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#### Baseline



# Seasonal variation in the bioaccumulation of potentially toxic metals in the tissues of *Astrangia poculata* in the northeastern United States

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### ABSTRACT

Astrangia poculata inhabits coasts near dense human populations in the northeastern United States and may be exposed to elevated pollutants. No studies have assessed heavy metal concentration in temperate corals despite their proximity to anthropogenic activity. We collected colonies four times in one year and analyzed coral tissue for As, Cd, Cr, Pb, and Zn. Most heavy metals except for As were 1.5–3.3 times lower in summer compared to other seasons. Pb, As, and Cd were three orders of magnitude higher than concentrations for other Narragansett Bay benthic species, suggesting that A. poculata bioaccumulates more readily and/or inhabits more contaminated areas of the Bay. Zn, Pb, and As had similar concentrations to tropical corals inhabiting anthropogenically polluted sites. While physiological impacts are unknown, this population of A. poculata may have a higher tolerance for heavy metal pollution than most scleractinians, making it an interesting candidate for future studies.

In the marine environment, heavy metals with known toxicities to corals often originate from anthropogenic sources (e.g., Berry et al., 2013; May et al., 2020). The effects of nearshore and coastal urbanization are sources of Zn, Cu, and Pb in corals (Chan et al., 2014; Esslemont, 2000; Nixon and Fulweiler, 2012). Similarly, antifouling paint used on boats contains Cu, Zn, Cd, Cr, and Pb, which can leach into marine environments and be incorporated by corals (Jones, 2007; Turner, 2010). In addition to recent pollution, perturbation of legacy contamination may reintroduce heavy metals to the water column that are subsequently taken up by corals (Whitall et al., 2014). Most prior studies on heavy metals in corals have occurred in the tropics, with limited work conducted on temperate corals despite their greater proximity to anthropogenic activity compared to most tropical reefs.

Heavy metals in seawater exist in dissolved or particulate forms (Wells et al., 1998, 2000) that can both be incorporated by corals (Howard and Brown, 1984). Metal uptake occurs by host tissue or symbionts, directly or indirectly incorporated into skeleton, and/or ingested during heterotrophy (Metian et al., 2015; Reichelt-Brushett and McOrist, 2003). Concentrations of heavy metals into coral compartments and rates of accumulation are likely controlled by a combination of environmental availability and coral physiology (Hédouin et al., 2016). Few studies have assessed seasonality in heavy metal

accumulation, even though both environmental conditions and coral physiology are known to exhibit seasonal variability (e.g., Brown et al.,

Evidence from the literature suggests that heavy metal accumulation may vary seasonally in coral tissue. For example, heavy metal concentration in suspended sediment was influenced by winds, suspended particulate loads, and tides during the dry season, but nearby corals were not evaluated (Fernandez et al., 2017). Heavy metals associated with wet/dry seasonality (Moyer et al., 2012) and upwelling periodicity (Delaney et al., 1993) are recorded in coral skeletons, but seasonal accumulation in coral tissues is less known. Toxicity tests showed greater coral survivorship in winter compared to summer (Hédouin et al., 2016), suggesting differential impacts of heavy metals across seasons. Assessing seasonal variation of heavy metals will address gaps in our knowledge of the persistence and rate of accumulation in coral tissues, while also providing a species-specific baseline for environmentally-relevant toxicity studies.

A. poculata is considered a hardy species that inhabits coastlines adjacent to dense human populations (Dimond et al., 2013; Grace, 2017), making it a good candidate for pollution studies. The most well-studied population of A. poculata lives within the northeast megalopolis of the United States. While the region has a large urban footprint (Nixon

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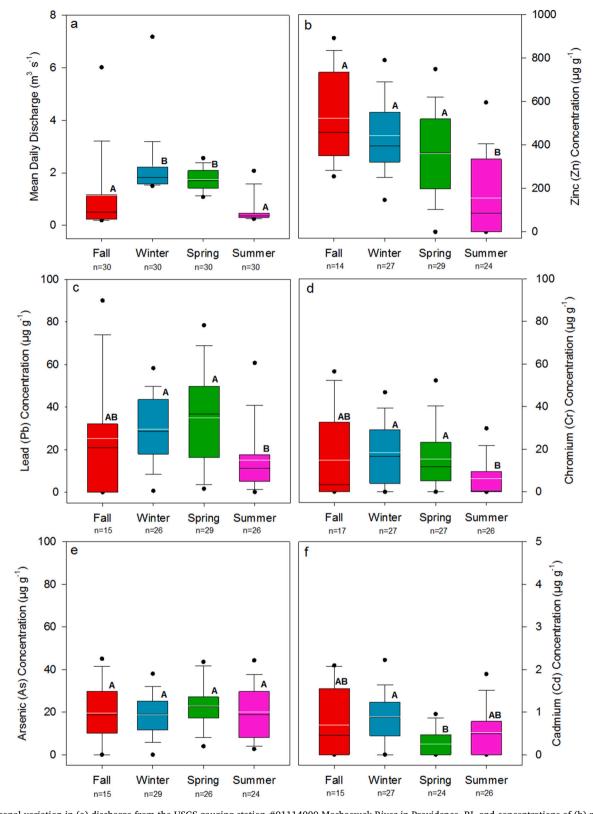


Fig. 1. Seasonal variation in (a) discharge from the USGS gauging station #01114000 Moshassuck River in Providence, RI, and concentrations of (b) zinc (Zn), (c) lead (Pb), (d) chromium (Cr), (e) arsenic (As), and (f) cadmium (Cd) in the tissue of *Astrangia poculata*. Boxplots indicate the first and third quartile ranges, mean (white line), median (black line), and outliers for each season. Post-hoc statistical significance among seasons is shown by different letters. Samples sizes are indicated on each x-axis. Note that y-axis values for each heavy metal differ by orders of magnitude.

Table 1
Mean seasonal range of heavy metals in the tissue of *Astrangia poculata* compared to published values for heavy metals of (a) tissue of benthic organisms; (b) seawater; (c) benthic surface sediments; (d) terrestrial inputs; and (e) atmospheric inputs in Narragansett Bay, Rhode Island. nd = no data, indicating the heavy metal was not analyzed.

Type of Sample	Units	Zn	Pb	Cr	As	Cd	Publication
a. Tissue of organisms							
A. poculata	μg/g	154.2-523.5	11.1-34.7	6.2 - 16.5	18.9-24.9	0.3-0.9	This study
Leaf samples, various marsh plant species	μg/g	91.3	1.3	nd	nd	nd	Rudin et al., 2017
Amphipod	ng/g	nd	1.6-1.8	nd	3.8-10.6	0.1-0.2	Chen et al., 2016 <sup>a</sup>
Crab	ng/g	nd	0.1-0.3	nd	7.0-7.5	0.1	
Fundulus sp.	ng/g	nd	0.3	nd	1.8 - 2.2	0.0-0.4	
Mussel	ng/g	nd	0.8 - 2.2	nd	7.9-9.7	0.3 - 3.7	
Shrimp	ng/g	nd	0.2-1.2	nd	3.2–7.3	0.2 - 0.4	
b. Seawater							
>0.45 μm	ng/kg	nd	118	nd	nd	nd	Nixon and Fulweiler, 2012b
<0.45 μm	ng/kg	nd	nd	144	nd	33.4	
>0.2 µm	nM/l	5.4	nd	nd	nd	nd	Wells et al., 2000 <sup>c</sup>
$< 0.2 \ \mu m$	nM/l	16.2	nd	nd	nd	nd	
<0.2 µm	nM/l	16.3	0.15	nd	nd	0.3	Wells et al., 1998 <sup>d</sup>
c. Benthic surface sediments							
	μg/g	nd	17.4-75.9	nd	1.2 - 8.8	0.1-0.5	Chen et al., 2016 <sup>a</sup>
	μg/g	nd	200	250	nd	10	Nixon and Fulweiler, 2012e
	μg/g	nd	12–25	nd	nd	nd	Santschi et al., 1984 <sup>f</sup>
d. Terrestrial inputs							
Rivers	metric tons/yr	134	6.8	nd	nd	nd	Bricker, 1993g
Direct sewage	metric tons/yr	59.1	3.3	nd	nd	nd	
e. Atmospheric inputs							
	metric tons/yr	3.9	5	nd	nd	nd	Bricker, 1993g

<sup>&</sup>lt;sup>a</sup> Species names not reported (except for *Fundulus* sp.), range reported for two locations.

and Fulweiler, 2012), no studies have assessed the uptake of anthropogenic pollutants in *A. poculata*, including heavy metals. We hypothesized that concentrations of heavy metals in the tissue of *A. poculata* will vary seasonally. To test this hypothesis, we (1) quantified baseline heavy metal concentrations in *A. poculata* coral tissue; and (2) compared seasonality of heavy metal bioaccumulation.

Colonies of *A. poculata* were collected at 14 m depth on SCUBA near Fort Wetherill State Park in Jamestown, RI ( $41^{\circ}28'40''N$ ,  $71^{\circ}21'24''W$ ) on 27 September 2018 (fall); 20 February 2019 (winter); 30 May 2019 (spring); and 7 August 2019 (summer). We randomly collected 20–30 colonies each season and analyzed chlorophyll *a* and *c2* (Chl *a* and *c2*) concentrations to quantify their level of symbiosis (reported in Trumbauer et al., 2021), since *A. poculata* exhibits a facultative symbiosis (Dimond and Carrington, 2007). Colonies were transported to Villanova University and frozen at -80 °C.

From nearby gauges, we retrieved environmental datasets to assess seasonal variation. Sea surface temperature (reported in Trumbauer et al., 2021), precipitation (NOAA station USW00014765) near Providence, RI, and discharge (USGS station 01114000) for the Moshassuck River that drains into the northern end of Narragansett Bay were obtained. Seasonal values were calculated from 24-h averages for each coral collection date and the 29 days prior, to create a 30-day sample set.

At Villanova University, we separated coral tissue from skeleton with an airbrush and deionized water. Tissue dried at 60  $^{\circ}\text{C}$  for 24 h and 7.56 mg  $\pm$  6.8 (mean  $\pm$  standard deviation) per colony was used for analyses. Tissue was acid digested in 100% trace metal grade nitric acid in a CEM Mars 6 Microwave Digestion System. We used the EPA 3051 method with a ramp time of 20–25 min to reach 175  $^{\circ}\text{C}$ , maintained for an additional 15 min. Extracts were diluted to 4% by volume and analyzed with an Agilent 7900 inductively coupled plasma mass spectrometer for As, Cd, Cu, Cr, Pb, and Zn. Each run contained 20–30 samples from

different collection dates, 3–4 blanks, replicates, drift checks, and NIST standard reference material (mussel tissue SRM 2976). Approximately 4% of samples from each date were analyzed in duplicate and in separate runs to verify consistency with repeated measurements. Cu did not produce repeatable measurements and was excluded from further analyses. Due to high variability, we eliminated outliers greater than the third quartile for each metal and season due to possible analytical contamination, resulting in the deletion of 30 out of 503 values or 5% of the total dataset.

On 8 June 2021 we collected suspended sediment from the site for metal analyses. At Villanova University, the suspended sediment was dried, digested, and analyzed for metals as described above for the coral tissue. We calculated biota-sediment accumulation factors (BSAFs) according to Szefer et al. (1999) for *A. poculata* by season (Supplementary Material).

Statistical analyses were conducted in base R 3.6.2 (R Core Team, 2020). We used Shapiro-Wilk normality tests to assess each metal by season, for all dates combined, and for each environmental dataset. Data were not normally distributed, including transformed data, therefore we used nonparametric statistics. We categorized samples by season and tested precipitation, discharge, and the concentration of each metal for significant differences using Kruskal-Wallis and pairwise Wilcoxon tests with a Benjamin and Hochberg false discovery rate for *p*-values (degrees of freedom = 3). Using the "Hmisc" package (Harrell and Dupont, 2020), we calculated Spearman correlation coefficients and *p*-values in correlation matrices among all heavy metals, Chl *a*, and Chl *c2*. We determined correlation coefficients for variables when categorized by season and pooled for the year. *p*-values were adjusted using a Bonferroni correction.

Throughout the year the overall heavy metal abundance was Zn > Pb  $\approx$  Cr  $\approx$  As > Cd and all heavy metals were present each season in the

b Values reported for East Passage.

<sup>&</sup>lt;sup>c</sup> Values reported for General Rocks.

<sup>&</sup>lt;sup>d</sup> Values reported for Lower Bay.

<sup>&</sup>lt;sup>e</sup> Values approximated from Fig. 9 for the lower Seekonk River.

<sup>&</sup>lt;sup>f</sup> Values approximated from Fig. 5 for Stations F and G.

 $<sup>^{\</sup>rm g}$  Values in metric tons/yr calculated from % total reported in Table 1 for 1985–1987.

Table 2 Mean seasonal range of heavy metals in the tissue of *Astrangia poculata* compared to published mean values for coral tissue from other species and locations. Units are  $\mu g/g$  of dry tissue weight. nd = no data, indicating the heavy metal was not analyzed, while 0.0 indicates the heavy metal was analyzed and below detection limit; arrows indicate the lowest ( $\downarrow$ ) or highest ( $\uparrow$ ) mean values for each heavy metal.

Coral species	Location	Zn	Pb	Cr	As	Cd	Publication
A. poculata	Narragansett Bay, Rhode Island	154.2–523.5	11.1–34.7↑	6.2–16.5	18.9-24.9	0.3-0.9	This study
Porites lutea	Kharg and Lark Coral Reefs, Persian Gulf, Iran	64.5-71.1	3.2-15	40.2-114.2	2.7–73.2↑	0.9 - 1.6	Ranjbar Jafarabadi et al.,
Acropora robusta		53.0-53.5	3.0-9.4	33.2-99.2	2.5-54.2	0.3-1.1	2018 <sup>a</sup>
Acropora valida		42.7-62.3	2.0 - 7.7	27.9-84.4	1.6-44.0	0.2-0.7	
Favia favus		20.5-33.4	0.2-1.4	13.0-57.4	0.2 - 19.6	0.1-1.2	
Favia speciose		16.3-26.5	0.2-1.4	10.6-42.5	0.2 - 16.5	0.1-0.9	
Platygyra daedalea		12.2-19.9	0.1-1.0	8.5-33.2	0.2 - 9.4	< 0.1-0.7	
Tubastraea coccinea	Yin-Yang Sea, Kueishan Islet, and Green Island, Taiwan	34.1–85.5	0.5–1.2	1.1–1.9	nd	0.6–12.1	Chan et al., 2014 <sup>b</sup>
Porites asteroides	Guánica Bay, Puerto Rico	9.4	0.1	0.0↓	1.4	0.3	Whitall et al., 2014
Agaricia tenuifolia	Bocas del Toro, Panama	30-80	nd	nd	13-18	2-5	Berry et al., 2013 <sup>c</sup>
Porites furcata		50-180	nd	nd	7–19	1–8↑	
P. asteroides	Jobos Bay, Puerto Rico	8.6	1.4	50.3	0.3	0.0↓	Whitall et al., 2011
P. asteroides	Vieques, Puerto Rico	3.4	0.1	0.2	0.2	0.2	Pait et al., 2010
P. asteroides	southwest Puerto Rico	6.1	<0.01↓	0.0↓	0.0↓	0.0↓	Pait et al., 2009
Lobophyllia corymbosa	Lakshadweep Archipelago, India	9.3	12.0	15.9	nd	1.5	Anu et al., 2007
Porites andrewsi		0.7↓	0.3	2.8	nd	0.0↓	
Montipora digitata		0.7↓	0.3	0.9	nd	0.2	
Acropora formosa		2.7	4.5	2.9	nd	0.6	
Psammocora contigua		2.0	20.7	4.4	nd	2.2	
Acropora tenuis	Magnetic and One Tree Islands, Australia	17.1–32.3	0.3	nd	1.1–1.6	< 0.1	Reichelt-Brushett and McOrist, 2003 <sup>d</sup>
Goniastrea aspera	Pioneer Bay, Nelly Bay, and Townsville	127.3–899.7↑	6.8 - 10.2	55.7-110.2	nd	2.0 - 3.3	Esslemont, 2000e
Pocillopora damicornis	Harbor, Australia	128.9–209.2	7.5–10.8	55.9–94.6	nd	2.8–6.0	
A. formosa		236.7	16.2	52.1	nd	4.4	
P. damicornis	Solitary Islands Marine Reserve, Australia	nd	2.2	4.0	nd	0.9	Esslemont et al., 2000f
Acropora nobilis	Heron Island, Australia	nd	0.7 - 2.6	nd	nd	0.2 - 1.6	Esslemont, 1999g
G. aspera		nd	2.0	nd	nd	0.4	
P. damicornis	Phuket, Thailand	182.0-608.0	nd	62.8-160.0	nd	nd	Howard and Brown, 1987

<sup>&</sup>lt;sup>a</sup> Mean values reported in Supplementary Material, range reported for two locations.

tissues of A. poculata corals from Narragansett Bay (Supplementary Material; Fig. 1). Zn was one order of magnitude more than Pb, Cr, and As, and three orders of magnitude more than Cd (Fig. 1). While bedrock can be a significant source of heavy metals (Zhai et al., 2003), it is an unlikely source in this case. The geology immediately surrounding our collection site is composed mostly of porphyritic granite, typically containing Cu, Mo, and Au, lacking notable quantities of Zn, Pb, Cr, As, or Cd (Candela, 1997; Nicholson et al., 2006). When pooled by season (Supplementary Material), there were positive correlations between Zn and Pb ( $\rho = 0.45$ ; p < 0.005); Zn and Cr ( $\rho = 0.33$ ; p < 0.005); Zn and As  $(\rho = 0.35; p < 0.005);$  and Pb and Cr  $(\rho = 0.62; p < 0.005).$  These correlations suggest similar sources throughout the year, especially for the three most abundant heavy metals Zn, Pb, and Cr. They are significant components of road dust from degraded building material, road surfaces, tires, brakes, clutches, and other vehicle parts (Adamiec et al., 2016; Davis et al., 2001) often transported from impermeable surfaces to nearshore marine and aquatic environments (Jeong et al., 2020; Mosley and Peake, 2001; Sebastiao et al., 2017).

There were significant seasonal differences among most heavy metals, except As, which remained constant at 20.4  $\mu g/g \pm 11.4$  throughout the year (Supplementary Material; Fig. 1e). The pattern of seasonality varied, as Zn was significantly lowest in summer compared to all other seasons (Fig. 1b). Pb and Cr also had low summer concentrations, but these were not significantly different from their respective fall concentrations (Fig. 1c–d). The pattern for Cd was unique, with

concentrations in the spring significantly lower than the winter (Fig. 1f). There were significant positive correlations between heavy metals in each season (Supplementary Material): in the fall between Cr and Pb ( $\rho=0.68;\ p<0.005);$  in the winter between Pb and Zn ( $\rho=0.58;\ p<0.005)$  and Pb and Cd ( $\rho=0.68;\ p<0.005);$  in the spring between Zn and As ( $\rho=0.54;\ p<0.005)$  and Pb and Cr ( $\rho=0.87;\ p<0.005);$  and in the summer between Zn and Cr ( $\rho=0.62;\ p<0.005)$  and Zn and As ( $\rho=0.61;\ p<0.001).$  In addition to shared annual sources, these correlations suggest some shared seasonal sources for heavy metals in A. poculata.

While there was no seasonal difference in precipitation (Supplementary Material), discharge from the nearby Moshassuck River was significantly higher in the winter and spring than the fall and summer (Supplementary Material; Fig. 1a). While runoff is often directly related to rainfall events (Jeong et al., 2020), evapotranspiration may vary seasonally and influence runoff. Runoff into Narragansett Bay likely followed the same seasonal pattern as river discharge and may have contributed to the seasonality of the three most abundant metals (Zn, Pb, Cr). Large rainfall events (outliers in Fig. 1a) may further explain the higher metal concentrations in coral tissue in fall than summer despite both seasons having similar discharge volumes. Seasonal range in heavy metals in *A. poculata* was large, emphasizing the importance of this subannual analysis for other coastal coral species.

Estimated BSAF values were highest for Zn, As, and Cd ranging from 2.14–3.24 during the fall and winter, while As and Zn were high in the spring and As and Cd were high in the summer (Supplementary

<sup>&</sup>lt;sup>b</sup> Range reported for three locations.

<sup>&</sup>lt;sup>c</sup> Mean values approximated from Fig. 2, range reported for five locations.

<sup>&</sup>lt;sup>d</sup> Mean values reported for method b (ICPMS), range reported for two locations.

 $<sup>^{\</sup>rm e}$  Mean values reported for extraction method A (water-pik) were converted from nM/g to  $\mu$ g/g; range reported for two locations.

<sup>&</sup>lt;sup>f</sup> Mean values approximated from Fig. 2.

<sup>&</sup>lt;sup>g</sup> Range reported for two locations.

h Range reported for three locations.

Material). Therefore, these metals are being bioaccumulated by *A. poculata* at concentrations higher than expected given concentrations found in suspended sediment. Both Cr and Pb had consistently lower BSAF values throughout the year, despite relatively high concentrations in the suspended sediment, indicating minimal bioaccumulation. Interestingly, BSAF values were lowest for all metals in summer when suspended sediment samples were collected, suggesting that there may also be seasonality in metal concentrations in the suspended sediment; thus, requiring further investigation.

Compared to other common benthic species from Narragansett Bay (Table 1a), the tissues of A. poculata incorporated at least three orders of magnitude more Pb, As, and Cd (Chen et al., 2016). Similarly, there was an order of magnitude more Zn and Pb in A. poculata than marsh plants (Rudin et al., 2017). The mixotrophic status of A. poculata (Trumbauer et al., 2021) may be partly responsible for the comparatively high levels of bioaccumulation. There was no effect of Chl a or c2 on the concentration of any metal in any season (Supplementary Material), indicating that bioaccumulation of heavy metals in A. poculata was not dependent upon autotrophy alone. Zn, Pb, Cd, and Cr were present in both dissolved and particulate forms throughout Narragansett Bay (Nixon and Fulweiler, 2012; Wells et al., 1998, 2000; Table 1b). Pb, Cr, As, and Cd were present in benthic surface sediments (Chen et al., 2016; Nixon and Fulweiler, 2012; Santschi et al., 1984; Table 1c), some that exceed probable effect levels (Macdonald et al., 1996). These may be incorporated by A. poculata when sediment is disturbed or resuspended. In south Narragansett Bay, more sediment was resuspended during fall and winter than at other times of the year (Oviatt and Nixon, 1975). This aligns with seasonal highs we observed in heavy metals in A. poculata tissue during those seasons (Fig. 1).

Since the 1860's historic coal mining and ore refining in the region introduced heavy metals into Narragansett Bay through at least the late 1970's (Bricker, 1993; Santschi et al., 1984). Zn and Pb likely peaked between 1950 and 1970 and have since been decreasing (Table 1d–e), however disturbance of older sediments containing legacy pollutants could contribute to enhanced heavy metal uptake by *A. poculata*. A military installation has been on the site of Fort Wetherill State Park since the 1800's, with fortifications remaining today, and may be a source of localized contamination. Former military activity and installations result in elevated heavy metal concentrations (especially Zn, Pb, Cu, Hg, and Cr) in local environments (reviewed in Beck et al., 2018; Broomandi et al., 2020) that bioaccumulate in nearshore organisms (Díaz et al., 2018).

High levels of environmental pollutants are expected at our collecting site because of the relatively high concentrations of heavy metals in A. poculata tissue compared to coral species from tropical locations (Table 2). Concentrations were most similar to corals inhabiting anthropogenically polluted locations (Esslemont, 2000; Howard and Brown, 1987; Ranjbar Jafarabadi et al., 2018). Not all sites with known polluted environments reported high heavy metal concentrations in corals (Chan et al., 2014; Pait et al., 2009; Whitall et al., 2014), suggesting that some corals may not readily bioaccumulate heavy metals. Concentrations of Pb and Zn in A. poculata tissue were the highest (26.7  $\mu g/g \pm 21.0$ ) and third highest (357.0  $\mu g/g \pm 227.1$ ) means reported, respectively, for tissue from any coral species to date (Table 2). Overall, our data indicate that this population of A. poculata is exposed to and/or is bioaccumulating higher concentrations of heavy metals compared to most other coral species. While A. poculata is considered a "hardy" coral species because of its cold tolerance, here we show that A. poculata may also have a high tolerance to heavy metals. More investigation on the impact, if any, of heavy metals on the Fort Wetherill coral population is warranted.

#### CRediT authorship contribution statement

**Wolfgang Trumbauer:** Conceptualization, Writing – original draft, Formal analysis. **Sean Grace:** Conceptualization, Field work, Writing –

review & editing. **Lisa Rodrigues**: Conceptualization, Resources, Writing – review & editing, Visualization, Supervision.

#### **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.marpolbul.2021.113180.

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