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Use of the sea hare (Aplysia fasciata) in marine pollution biomonitoring of harbors and bays

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Use of the sea hare (*Aplysia fasciata*) in marine pollution biomonitoring of harbors and bays

Abstract

Our study evaluated heavy metal concentrations in soft tissues of sea hare, *Aplysia fasciata*, from the Lower Laguna Madre, Texas. Heavy metals in tissues followed Se > As > Pb > Cd. Concentrations ranged As (BDL-28.08), Cd (BDL-5.50), Pb (BDL-12.85) and Se (4.25-93.43 ppm). Median As, Cd, Pb, and Se tissue levels exceeded exposure levels. Significant relationships occurred in metal-metal (As-Cd, As-Pb, Cd-Pb, Cd-Se, and Pb-Se), metal-tissue (significant Se uptake by inhalant and exhalant siphons and As in the hepatopancreas), and metal-metal within tissue (As-Pb in the hepatopancreas and Cd-Pb in the digestive cecum) analyses ($p<0.05$). Bioaccumulation factors (BAF) suggested the inhalant siphon, hepatopancreas, and digestive cecum function as macroconcentrators of Cd, hepatopancreas and digestive cecum as macroconcentrators of Pb, and all tissues were deconcentrators for As and Se. As a bioaccumulator of heavy metals, *Aplysia* was evaluated as a bioindicator of marine pollution in harbors and bays.

Keywords

bioaccumulation; harbors, heavy metals, sea hares, Texas

Highlights

- Findings of metal concentrations in sea hare tissues
- Median concentrations for As, Cd, Pb, and Se in tissues exceeded state and national exposure levels
- Significant differences found among metal-metal, metal-tissue, and metal-metal within tissue bioaccumulation
- Sea hare tissues bioaccumulate through macroconcentration of Cd and Pb and deconcentration of As and Se
- Consideration of using sea hares as a bioindicator to monitor marine pollution in harbors and bays

1. Introduction

The evaluation of marine ecosystem health involves the use animal bioindicator species, whose spatial and temporal occurrence and metal accumulation in their tissues can identify pollutant contamination and the presence of environmental stressors (Boening, 1999; McCarthy and Shugart, 1990; Yusof et al., 2004). The most common targeted metals of marine pollution monitoring include, arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) (Chiarelli and Roccheri, 2014). Other metals monitored in marine ecosystems include copper (Cu), selenium (Se) and tin (Sn) (Kennish, 1998). Marine invertebrates are particularly useful bioindicators of heavy metal pollution, and the ability to bioaccumulate metals can vary among taxonomic groups (Rainbow, 1995, 2002).

Whereas, molluscs need to bioaccumulate and maintain essential metals in their tissues (e.g., Cu and Zn), other non-essential metals (As, Cd, Pb, and Se) disrupt their metabolism and physiology (Jakimska et al., 2011a; 2011b). Such heavy metals are the focus of marine pollution monitoring, and marine molluscs are reliable bioindicators of these metals (Gupta and Singh, 2011; Rittschof and McClellan-Green, 2005; Zhou et al., 2008). One potential mollusc includes the sea hare, *Aplysia sp*. (Gastropoda, Anaspidea), which has received intensive research on its neurobiology, toxicology, chemical defense, and bioactive molecules (Kamiya et al., 2006; Kicklighter et al., 2005; Nusnbaum and Derby, 2010; Rittschof and McClellan-Green, 2005; Takeda, 1992). The ability of the sea hare to be used in monitoring marine ecosystem health has been explored, and more recent studies involve investigations with *Aplysia maricultures* (Jarvis et al., 2015; Patel et al., 1973).

An early study by Phillips (1917) found *Aplysia* “liver” (i.e., hepatopancreas) to contain traces of Cu, Fe, Mn, and Zn. Additionally, *Aplysia* tissues (e.g., gill and hepatopancreas) are known to accumulate polychlorinated biphenyl (PCBs), Ag, and As in the laboratory (Bianchini et al., 2007; Jahan-Parwar et al., 1990; Shiomi et al., 1988). Similar to polychaetes, gastropods, and bivalves, the sea hare also is known to biomethylate arsenic as tetramethylarsonium ion (Me_4As^+) and differentially store metals among tissues (Bianchini et al., 2007; Fattorini and Regoli, 2004; Morita and Edmonds 1992). For example, Co, Cu, Fe, Mn, Ni, Sr, and Zn differentially accumulate in tissues (e.g., reproductive, intestine, and hepatopancreas) of field collected *A. benedicti* (Patel et al., 1973). Most recently, Jarvis et al. (2015) reports metal (Cd, Cu, Ni, Pb, and Zn) accumulation effects on *A. californica* growth for laboratory-cultured individuals. When sea hares were fed different metal concentrations of green (*Ulva lactuca*) and red (*Agardhiella subulata*) seaweed exposed to metals, they found variation in *Aplysia* organ bioaccumulation.

The ability of *Aplysia* to bioaccumulate metals and pollutants in its tissues warrants its consideration as a potential marine bioindicator animal of bays, inlets, and harbor areas of the Laguna Madre of south Texas, USA. This hypersaline lagoon is identified as one of the region’s most susceptible estuaries to marine pollution (Biggs, et al., 1989). In a harbor polluted with As, Cd, Pb, and Se, and where sea hares regularly congregate, we investigated the bioaccumulation of these non-essential and borderline metals in *A. fasciata* tissues through its saltwater exposure pathways. Through our examination, we evaluated sea hare potential as a biomonitor of marine harbor ecosystem health.

2. Methods and materials

A. fasciata (n=5) were collected in May 2012 from the entrance to Port Isabel Harbor, Cameron County, Texas, USA (26.063079, -97.209248) (Fig. 1). This harbor exists in the Lower Laguna Madre (LLM), which "comprises the south Texas estuary bounded by the barrier island, South Padre Island, and extending from near Brownsville north to the Land Cut, and intersected by Mansfield Pass at Port Mansfield" (BBEST, 2012:3-1). The Laguna Madre is one of the few hypersaline estuaries in the world and has an average salinity of 35 to 45 ppt (Tunnell and Judd, 2002). The high salinity of the lagoon results from low freshwater inputs, high evaporation, shallow water, and its small connectivity with the Gulf of Mexico (BBEST, 2012; TDWR, 1983).

The harbor collection site was chosen based on its greater than eighty years of use supporting commercial fishing, petroleum industry, and recreational boating and tourism. Based on these activities and surrounding land use, the major and potential sources of metals in LLM waters includes agricultural use of As in herbicides and cotton desiccants, Cd from fertilizer, municipal wastewater, industrial releases, or landfills, Pb released from regional refineries and petroleum companies, and Se from industrial waste and sediment transfer (ATSDR, 2007a, 2007b, 2012; GESAMP, 1988; National Research Council, 1976). Additionally, our field observations found this harbor location to contain a large, regularly occurring gathering of sea hares in the LLM. This is similar to Audesirk (1979), who also reported changes in sea hare gatherings and seasonal abundance.

Physiochemical field measurements (temperature, pH, salinity) were taken at the collection site using a Hydrolab Quanta Multi-probe sonde (OTT Hydromet, Germany). Surface ocean water grab samples were also taken and placed in new, pre-cleaned, high-density polyethylene bottles and stored on ice during transport. At the lab, water samples were filtered at 0.45 µm and stored unacidified and frozen at -20 ˚C until tested at room temperature (22.5 ˚C) (see Avanzino and Kennedy 1993; Batley and Gardner, 1977; USEPA, 1983).

All collected *Aplysia* specimens were placed in separate polyethylene bags, transported on ice, and stored at -20˚C until thawed and dissected. On dissection, sea hare bodies were defrosted to room temperature, and all dissection blades were rinsed with distilled water to avoid contamination. Soft tissues extracted included the inhalant (IS) and exhalant (ES) siphons, hepatopancreas (HP), and digestive cecum (DC). Tissue sample preparation included freeze-drying and pulverization. Approximately 0.2 g of dried tissue was digested on a hot block with Trace Metal grade 70% nitric acid (NHO_3) for 40 min at 95°C. After digestion, samples were diluted with Millipore (Millipore Sigma Aldrich, Billerica, MA) purified water and stored in 50 mL polypropylene vials until analysis. At the least, three replicates per each of the four tissue types from each sea hare body were analyzed. Concentrations of As, Cd, Pb, and Se were determined using inductively couple plasma optical emission spectroscopy (ICP-OES) (PerkinElmer Optima 8300). Analyses were performed in triplicate using calibration curves maintained at a R^2 value of 0.99 or better. Limits of detection were As 0.01, Cd 0.0001, Pb 0.0015, and Se 0.0088 ppm, and values below detection limits were identified as BDL.

Statistical analysis was conducted using JMP 11.2 (SAS, Cary, NC). Summary statistics (mean, standard deviation, median, standard error) were calculated for each metal analyzed for a total of 80 measurements. Median dissolved metal values for water and tissue samples were compared to the saltwater chronic and acute criteria of the Texas Aquatic Life Surface Water Risk-Based Exposure Limits (SWRBEL) (TCEQ, 2014a, 2014b). Additionally, comparisons were made to the saltwater criterion continuous concentration (CCC) and criteria maximum concentration (CMC) set by the USEPA National Recommended Water Quality Criteria Aquatic Life for surface saltwater and applicable updates (Clean Water Act of 1972, 33 USC §304(a); Lemley, 2002; USEPA, 2017).

The potential effect of any individual sea hare specimen on resulting metal concentrations was evaluated using one-way analyses of variance (ANOVA). The absence of an effect supported further use of Spearman's ρ to determine metal-metal correlations and two-way ANOVA to examine metal-tissue, and metal-metal within tissue relationships. Where metal concentrations were indicated to be significantly different, least square means (LS-Means) were used to develop linear estimates for the post-hoc Tukey Highest Significant Difference (HSD) test to identify the significance between metal-tissue combinations. Significance was determined at $p < .05$ or lower for all statistical tests.

Our analysis involved investigating the bioaccumulation or metal uptake by sea hares and considered potential exposure pathways (e.g., dermal, ingestion, and respiration) (Spacie et al., 1995). The mean bioaccumulation factor in water (BAF_w) for each tissue was calculated as:

$$BAF_w = C_t / C_w \qquad (1)$$

where C_t ($\mu g\ g^{-1}$ wet weight (ppm)) is the metal concentration in tissue, and C_w ($\mu g\ L^{-1}$) is the metal concentration in surrounding saltwater (Dallinger, 1993; USEPA, 2000a). For this study, BAFs represent field-measured values. No food-chain multipliers (FM) were applied because *Aplysia* metal exposure in the LLM is from multiple sources, such as bottom sediments, water, and food (e.g., sea algae). The use of BAFs is more applicable to our study than bioconcentration (BCF) or biomagnification (BMF) factors, because this measure considers all potential exposure pathways rather than just dermal and respiration (i.e., BCF) or ingestion (i.e., BMF) (USEPA, 2000a).

3. Results

Physiochemical field properties of the surface water at the LLM site of sea hare capture were as follows: temperature 28.7 °C, pH 8.00, and salinity 35.48 ppt. pH and salinity agreed with historical and recent reported levels (https://waterdatafortexas.org/coastal/sites/JARD). Table 1 presents a summary of ICP-OES results for As, Cd, Pb, and Se among water and tissues. Tables 2-4 provide details on the relationships among metal-metal, metal-tissue, and metal-metal within tissues.

3.1. Metal-Metal Relationships

Positive significant correlations occurred for As-Cd, As-Pb, Cd-Pb, and negative correlations occurred for Cd-Se and Pb-Se (Table 2). These metal-metal relationships were found to be significant (F = 36.8105, df = 3, $p < 0.0001$).

3.2. Metal-Tissue Relationships

Concentrations of As (F= 20.42, $p<0.001$), Cd (F= 17.57, $p<0.001$), Pb (F= 22.82, $p<0.001$), and Se (F= 5.01, $p=0.012$) significantly differed by tissue type (Table 3). The inhalant and exhalant siphons exhibited high bioaccumulation of Se (Table 1). All tissue types showed less affinity to uptake Cd and Pb.

3.2.1. As

As concentrations among tissues varied from BDL to 28.08 µg g^{-1} wet wt. (ppm). Whereas the exhalant siphons contained the least mean concentrations, the highest mean As was found in the hepatopancreas (Table 1). The exhalent siphons displayed an inconsistency in As uptake with 40% of the specimens unable to bioaccumulate the metal. Overall the mean concentrations of the tissues were HP > DC > IS > ES. Median tissue values were above the Texas SWRBEL chronic criteria for As (0.078 ppm) saltwater exposure (Fig. 2).

3.2.2. Cd

Cd concentrations among tissues varied from BDL to 5.50 µg g^{-1}wet wt. (ppm). Whereas the inhalant siphon contained the least mean concentrations, the highest mean Cd was found in the digestive cecum (Table 1). Cd uptake for the exhalent siphons were below detection limit. Overall the mean concentrations of the tissues were DC > HP > IS > ES. Median tissue values, except the inhalant and exhalant siphons, were above the Texas SWRBEL chronic criteria for Cd (0.009 ppm) in saltwater (Fig. 2).

3.2.3. Pb

Pb concentrations in each sample varied from BDL to 12.84 µg g^{-1} wet wt. (ppm). Whereas the inhalant and exhalant siphons had values below detection limits, the digestive cecums had the highest mean Pb (Table 1). Overall the mean concentrations of the tissues were

DC > HP > IS = ES. Except for the inhalant and exhalant siphons, all other median tissue values were above the Texas SWRBEL chronic criteria for Pb (0.005 ppm)in saltwater (Fig. 2).

3.2.4. Se

Se concentrations in each sample varied from 4.25 to 93.43 µg g^{-1} wet wt. (ppm). Whereas the digestive cecums contained the least mean concentration, the highest mean Se was found in the inhalant siphons (Table 1). Overall the mean concentrations of the tissues were IS > ES > HP > DC. Levels of all tissues were above the Texas SWRBEL chronic criteria for Se (0.564 ppm) in saltwater (Fig. 2).

3.2.5. Overall metal-tissue relationships

The effects of individual sea hare specimens on metal-tissue relationships was non-significant ($p<0.05$). This allowed for the removal of specimen information to avoid conflating the degrees of freedom. The overall ANOVA was significant (F = 12.11, df = 79, $p < 0.0001$). Additionally, we found significant metal-tissue interactions (F = 7.54, df = 9, p <0.0001). Notably, the inclusion of metal-tissue combinations resulted in a direct evaluation of non-significant tissue-tissue interactions (F = 1.09, df = 3, $p = 0.3611$).

Results of the post-hoc LS-Means and Tukey Highest Significant Difference (HSD) found significant relationships between metal-tissue combinations (Table 3). These data likely suggest a different exposure pathway for selenium bioaccumulation in IS and ES tissues, which significantly differed from all metal-tissue pairing. Low Cd and Pb levels in the IS and ES tissues also suggested the existence of differential bioaccumulation between these two tissues and others. However, the high variation in the data resulted in only a significant difference between As in HP tissues. Increasing the sample size may further parse out differences with these Cd and Pb in HP and siphon tissues. All other paired metal-tissue combinations were not significantly different, although the LS-Means values range from 2.54-22.03. This may suggest that increasing the sample size in future research could identify further possible differences.

3.3. Metal-Metal Relationships within Tissues

Overall, the highest mean metal concentrations accumulated in *Aplysia* followed Se > As > Pb > Cd (Table 1). Positive significant correlations for As-Pb existed for the hepatopancreas and Cd-Pb for the digestive cecum at $p < 0.05$ (Table 4). All three of these correlations are for non-essential metals to organism metabolism or physiology and are reported to occur in the livers of organisms (Sharma and Shupe, 1977). The bioaccumulation of As in the sea hare is notable, for bioaccumulation of this metal in aquatic organisms is less common (NRC, 1977).

The bioaccumulation of lead varies among molluscs and especially bivalves (Saavedra et al. 2004). Arsenic has an affinity to bond with lead producing lead arsenate, which was historically used as an agricultural insecticide in the Rio Grande Valley, Texas (TCPS, 1999). Furthermore, the relationship occurring for Cd-Pb is expected, because these two metals occur naturally together in marine environments (Thornton, 1992). Whereas cadmium tends to bioaccumulate in the liver of aquatic organisms, lead varies in its occurrence in liver tissues (Pradit et al. 2013).

4. Discussion

Marine invertebrate tissues accumulate metals through exposure to water, sediments, and biota (Rainbow, 1995). This study investigated the ability of *A. fasciata*, which congregates regularly in a Texas harbor, to serve as a bioindicator of metals in marine waters through metal bioaccumulation.

4.1. Presence of Metals in Water

LLM Madre waters, similar to other regions along the Gulf of Mexico, contain metals including As, Cd, Pb, and Se (Davis et al., 1995; Montagna and Palmer, 2012). We found LLM water at the sea hare capture site to display levels Pb > Cd > As = Se (Table 1). The concentrations of these metals in LLM waters are historically low (TDWR, 1983; Wells et al. 1988; Withers et al., 2004). Recognizing that temperature, pH, and salinity can affect heavy metal concentrations in water, the LLM conditions we reported are conducive to harbors and bays having similar or lesser amounts of year-round warm, alkaline sea water (Denton and Burdon-Jones, 1981; Frazier, 1979).

The water samples collected in our LLM study contain concentrations below detection limits (BDL) for As and Se (Table 1). The low As levels are comparable to ranges (0-0.01 ppm) reported by Withers et al. (2004), USEPA Storage and Retrieval Data Warehouse (STORET), and Water Quality Exchange Database (WQX) data. Additionally, when compared to historic levels, the current water chemistry shows As and Se to be similar or less (Wells et al. 1988). The values meet Texas surface water quality standards and USEPA national chronic and acute benchmarks for at least As and Se (TCEQ, 2014a, 2014b; USEPA, 2017). However, the Cd and Pb water values we measured were higher than data ranges (where Cd 0 ppm and Pb 0-0.006 ppm) reported by Withers et al. (2004), STORET, and WQX. Cd and Pb levels also exceeded historical levels and state and national criterion (TCEQ, 2014a, 2014b; USEPA, 2017; Wells et al. 1988).

We found both significant positive and negative relationships between metals (Table 2). Cd-Pb are known to occur naturally together, and Cd-Se and Pb-Se mixtures are counteractive (Alexander, 2015; Nordberg et al., 2015b). Elemental interactions among As, Cd, and Pb are limited except in the context of *in vivo* studies and toxicity dosage (Nordberg et al., 2015a). Whereas competitive metal interactions are known (e.g., between Ca, Mg, and Na), more often metal-metal relationships for As, Cd, and Pb are lesser known and instead used to predict the combined exposure toxicity to organisms and effects on tissues (Wu et al., 2016). For example, mixtures of As-Cd, As-Pb, and Pb-Cd can be more toxic than each individual metal and result in biochemical, histological, and physiological changes (see Table 2, Wu et al., 2016).

The bioavailability of metal mixtures are also environmental dependent. For example, high salinity increases the availability of inorganic ions and decreases fee metal ions (e.g., Cd and Pb) (Piazza et al. 2016). However, we found Cd and Pb to have the higher levels in LLM water (Table 1). The prevalence of Cd and Pb in the LLM is most likely because of their low solubility and reflects the history of industrial activity in Port Isabel Harbor (USACE, 1980).

4.2. Tissue Bioaccumulation and Exposure Pathways

Metal pathways in marine invertebrates include direct absorption through dermal contact, ingestion, and digestion from available food and the ambient habitat (Wang and Fisher, 1999). Based on the natural history, ecology, and behavior of sea hares, potential direct exposure pathways include, 1) dermal contact with sediment and water, 2) ingestion of water, and 3) ingestion of food. With the presence of metals in waters of the LLM, it is important to identify the exposure pathways for of As, Cd, Pb, and Se in *Aplysia* (Sharma et al., 1999).

The marine plants and animals of the Laguna Madre are known to contain metals in their tissues (Custer and Mitchell, 1983; Whelan et al., 2005). However, the tissues of marine animals vary in metal bioaccumulation based on taxa, physiology, trophic level, environmental condition of habitats, and exposure pathways (Jakimska et al., 2011b). Among crustacea, lobsters and crabs can accumulate more Pb than prawns; however, prawns can accumulate more Cd (Jamikska et al., 2011). When comparing feeding methods, herbivorous molluscs accumulate Pb greater than herbivorous and carnivorous crustaceans or fish (Jamikska et al., 2011a, 2001b).

Aplysia in the LLM are herbivorous grazers on macrophytic algae and commonly feed and swim in the shoreline waters and jetties, and then migrate to shallow bays during the spawning season (Britton and Morton, 1989; Hamilton et al., 1982; Kandel 1979). Laguna Madre *Aplysia* occur year-round, however juvenile swimming activity is highest after overwintering (Strenth and Blankenship, 1991). During our study, we observed high numbers of adult sea hares in April-May as animals migrated into calmer harbor and bay areas of Port Isabel, Texas to feed on dominant occurring green algae. Known for its pelagic free swimming, *Aplysia* also crawl and burrow in sediments (Carefoot, 1987; Susswein et al., 1983, 1984). However, the potential of sea hare exposure to As, Cd, and Pb from bottom sediments in the LLM is unexpected, because all known mean values (As BDL, Cd BDL, and Pb 1.41±1.55 μ g^{-1} (ppm)) from the area are below Texas state sediment contaminant levels (As 8.2, Cd 1.2, and Pb 46.7 ppm) (TCEQ, 2006; Deyoe, H., unpublished results). However, the possibility for Se exposure from surrounding sediments is possible with mean Se values (8.13±3.63 > Se 2.0 ppm) found to be greater than the sediment criteria (Lemley, 2002).

Previous studies of terrestrial and marine slugs show that metals differentially can accumulate in their tissues (Hernández-Almaraz et al., 2014; Triebskorn and Köhler, 1996). For example, Dallinger et al. (1989) found Cd and Pb in the hepatopancreas, while Li et al. (2009) found Cd in reproductive tissues and Pb in muscles. In *Aplysia*, metal accumulation affects embryo development and growth (Bidwell et al., 1986; Jarvis et al., 2015). Moreover, differential accumulation occurs among *Aplysia* organs (Jarvis, et al., 2015). Our results demonstrate that the inhalant and exhalant siphons, hepatopancreas, and digestive cecum of the sea hare differentially uptake and bioaccumulate As, Cd, Pb, and Se (Tables 1, 3-4).

Whereas continuous dermal contact with saltwater occurs in the inhalant and exhalant siphons, ingestion occurs in the mouth followed by digestion and absorption in the heptapancreas and digestive cecum. We found inhalant and exhalant siphons to bioaccumulate As and Se (Table 1 and 3). The sea hare inhalant (i.e., upper or anterior respiratory opening) siphon is an extension of the mantle that draws water into the mantle cavity and over the gills (Bebbington, 1974). Contrarily, the exhalant (i.e., lower, anal, or pallial siphon) encircles the anus and discharges water and fecal pellets from the mantle cavity (Purchon, 1977; Rudman, 2002). It is unknown whether the accumulation of metals in the exhalant siphon results from dermal contact with water or fecal pellets released or both.

Additionally, our results demonstrate that the sea hare hepatopancreas and digestive cecum bioaccumulate As, Cd, Pb, and Se (Table 1 and 3) through the ingestion pathway of seawater and food. Not a true liver, the hepatopancreas (i.e., digestive gland) of *Aplysia* functions as a combined liver and pancreas by producing digestive enzymes and absorbing food (Bidder, 1966; Coelho et al., 1998; Goddard and Martin, 1966; Lobo-da-Cuhna and Batista-Pinto, 2003). Embedded in the hepatopancreas, the digestive cecum is an accessory compartment of the stomach and functions to condense waste into fecal rods (Lobo-da-Cuhna and Batista-Pinto, 2003; Ruppert et al., 2004). The invertebrate hepatopancreas and digestive cecum are known to accumulate Cd more than Pb (Berandha et al., 2010; Ghiretti, 1966; Roland and Shivers, 1987). Our results are complimentary and show the ability of these organs to bioaccumulate As and Se as well (Table 1 and 3).

Overall, we found the metal bioaccumulation of hepatopancreas and digestive cecum tissues to be above previously reported As, Cd, Pb, and Se median concentrations for LLM water (Wells et al., 1988; Whelan, unpublished data) and Texas and national criteria (Fig. 2). The inhalant and exhalant siphons also contained As and Se levels above water and criteria levels. Whereas trace levels of Cd occurred in the inhalant siphon, Cd was undetectable in the exhalant siphon (Table 1). Both siphons displayed undetectable levels of Pb.

4.3. Characterization of Tissue Bioaccumulation

Similar to organisms, the tissues of molluscs can be characterized regarding their capacity to bioaccumulate metals and the concentration of metals existing in ambient seawater (Yap and Cheng, 2013). Dallinger (1993) recognized bioaccumulation factors (BAFs) characterizing three different bioacummulators, deconcentrators ($BAF<1$), microconcentrators ($1<BAF<2$), and macroconcentrators ($BAF>2$). The results suggest that *Aplysia* tissues vary overall in their bioaccumulation for As ($2,615.6^{-1}$-508.6^{-1}), Cd (0-40.7), Pb (0-48.5), and Se

(792.6^{-1}-188.0^{-1}) (Table 1). The digestive cecum has the greatest values for Cd, Pb, and Se. whereas the exhalant siphon the greatest value for As. The inhalant siphon, hepatopancreas, and digestive cecum all represent tissue that function as macroconcentrators of Cd ($BAF_{Cd} >2$). Moreover, the hepatopancreas and digestive cecum also macroconcentrate Pb ($BAF_{Pb} >2$). All four tissues act as deconcentrators for As and Se.

The results of our study contrast to other molluscan findings. For As, molluscs (bivalves) demonstrate a wide BAF range (623.9-8,382) and mean BAF range (880-8,400 mean) (USEPA, 2003). DeForest et al. (2007) found that the geometric BAF means and ranges for saltwater were consistently greater than freshwater for As 10,808 (1,423-26,866), Cd 27,666 (65-680,000), Pb 16,159 (10-4,000,000), and Se 21,634 (3,947-130,000). Our results showed negative As and Se values

4.4. Aplysia as a Bioindicator Organism

Researchers review the numerous qualities of good marine invertebrate bioindicator organisms (Boening, 1999; Burger and Gochfeld, 2001; Carignan and Villard, 2002; Edwards et al., 1996; Holt and Miller 2011; Pan et al., 2011; Rainbow 1995, and references therein; Zhou et al. 2008). Because molluscs, in particular bivalves, bioaccumulate a wide range of heavy metals, they are useful as bioindicators (Gupta and Singh, 2011; Rittschof and McClellan-Green, 2005; Usero et al., 1997; Wang et al. 2005; Zhou et al., 2008). The many qualities that molluscs exhibit make them useful to monitor ecosystem health and exposure pathways toward human consumption (Eisler, 2010; Rittschof and McClellan-Green, 2005). The variability in the soft tissues of bivalves and gastropods to accumulate heavy metals make molluscs appealing as potential biomonitors of pollution presence (Yap et al. 2010). However, the use of invertebrates as bioindicators heads caution, because of their responses to disturbances in localized environments (Carignan and Villard, 2002). We contend that this quality of *A. fasciata* to reflecting local marine pollutants may be helpful in monitoring fragile ecosystems, such as the LLM as a hyposaline lagoon.

In selecting a biomonitor, it is desirable that the organism exhibits several characteristics and inheritably not all criteria will be met (Rainbow 1995). Molluscs are considered good bioindicators based on their feeding, growth, reproduction, and physiological responses to metal accumulation (Bryan et al., 1983). Table 4 provides the applicable characteristics and justifications or limitations for *A. fasciata* that support its consideration as a marine biomonitoring organism for harbors and bays. Complementary to Table 5, the body *A. fasciata* consists entirely of soft tissues, which makes it desirable by avoiding known differences in metal bioaccumulation between soft and hard tissues (e.g., operculum or shell) of other molluscs (Yap et al. 2009). Additionally, its lack of a shell and the pelagic swimming behavior of this sea hare lessens concerns in metal variability associated with bivalve opening and closing and regular burrowing (Byrne and Halloran, 2001; USEPA, 2000b). As an herbivorous grazer of green and red algae, *A. fasciata* siphons and digestive tissues examined in this study are more likely to reflect metal concentrations in water contrary to the sediment levels reflected among deposit feeders (e.g., *Nassarius*) (Newman and McIntosh, 1982).

In conclusion, our investigation supports that the sea hare does uptake As, Cd, Pb, and Se and exhibits differential bioaccumulation among its soft tissues. Moreover, sea hare overall tissue concentrations are higher than previous and currently reported saltwater values and benchmarks. Because these four metals are priority USEPA surface saltwater pollutants, *Aplysia* is a good candidate as a bioindicator that is worthy of consideration in marine pollution monitoring of harbors and bays.

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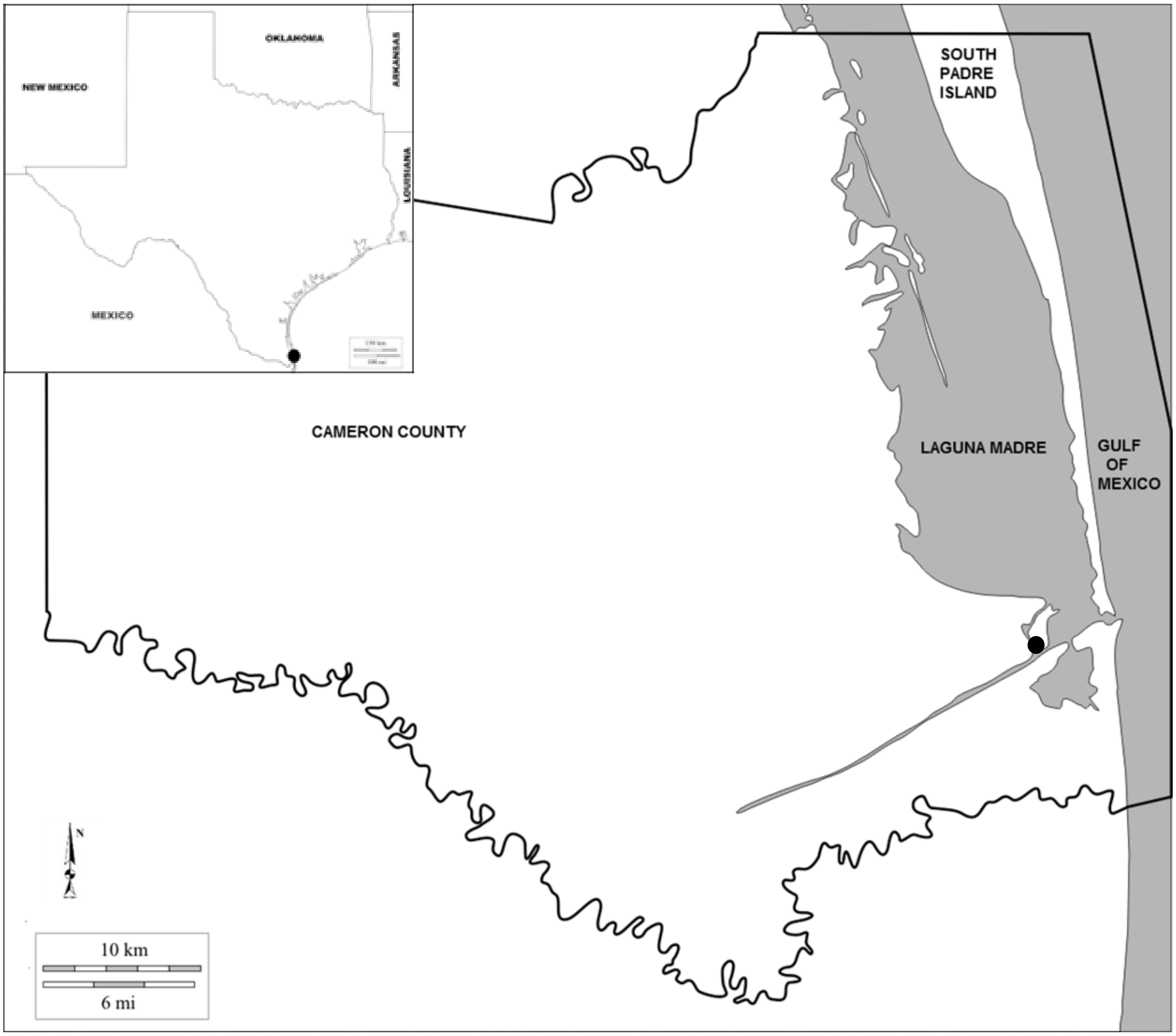
Figure Captions

Fig. 1. Location of the Lower Laguna Madre and Gulf of Mexico, Texas.

Fig. 2. Median concentrations for As, Cd, Pb, and Se in sea hare *Aplysia fasciata* tissues compared to Texas Aquatic Life Surface Water Risk-Based Exposure Limits (SWRBEL) (TCEQ, 2014a, 2014b; USEPA 1996). Notes: IS (inhalant siphon). ES (exhalent siphon). HP (hepatopancreas). DC (digestive cecum). Dash line (saltwater benchmark).


OKLAHOMA
NEW MEXICO
ARKANSAS
LOUISIANA
MEXICO
SOUTH PADRE ISLAND
CAMERON COUNTY
LAGUNA MADRE
GULF OF MEXICO
N
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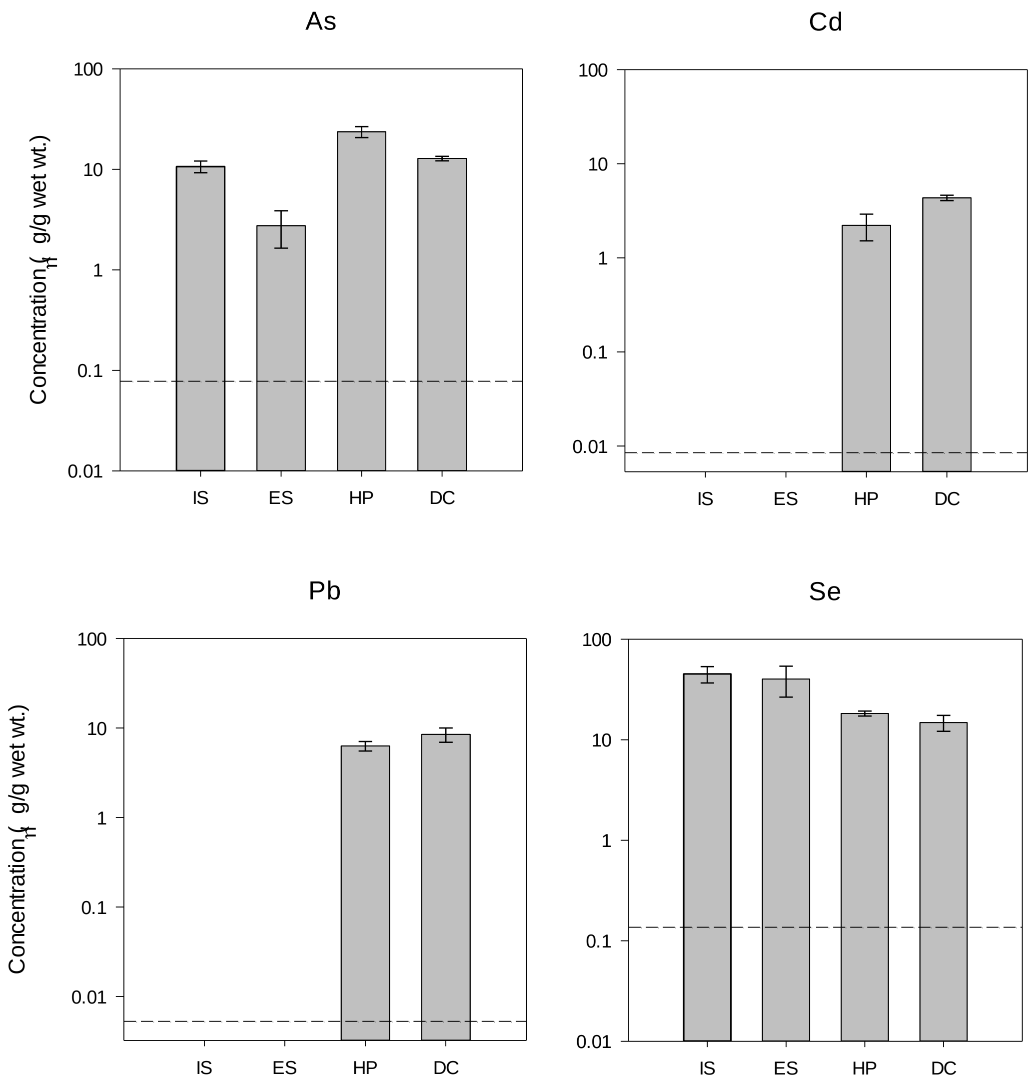
As
Cd
Pb
Se
Concentration (µg/g wet wt.)
100
10
1
0.1
0.01
IS
ES
HP
DC

Table 1.
As, Cd, Pb, and Se metal concentrations (ppm) in water and tissues (µg g^{-1} wet wt. (ppm)) and BAF values for the

mottled sea hare (*Aplysia fasciata*).

Tissue	Water	Inhalant siphon	Exhalant siphon	Hepatopancreas	Digestive cecum
As	<0.0010[a]	10.60 ± 3.15 (5.92-13.70) -2,120.0	2.54 ± 2.49 (0-5.05) -508.6	22.02 ± 6.69 (10.59-28.08) -4,405	13.08 ± 1.47 (11.61-15.52) -2,615.6
Cd	0.0990	0.72 ± 1.04 (0-2.28) 7.3	BDL	2.86 ± 1.57 (1.45-5.50) 28.9	4.03 ± 0.64 (3.21-4.61) 40.7
Pb	0.1603	BDL	BDL	6.06 ± 1.70 (3.45-7.88) 37.2	7.99 ± 3.47 (3.96-12.85) 48.5
Se	<0.0010[a]	45.97 ± 18.61 (22.83-71.70) -792.6	44.34 ± 30.50 (16.58-93.43) -764.5	17.13 ± 2.29 (13.79-19.11) -295.4	10.90 ± 5.98 (4.25-15.56) -188.0

BDL (Below detection limit)
Mean ± standard deviation (range)
BAF (bioaccumulation factor)

Table 2.

Spearman’s ρ correlations among metal concentrations.

	As	Cd	Pb	Se
As	1.0			
Cd	0.69*	1.0		
Pb	0.70*	0.88*	1.0	
Se	-0.37	-0.73*	-0.75*	1.0

*Significant at *p*<0.001

Table 3.
Results of ANOVA and Tukey's test for metal concentrations affected by mottled sea hare (*Aplysia fasciata*) tissues.

Parameter (F-test, Sig.)	Tissue	LSMean±SE
As (20.42, <0.0001)	Inhalant siphon	10.60 ± 1.77^{ab}
	Exhalant siphon	2.54 ± 1.77^{ab}
	Hepatopancreas	22.03 ± 1.77^{b}
	Digestive cecum	13.08 ± 1.77^{ab}
Cd (17.57, <0.0001)	Inhalant siphon	0.72 ± 0.44^{a}
	Exhalant siphon	0.00 ± 0.44^{a}
	Hepatopancreas	2.86 ± 0.44^{ab}
	Digestive cecum	4.03 ± 0.44^{ab}
Pb (22.82, <0.0001)	Inhalant siphon	0.00 ± 0.86^{a}
	Exhalant siphon	0.00 ± 0.86^{a}
	Hepatopancreas	6.06 ± 0.86^{ab}
	Digestive cecum	7.99 ± 0.86^{ab}
Se (5.01, 0.012)	Inhalant siphon	45.97 ± 8.12^{c}
	Exhalant siphon	44.34 ± 8.12^{c}
	Hepatopancreas	17.13 ± 8.12^{ab}
	Digestive cecum	10.90 ± 8.12^{ab}

For all ANOVA F-tests, the df = 9. LSMeans (least square means) sharing the same superscript are not significantly different from each other.

Table 4.

Spearman’s ρ coefficients between metal-metal concentrations and tissues

Inhalant siphon		Exhalant siphon		Hepatopancreas		Digestive cecum	
M-M	Corr	M-M	Corr	M-M	Corr	M-M	Corr
As-Cd	0.67	As-Cd	id	As-Cd	0.60	As-Cd	0.70
As-Pb	id	As-Pb	id	As-Pb	0.90*	As-Pb	0.60
As-Se	0.00	As-Se	0.15	As-Se	0.50	As-Se	-0.60
Cd-Pb	id	Cd-Pb	id	Cd-Pb	0.70	Cd-Pb	0.90*
Cd-Se	-0.45	Cd-Se	id	Cd-Se	0.30	Cd-Se	0.39
Pb-Se	id	Pb-Se	id	Pb-Se	0.70	Pb-Se	0.74

id (insufficient data)

*significant at $p < 0.05$

Table 5.

Applicable biomonitoring characteristics and qualities of *Aplysia* that support their use as a biomonitor organism

Characteristic and qualities	**Justification or limitation**
Abundant, common, and wide-ranging	**Found in abundance and common in the Gulf of Mexico and its bays and harbors**
Ability to accumulate high levels of toxins or metals / Accumulates high concentration of metals compared to average ambient water concentration	**Results in this paper support its ability to bioaccumulate metals in its tissues at high levels**
Readily available, surveyed, and testable	**Easy to collect specimens swimming on surface with net**
Strong net accumulator compared to other potential organisms	**Ability to accumulate similar to other mollusks**
Reliable identification	**Large size and easily recognizable characteristics**
Available knowledge of anatomy, physiology, ecology, and natural history	**A herbivorous browser on green algae; Pelagic swimmer; Siphons in continuous contact with seawater.**
Long lived	**Short life span of approximately 8 mo - 1 yr**
Provide sufficient tissue for testing	**Large body and organ size provides for testing**
Resistant to handling stress and adaptable to laboratory conditions	**Rich history of ability to be raised and kept in laboratories**
Tolerant to environmental exposure and conditions	**Ability to live in hypersaline conditions and shallow waters of the Lower Laguna Madre; Occurs in harbors and bays**
Important to ecosystem function	**Important to food web and food chain to marine turtles protected in Gulf of Mexico**
Slow and limited range of movement or sedentary or sessile	**Although an active swimmer, tends to remain in harbor and coastal areas after seasonal arrival and until death**

Characteristics and qualities derived from Audesirk (1976); Carignan and Villard, 2002; Gev et al., (1984); Pan et al. (2011): Rainbow (1995); and Rittschof and McClellan-Green (2005).