Latin American Journal of Aquatic Research, 54(3): 358-369, 2026
DOI: 10.3856/vol54-issue3-fulltext-3334

Research Article

Metals in *Phragmatopoma attenuata* Hartman, 1944 (Annelida, Sabellariidae) worm reefs and beach sand, Gulf of Nicoya estuary, Pacific, Costa Rica

José A. Vargas[1,2], Johan Molina[3], Rebeca Cambronero[2] & Jeffrey A. Sibaja-Cordero[1,2]
[1]Centro de Investigación en Ciencias del Mar y Limnología (CIMAR)
Universidad de Costa Rica, San José, Costa Rica
[2]Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica
[3]Centro de Investigación en Contaminación Ambiental (CICA)
Universidad de Costa Rica, San José, Costa Rica
Corresponding author: Jeffrey A. Sibaja-Cordero (jeffrey.sibaja@ucr.ac.cr)

ABSTRACT. Sabellariid annelid polychaete worms build reefs by cementing sand grains in their tubes. In the Gulf of Nicoya estuary, Costa Rica, *Phragmatopoma attenuata* worms construct reefs on and between rocks used in groins. The objective of this study was to investigate whether these worms select sediment grain sizes and trace metals in the building of their reefs. A total of 12 sand samples were collected (three each from three reefs and three from beach sand) to compare the beach sand to the composition of the sand tubes. Samples were sieved through a series of nested Standard Sieve Series with 2,000 and 62 µm mesh sizes as the upper and lower limits for the sand fractions. A total of 12 metals: Al, Cd, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Sn, and Zn were analyzed by Inductively Coupled Plasma Mass Spectrometry. The results indicate the following decreasing order of concentrations (µg g^{-1} dry weight): Al > Fe > Mg > Mn > Cu > Zn > Cr > Ni > Pb > Hg > Sn > Cd. It was concluded that: 1. Metal concentrations were expected for a non-industrialized estuary. 2. *P. attenuata* worms selected for medium grain size fractions between 300 and 850 µm. 3. Worms also seem to prefer sand containing relatively higher levels of calcium carbonate, cadmium, and manganese when compared to those in beach sand.

Keywords: tube building; trace metal accumulation; animal behavior; ecosystem engineers; sediments; sandy beach

INTRODUCTION

The marine and estuarine sabellariid annelids include species that build reefs by cementing sand grains together with a protein produced by the worms (Zhao et al. 2006). Worm reefs are important structures that increase habitat heterogeneity and provide refuge for a diverse associated fauna, with crustaceans being common (Lane-Medeiros et al. 2021). In addition, the worms' feeding activities contribute to the community's trophic dynamics. In this sense, reef-building polychaetes are considered ecosystem engineers (Bruschetti 2019).

Fauchald (1977) reported the presence of seven species of tube-building sabellariid worms on the Pacific coast of Panama. Four valid species are *Phragmatopoma attenuata* Hartman, 1944; *Idanthyrsus armatus* Kinberg, 1866; *I. crestus* Chamberlin, 1919 (as *I. pennatus* (Peters, 1854)); and *Sabellaria moorei* Monro, 1933. Another three are questionable records of *Sabellaria*, as noted by Chávez-López & Bastida-Zavala (2021): *S. floridensis* Hartman, 1944; *S. alcocki* Gravier, 1906; and *S. spinulosa* Leuckart, 1849. Barrios et al. (2009) report *I. cretus* Chamberlin, 1919, for Culebra Bay, Costa Rica. More recently *Phragmatopoma villalobosi* Chávez-

Associate Editor: Paulo Paiva

López, 2020, has also been described for the Pacific coast of Costa Rica by Chávez-López (2020).

Additionally, *P. attenuata* has been recorded along the Pacific coast of Costa Rica by Sibaja-Cordero & Vargas-Zamora (2006) and Dean (2009). In Culebra Bay (Gulf of Papagayo), the species was found in the most exposed rocky coast (Sibaja-Cordero & García-Méndez 2014). In Caldera Bay (Gulf of Nicoya estuary), it was present in the low and mid-littoral zones with a mean cover of around 40% (Sibaja-Cordero & Vargas-Zamora 2006). Vargas et al. (2024) reported colonies of this genus at Ostional Beach, Junquillal Bay, both in the Gulf of Papagayo and in the port city of Puntarenas (Gulf of Nicoya), with 30 associated invertebrate species.

Reports on the concentration of metals on tropical estuarine beach sands and in worm reefs are scarce. Chouikh et al. (2022) found that the Moroccan species *Sabellaria alveolata* (Linnaeus, 1767) can bioaccumulate trace metals in its tissues. Deias et al. (2023) found that trace metals in seawater were bioaccumulated in the biocement produced by *S. alveolata* worms. These studies indicate that sabellariid tubes may be used to assess environmental pollution (Dean 2008).

Information on the granulometric characteristics of worm reefs in general and of particle selection for tube construction in particular is also scarce. In this context, the report by Main & Nelson (1988) on *Phragmatopoma lapidosa* Kinberg, 1866, from Florida is a notable exception. More recently, Santos-Mella et al. (2017) from the Dominican Republic and Aviz et al. (2019) from Brazil provide grain-size data from worm reefs (*Phragmatopoma caudata* Kroyer in Mörch, 1863; *Sabellaria wilsoni* Lana & Gruet, 1989, respectively) and adjacent beaches. Lisco et al. (2020) have also studied particle composition of reefs and tubes of European *S. alveolata*.

In Costa Rica, data on the concentrations of Fe, Zn, Cu, and Pb are available for the Puntarenas beach sand (García-Céspedes et al. 2004) and for a sand flat further up the Gulf of Nicoya estuary (Vargas et al. 2016). Thus, the objectives of this study were to determine the concentrations of metals and grain-size parameters in beach sand and in sand at three heights within intertidal reefs built by *P. attenuata*. Our null hypotheses were: 1-the *P. attenuata* worms do not accumulate trace metals when compared to those found in beach sand, and 2-the *P. attenuata* worms are not selective for certain grain sizes to build the reefs when compared to beach sand composition.

MATERIALS AND METHODS

Study area

The Gulf of Nicoya is an estuary (Voorhis et al. 1983) on the Pacific coast of Costa Rica (10°N, 85°W). The port city of Puntarenas is located along a 10 km-long sandbar on the mid-upper estuary. In this region, salinity varies seasonally (dry *vs*. rainy) around 30, and water temperature is approximately 30°C year-round (Voorhis et al. 1983). Littoral currents transport sediment and debris from the Barranca River, which drains agricultural lands and flows over rocks of diverse geological origins (Denyer et al. 2004). The city is a source of urban runoff and is the base of operations for the artisanal fishing fleet, tourist boats, and cruise ships. The north shore of the bar faces a small estuary bordered by mangrove forests. The southern shore faces the gulf and receives year-round beach tourism. The dark gray sand beach is cleaned frequently to remove plastic and organic debris (Sibaja-Cordero & Gómez-Ramírez 2022). The tip of the sand bar (La Punta) is protected from beach erosion by five rock groins (09°58'29.5''N, 84°51'00.0''W) made of rocks brought from far inland. Waves around 1 m height break at this beach, stirring up sand.

Worm reefs were found growing on the rock groins (Figs. 1a-b). Samples were collected at low tide (tidal range: 3 m) on September 20, 2020. The worm colonies grow on rocks above the sand beach and remain exposed for about 2 h during low tide (Figs. 1b, d). The worm tubes grow in proximity to one another (Figs. 1c, 2a).

Species identification

It is not the objective of this study to provide a review of the taxonomic status of the species cited above. However, we include data to support the identification of the Puntarenas specimens as *P. attenuata*. Identification was based on external anatomy (Fig. 2b), the structure of the operculum (Figs. 2c-d), and the outer opercular paleae (Figs. 2e-f). These structures were found to be consistent with the annotations and illustration in Plate 38 of Hartman (1944) and Fauchald (1977). Moreover, the uncini in our specimens have six pairs of teeth, in contrast to the seven pairs in *P. villalobosi* and *Phragmatopoma* sp. from Costa Rica described by Chávez-López (2020). *P. attenuata* has been reported in Ecuador and Colombia (Hartman, 1944), Panama (Fauchald, 1977), and Costa Rica (as previously cited). Voucher specimens are deposited in the Polychaete Collection of the Museum of Zoology,

Figure 1. a) Rock groin for control of sand erosion at the tip of the Puntarenas Peninsula, low tide, mid-upper Gulf of Nicoya estuary. Note the dark gray color of the sand, b) arrows point to the upper level for the growth of worm reefs, c) close view of colony showing individual tubes, d) arrows point to the four levels at which samples were collected. Beds of small white barnacles also colonize rocks. All photographs taken by J.A. Vargas.

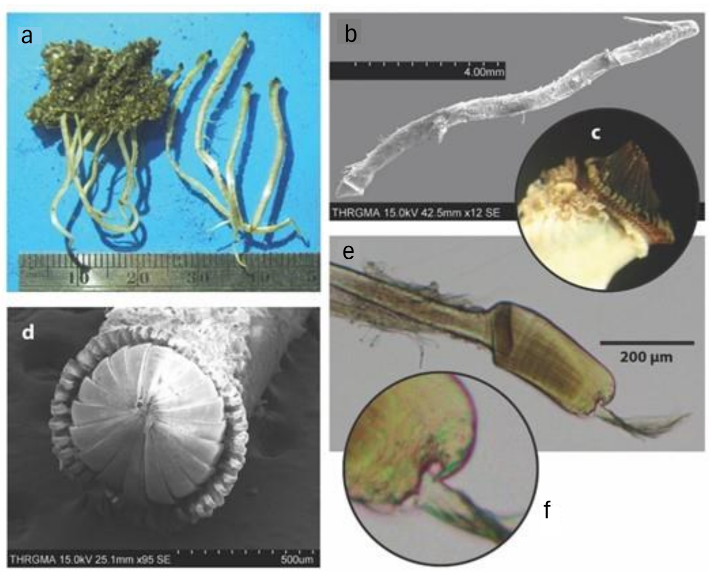

Figure 2. a) A group of six *Phragmatopoma attenuata* worms partially protruding from their tubes, and four extracted whole worms. Note the black color of the opercula, b) Scanning Electron Microscope (SEM) photograph of a whole worm showing external anatomy, c) side view of operculum of a live specimen, d) front SEM view of the asymmetrical operculum, e) morphology of the outer opercular paleae with palmately filamentous appendage directed at right angle to the shaft, f) close view of the paleae with details of the tip and hook. Photographs a-b, d, by J.A. Vargas, c, e-f, by J. Sibaja-Cordero.

University of Costa Rica (MZUCR-2506-01, MZUCR-2501-01, MZUCR-2505-01).

Trace metal concentrations

Samples for trace metal analysis were collected by inserting a plastic corer (5 cm in diameter) 15 cm into the colony substrate and beach sand. Samples were also taken from three reefs at three elevation levels, each 50 cm apart: 0 to 50, 50 to 100, and 100 to 150 cm above the beach (Fig. 1d). A total of 12 samples (nine from the reefs and three from beach sand) were collected and stored in previously acid-washed sealable dry plastic bags. The reef fragments were later disaggregated with a plastic hammer, and the worms and associated fauna were removed under a dissecting microscope.

Digestion and metal analyses were performed at the Center for Research on Environmental Pollution (CICA, by its Spanish acronym) of the University of Costa Rica. For digestion, 1 g of sample was added to Teflon® vessels along with 6 mL of concentrated ultrapure HNO_3 and 2 mL of concentrated ultrapure HCl. Microwave-assisted digestion (U.S. EPA Method 3051, 2007) was performed with a MARS 6 Microwave Accelerated Reaction System (CEM Corp., Matthews, NC, USA). After digestion, samples were brought to a final volume of 50 mL in volumetric flasks with deionized water and filtered through 0.45 µm surfactant-free cellulose acetate (SFCA) syringe filters.

A total of 12 metals: Al, Cd, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Sn, and Zn were analyzed by Inductively Coupled Plasma Mass Spectrometry using an Agilent 7500 cx ICP-MS (Agilent Technologies, Santa Clara, CA, USA). A Collision Cell mode was applied with a He flow rate of 4 mL min^{-1} to reduce or eliminate interference from polyatomic molecules, in accordance with the EPA 6020 B and SM 3125 B methods. For calibration curves, Supelco® Sigma-Aldrich C and Certified Reference Material Periodic Table mix 1 & 3 were used. All analyses were conducted in triplicate. Concentrations are expressed in µg g^{-1} dry weight (dw).

Differences were tested for mean concentrations (µg g^{-1} dw) of metal elements in beach sediments and in tube sediments at low, mid, and upper intertidal levels. The mean values of each metal concentration were compared using a two-way ANOVA without replication in R. Three samples per level constitute a repeated-measures factor (transect) nested within the fixed factor (level) (Quinn & Keough 2002, Crawley 2013). Mean concentrations with their 95% confidence intervals for metals were represented as bars in the graph (Fig. 2) using the ggplot2 package in R (Chang 2019).

For comparison, Table 1 includes data on trace metals from sand beaches in the Pacific coast of Mexico by Jonathan et al. (2011) and from Florida (USA) by Macdonald et al. (1996), who measured the threshold effect level (TEL) (estimates of the concentration of a particular metal at which a physiological effect begins to be produced in an organism).

Grain size

Granulometric analysis was conducted on triplicate 100 g oven-dried (60°C) sediment samples from the sandy beach and the worm reefs, for a total of 12 samples. Samples were sieved through a series of nested Standard Sieve Series with 2,000 and 62 µm mesh sizes as the upper and lower limits for the sand fractions, respectively. The package 'rysgran' in R (Gilbert et al. 2012) was used to analyze sediment data to obtain asymmetry, sorting, kurtosis, and mean grain size, following Folk & Ward (1957). The sediment fraction data were graphed as a cumulative frequency curve, with percentage on the y-axis and grain size (µm) on the x-axis. Total organic matter (TOM) and $CaCO_3$ contents of the homogenized sediments were also determined in triplicate by using the loss on ignition method by Dean (1974). Five grams of dry sediment were placed in a crucible and burned at 550°C for TOM content and subsequently at 1,000°C for $CaCO_3$ content. TOM and carbonate content were analyzed using two-way ANOVA without replication (Quinn & Keough 2002, Crawley 2013). Assumptions of residual normality and homoscedasticity were checked for each ANOVA test using the Shapiro-Wilk and Bartlett tests, respectively, and graphical inspection of residuals (Crawley 2013).

RESULTS

Metal concentrations

Mean concentrations with their 95% confidence intervals for the 12 metals analyzed are shown (Fig. 3). Aluminum was the most abundant ($25.03 \pm 0.94 \times 10^3$ µg g^{-1} dw) and cadmium the least (0.049 ± 0.001 µg g^{-1} dw). The order of decreasing concentrations (µg g^{-1} dw) was Al > Fe > Mg > Mn > Cu > Zn > Cr > Ni > Mg > Pb > Hg > Sn > Cd. The maximum and minimum concentrations of metals found in sand samples from worm tubes and beach sand are included (Table 1). The concentrations of the metals were below the TEL except for copper (Table 1). The Puntarenas metal concentrations were similar to those reported in a sandy beach of the Pacific of Mexico (Table 1).

Table 1. Maximum and minimum concentrations (μg g^{-1} dw) of metals found in sand and worm tubes samples. Puntarenas Peninsula, Gulf of Nicoya estuary, Pacific Costa Rica, 2020. ND: no data. *Threshold effect level, according to Macdonald et al. (1996, Table 1, pp. 258). **Range. Acapulco sandy beach, Pacific, Mexico (Jonathan et al. 2011, Table 1, pp. 849).

Metal	Type	Maximum	Minimum	Threshold Effect Level*	Range, Acapulco Beach**
Al	Tubes	25.03 ± 0.94×10³	12.13 ± 0.45×10³	ND	ND
	Sand	19.99 ± 0.74×10³	19.18 ± 0.70×10³		
Cd	Tubes	0.049 ± 0.001	0.033 ± 0.001	0.68	0.10-12.51
	Sand	0.029 ± 0.001	0.018 ± 0.001		
Cu	Tubes	34.52 ± 0.52	27.30 ± 0.41	18.7	0.76-26.97
	Sand	36.90 ± 0.55	33.92 ± 0.51		
Cr	Tubes	11.58 ± 0.15	6.95 ± 0.09	52.3	0.64-105.50
	Sand	13.33 ± 0.17	7.58 ± 0.10		
Fe	Tubes	11.03 ± 0.26×10³	8.28 ± 0.19×10³	ND	1072-27190
	Sand	12.90 ± 0.30×10³	9.99 ± 0.23×10³		
Hg	Tubes	0.0580 ± 0.0006	0.0096 ± 0.0003	0.13	ND
	Sand	0.0297 ± 0.0005	0.0098 ± 0.0004		
Mg	Tubes	8.58 ± 0.44×10³	5.69 ± 0.18×10³	ND	ND
	Sand	8.68 ± 0.25×10³	8.35 ± 0.24×10³		
Mn	Tubes	1296 ± 33	697 ±17	ND	6.85-586
	Sand	575 ± 14	513 ±13		
Ni	Tubes	12.32 ± 0.30	6.54 ± 0.12	15.9	0.33-16.35
	Sand	12.67 ± 0.24	9.58 ± 0.18		
Pb	Tubes	3.16 ± 0.05	2.23 ± 0.04	30.2	0.13-24.46
	Sand	2.80 ± 0.4	2.35 ± 0.4		
Sn	Tubes	0.045 ± 0.003	0.011 ± 0.003	ND	ND
	Sand	0.053 ± 0.003	0.033 ± 0.003		
Zn	Tubes	24.46 ± 071	18.11 ± 0.53	124	3.54-96.23
	Sand	28.53 ± 0.83	21.75 ± 0.63		

Differences in mean metal concentrations were found between the beach sand and the sabellariid worm colonies for three metals (dark gray bars in Fig. 3 and Fig. S1): cadmium content was higher in the worm reefs than in beach sand, but concentrations show lower values (Fig. 2, F = 6.7, df = 3/6, P = 0.024). The mean concentration of manganese was higher in reefs than in beach sand (Fig. 2, F = 4.9, df = 3/6, P = 0.047). Iron had lower mean concentrations in reefs than in beach sand (Fig. 2, F = 5.5, df = 3/6, P = 0.038). The means for each metal were similar among the three intertidal levels for the sabellariid worm reefs (ANOVA, Tukey test, P > 0.05). Copper (F, P = 0.106), zinc (F, P = 0.091), and magnesium (Kruskal-Wallis, P = 0.108) had a trend of lower concentrations in reefs than in beach sand (light gray bars in Fig. 3 and Fig. S1).

Total organic matter, $CaCO_3$, and grain size

The worm reefs had a lower mean value of total organic matter (2.40 - 2.67%, Table 2) than in beach sand (2.83%, Table 2), and the three tidal levels of reefs sampled were not significantly different (Tukey, P > 0.05). $CaCO_3$ was significantly lower in beach sand (2.66-2.91%) than in sabellariid reefs (3.78-12.48%) (t-Student = 3.98, beach sand n = 3, pooled colonies n = 9, P < 0.004) (Table 2).

The mean grain size did not differ between the beach sand and the worm tubes because of the wider variation of the granulometric parameter between samples (Table 2). Moreover, the cumulative curve for the reefs is leptokurtic, with a peak near the mean grain size (around 349 μm), while the beach sand is mesokurtic (Fig. 4). This shows that worms selected medium-grain-size fractions from the sand between 300 and 850 μm. Additionally, the sand grains in the reefs tended to be more well-sorted (with more similar sizes of their grains) than beach sand (with more grain size variability) (Table 2). The reefs also contained fewer fine sand grains (250 and 125 μm) than the beach sand (Fig. 4). Additionally, the worm reef sand had a smaller percentage of fine sand (<35%) compared to ~30-45% in beach sand (Fig. 4). This indicates the worms

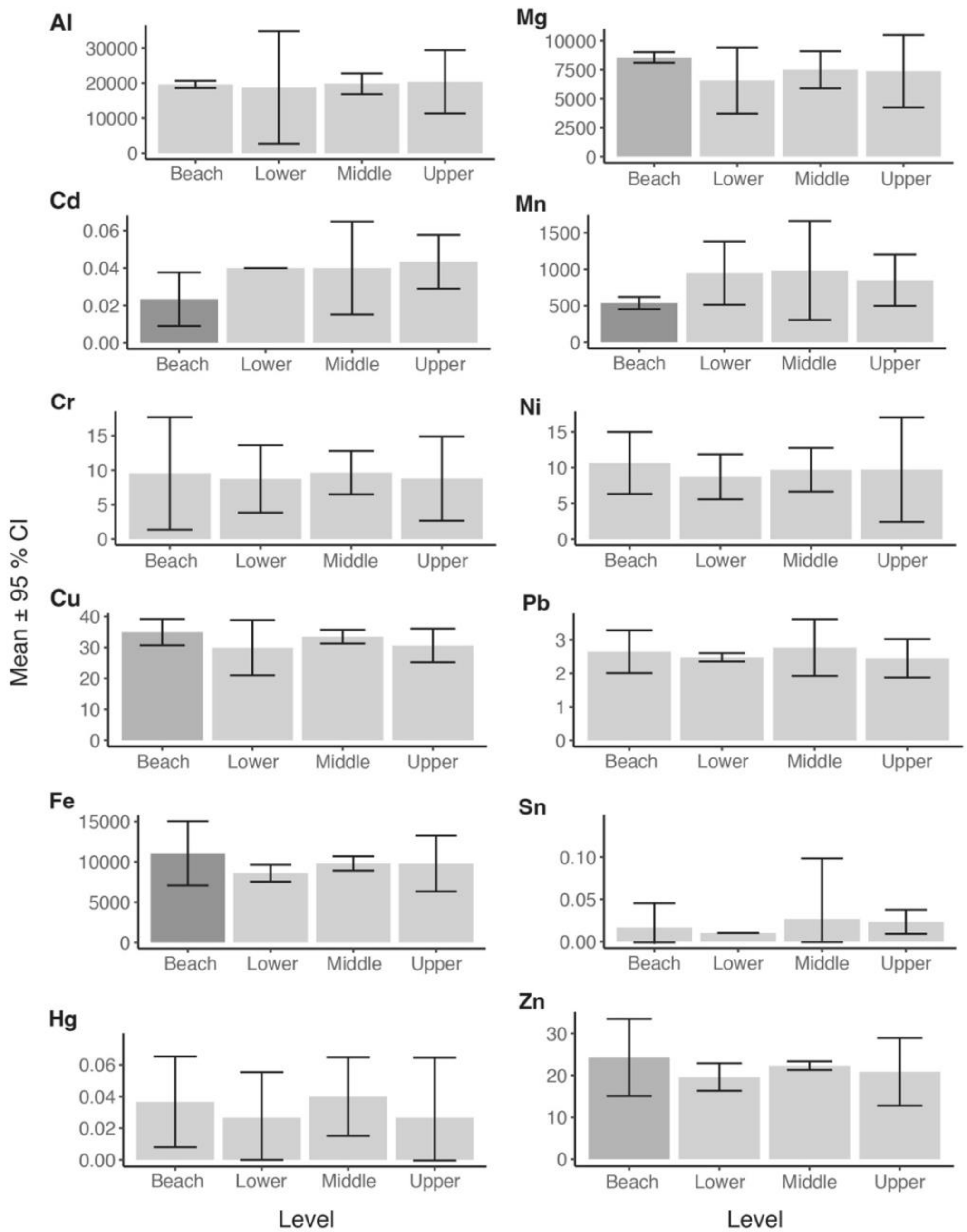

Figure 3. Metal concentrations (µg g^{-1} dw) for Al, Cd, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Sn, and Zn analyzed by Inductively Coupled Plasma Mass Spectrometry. Bars indicate the mean ± 95% confidence interval (CI) for samples collected at the four levels. Dark gray bars indicate mean differences ($P < 0.05$). Light gray bars indicate a trend.

select for coarser fractions of sand (300 to 850 µm) than is present in the beach sand.

Associated fauna

When the reef fragments were disaggregated, almost 30 species of crustaceans were found. Polychaetes, such as phyllodocids and nereids, were also found. Anemones, flatworms, sipunculids, and bivalve mollusks were also found but not identified. Patches covered the rocks (Fig. 1d) of small barnacles, *Chthamalus panamensis* Pilsbry, 1916, and the reefs seem to overgrow the patches**.**

DISCUSSION

In this study, we tested two hypotheses: first, that the worms do not accumulate trace metals at levels higher than those found in beach sand. Second, the worms are

Table 2. Mean values of granulometric parameters for three sand samples, along with the overall mean at each sampling level: beach and the elevation levels (lower, middle, upper) of the worm colonies. Mean total organic matter and calcium carbonate content for each sampling level. Puntarenas Peninsula, Gulf of Nicoya estuary, Pacific, Costa Rica, 2020. *Moderately sorted. **Moderately well sorted. ^{P}Positive, ^{N}Negative, ^{M} Mesokurtic, ^{L}Leptokurtic.

Level	Mean grain size (µm)	Sorting (phi)	Skewness (phi)	Kurtosis (phi)	Total organic matter (%) (min-max)	CaCo3 (%) (min-max)
Beach	311.28	0.89*	- 0.12^{N}	0.95^{M}	2.83	2.81
	(288.72 / 309.82 / 335.31)	(0.87 / 0.88 / 0.91)	(-0.06 / -0.14 / -0.15)	(0.90 / 0.92 / 1.02)	(2.74-3.01)	(2.66-2.91)
Lower	332.64	0.82*	0.25^{P}	1.10^{M}	2.44	7.98
	(301.28 / 339.24 / 357.39)	(0.72 / 0.84 / 0.91)	(0.18 / 0.27 / 0.29)	(1.04 / 1.05 / 1.20)	(2.40-2.67)	(3.78-12.44)
Middle	354.32	0.75*	0.28^{P}	1.31^{L}	2.32	6.23
	(349.21 / 354.3 / 359.46)	(0.71 / 0.75 / 0.79)	(0.23 / 0.30 / 0.31)	(1.25 / 1.33 / 1.34)	(2.24-2.37)	(4.90-8.30)
Upper	359.99	0.69**	0.21^{P}	1.17^{L}	2.42	5.86
	(297.98 / 339.24 / 442.76)	(0.65 / 0.69 / 0.73)	(0.15 / 0.24 / 0.24)	(1.01 / 1.21 / 1.28)	(2.33-2.48)	(4.11-9.12)

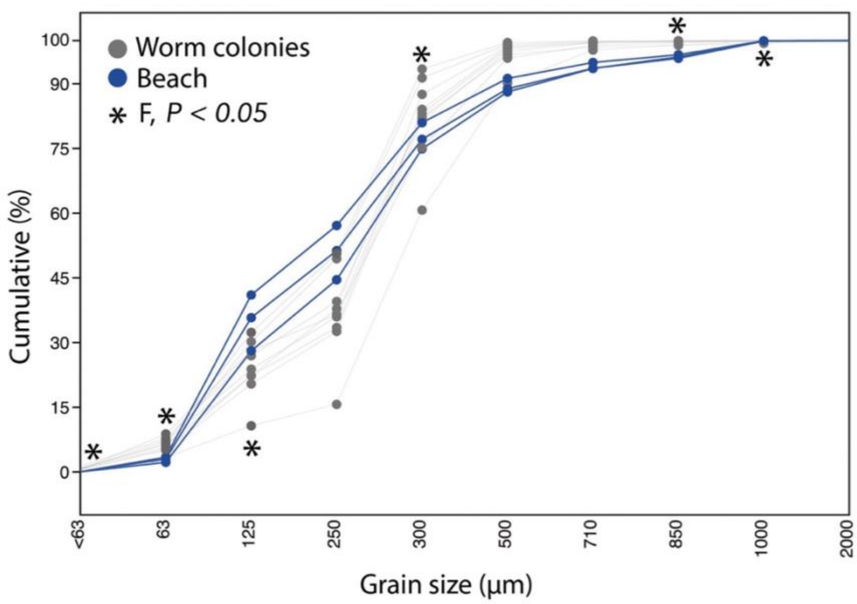

Figure 4. Grain size analysis. Cumulative frequency curves, with the percentage as the y-axis and the grain size in micrometers (µm) as the x-axis. Puntarenas Peninsula, Gulf of Nicoya estuary, Pacific Costa Rica, 2020.

not selective about grain size when building reefs, unlike beach sand composition.

As for the first hypothesis, our results indicate that cadmium and manganese occur at higher concentrations in worm reefs than in beach sand, while other metals show no clear trend. Iron had a lower mean concentration in reefs than in beach sand. The study by Stewart et al. (2004) found that the tube cement in *Phragmatopoma californica* (Fewkes, 1889) contains high concentrations of Ca and Mg, and other sabellariid tubes contain traces of Al, Fe, Mn, and Zn. The study by Hofman et al. (2018) in the same species reported that the adhesive material contains traces of Mg, Ca, Fe, Zn, and Mn. These ions likely play a role in the complexation process. Specifically, these ions can form ionic bonds or coordinate with dihydroxyphenylalanine (DOPA), an amino acid known for its adhesive properties.

There is little data on metal concentrations in sabellariid worm sand tubes in the eastern Tropical Pacific, and the same applies to studies of the sedimentary composition of worm tubes. In this context, it is important to note that worms may also obtain trace metals from seawater. Deias et al. (2023)

found a relationship between trace metals in seawater and bioaccumulation in cement produced by Mediterranean *S. alveolata* worms that bind tube sediment particles. The relatively small accumulation of Cd in the tubes of *P. attenuata*, compared with that in beach sand (Table 1), warrants future investigation. Concentrations of Cd on Amazonian oceanic sandy beaches (0.2 ± 0.6 to 0.4 ± 0.77 µg g^{-1} dw) were similar to those reported in other studies in which sources of pollution, such as sewage discharges, fertilizers, and industrial waste, were identified (Vilhena et al. 2021). The proximity of the city of Puntarenas and of small creeks carrying fertilizers and other compounds from agricultural lands may be a possible source of Cd and other metals. For comparison, in an earlier study of trace metals in subtidal sediments in front of the port of Puntarenas, the concentration of Cd was found to be 0.096 µg g^{-1} dw (Dean et al. 1986) and was considered as expected of non-industrialized tropical estuaries as well as for other metals (Fe = 6283.42, Zn = 130.06, Cu = 3.43, Pb = 3.12 µg g^{-1} dw).

For the second hypothesis, *P. attenuata* selects grains with mesh sizes between 300 and 850 µm, more than those present in beach sand. Worms used $CaCO_3$ grains nearly four times the concentration found in beach sand. The species selected coarser grains with higher CaCO3 content than those in the adjacent beach. A reduction in the selection of terrigenous sand by *Phragmatopoma* likely leads to a lower iron (Fe) content in the reefs compared to the beach sand. This selection enhanced the tube's structural integrity and stability by minimizing gaps (Stewart et al. 2017). These grains bind effectively when they are the correct size to fit within the worm-building organs, about 500 µm, for example, in *P. californic*a (Stevens et al. 2007). The early study by Main & Nelson (1988) on *P. lapidosa* from Florida provided data on the sedimentary composition of worm tubes and found that $CaCO_3$ particles ranging from 0.25 to 0.50 mm were used to construct the tubes. Bremec et al. (2013) found that worm tubes of *Sabellaria nanella* Chamberlin, 1919 from Argentina contain a higher percentage (35.5%) of medium-sized grains than beach sediments (16.6%).

In the Dominican Republic, *P. caudata* was found to have tubes composed of 36.6% coarse sand (500 to 2,000 µm). In contrast, the beach sand contained only 0.75% of this grain fraction, indicating selection by the worms (Santos-Mella et al. 2017). In northern Brazil, research on *S. wilsoni* by Aviz et al. (2019) reports significant differences in grain size between beach and worm reef sediments, with higher percentages of silt, clay, coarse sand, and organic matter in the reef sediments. Similar results were observed for the colonies and beach sand in the present study: the retained fractions on the 250- and 125-µm mesh sieves were lower in the colonies than in the beach sand.

Bremec et al. (2013), working with *S. nanella* from Argentina, found that organic matter was higher in the worm tubes (7%) than on the beach (3%). In our study, the worms showed slightly lower organic matter concentrations in the tubes than in the beach sand. A possible explanation could be related to the structure of the tubes. The species *P. californica* uses minuscule glue spots to attach sediment particles as it builds the tube tip, resulting in a solid foam structure (Wang & Stewart 2012). Other species, such as *S. alveolata*, use glue spots to attach shell fragments and produce an organic inner layer within their tubes (Buffet et al. 2018). The sand obtained from the water column by *P. attenuata* may be cleaner than the sand deposited on the beach, which contains interstitial biota between the grains. Additionally, several species of *Phragmatopoma* capture biotic material, such as shell fragments, foraminiferal tests, sponge spicules, and echinoid spines, to incorporate into their tubes (Jensen & Morse 1988). The selection of these materials explains the higher percentage of calcium carbonate in the tubes compared to beach sand.

It is pertinent to note that particle-size selection is not exclusive to Sabellariid worms. The study by Noffke et al. (2009) on *Owenia fusiformis* Delle Chiaje, 1844 (Oweniidae) indicates that this polychaete actively selects medium- to fine-sand particles for tube construction, rather than the very fine particles found in adjacent sediments.

Beach sand metals

The survey by García-Céspedes et al. (2004) at three locations along the Pacific coast of Costa Rica yielded maximum values of Cu (128 µg g^{-1} dw, Golfo Dulce embayment), Fe (7.33%, Gulf of Papagayo), Pb (8.2 µg g^{-1} dw, Gulf of Nicoya and Golfo Dulce), and Zn (109.4 µg g^{-1} dw, Golfo Dulce). The Gulf of Papagayo, a relatively pristine site with no significant sources of pollution, had the lowest maximum concentrations of Cu (84.7 µg g^{-1}), Fe (7.33%), Pb (3.8 µg g^{-1} dw), and Zn (96.9 µg g^{-1} dw), which were most likely attributable to natural sources. The study by Vargas et al. (2016) at the Cocorocas sand flat, west of the port of Puntarenas, found that the sediment included fine sands (29.2%) and very fine sands (41.7%), with 3% organic matter. Metal concentrations were Fe (46 mg g^{-1} dw), Mn (413 µg g^{-1}), Zn (63 µg g^{-1}), Cu (36.2 µg g^{-1}), Cr (31.5 µg g^{-1}), Pb (21.1 µg g^{-1}), Ni (16.1 µg g^{-1}), and Cd (1.1 µg g^{-1}).

These concentrations were also considered to be within the range expected for a non-industrialized estuary. Aluminum had higher concentrations in the present study (~12 to 25×10^3 µg g^{-1} dw). It was similar to values observed in the Seine, France (~15 to 32×10^3 µg g^{-1} dw), which were attributed to the use of sacrificial anodes in vessels (Gabelle et al. 2012). However, aluminum has a high natural abundance as aluminosilicates in the fine-grain fraction of sediment (Windom et al. 1989).

The question arises whether the Puntarenas beach sands collected in 2020 contain relatively high levels of metals. Macdonald et al. (1996) provided guidelines for evaluating the effects of metals in coastal sedimentary environments, revising the concept of TEL, an estimate of the concentration at which a physiological effect begins to occur. Several of these concentrations are included in Table 1 for comparison. The Puntarenas sands appear below those levels, except copper (8.19-18.75 µg g^{-1} dw over the threshold). The survey by Buzzi et al. (2022) includes data from 44 studies worldwide on tourist beach sands. It illustrates the variability in metal positions when ordered from most to least abundant. They included the survey by Jonathan et al. (2011) for sand beaches in Acapulco, on the Pacific coast of Mexico (16°50′N - 99°52′W), and the sequence was: Fe > Mn > Zn > Cr > Cu > Pb > Ni > Cd. Their concentration range is also included for comparison in Table 1. They indicate that sources of metals are related in part to tourist activities, hotel operations, construction, boat operations and maintenance, and the disposal of electronic equipment. Metal concentrations of Cd, Cr, Fe, Ni, and Mn were slightly above the United States Environmental Protection Agency's 2001 limit. In this sense, the concentrations found in sands at the tip of the port of Puntarenas may include metals from anthropogenic sources as well as those of geological origin, but at levels expected for non-industrialized estuaries, as previously mentioned.

There is no data on the tolerance of sabellariid worms to metal contamination. Reports for other polychaetes indicate that certain species can take up metals, detoxify them, and store some in less toxic forms (Dean 2008). Moreover, organic matter in the sediment is important, as high levels often lead to greater metal accumulation. The relatively low concentration (3%) of total organic matter in Puntarenas beach sand may be an important factor to explore in future studies. The accumulation of metals in other intertidal species has been studied in the muricid snail *Acanthais brevidentata* (W. Wood, 1828), which lives on rocks at the Puntarenas port and the nearby Caldera port (Vargas et al. 2015). At Caldera, *A. bredidentata* has been found to exhibit imposex (the imposition of male sexual characteristics on female gastropod mollusks) due to the presence of Sn compounds (Gravel et al. 2006). Individuals of *Phragmatopoma* in Puntarenas are constantly exposed to these trace metals within their tubes. In this context, determining metal concentrations in the tissues of *Phragmatopoma* spp. worms are relevant.

Credit author contribution

J.A. Vargas: conceptualization, funding acquisition, sampling, species identification, writing - original draft, review, and editing; J. Molina: trace metal concentration analysis, writing - original draft, review, and editing, and review;, R. Cambronero: granulometric analysis, writing - original draft, review, and editing; J.A. Sibaja-Cordero: conceptualization, funding acquisition, sampling, species identification, statistical analysis, writing - original draft, review, and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Sergio Aguilar for preparing the figures and Davis Morera for his help in the fieldwork. This research was made possible by a grant to project C2104 of CIMAR, University of Costa Rica. We thank Harlan Dean, the editor, Paula Paiva, and two reviewers for their valuable feedback and suggestions on this manuscript.

REFERENCES

Aviz, D., Figueira-Da Silva, R. & Rosa-Filho, J.S. 2019. *Sabellaria wilsoni* (Polychaeta: Sabellariidae): an ecosystem engineer and promoter of zoobenthos diversity in the Brazilian Amazon coast. Journal of the Marine Biological Association of the United Kingdom, 99: 1099-1109. doi: 10.1017/S00253154 18001157

Barrios, L.M., Chambers, S.J., Ismail, N., et al. 2009. Distribution of *Idanthyrsus cretus* (Polychaeta: Sabellariidae) in the Tropical Eastern Pacific and application of PCR-RAPD for population analysis. Zoosymposia, 2: 487-503. doi: 10.11646/zoosymposia.2.1.34

Bremec, C., Carcedo, C., Piccolo, M.C., et al. 2013. *Sabellaria nanella* (Sabellariidae): from solitary subtidal to intertidal reef-building worm at Monte Hermoso, Argentina (39°S, south-west Atlantic). Journal of the Marine Biological Association of the United Kingdom, 93: 81-86. doi: 10.1017/S0025315412000550

Bruschetti, M. 2019. Role of reef-building, ecosystem engineering polychaetes in shallow water ecosystems. Diversity, 11: 168. doi: 10.3390/d11090168

Buffet, J.P., Corre, E., Duvernois-Berthet, E., et al. 2018. Adhesive gland transcriptomics uncovers a diversity of genes involved in glue formation in marine tube-building polychaetes. Acta Biomaterialia, 72: 316-328. doi: 10.1016/j.actbio.2018.03.037

Buzzi, N.S., Menendez, M.C., Truchet, D.M., et al. 2022. An overview on metal pollution on touristic sandy beaches: Is the COVID-19 pandemic an opportunity to improve coastal management? Marine Pollution Bulletin, 174: 113275. doi: 10.1016/j.marpolbul.2021.113275

Chang, W. 2019. R Graphics Cookbook. R-graphics. [https://r-graphics.org]. Reviewed: July 22, 2024.

Chávez-López, Y. 2020. New species and new records of *Phragmatopoma* (Polychaeta: Sabellariidae) from Tropical America. Zootaxa, 4845: 301-330. doi: 10.11646/zootaxa.4845.3.1

Chávez-López, Y. & Bastida-Zavala, J.R. 2021. Sabellariidae. In: de León-González, J.A., Bastida-Zavala, J.R., Carrera-Parra, L.F., et al. (Eds.). Anélidos marinos de México y América tropical. Universidad Autónoma de Nuevo León, Monterrey, pp. 695-715.

Chouikh, N.E., Gillet, P., Cheggour, M., et al. 2022. Bioaccumulation of trace metals in *Sabellaria alveolata* (Annelida: Polychaeta) and their controlling factors along the Essaouira Protected Coastal Area (Atlantic Coast of Morocco). Ocean Science Journal, 57: 224-238. doi: 10.1007/s12601-022-00057-z

Crawley, M.J. 2013. The R book. Wiley, New York.

Dean, W.E. 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition; comparison with other methods. Journal of Sedimentary Research, 44: 242-248. doi: 10.1306/74D729D2-2B21-11D7-8648000102C1865D

Dean, H.K. 2008. The use of polychaetes (Annelida) as indicator species of marine pollution: a review. Revista de Biología Tropical, 56(S4): 11-38. doi: 10.15517/rbt.v56i4.27162

Dean, H.K. 2009. Polychaetes and echiurans. In: Wehrtmann, I.S. & Cortés, J. (Eds.). Marine biodiversity of Costa Rica, Central America. Monographiae Biologicae, 86: 122-159, 181-191.

Dean, H.K., Maurer, D., Vargas, J.A., et al. 1986. Trace metal concentrations in sediment and invertebrates from the Gulf of Nicoya, Costa Rica. Marine Pollution Bulletin, 17: 128-131. doi: 10.1016/0025-326X(86)90418-2

Deias, C., Guido, A., Sanfilippo, R., et al. 2023. Elemental fractionation in Sabellariidae (Polychaeta) biocement and comparison with seawater pattern: A new environmental proxy in a high-biodiversity ecosystem? Water, 15: 1549. doi: 10.3390/w15081549

Denyer, P., Cárdenas, G. & Kruse, S. 2004. Registro histórico y evolución de la barra arenosa de Puntarenas, Golfo de Nicoya, Costa Rica. Revista Geológica de América Central, 31: 45-59.

Fauchald, K. 1977. Polychaetes from intertidal areas in Panama, with a review of previous shallow-water records. Smithsonian Contributions to Zoology, 221: 1-81. doi: 10.5479/si.00810282.221

Folk, R.L. & Ward, W.C. 1957. Brazos River bar (Texas): a study in the significance of grain size parameters. Journal of Sedimentary Research, 27: 3-26. doi: 10.1306/74D70646-2B21-11D7-8648000102C1865D

Gabelle, C., Baraud, F., Biree, L., et al. 2012. The impact of aluminum sacrificial anodes on the marine environment: a case study. Applied Geochemistry, 27: 2088-2095. doi: 10.1016/j.apgeochem.2012.07.001

García-Céspedes, J., Acuña-González, J.A. & Vargas-Zamora, J.A. 2004. Metales traza en sedimentos costeros de Costa Rica. Revista de Biología Tropical, 52(S2): 51-60.

Gilbert, E.R., Camargo, M.G. & Sandrini-Neto, L. 2012. Rysgran: Grain size analysis, textural classifications and distribution of unconsolidated sediments. R Package Documentation. [https://rdrr.io/cran/rysgran/man/rysgran-package.html]. Reviewed: July 22, 2024.

Gravel, P., Johanning, K., McLachlan, J., et al. 2006. Imposex in the intertidal snail *Thais brevidentata* (Gastropoda: Muricidae) from the Pacific coast of Costa Rica. Revista de Biología Tropical, 54(S1): 21-26. doi: 10.15517/rbt.v54i1.26829

Hartman, O. 1944. Polychaetous annelids. Part. VI Paraonidae, Megalonidae, Longosomidae, Ctenodrilidae, and Sabellariidae. Allan Hancock Pacific Expeditions, 10: 311-389.

Hofman, A.H., van Hees, I.A., Yang, J., et al. 2018. Bioinspired underwater adhesives by using the supramolecular toolbox. Advanced Materials, 30: 1704640. doi: 10.1002/adma.201704640

Jensen, R.A. & Morse, D.E. 1998. The bioadhesive of *Phragmatopoma californica* tubes: a silk-like cement containing L-DOPA. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology, 158: 317-324. doi: 10.1007/BF00695330

Jonathan, M.P., Roy, P.D., Thangadurai, N., et al. 2011. Metal concentrations in water and sediments from tourist beaches of Acapulco, Mexico. Marine Pollution Bulletin, 62: 845-850. doi: 10.1016/j.marpolbul.2011.02.042

Lane-Medeiros, L., Puppin-Gonçalves, C.T., Rocha, M.A.L.D., et al. 2021. Macrocrustaceans associated with reefs of *Phragmatopoma caudata* Krøyer in Mörch, 1863 (Polychaeta: Sabellariidae) and rocky shore in the northeastern Brazil. Papéis Avulsos de Zoologia, 61: e20216119. doi: 10.11606/1807-0205/2021.61.19

Lisco, S.N., Acquafredda, P., Gallicchio, S., et al. 2020. The sedimentary dynamics of *Sabellaria alveolata* bioconstructions (Ostia, Tyrrhenian Sea, central Italy). Journal of Paleogeography, 9: 1-18. doi: 10.1186/s42501-019-0050-6

Macdonald, D.D., Carr, R.S., Calder, F.D., et al. 1996. Development and evaluation of sediment quality guidelines for Florida coastal waters. Ecotoxicology, 5: 253-278. doi: 10.1007/BF00118995

Main, M.B. & Nelson, W.G. 1988. Sedimentary characteristics of sabellariid worm reefs (*Phragmatopoma lapidosa* Kinberg). Estuarine Coastal & Shelf Science, 26: 105-109. doi: 10.1016/0272-7714(88)90015-7

Noffke, A., Hertweck, G., Kröncke, I., et al. 2009. Particle size selection and tube structure of the polychaete *Owenia fusiformis*. Estuarine, Coastal and Shelf Science, 81: 160-168. doi: 10.1016/j.ecss.2008.10.010

Quinn, G.P. & Keough, M.J. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge.

Santos-Mella, F., Contreras-Puigbó, A., Pugibet, E., et al. 2017. Ecología y biodiversidad de un arrecife formado por *Phragmatopoma caudata* Krøyer in Mörch (Canalipalpata: Sabellariidae) en República Dominicana. Novitates Caribaea, 11: 69-78. doi: 10.33800/nc.v0i11.16

Sibaja-Cordero, J.A. & García-Méndez, K. 2014. Variación espacial y temporal de los organismos de un intermareal rocoso, Bahía Panamá, Pacífico Norte, Costa Rica. Revista de Biología Tropical, 62(S4): 85-97. doi: 10.15517/rbt.v62i4.20034

Sibaja-Cordero, J.A. & Gómez-Ramírez, E.H. 2022. Marine litter on sandy beaches with different human uses and waste management along the Gulf of Nicoya, Costa Rica. Marine Pollution Bulletin, 175: 113392. doi: 10.1016/j.marpolbul.2022.113392

Sibaja-Cordero, J.A. & Vargas-Zamora, J.A. 2006. Zonación vertical de epifauna y algas en litorales rocosos del Golfo de Nicoya, Costa Rica. Revista de Biología Tropical, 54(S1): 49-67. doi: 10.15517/rbt.v54i1.26834

Stevens, M.J., Steren, R.E., Hlady, V., et al. 2007. Multiscale structure of the underwater adhesive of *Phragmatopoma californica*: a nanostructured latex with a steep microporosity gradient. Langmuir, 23: 5045-5049. doi: 10.1021/la063765e

Stewart, R.J., Wang, C.S., Song, I.T., et al. 2017. The role of coacervation and phase transitions in the sandcastle worm adhesive system. Advances in Colloid and Interface Science, 239: 88-96. doi: 10.1016/j.cis.2016.06.008

Stewart, R.J., Weaver, J.C., Morse, D.E., et al. 2004. The tube cement of *Phragmatopoma californica*: a solid foam. Journal of Experimental Biology, 207: 4727-4734. doi: 10.1242/jeb.01330

Vargas, J.A., Vargas-Castillo, R. & Sibaja-Cordero, J.A. 2024. Crustaceans in *Phragmatopoma* sp. (Polychaeta: Sabellariidae) intertidal reefs, Pacific, Costa Rica. UNED Research Journal, 16: e4926. doi: 10.22458/urj.v16i1.4926

Vargas, J.A., Acuña-González, J., Gómez, E., et al. 2015. Metals in coastal mollusks of Costa Rica. Revista de Biología Tropical, 63: 1007-1019. doi: 10.15517/rbt.v63i4.18143

Vargas, J.A., Acuña-González, J., Vásquez, F., et al. 2016. Brachiopods, sipunculans, enteropneusts, and metals from two estuarine tidal flats, Pacific, Costa Rica. Revista de Biología Tropical, 64: 1311-1331. doi: 10.15517/rbt.v64i3.23251

Vilhena, J.C., Amorim, A., Ribeiro, L., et al. 2021. Baseline study of trace element concentrations in sediments of the intertidal zone of Amazonian oceanic beaches. Frontiers in Marine Science, 8: 671390. doi: 10.3389/fmars.2021.671390

Voorhis, A.D., Epifanio, C.E., Maurer, D., et al. 1983. The estuarine character of the Gulf of Nicoya, an embayment on the Pacific coast of Central America. Hydrobiologia, 99: 225-237. doi: 10.1007/BF00008774

Wang, C.S. & Stewart, R.J. 2012. Localization of the bioadhesive precursors of the sandcastle worm, *Phragmatopoma californica* (Fewkes). Journal of Experimental Biology, 215: 351-361. doi: 10.1242/jeb.065011

Windom, H.L., Schropp, S.J., Calder, F.D., et al. 1989. Natural trace metal concentrations in estuarine and coastal marine sediments of the southeastern United States. Environmental Science & Technology, 23: 314-320. doi: 10.1021/es00180a008

Zhao, H., Sun, C., Stewart, R.J., et al. 2006. Cement proteins of the tube building polychaete *Phragmatopoma californica*. Journal of Biological Chemistry, 280: 42936-42944. doi: 10.1074/jbc.M508457200

Received: September 23, 2025; Accepted: March 19, 2026

SUPPLEMENTARY MATERIAL

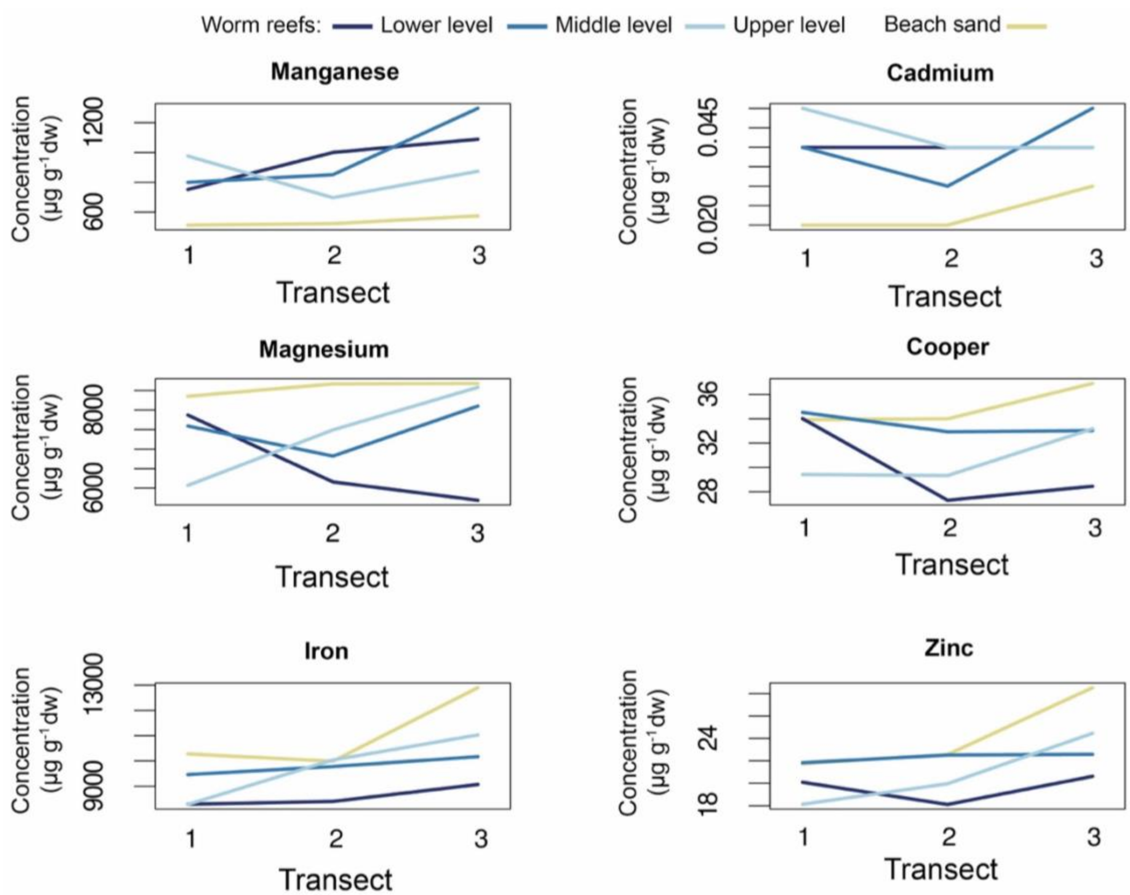

Figure S1. Differences in metal concentrations (µg g^{-1} dw) observed between sabellarid worm reefs at three intertidal levels and the adjacent sand for each sampled transect.