Fig. 4. Stable nitrogen isotope ($\delta^{15}N$, ‰) values versus log-transformed A) arsenic (As), B) zinc (Zn), C) manganese (Mn), D) copper (Cu), E) chromium (Cr), and F) selenium (Se) concentrations (µg/g dry weight) measured in mangrove mollusc tissue. Only significant linear regressions are plotted (Table 2).

concentrations are also lower than in southern Thailand where municipal areas, industrial zones, and dockyard areas had the highest sediment contamination by heavy metals, particularly Pb and As, while shrimp farming and traditional land uses such as salt flats, paddy fields, orchards, and mangrove forests showed low levels of metals (Sowana et al., 2011). Overall, at these other tropical Asian embayments, mangrove elemental concentrations are much higher than KKB sediments particularly for Pb and Cu (e.g., Ong Che, 1999); however, the sediments of industrially-polluted bays most closely resemble shrimp drainage pond sediments suggesting these anthropogenic settling ponds are having some effect at retaining aquaculture metal(loid) waste.

4.2. Mangrove mollusc diet interpreted from carbon and nitrogen stable isotopes

Mangrove molluscs in KKB capture a broad range in $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 3), representing varying diets and niche habitats within coastal ecosystems (Demopoulos et al., 2008). Among the primary consumers (*Littoraria* sp., *N. lineata*, *C. obtusa*, *E. aurisjudae*), a gradient in $\delta^{13}C$ and $\delta^{15}N$ values reflects different diets and feeding locations in the mangrove, including on the forest floor grazing algae/leaf litter in sediment (*E. aurisjudae*, *C. obtusa,* sometimes *N. lineata*) versus on tree trunks grazing epiphytic algae on bark and leaves (*Littoraria* sp.). Omnivory in *I. ephippium*, including the consumption of marine plankton (Chen and Folt, 2000), results in a higher trophic position than primary consumer gastropods (Fig. 3). Given a similar *I. ephippium* $\delta^{13}C$ signature to marine KKB particulate organic matter (POM) and canal POM, tree oysters are likely filtering food resources from this OM pool (Fig. 3B). *Thais gradata* had the highest $\delta^{15}N$ values, representative of their predatory diet on barnacles, oysters, and mussels (Blackmore and Morton, 2002). Molluscs with a greater proportion of their diet originating from a marine ecosystem will have more enriched tissue $\delta^{13}C$ signatures relative to molluscs consuming mangrove primary producers, as marine primary producers have enriched $\delta^{13}C$ values relative to terrestrial organic matter (Bouillon et al., 2008). Our two mollusc species with the highest trophic positions (*T. gradata* and *I. ephippium*) happen to also have the greatest proportion of their diet from marine KKB; thus, this yielded a significant correlation between $\delta^{13}C$ and $\delta^{15}N$ values across mollusc samples (Fig. 3A).

Understanding the diet and habitat location of molluscs is important

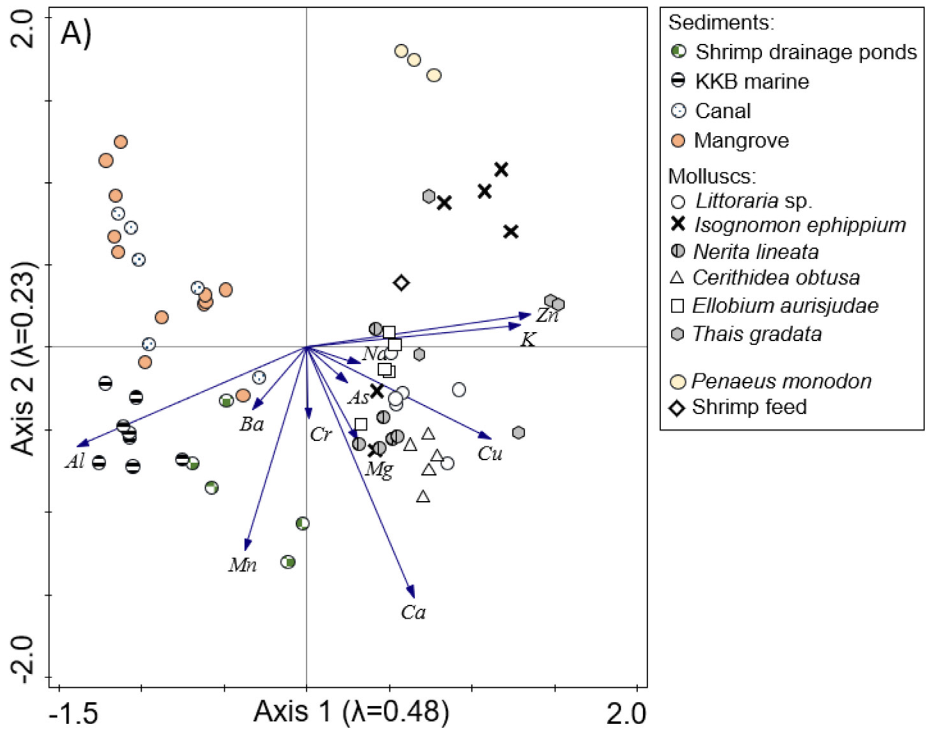

Fig. 5. Principal components analysis (PCA) axis 1 and 2 for including all sediment, mollusc, and farmed shrimp samples. Samples were log-transformed prior to PCA analysis to equalize the variance among the different elements. Elements were included in the PCA if > 50% of the samples fell above the detection limit.

for determining their potential exposure to aquaculture effluent elemental contamination. The substantially enriched (+4–5‰) snail $\delta^{13}C$ signatures relative to the mangrove tree leaf $\delta^{13}C$ values indicates that the molluscs with lower trophic positions (e.g., *Littoraria* sp., *N. lineata*) are not solely consuming mangrove vegetation or leaf detritus (Fig. 3B). *Littoraria* sp. are generalist herbivores, that easily shift diet depending on food availability (Christensen et al., 2001; Alfaro, 2008), feeding on mangrove tree bark (Lee et al., 2001), tree leaves (Thanh-Nho et al., 2019), and marine fungi from the surface of *Rhizophora* prop roots (Kohlmeyer and Bebout, 1986). These niche habitat differences across the gastropods indicate that their exposure to shrimp farm effluent and metal(loid)s that settle in mangrove sediments is different (i.e., the bioavailability of metals will differ for each mollusc species). For instance, the confinement of *Littoraria* sp. to mangrove trees and prop roots will reduce its exposure to shrimp farm effluent flushed into the mangrove at high tide compared to the snails grazing on the forest floor at low tide. Hence, *Littoraria* tissue metal bioaccumulation is more prone to trace element uptake and accumulation that occurs in *Rhizophora* leaves (Thanh-Nho et al., 2019). There is a higher propensity for *E. aurisjudae* and *C. obtusa*, which graze at that mud surface, to be exposed to elemental contamination released in shrimp farm effluent. Furthermore, the sedentary position of *I. ephippium* near the forest floor as well as their passive feeding strategy would also increase their exposure to metal(loid)s released in aquaculture effluent. Although *E. aurisjudae* are grazing on the forest floor, likely consuming mangrove leaf litter, we note that *Ellobium* tissue demonstrated a $\delta^{13}C$ enrichment of 5‰ from mangrove leave $\delta^{13}C$ values, and a mean $\delta^{15}N$ increase of 2.5‰ from leaves. These high discrimination factors appear to be common for mangrove organisms consuming mangrove leaf litter (Kristensen et al., 2008). In controlled feeding experiments with *E. aurisjudae*, the mean difference in $\delta^{13}C$ and $\delta^{15}N$ values from mangrove leaves to animals was 5.3 ± 0.3‰ and 4.2 ± 0.2‰, respectively (Teoh et al., 2018), demonstrating large trophic discrimination factors like those reported for mangrove crabs consuming mangrove leaf litter (+5‰ for $\delta^{13}C$; Kristenson et al., 2008). Hence, it is challenging to determine mollusc diet from stable isotopes and the use of commonly reported isotopic fractionation values for consumers, and observations on where molluscs occur in mangroves aid determination of exposure pathways to effluent.

4.3. Varying elemental concentrations between mollusc species

Mollusc elemental concentrations were lower than in invertebrates found in polluted global estuaries, such as in the Netherlands (Van den Broeck et al., 2010) and China (Pan and Wang, 2012), as well as streams receiving acid mine drainage (for Cu and Fe; Quinn et al., 2003). The KKB mollusc elemental concentrations are comparable to mollusc metal(loid) concentrations in less polluted Asian coastal systems (e.g., *T. gradata* from an estuary in Borneo; Proum et al., 2016), but also to molluscs collected in a mangrove downstream of Ho Chi Minh City, Vietnam (Thanh-Nho et al., 2019). Thanh-Nho et al. (2019) represents the most comparable study to our own with the same species sampled and same elemental concentrations measured. Here we see that our mollusc tissue [Mn] and [As] are within a similar concentration range for *C. obtusa*, *N. lineata*, and *Littoraria* sp., but we have species-specific differences in Cu with elevated [Cu] in nerites from KKB. Interestingly, [Cr] are higher in all mollusc species sampled in KKB compared to the mangrove adjacent to Ho Chi Minh City. As elevated Cr was measured in the shrimp drainage pond sediments (Fig. 2), this metal may be a specific indicator of aquaculture and sewage contamination versus industrial urban runoff. However, elevated metal (loid) concentrations were not spatially influenced by transect type, i.e., reference *vs*. impact, and thus it cannot be concluded that intensive aquaculture is elevating metal(loid) concentrations in mangrove molluscs. We see that many metal(loid)s decline in molluscs with increasing distance from the shrimp farms (Fig. S3). However, mollusc species occurrence varied along the intertidal zone (e.g., *T. gradata* was more common towards the seaward edge of the mangrove) such that molluscs of higher trophic position were generally sampled further from the shrimp farms. Due to biomagnification, metal(loid) concentrations were thus elevated in higher trophic level molluscs and we could not address whether metal(loid)s increase or decrease in molluscs moving away from the shrimp farms. To evaluate an impact of this research question only one mollusc species that is common along the mangrove intertidal zone should be sampled at greater frequency (i.e., more than every 50 m) and at higher abundances to achieve a large sample size capable of determining how bioaccumulation changes along the intertidal zone.

In KKB, sediment and mollusc element concentrations were not correlated, and thus given different metal concentrations (Zn, As, Cu, Mn, and Cd) between molluscs species, other driving factors (e.g., physiology, exposure pathway and diet) are contributing to the levels of metal bioaccumulation. However, the multivariate ordinations yield some similarities between mollusc tissue and sediment metal concentrations. In the KKB mangrove, *E. aurisjudae* had high concentrations of Pb, Al, and Fe; metals that distinguish shrimp aquaculture sediments from mangrove and marine sediments, and thus could be considered better tracers of aquaculture effluent (Fig. 2, Fig. 5). Some gastropods can be especially good trace element accumulators due to their feeding habits of taking in sediment-bound and organic matter-adsorbed metals (Leppanen, 1995). Ellobiid gastropods have a specialized gizzard and stomach system that allows a high efficiency of assimilation of consumed plant matter in sediments (Morton, 1955). Thus, this would also allow these snails to process more metal(loid)s bound to organic matter in sediments including those elements that may be released in shrimp farm effluent and rapidly settle in the mangrove. It would be informative for future biomonitoring studies using Ellobiid gastropods to also examine organ-specific metal(loid) accumulation.

4.4. Trophic dynamics of elements in KKB mangrove molluscs

4.4.1. Zn, As, and Cu

The significant increase in [Zn] and [As] with increasing $\delta^{15}N$ values demonstrates the ability of elements to biomagnify in species with a higher trophic position in the food web. Tree oysters had high [As] and [Zn] (Fig. 4), and as filter feeders, high metal(loid) accumulation could be due to both their diet and exposure to shrimp pond effluent as they are sedentary and submerged at high tide. Oysters can be net metal (loid) accumulators, and are shown to have elevated [Zn]; with *I. ephippium* in KKB demonstrating the highest [Zn] (Phillips and Yim, 1981; Rainbow, 1995; Blackmore, 2001; Marín-Guirao et al., 2008). In passive predators, like *I. ephippium*, and active predators, like *T. gradata*, transfer of trace metals is controlled by the quantity of metal accumulated in the prey, which is a result of the physiological detoxification process favored by the prey species (Blackmore, 2001). Thus, dietary exposure is a good predictor of elemental biomagnification, with *T. gradata* [Zn] explained by their diet of barnacles which usually accumulate Zn with no significant excretion (Blackmore and Morton, 2002; Wang and Ke, 2002; Rainbow and Wang, 2005). Zooplankton, a component of *I. ephippium* diet, take up metal(loid)s directly from water, with often the highest [As] in planktivorous species that feed directly on metal-enriched zooplankton (Chen and Folt, 2000). Overall, predatory molluscs contain the highest [As] relative to the detritus and algae eating snail species, demonstrating significant biomagnification in the KKB mangrove (Goessler et al., 1997; Khokiattiwong et al., 2009; Shilla et al., 2019). In general, total [As] and [Zn] in KKB molluscs are not of toxicological concern. Furthermore, most As in seafood, like oysters, is found in organic forms, which are less toxic than the inorganic forms, and generally believed to be non-carcinogenic (Guo, 2002).

Copper is an essential element to living organisms, although it can be toxic when high concentrations are consumed. Cu biomagnification in snails and food webs is usually observed, with gastropods considered as “macro-concentrators” species for Cu (Marín-Guirao et al., 2008; Nica et al., 2012). Indeed, the PCA highlights that *N. lineata* and *C. obtusa* are distinguished by high [Cu] (Fig. 5). Some species of gastropods require higher body Cu as it is a constituent of the respiratory pigment hemocyanin (Dallinger et al., 2005). Bivalves do not have hemocyanin and so gastropods commonly have high [Cu] in comparisons across molluscs, indicating the physiological importance of this metal to *N. lineata* and *C. obtusa* (Blackmore, 2001). Here, we did not observe Cu biomagnification among the molluscs. This is likely due to high gastropod demands for Cu, and indeed we see the lowest [Cu] are measured in *I. ephippium*. Predatory mangrove gastropods have previously presented with high [Cu] relative to primary consumer gastropods (Blackmore and Morton, 2002), although here, *T. gradata* did not have significantly higher [Cu] than other gastropod species in our study indicating that their main diet of barnacles is also likely not high in Cu.

4.4.2. Mn, Cr, Se, Pb, and Fe

Over a range of aquatic ecosystems Mn generally demonstrates no significant increase or decrease with trophic level; however, in a Tanzanian mangrove estuary, Mn was found to biodilute, although this trend was insignificant (Shilla et al., 2019). *Cerithidea obtusa*, also sediment grazers like *E. aurisjudae*, demonstrated the highest mean [Mn] (after *I. ephippium* reference samples). Some evidence suggests that due to the organic sediment beneath *Rhizophora* stands, reductive dissolution of Fe-Mn oxihydroxides by bacteria during organic matter decay is a source of dissolved trace elements in pore-waters, these elements being more bioavailable (Thanh-Nho et al., 2019). This may indicate why a lower trophic organism like *C. obtusa* could accumulate more Mn, contributing to a significant biodilution in Mn amongst mangrove molluscs.

Domestic and industrial products are rich in Cr; although the bioavailability of Cr is controlled by its speciation and redox behavior with Cr(VI) more toxic than Cr(III) which is relatively non-toxic to organisms and is an essential micronutrient for mammals (Pan and Wang, 2012). In KKB, [Cr] concentrations of molluscs were generally high compared to other mangroves impacted by anthropogenic activities; and we observe that some lower trophic organisms, particularly *C. obtusa*, again have higher tissue Cr. This may indicate that this metal is more bioavailable on the forest floor. Tree oysters had lower [Cr], likely due to their low assimilation efficiencies of this metal (Wang and Fisher, 1999). In contrast, Se concentrations were significantly different between mollusc species and notably, *I. ephippium* had the highest [Se] (Fig. 4F, Fig. 5). Like for Zn, *I. ephippium* are net accumulators of Se with many studies measuring high [Se] in bivalves (Pan and Wang, 2012).

Lead is also a cumulative metabolic poison (Pan and Wang, 2012). Low [Pb] in marine organisms often occurs due to lower bioavailability of dissolved and dietary Pb, and even in areas with mine drainage pollution, the bioaccumulation of Pb in mussels is slow (Riget et al., 1997). However, there can be good correspondence between sediment and animal Pb patterns indicating that sediment is a Pb source for mangrove organisms. Given that Pb is a persistent, immobile element with low solubility, mud grazers would have higher risks to Pb exposure. Similarly, *E. aurisjudae* also has notably higher tissue [Fe] which can could be traced to its high ingestion efficiency of organic matter in sediments (Morton, 1955).

5. Conclusion

We found low elemental concentrations in farmed shrimp and pellet food, but high elemental concentrations in drainage pond sediments (similar to other aquaculture, sewage, and industrially polluted sediments across Thailand and SE Asia), and thus we conclude the sheer volume of shrimp reared in high intensity aquaculture yields considerable elemental accumulation in pond sediments. However, to some extent the management ponds capture metal(loid) contamination from the effluent before it enters the marine environment and we find that both the mangrove sediments and mangrove mollusc metal(loid) concentrations do not indicate signs of contamination from aquaculture.

Carbon and nitrogen stable isotopes of the mangrove mollusc food web indicate large differences in diets across mollusc species, with molluscs consuming marine diets having higher trophic positions. In the KKB mangrove, molluscs biomagnify As and Zn, whereas Mn appears to biodilute with increasing trophic position. Elevated [Cu] in *N. lineata* can largely be related to the physiological importance of Cu to some species of gastropods. *Ellobium aurisjudae* and *C. obtusa*, mud-dwelling gastropods, are distinguished from other molluscs by higher tissue metal concentrations of those elements also elevated in the shrimp drainage pond sediments (e.g., Pb, Fe, Al, Cr); and thus we identify these common gastropods as best probable bioindicators of aquaculture contamination in southeast Asian mangroves.

CRediT authorship contribution statement

Alison H. Hong: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Kathryn E. Hargan:** Conceptualization, Data curation, Formal analysis, Visualization, Writing - review & editing. **Branwen Williams:** Conceptualization, Funding acquisition, Supervision. **Bunlung Nuangsaeng:** Supervision, Methodology. **Sarawut Siriwong:** Methodology, Project administration. **Pisut Tassawad:** Methodology, Validation, Project administration. **Chatdanai Chaiharn:** Methodology. **Marc Los Huertos:** Conceptualization, Funding acquisition, Methodology, Validation.

Declaration of Competing Interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106364.

References

Alfaro, A.C., 2008. Diet of *Littoraria scabra*, while vertically migrating on mangrove trees: Gut content, fatty acid, and stable isotope analyses. Estuar. Coast. Shelf Sci. 79, 718–726.

Blackmore, G., 2001. Interspecific variation in heavy metal body concentrations in Hong Kong marine invertebrates. Environ. Pollut. 114, 303–311.

Blackmore, G., Morton, B., 2002. The influence of diet on comparative trace metal cadmium, copper and zinc accumulation in *Thais clavigera* (Gastropoda: Muricidae) preying on intertidal barnacles or mussels. Mar. Pollut. Bull. 44, 870–876.

Bouillon, S., Connolly, R.M., Lee, S.Y., 2008. Organic matter exchange and cycling in mangrove ecosystems: recent insights from stable isotope studies. J. Sea Res. 59, 44–58.

Bordean, D.-M., Nica, D.V., Harmanescu, M., Banatean-Dunea, I., Gergen, I.I., 2014. Soil manganese enrichment from industrial inputs: a gastropod perspective. Plos One 9, e85384.

Chen, C.Y., Folt, C.L., 2000. Bioaccumulation and diminution of arsenic and lead in a freshwater food web. Environ. Sci. Technol. 34, 3878–3884.

Cheung, K.C., Wong, M.H., 2006. Risk assessment of heavy metal contamination in shrimp farming in Mai Po Nature Reserve, Hong Kong. Environ. Geochem. Health 28, 27–36.

Cheng, Z., Man, Y.B., Nie, X.P., Wong, M.H., 2013. Trophic relationships and health risk assessments of trace metals in the aquaculture pond ecosystem of Pearl River Delta, China. Chemosphere 90, 2142–2148.

Christensen, J.T., Sauriau, P.-G., Richard, P., Jensen, P.D., 2001. Diet in mangrove snails: preliminary data on gut contents and stable isotope analysis. J. Shellfish Res. 20 (1), 423–426.

Croteau, M.-N., Luoma, S.N., Stewart, A.R., 2005. Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. Limnol. Oceanogr. 50, 1511–1519.

Dallinger, R., Chabicovsky, M., Hodl, E., Prem, C., Hunziker, P., Manzl, C., 2005. Copper in Helix pomatia (Gastropoda) is regulated by one single cell type: differently responsive metal pools in rhogocytes. Am. J. Physiol. Regul. Integr. Comp. Physiol. 289, R1185–R1195.

Defew, L.H., Mair, J.M., Guzman, H.M., 2005. An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Pacific Panama. Mar. Pollut. Bull. 50, 547–552.

Demopoulos, A.W.J., Cormier, N., Ewel, K.C., Fry, B., 2008. Use of multiple chemical tracers to define habitat use of Indo-Pacific mangrove crab, *Scylla Serrata* (Decapoda: Portunidae). Estuaries Coasts: J CERF 31, 371–381.

Elder, J.F., Collins, J.J., 1991. Freshwater molluscs as indicators of bioavailability and toxicity of metals in surface-water systems. Rev. Environ. Contamination Toxicol. 122, 37–79.

Ghasemi, A., Zahediasl, S., 2012. Normality tests for statistical analysis: A guide for non-statisticians. Int. J. Endocrinol. Metab. 10 (2), 486–489.

Gibson, R.N., Atkinson, R.J.A., Gordon, J.D.M., 2006. Role, routes and effects of manganese in crustaceans. Oceanogr. Marine Biol.: Annual Rev. 44, 61–83.

Goessler, W., Maher, W., Irgolic, K., Kuehnelt, D., Schlagenhaufen, C., Kaise, T., 1997. Arsenic compounds in a marine food chain. Fresenius' J. Anal. Chem. 359, 434–437.

Goldburg, R., Naylor, R., 2005. Future seascapes, fishing, and fish farming. Front. Ecol. Environ. 3 (1), 21–28.

Guo, H.-R., 2002. Cancer risk assessment for arsenic exposure through oyster consumption. Environ. Health Perspect. 110 (2), 123–124.

Gradlund, S., Bengtsson, B., 2001. Chemicals and biological products used in south-east Asian shrimp farming, and their potential impact on the environment-a review. Sci. Total Environ. 280, 93–131.

Hargan, K.E., Williams, B., Nuangsaeng, B., Siriwong, S., Tassawad, P., Chaiharn, C., McAdoo, B., Los Huertos, M., 2020. Understanding the fate of shrimp aquaculture effluent in a mangrove ecosystem: aiding management for coastal conservation. J. Appl. Ecol. https://doi.org/10.1111/1365-2664.13579.

Hashmi, M.I., Mustafa, S., Tariq, S.A., 2002. Heavy metal concentrations in water and tiger prawn (*Penaeus monodon*) from grow-out farms in Sabah. North Borneo. Food Chem. 79, 151–156.

Hernroth, B., Baden, S.P., Holm, K., Andre, T., Soderhall, I., 2004. Manganese induced immune suppression of the lobster *Nephrops norvegicus*. Aquatic Toxicol. 70, 223–231.

Hornung, R.W., Reed, L.D., 1990. Estimation of average concentration in the presence of nondetectable values. Appl. Occup. Environ. Hyg. 5, 46–51.

Ikemoto, T., Tu, N.P.C., Okuda, N., Iwata, A., Omori, K., Tanabe, S., Tuyen, B.C., Takeuch, I., 2008. Biomagnification of trace elements in the aquatic food web in the Mekong Delta, South Vietnam using stable carbon and nitrogen isotope analysis. Arch. Environ. Contam. Toxicol. 54, 504–515.

Khokiattiwong, K.S., Kornkanitnan, N., Goessler, W., Kokarnig, S., Francesconi, K.A., 2009. Arsenic compounds in tropical marine ecosystems: similarities between mangrove forest and coral reef. Environ. Chem. 6, 226–234.

Kohlmeyer, J., Bebout, B., 1986. On the occurrence of marine fungi in the diet of *Littorina angulifera* and observations on the behavior of the periwinkle. Marine Ecol. 7, 333–343.

Kristensen, E., Bouillon, S., Dittmar, T., Marchand, C., 2008. Organic carbon dynamics in mangrove ecosystems: A review. Aquatic Botany 89, 201–219. https://doi.org/10.1016/j.aquabot.2007.12.005.

Lee, O.H.K., Williams, G.A., Hyde, K., 2001. The diets of *Littoraria ardouiniana* and *L. melanostoma* in Hong Kong mangroves. J. Mar. Biol. Assoc. U.K. 81, 967–973.

Leppanen, M., 1995. The role of feeding behavior in bioaccumulation of organic chemicals in benthic organisms. Ann. Zool. Fennici. 32, 247–255.

MacFarlane, G.R., Koller, C.E., Blomberg, S.P., 2007. Accumulation and partitioning of heavy metals in mangroves: A synthesis of field-based studies. Chemosphere 69, 1454–1464.

Marchand, C., Lallier-Vergès, E., Allenbach, M., 2011. Redox conditions and heavy metals distribution in mangrove forests receiving effluents from shrimp farms (Teremba Bay, New Caledonia). J. Soils Sediments 11, 529–541.

Marín-Guirao, L., Lloret, J., Marin, A., 2008. Carbon and nitrogen stable isotopes and metal concentration in food webs from a mining-impacted coastal lagoon. Sci. Total Environ. 393, 118–130.

Matthews, B., Mazumder, A., 2008. Detecting trophic-level variation in consumer assemblages. Freshwater Biol. 53, 1942–1953.

Mendiguchía, C., Moreno, C., Mánuel-Vez, M.P., García-Vargas, M., 2006. Preliminary investigation on the enrichment of heavy metals in marine sediments originated from intensive aquaculture effluents. Aquaculture 254, 317–325.

Molnar, N., Welsh, D.T., Marchand, C., Deborde, J., Meziane, T., 2013. Impacts of shrimp farm effluent on water quality, benthic metabolism and N-dynamics in a mangrove forest (New Caledonia). J. Aquac. Res. Development 5, 262–275.

Montgomery, J.R., Price, M.T., 1979. Release of trace metals by sewage sludge and the subsequent uptake by members of a turtle grass mangrove ecosystem. Environ. Sci. Technol. 13, 546–549.

Morton, J.E., 1955. The evolution of the Ellobiidae with a discussion on the origin of the Pulmonata. J. Zool. 125, 127–168.

Nascimento, J.R., Sabadini-Santos, E., Carvalho, C., Keunecke, K.A., Edison, R.C., Bidone, D., 2017. Bioaccumulation of heavy metals by shrimp (*Litopenaeus schmitti*): A dose–response approach for coastal resources management. Mar. Pollut. Bull. 114, 1007–1013.

Nemati, K., Abas, R.B., Bakar, N.K.A., 2009. Investigation of heavy metals mobility in shrimp aquaculture sludge – comparison of two sequential extraction Procedures. Microchemical J. 91, 227–231.

Nica, D.V., Bura, M., Gergen, I., Harmanescu, M., Bordean, D.-M., 2012. Bioaccumulative and conchological assessment of heavy metal transfer in a soil-plant-snail food chain. Chem. Central J. 6, 55.
Ong Che, R.G., 1999. Concentration of 7 heavy metals in sediments and mangrove root samples from Mai Po, Hong Kong. Mar. Pollut. Bull. 39, 269–279.
Pan, K., Wang, W.-X., 2012. Review: Trace metal contamination in estuarine and coastal environments in China. Sci. Total Environ. 421–422, 3–16.
Phillips, D.J.H., Yim, W.W.-S., 1981. A comparative evaluation of oysters, mussels and sediments as indicators of trace metals in Hong Kong waters. Mar. Ecol. Prog. Ser. 6, 285–293.
Proum, S., Santos, J.H., Lim, L.H., Marshall, D.J., 2016. Metal accumulation in the tissues and shells of *Indothais gradata* snails inhabiting soft and hard substrata in an acidified tropical estuary (Brunei, South East Asia). Reg. Stud. Marine Sci. 8, 487–497.
Quinn, M.R., Feng, X., Folt, C.L., Chamberlain, C.P., 2003. Analyzing trophic transfer of metals in stream food webs using nitrogen isotopes. Sci. Total Environ. 317, 73–89.
Van den Broeck, H., De Wolf, H., Backeljau, T., Blust, R., 2010. Effect of metal accumulation on metallothionein level and condition of the periwinkle *Littorina littorea* along the Scheldt estuary (the Netherlands). Environ. Pollut. 158, 1791–1799.
Zhou, Q., Zhang, J., Fu, J., Shi, J., Jiang, G., 2008. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Analytica Chimica Acta 606, 135–150.
Rainbow, P.S., 1995. Biomonitoring of heavy metal availability in the marine environment. Mar. Pollut. Bull. 31, 183–192.
Rainbow, P.S., Wang, W-X., 2005. Trace metals in barnacles: the significance of trophic transfer. Sci. in China Ser. C Life Sci. 48 (Supp. I), 110–117.
Revenga, J.E., Campbell, L.M., Arribere, M.A., Guevara, S.R., 2012. Arsenic, cobalt and chromium food web biodilution in a Patagonia mountain lake. Ecotoxocol. Environ. Safety 81, 1–10.
Riget, F., Johansen, P., Asmund, G., 1997. Uptake and release of lead and zinc by blue mussels. Experience from transplantation experiments in Greenland. Mar. Pollut. Bull. 34 (10), 805–815.
Shilla, D., Pajalab, G., Routh, J., Dariob, M., Kristoffersson, P., 2019. Trophodynamics and biomagnification of trace metals in aquatic food webs: The case of Rufiji estuary in Tanzania. Appl. Geochem. 100, 160–168.
Sowana, A., Shrestha, R.P., Parkpian, P., Pongquan, S., 2011. Influence of coastal land use on soil heavy-metal contamination in Pattani Bay Thailand. J. Coastal Res. 27 (2), 252–262.
Tam, N.F.Y., Wong, Y.S., 2000. Spatial variation of heavy metals in surface sediments of Hong Kong mangrove swamps. Environ. Pollut. 110, 195–205.
Thanh-Nho, B., Marchand, C., Strady, E., Huu-Phat, N., Nhu-Trang, T.-T., 2019. Bioaccumulation of some trace elements in tropical mangrove plants and snails (Can Gio, Vietnam). Environ. Pollut. 248, 635–645.
Thimdee, W., Deein, G., Sangrungruang, C., Nishioka, J., Matsunaga, K., 2003. Sources and fate of organic matter in Khung Krabaen Bay (Thailand) as traced by $\delta^{13}C$ and C/N atomic ratios. Wetlands 23 (4), 729–738.
Teoh, H.W., Sasekumar, A., Ismail, M.H., Chong, V.C., 2018. Trophic discrimination factor and the significance of mangrove litter to benthic detritivorous gastropod, *Ellobium aurisjudae* (Linnaeus). J. Sea Res. 131, 79–84.
Tu, N.P.C., Agusa, T., Ha, N.N., Tuyen, B.C., Tanabe, S., Takeuchi, I., 2011. Stable isotope-guided analysis of biomagnification profiles of arsenic species in a tropical mangrove ecosystem Mar. Pollut. Bull. 63, 124–134.
U.S. EPA (U.S. Environmental Protection Agency)., 1996. Method 3050B: Acid Digestion of Sediments, Sludges and Soils. Revision 2. Washington, DC.
U.S. EPA (U.S. Environmental Protection Agency)., 2007. EPA Method 7471B (SW-846): Mercury in Solid or Semisolid Wastes (Manual Cold-Vapor Technique). Revision 2. Washington, DC.
U.S. EPA (U.S. Environmental Protection Agency)., 2014. Method 6020B (SW-846): Inductively Coupled Plasma-Mass Spectrometry. Revision 2. Washington, DC.
Vander Zanden, M.J., Rasmussen, J.B., 2001. Variations in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. Limnol. Oceanogr. 46, 2061–2066.
Wang, W.-X., Fisher, N.S., 1999. Delineating metal accumulation pathways for aquatic invertebrates. Sci. Total Environ. 237, 459–472.
Wang, W.-X., Ke, C., 2002. Dominance of dietary intake of cadmium and zinc by two marine predatory gastropods. Aquatic Toxicol. 56, 153–165.