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Accumulation and effects of microplastic fibers in American lobster larvae (*Homarus americanus*)

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ABSTRACT

The effects of microplastic fibers (MPF) on the survival, molting and oxygen consumption rates of larval (I-III) and post-larval (IV) stages of the American lobster, *Homarus americanus*, were quantified as a function of MPF concentration and food availability. Only the highest MPF concentration decreased early larval survival. MPF did not affect the timing or rate of molting across MPF treatments. While all larval and post-larval stages accumulated MPF under the cephalothorax carapace, stage II larvae and stage IV post-larvae showed the highest and lowest accumulation, respectively. MPF ingestion increased with larval stage and with MPF concentration; under starvation conditions, stage I larvae only ingested them at low MPF concentrations. Oxygen consumption rates were lower only in later larval stages when exposed to high MPF concentrations. Combined, our results indicate that MPF interactions and effects on American lobster larvae are dependent on larval stage, MPF concentration, and presence of food.

1. Introduction

The world's oceans are littered with large plastic debris. The discovery of staggering amounts of plastics collecting in the gyres of the North Pacific and Atlantic Oceans as well as along our coasts focused growing attention on determining the fate of these plastics as they break into smaller pieces. Microplastics (smaller than 0.5 mm) are likely the most numerically abundant plastic debris in the ocean today (GESAMP, 2016). Microplastics are known to accumulate in the coastal pelagic zones of the Atlantic Ocean. Indeed, seawater from the Gulf of Maine, among other regions, contained microplastics in 61% of the samples, and microplastic fibers (MPF) were the dominant form (91%) in all samples (Barrows et al., 2018). MPF are perhaps the most abundantly manufactured nanomaterial (by)-product (Hartline et al., 2016). Fibers were originally identified in sediment (Browne et al., 2010; Mathalon and Hill, 2014) and air samples (Dris et al., 2016) in the North Atlantic Ocean, but are now reported in seawater globally, thought largely to be introduced through garment washing cycles, degraded fishing gear, and sewage treatment systems (Hartline et al., 2016).

Most microplastics result from the fragmentation and degradation of larger pieces of plastic (Brandon et al., 2016); they eventually break down in size into nanoplastics (smaller than 0.1 mm), entering firmly into the size range of zoo-, phyto- and bacterio-plankton. These microscopic animals, algae and bacteria are key food items for larval fish and crustaceans living in the upper water column. Ingestion of plastic fragments can affect all levels of the marine food chain, from microalgae to zooplankton (including larval lobster and fish) and filter feeders (such as clams, mussels and oysters) (GESAMP, 2016; SAPEA, 2019). Microplastics are similar in size to planktonic food items such that animals cannot discriminate between nutritious food and anthropogenic debris (Moore et al., 2001); especially if microplastic particles are much less abundant than the planktonic prey items. In addition, when plastics aggregate into larger particles, larger animals might feed on them directly, mistaking them for prey or selectively feeding on microplastics in place of food (Moore, 2008). Microplastics have been found in the stomachs and intestines of almost all marine organisms, including fish, shrimp, langoustines, crabs, mussels, oysters and other invertebrates (e.g., Thompson et al., 2004; Devriese et al., 2015; Sussarellu et al., 2016; Welden and Cowie, 2016a), as individual

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particles or MPF strands and balls (e.g., Murray and Cowie, 2011; Wójcik-Fudalewska et al., 2016). Most studies report the presence of microplastics in various invertebrates, but rarely their physiological and/or ecological effect(s). Ingested microplastics can have adverse consequences by disrupting feeding, digestion and, ultimately, growth of individual marine organisms in laboratory experiments (GESAMP, 2016). Watts et al. (2014) showed that shore crabs (*Carcinus maenas*) will not only ingest microplastics along with food (evidence in the foregut), but will bring plastics into the gill cavity where their ventilation mechanism is located. This has also been shown for mussels and oysters (Tibbetts, 2015 and refs. therein). More recently, some animals have been shown to dispose of microplastics by behavioral and/or physiological adaptations used to remove unwanted particles, such as through feaces, pseudofaeces, regurgitation, and ecdysis (Saborowski et al., 2019; Woods et al., 2018), though closely related species often appear incapable of doing so (e.g., Welden and Cowie, 2016b; Gray and Weinstein, 2017).

Newly hatched larvae of the American lobster are found near the surface in the upper epipelagic layer at night (Harding et al., 1987), where MPF concentrations can be several orders of magnitude greater than the average water-column concentrations (GESAMP, 2016). In this study, we investigate the effects of increasing MPF concentrations on the survival, molting, ingestion and oxygen consumption rates as proxies for individual physiological performance at all larval stages of the American lobster, *Homarus americanus*. The American lobster is a species of ecological, cultural and commercial relevance in coastal waters. It is the most valuable single-species fishery in the United States (US) where the state of Maine harvests 80% of total US landings (NOAA, 2017). Since other fisheries, such as groundfish, have been widely depleted, lobster comprises nearly three-quarters of Maine's fishery revenue (Maine Department of Marine Resources (DMR), 2017), and the state's coastal economy is perilously dependent on this single fishery (Steneck et al., 2011). The abundance and ubiquity of microplastic contaminants in marine systems could pose a serious threat to lobster ecology, health, and development.

2. Materials and methods

2.1. Sample collection and food supply

Midcoast Maine ovigerous female *Homarus americanus* (107–136 mm carapace length) were harvested by local fishermen and the Maine Department of Marine Resources in June 2019. Lobsters were held in hatchery tanks (2 m diameter by 0.75 m deep) at the University of Maine's Darling Marine Center seawater facility with continuously flowing, coarsely-filtered seawater (Waller et al., 2017). Hatchery tanks were examined every morning for fresh stage I larvae; these were immediately transported the short distance to Bigelow Laboratory for Ocean Sciences. At Bigelow, ~250 larvae were placed in bubbled 20 L rearing tanks filled with 0.2 μm filtered and UV-sterilized seawater (FSW). Larvae were fed daily with excess amounts (>20:1) of two-day old *Artemia salina*. Tanks were bubbled vigorously with ambient air to minimize cannibalism and ensure the availability of later stage larvae. Water changes occurred every two to three days and all tanks and experimental jars were kept in a temperature-controlled room at 16 °C in a 12:12 h light cycle (Waller et al., 2017). Three larval stages (I-III) and one post-larval stage (IV) were reared in the laboratory and used in the various experimental designs described below.

2.2. Microplastic fibers characterization and quality control

MPF were generated by shearing a neon pink polyethylene terephthalate (PET) fleece following the methods of Woods et al. (2018). The MPF averaged <0.5 mm in length (459 ± SE 2.25 μm; n = 6378) (Woods et al., 2018). This size distribution was selected to match the average MPF length from natural Gulf of Maine seawater samples near our sampling location (M.N. Woods, Shaw Institute, unpubl. data, 2014–2017). A MPF stock suspension was prepared using a 1:10 ratio of 10% Simple Green (SG; Simple Green® All-Purpose Cleaner, Concentrated – contains C9–11 ethoxylated alcohols as a surfactant) and FSW, coating the fibers in SG before adding FSW to reduce clumping of fibers. Concentrations were estimated via 10 mL subsamples under a compound microscope by inverting the jar slowly a minimum of four times to homogenize the solution (Woods et al., 2018). To prevent microplastic contamination from unintentional sources, all equipment, jars, and buckets were rinsed 3× with tap water and then 3× with ultra-pure Milli-Q water, and kept covered at all times, including during experiments (Phuong et al., 2017). In addition, all glass labware, white lab coats, and non-pink disposable gloves were used at all times. For all experiments, any fibers detected in our controls that matched our manufactured neon pink microfibers were averaged and subtracted from our results (Vandermeersch et al., 2015). The average MPF number found in the Gulf of Maine is 3–10 MPF L^{-1} (Barrows et al. 2017; 2018). MPF concentrations decrease exponentially with depth (Reisser et al., 2015; Kooi et al., 2016), suggesting that near-surface layer concentrations can be orders of magnitude higher than the average water column concentration. In this study, the experimental MPF concentrations were selected to simulate elevated MPF abundance found in the near-surface layer in Gulf of Maine coastal waters as well as point-source effluents or hotspots (GESAMP, 2016).

2.3. Laboratory exposure assays

2.3.1. Larval survival and developmental rates

Larval survival from stage I to stage II was tested by placing 10 fed stage I larvae in a single 4 L glass jar filled with 3 L of 0.2 μm UV-sterilized FSW. Each jar was vigorously bubbled to 1) minimize cannibalism and 2) to keep MPF in suspension. Jars were inoculated with MPF stock suspension at four MPF concentrations including a zero control (0, 1, 10 and 25 MPF mL^{-1}) with 4 replicate jars per treatment. Larvae were fed daily with 40 L^{-1} *A. salina* in each jar. Over the course of ten days, the number of live or dead larvae and the number of fresh molts in each jar were recorded daily. At the end of ten days, larvae were individually examined under a compound microscope to confirm their current larval stage and to count the number of MPF under their cephalothorax carapace or ingested in their foregut, which is visible through their cephalothorax carapace at these early larval stages. This experiment was repeated under starvation conditions (without food) at three MPF concentrations (0, 1, and 25 MPF mL^{-1}). Larval survival rates were estimated as the slope of a linear regression fit, between the inflexion point of the 25 MPF mL^{-1} treatment with respect to the control and day 10.

2.3.2. Ingestion and accumulation rates

A second type of experiment was conducted to assess MPF ingestion and/or accumulation under the cephalothorax carapace over time in the larval stages II and III and the post-larval stage IV; note that ingestion and accumulation in stage I larvae is described above. Single larvae were each placed in a glass jar with 300 mL of 0.2 μm UV-sterilized FSW; low bubbling was used to provide oxygen and maintain MPF in suspension. All larvae were fed *A. salina* in similar amounts as in the previous experiments (Section 2.3.1). Three MPF concentrations, including a zero control (0, 1, 25 MPF mL^{-1}), were tested with 10 to 20 replicates per concentration, depending on the availability of larvae at each stage. Each experiment lasted five days. Each larva was examined daily under a compound microscope for the number of MPF accumulated under the cephalothorax carapace and/or ingested in the foregut, with the exception of stage IV post-larvae. Over the 5 day-period, the highest MPF count for each larva at each time point was recorded; these values were then averaged for each treatment to obtain a mean maximum number count for stages II and III. At stage IV, the cephalothorax carapace became too dense and opaque to get accurate counts of MPF in

the foregut and under the cephalothorax carapace; thus, these daily counts are a minimum estimate. After five days, stage IV post-larvae were immediately placed in individual tin boats and dried (at 37.5 °C for 48 h). Afterwards, the larval foregut was dissected and removed from the rest of the body by pinning the tail down with curved tweezers in one hand and pulling or breaking off appendages and outer carapace with straight tweezers, in the other hand, until the foregut was free. The number of MPF inside the foregut were either counted immediately under the microscope, or placed in 2 mL of DI water in a 10 mL scintillation vial and vortexed to break apart larger clumps before enumeration. Ingestion and accumulation rates were estimated as the slope of a linear regression fit with respect to time elapsed for each experiment, as described above.

2.3.3. Oxygen consumption rates

Dissolved oxygen concentrations were measured with a Clark-type oxygen microelectrode (Unisense; Aarhus, Denmark). Oxygen consumption rates (OCR) were calculated from the change in oxygen concentration over the duration of the measurements, as fitted with a least-squares linear regression. Oxygen concentrations were measured in a 4.8 mL chamber submerged in a water bath (VWR Scientific model MV 7LR-2000) at 16 (±0.02) °C. The glass chamber was sealed with a ground glass stopper equipped with a 400 µm hole to accommodate the electrode. Measurements were made at 1 Hz for up to 2 h; the oxygen concentrations within the chambers never decreased by >50% below saturation (Waller et al., 2017). All OCRs were measured on individual larvae exposed to 25 MPF mL^{-1} for five days. Control OCR measurements were collected from unexposed larvae. The number of replicates for both controls and exposed larvae ranged from 6 to 12 due to complications with timing of molting and/or overall survival during experiments. An additional control (S-control) was run with non-MPF-exposed stage III larvae kept in the experimental containers for two weeks, molting into stage IV post-larvae, for which OCR and dry weights were also measured. Seawater control OCRs were also measured using water from larval containers to account for any microbial oxygen consumption. The mass-specific OCR was calculated from OCR corrected by the seawater control, divided by individual larval dry weight (Waller et al., 2017).

The linear response of each electrode was calibrated with 0.2-µm filtered seawater bubbled for a minimum of 1 h to set the 100% dissolved oxygen calibration point. The anoxic calibration point was determined by placing seawater into a silicone tube that was immersed in a solution of 0.1 M sodium ascorbate and 0.1 M sodium hydroxide for over 4 h. The 95% response time of the sensor was below 1 s (Fields et al., 2014).

2.4. Statistical analysis

Experimental survival rates were determined with least-square regression starting (slope ± standard error, SE) when survival deviated from the controls. A *t-test* was used to compare the experimental larval survival rates relative to controls and between specific larval stages (Sokal and Rohlf, 1995). MPF accumulation under the cephalothorax carapace and the ingestion of MPF, as a function of larval stage and MPF concentration, were analyzed with a two-way ANOVA with replication with a post hoc pairwise multiple comparison (Holm-Sidak method). The rates of MPF accumulation and ingestion as well as the oxygen consumption and weight change rates were determined with a least-square linear regression. Statistical analysis was done using JMP software (SAS Institute, Inc. V.14.3.0). Values were considered statistically different when $p < 0.05$.

3. Results

All larvae and post-larvae accumulated MPF under their cephalothorax carapace and/or ingested them (Fig. 1) to varying degrees as a function of larval stage and, when tested, food availability. The presence of MPF affected survival, molting, and oxygen consumption rates differently at each larval and post-larval stage.

3.1. Larval survival rates (stage I & II)

The highest MPF concentration of 25 MPF mL^{-1} significantly decreased larval survival ($-0.72 \pm 0.28\%\ d^{-1}$) with respect to the control treatment ($-0.35 \pm 0.12\%\ d^{-1}$; *t-test*, $p < 0.01$) (Table 1) over time starting on day 7, when food was present, for stage I to stage II

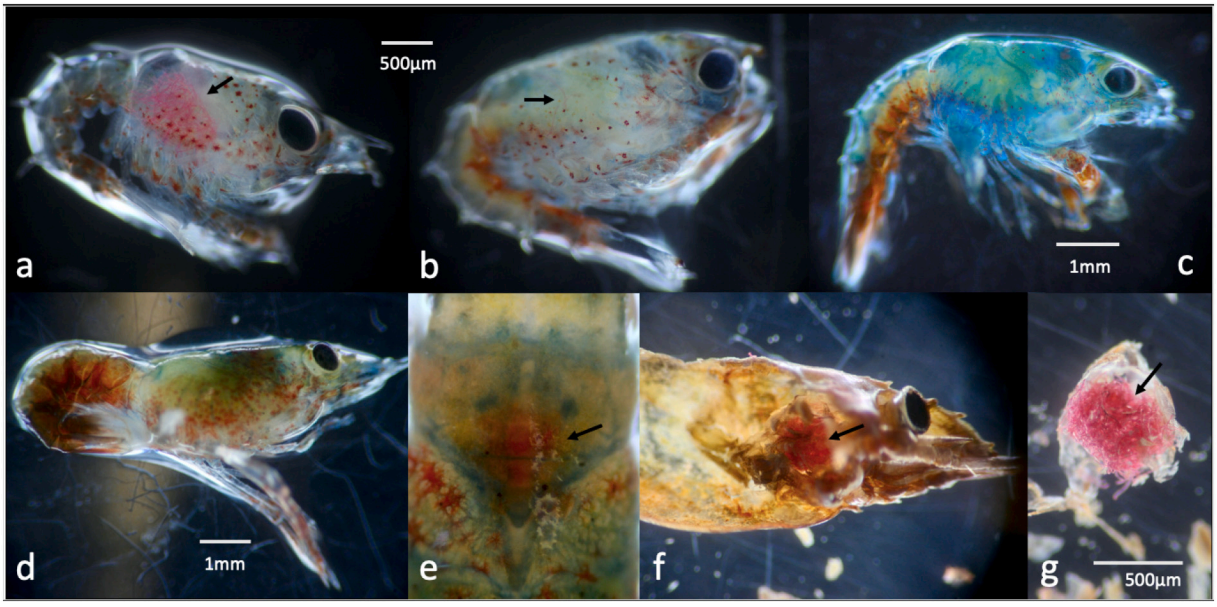

Fig. 1. Larval (a) stage I, (b) stage II, (c) stage III, and (d) post-larval stage IV. Black arrows show the location of pink MPF under the cephalothorax carapace: (a) a clump and (b) a few individual fibers in larval stages I and II, respectively, as well as (e) a clump in a stage IV post-larva foregut seen through the cephalothorax carapace (view from above), (f) a stage IV post-larva dried and partially dissected with a large clump seen in foregut, and (g) a dissected foregut of a dried stage IV post-larva filled with a large, tangled clump. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Survival and molting rates (standard error, SE) for stage I and into stage II lobster larvae with and without food. (−) no data.

Survival rate (% d^{-1})		
MPF mL^{-1}	+ Food	- Food
0	−0.35 (0.12)	−0.60 (0.11)
1	0 (0)	−0.22 (0.13)
10	−0.38 (0.11)	- (−)
25	−0.72 (0.28)	−0.90 (0.26)

Molt rate (% d^{-1})		
MPF mL^{-1}	+ Food	- Food
0	0.95 (0.39)	0 (0)
1	0.92 (0.37)	0 (0)
10	0.95 (0.21)	- (−)
25	1.38 (0.26)	0 (0)

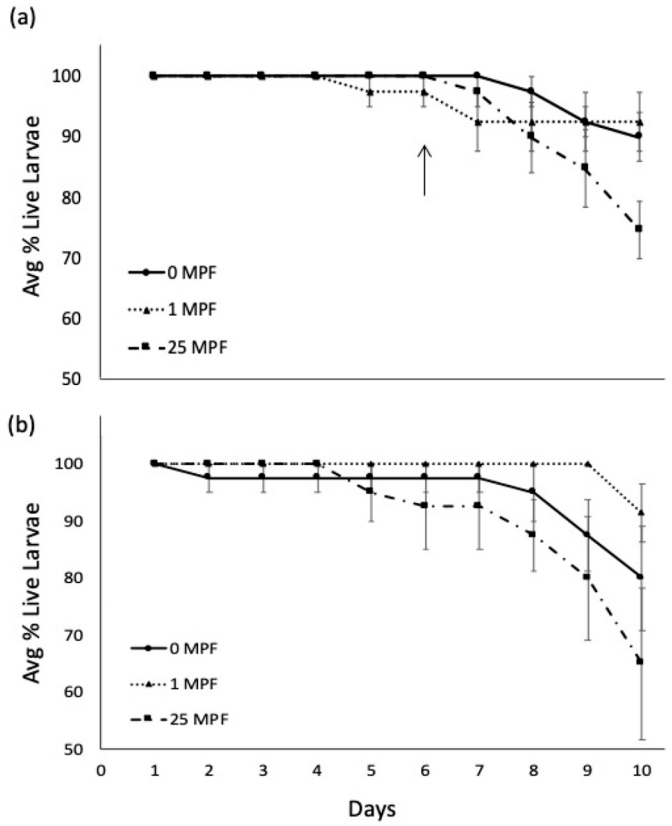

Fig. 2. Mean larval survival (mean ± standard error) (for stage I and into stage II larvae) over time at three MPF treatments (a) with and (b) without food (4 replicate jars per treatment with 10 larvae/jar). The arrow indicates the onset of molting from stage I to II for fed larvae; starved larvae did not molt.

larvae (Fig. 2a). Starved larvae exposed to 25 MPF mL^{-1} started dying two to three days earlier than fed larvae exposed to the same treatment (Fig. 2a,b), beginning on day four, and continued dying at a significantly faster rate (−0.9 ± 0.26% d^{-1}) than the fed larvae (*t-test*, $p < 0.05$) over the next six days. The survival rates of fed larvae exposed to 1 MPF mL^{-1} and 10 MPF mL^{-1} did not differ significantly from the controls (*t-test*, $p < 0.05$) (Table 1). Molting began on day 6 and lasted through day 10, regardless of MPF concentration; there was no significant difference in the timing or rate of molt for stage I to II larvae across MPF treatments (Table 1, *t-test*, $p > 0.05$);

Under starvation conditions (Fig. 2b), larval survival at increasing

Table 2
Mean maximum (Max) number count of MPF ingested and accumulated under cephalothorax carapace (and standard error, SE) at each larval stage for each MPF concentration tested. MPF counts were made after ten and five days for larval stage I and stage IV post-larvae, respectively, while daily counts were taken for stages II and III. Stage I larvae were observed with and without food while later stages were all fed. The daily rates of ingestion (MPF_{ing}) and accumulation (MPF_{accum}) at 25 MPF mL^{-1} are also shown for each larval stage. (−) no data.

		Stage I		Stage II	Stage III	Stage IV
		+Food	-Food	+Food		
MPF mL^{-1}		Max average (SE)				
Max MPF Ingested (# $larva^{-1}$)	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	1	0 (0)	0.11 (0.11)	0 (0)	0.33 (0.33)	8.11 (2.89)
	10	0 (0)	- (−)	- (−)	- (−)	- (−)
	25	1.86 (1.53)	0.20 (0.13)	1.38 (0.38)	2.33 (0.62)	119.71 (22.94)
MPF_{ing} mL^{-1} d^{-1}	25	0.19 (0.15)	0.02 (0.01)	0.39 (0.10)	0.89 (0.23)	23.94 (4.59)
Max MPF Accumulated (# $larva^{-1}$)	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	1	0 (0)	0.33 (0.33)	0 (0)	0 (0)	0 (0)
	10	1.40 (0.51)	- (−)	- (−)	- (−)	- (−)
	25	3.78 (1.04)	0 (0)	3.13 (1.08)	0.89 (0.54)	0.10 (0.10)
MPF_{accum} mL^{-1} d^{-1}	25	0.38 (0.10)	0 (0)	1.62 (0.60)	0.37 (0.27)	0.02 (0.02)

MPF exposure concentrations showed similar trends as when food was present; however, there was no significant difference among MPF treatments (*t-test*, $p < 0.05$). Larvae did not molt from stage I into stage II in the absence of food (Table 1).

3.2. Accumulation of MPF

3.2.1. Microplastic fiber accumulation under the cephalothorax carapace

MPF accumulated under the cephalothorax carapace of stage I larvae over the course of ten days when exposed to low MPF concentrations (1 MPF mL^{-1}) only under starvation conditions (0.33 ± SE 0.33 MPF $larva^{-1}$, Table 2). In the presence of food, MPF accumulation under the cephalothorax carapace in stage I larvae increased significantly with increasing MPF concentration (0.154 ± 0.004 MPF $larva^{-1}$ (MPF mL^{-1}) $^{-1}$, *t-test*, $p < 0.01$; Fig. 3a). On the other hand, MPF accumulated at high MPF concentrations (25 MPF mL^{-1}) only in the presence of food (e.g., 3.78 ± SE 1.04 MPF $larva^{-1}$ for stage I, Table 2).

MPF accumulation under the cephalothorax carapace also changed as a function of time in all larval stages, at both high and low MPF concentrations (Table 3). An example is shown in Fig. 3b for stage II larvae for which MPF accumulation over five days at 25 MPF mL^{-1} reached a peak at day two; no increase in MPF numbers over time was seen at 1 MPF mL^{-1} for this larval stage. The percent of larvae that accumulated MPF under the cephalothorax carapace changed among larval stages (Fig. 4a). Stage II had the highest number of larvae with MPF under the cephalothorax carapace at both 1 MPF mL^{-1} (21%) and 25 MPF mL^{-1} (80%) while stage IV post-larvae had the least (0% and 1%, respectively). The mean maximum number count of MPF accumulated under the cephalothorax carapace decreased significantly with stage (two-way ANOVA; $F_{3,84} = 2.98$, $p = 0.037$; Table S1a) and increased significantly with MPF concentration ($F_{2,84} = 17.48$, $p < 0.001$; Table S1a) with a significant interactive effect ($F_{6,84} = 3.57$, $p = 0.004$; Table S1a). The post-hoc test found no significant difference

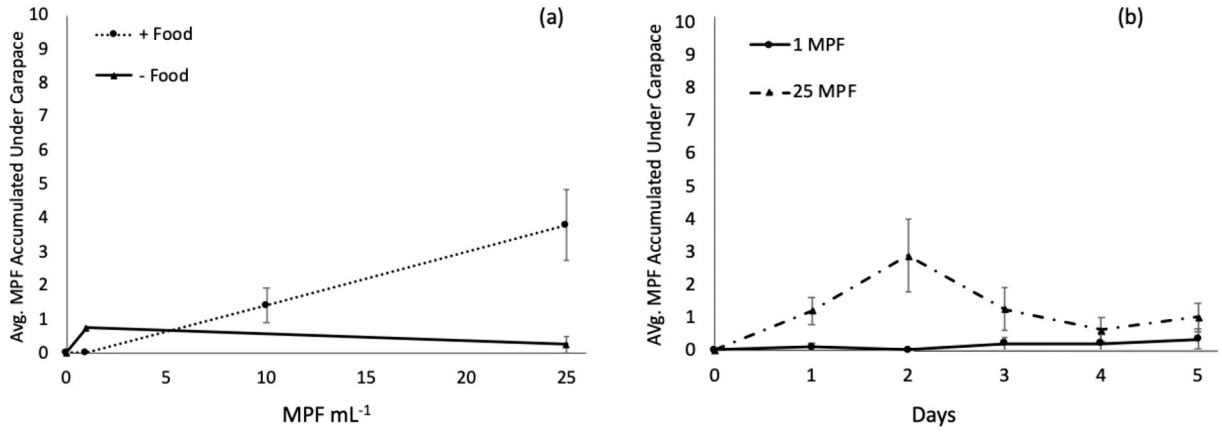

Fig. 3. MPF accumulated under the cephalothorax carapace (average ± SE) as a function of (a) MPF concentration at stage I with (0.154 ± 0.004 MPF larva^{-1}(MPF mL^{-1})$^{-1}$ and without food and (b) time at stage II, with food and at two MPF concentrations.

Table 3
Daily average (standard error) MPF ingested and accumulated under cephalothorax carapace per larva at stages II-IV exposed to 25 MPF mL^{-1}. Stage IV prior to day 5 are minimum estimates. (−) not detected.

Days	Ingestion (MPF larva^{-1})			Accumulation (MPF larva^{-1})		
	Larval stage			Larval stage		
	II	III	IV	II	III	IV
1	0.10 (0.18)	0.20 (0.13)	–	1.20 (0.42)	0 (0)	–
2	0 (0)	1.25 (0.49)	–	2.88 (1.11)	0.63 (0.63)	–
3	0.38 (0.30)	1.50 (0.73)	18 (−)	1.25 (0.65)	0.63 (0.37)	–
4	1.00 (0.76)	0.71 (0.28)	10 (10)	0.63 (0.38)	0 (0)	0 (0)
5	0.14 (0.24)	1.00 (0.63)	119.71 (22.94)	1.00 (0.44)	0.17 (0.17)	0 (0)

in maximum number of MPF under the cephalothorax carapace between concentrations of 0 and 1 MPF mL^{-1} for any of the stages (Table S1b). At MPF concentrations of 25 mL^{-1}, there was no significant difference in accumulation between stage I and stage II larvae or between stage III and stage IV post-larvae (Table S1b); however, stage I and stage II (~3–4 MPF/larva) did differ significantly from stage III and stage IV (~ ≤ 1 MPF/larva) (Fig. 5a).

3.2.2. Microplastic fiber ingestion

Ingestion of MPF by stage I larvae increased with increasing MPF concentrations in the presence of food (Fig. 3); indeed, stage I fed larvae ingested 10-fold more MPF per day than starved larvae (0.02 vs. 0.20 max MPF mL^{-1} d^{-1}, respectively) (Table 2). However, when exposed to low concentrations (1 MPF mL^{-1}) stage I larvae only ingested MPF under starvation conditions (Table 2). This pattern was similar to the exhibited MPF accumulation under the cephalothorax carapace described earlier. It should be noted that no MPF were observed on the *A. salina* prey. The number of ingested MPF increased significantly with stage (two-way ANOVA; $F_{3,78} = 26.34$, $p < 0.001$; Table S2a) and increased significantly with MPF concentration ($F_{2,78} = 24.59$, $p < 0.001$; Table S2a) with a significant interactive effect ($F_{6,78} = 22.26$ $p < 0.001$; Table S2a). (Fig. 5b). Stage III larvae (10% of all individuals) began ingesting MPF at the low MPF concentration (1 MPF mL^{-1}), whereas no larvae ingested MPF in the low MPF concentration at stage I and stage II (Fig. 4b). Similarly, the mean maximum count of ingested MPF increased significantly (*post-hoc-test*; Table S2b) from larval stage III (approx. 0.5–2 MPF larva^{-1}) to stage IV post-larvae (approx. 7–120 MPF larva^{-1}) at both MPF concentrations (Table 2), but was not different among larval stages I, II, and III (Fig. 5b). All exposed stage IV post-larvae ingested MPF (Fig. 4b). The number of ingested MPF also changed as a function of time over 5–10 days when exposed to 25 MPF mL^{-1} for stages II-IV (Table 3); MPF in the guts of stage I larvae were not counted until after 10 days (Table 2).

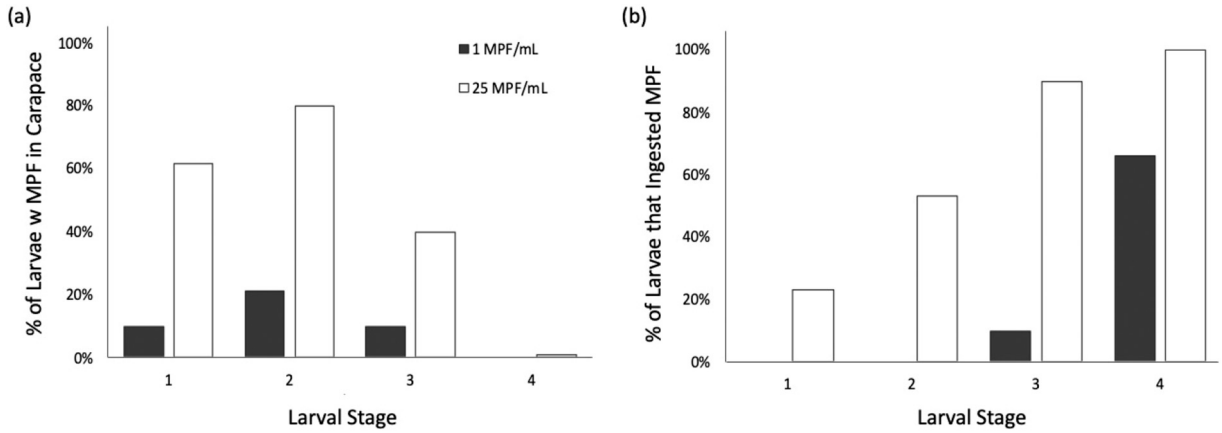

Fig. 4. Percent of larvae (stage I to IV) that (a) accumulated MPF under their cephalothorax carapace or (b) ingested MPF in the presence of food.

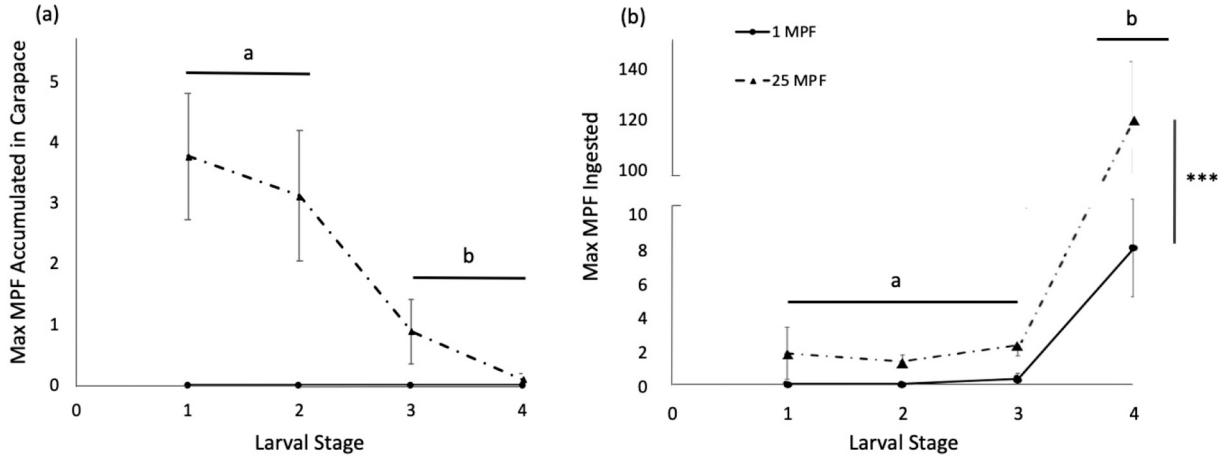

Fig. 5. Maximum average MPF per larva (average ± SE) (a) accumulated under the cephalothorax carapace and (b) ingested in the presence of food (stages I to IV; $n = 9$ to 19). Horizontal lines denote larval stages with no significant differences. Different letters above the line represent significant differences between stages ($p < 0.05$). Vertical line in panel (b) shows significantly higher ingestion rate in stage IV post-larvae at concentrations of 25 MPF mL^{-1} compared to 1 MPF mL^{-1}.

3.3. Oxygen consumption rates

For larval stage III and stage IV post-larvae, oxygen consumption rates (OCR) were significantly lower in exposed larvae (25 MPF mL^{-1}) (0.028 and 0.035 μmol O_2 mg^{-1} h^{-1}, respectively) than non-exposed control larvae (0.047 and 0.051 μmol O_2 mg^{-1} h^{-1}, respectively; *t-test*, $p < 0.05$) (Fig. 6a). OCR of stage I and II larvae were not significantly different between exposed and control larvae. Average dry weights were lower in exposed larvae of stages I and III compared to controls (Fig. 6b), but not different for larval stage II and stage IV post-larvae. As a secondary control, a separate set of stage IV post-larvae, kept in similar experimental container conditions (same glass jars for 14 days, but not exposed to MPF), were used as a system control (S-control). These S-control stage IV post-larvae had significantly lower average dry weight (*t-test*, $p < 0.01$) than both the MPF-exposed and the original control larvae (maintained in a rearing tank) (Fig. 6b). Average OCR of the S-control stage IV post-larvae was also lower than that of MPF-exposed and original control larvae after five days (Fig. 6a).

4. Discussion

Lobster larvae and post-larvae accumulated MPF throughout these early developmental stages (Fig. 1). Accumulation occurred under the cephalothorax carapace and within the animal's gastrointestinal tract, likely resulting in decreased survival and oxygen consumption rates. The survival rates of stage I and II larvae decreased as exposure time to the MPF increased (Fig. 2). These results suggest that the damaging effects of MPF may require multiple days of exposure (chronic), which would be the case in their natural environment, rather than short-term or acute exposure (<10 days). Early stage I larvae (< 6 days post hatching) experienced no measurable change in survival in the presence of MPF, with or without food (Table 2). However, at 7–10 days post hatching, the survival rate decreased in the presence of MPF, especially at 25 MPF mL^{-1}. Survival rate decreased further in the absence of food (Table 1) suggesting that even low MPF levels may augment the deleterious effects of other environmental stressors, such as low food conditions or high temperature (Lavalli and Factor, 1995; Niemisto, 2019). These results are similar to the findings for other small crustaceans (Cole et al., 2015; Lee et al., 2013) who observed higher mortality rates of marine copepods exposed to microplastic beads. The underlying mechanisms that caused the higher mortality rates in those studies are unknown.

The accumulation of MPF throughout the larval developmental stages is consistent with previous work reporting microbeads intake in many planktonic crustaceans (Setälä et al., 2014; de Sá et al., 2018; Watts et al., 2014). However, the amount, location and process of MPF accumulation are dependent on the stage of the lobster, the concentration of MPF and the presence of food (Figs. 3b, 4a, 5a). Accumulation occurred primarily under the cephalothorax carapace and within the animal's digestive tract. In larval stages I - III, we found high numbers of MPF accumulated under the cephalothorax carapace in the region of the developing gill folds (Fig. 1a). American lobster larvae

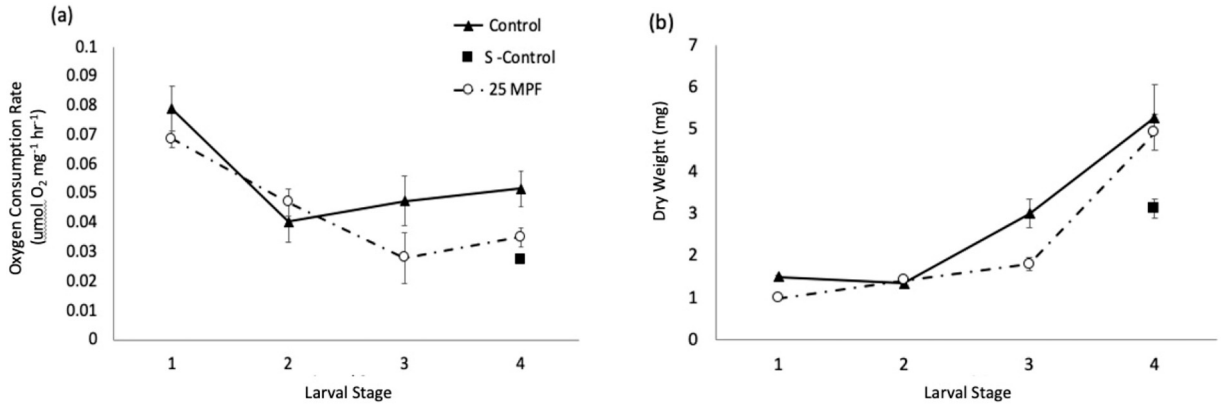

Fig. 6. (a) Oxygen consumption rate (OCR) and (b) dry weight of lobster larvae at stages I to IV, with MPF (25 MPF mL^{-1}) and without (control, S-control; see text for explanation). Mean (± SE) shown for treatments and controls.

ventilate their gills using their scaphognathite (Herrick, 1911). During ventilation, MPF and other debris in seawater pass through the inter-carapace space and aggregate around the gill structures. Accumulation was highest in stage I and II larvae, with MPF found in 60% and 80% of the animals, respectively (Fig. 4a), and increased as a function of increasing MPF concentration. This amount is likely to be a conservative estimate of the total number of animals that experienced MPF aggregates under the cephalothorax carapace. Our data show that the amount of MPF trapped under the cephalothorax carapace is dynamic and larvae are able to release trapped MPF, especially as larval development progresses (Table 3). The mechanisms, if any, for clearing foreign particles from the regions surrounding the gills are unknown for American lobster larval forms. This may occur through processes such as the passive or active use of setae and specialized appendages, changes in the ventilation patterns over the gill structures, or through molting. For example, some larval and small adult decapod crustacean species use a complex arrangement of setae for cleaning foreign particles from the gills, including marine shrimps, clawed lobsters and crayfish (Middlemiss et al., 2015). In juvenile European lobsters, such setae-based cleaning appears to rely on appendage locomotion, or water flow through the gill chamber (Middlemiss et al., 2015). Certain adult crabs can clear small accumulations of detritus in the region by the epipodite flagella of the maxillipeds, when reaching under the carapace to dislodge obstructions. Alternatively, heavier blockages can induce a flow reversal of the ventilation (Cumburlidge and Uglow, 1978). Here, the animal alters the flow rate and direction in an attempt to pass the objects. In particularly tenacious blockages, crabs can decrease and even stop ventilation of the gills until the animal can relocate into cleaner waters.

Modification of the flow over the gills may not be without consequence. For the larvae and post-larvae in this study, the accumulation of MPF under the cephalothorax carapace likely resulted in a significant reduction in the oxygen consumption rates (OCR), due to decreased flow rates and/or ventilation time during back flushing. Physiologically, we found larval stage III and stage IV post-larvae to have similar, significantly depressed OCR with respect to their controls (i.e., likely resulting from a treatment effect rather than a developmental difference) while no impact of MPF was observed on the weight-normalized OCR at high MPF concentrations for larval stages I and II (Fig. 6a). Early stage I and II larvae may lack the necessary strength to produce a strong enough gill-current to remove MPF as efficiently as larval stage III and stage IV post-larvae, which could explain the significant reduction in MPF under the cephalothorax carapace of the latter larval stages (Table 2, Fig. 5a). These results suggest that at low metabolic demand, respiration was not severely impaired for early lobster larval stages, even at high MPF concentrations. The decreasing levels of OCR in larval stage III and stage VI post-larvae after five days suggest a different mechanism than for the earlier larval stages. Stage I and II larvae are poor swimmers while stage II larvae are also reported to be less active than other stages (Factor, 1995; Herrick, 1911); therefore, it is plausible that their energetic demands are low enough that their oxygen consumption is not affected by the accumulation of MPF under their cephalothorax carapace. This lack of an effect on larval respiration disagrees with data for other adult crustaceans, such as the shore crab *Carcinus maenas*, which shows a longer microplastic (MP) external retention through inspiration across the gills than by ingestion of experimentally MP-contaminated food (Watts et al., 2014). The effect of plastic accumulation under the carapace for energetically demanding conditions (rapid swimming), such as by post-larval stage IV, is unknown.

Alternatively, molting may release the accumulated MPF when the exoskeleton softens and is then shed (Middlemiss et al., 2015; Welden and Cowie, 2016b). Middlemiss et al. (Middlemiss et al., 2015) noted that bacteria growing on gill setae were lost with every molt and especially during molting from megalopa larvae (stage IV) to juveniles (stage V) of the European lobster; their gill-cleaning structures being described as rudimentary in early larval stages. This morphological development suggests that the American lobster larval stages I and II (examined herein) may not have any gill setae, that setae may be in development, or that they may be lost due to high molt frequency, i.e., indicating less developed gills. Whereas food availability clearly had a very strong effect on molting (i.e., no food, no molt), increasing MPF concentrations accelerated the molt rate for fed stage I larvae only when exposed to the highest experimental MPF concentration (Table 1). If MPF loss were also due to molting in American lobster larvae as reported for its European counterpart, then we would expect the shortest larval stage (i.e., stage I) to accumulate the least amount of MPF. Instead, stage III larvae had the least MPF accumulation despite being the larval stage with the longest duration (Fig. 5a). Alternatively, Middlemiss et al. (Middlemiss et al., 2015) suggest the intriguing possibility of an interaction between bacterial proliferation in respiratory structures of later larval stages and molting, potentially negatively affecting respiration and thus body mass.

The transition from larval stage II to stage III shows a clear shift in the interaction between larvae and MPF. Whereas the presence of MPF resulted mostly in accumulation under the cephalothorax carapace of stage I and II larvae (Fig. 4a), stage III larvae begin to show MPF ingestion, even at near-ambient concentrations (Fig. 4b). Because the mean maximum number count of MPF ingested by stage III larvae differed significantly from stage I and II larvae at high MPF concentrations (25 MPF mL^{-1}) (Fig. 5a), increased swimming ability and purposeful MPF prey-type interactions may be important elements controlling MPF ingestion by later lobster larvae. This is clearly shown by the large increase in MPF ingestion by the stage IV post-larvae, both at near-ambient and high MPF concentrations; 8–100 fold higher in number and 20-fold higher per-day rate, respectively (Fig. 5b and Table 2), compared to the earlier larval stages. Indeed, stage IV post-larvae, having undergone a metamorphosis (Herrick, 1911), are significantly better swimmers and have voracious appetites, seeking out their prey rather than opportunistically bumping into it (Factor, 1995), which makes them active players in the ocean surface layer and in our experimental system. Better maneuverability might also facilitate intentional perceived-prey encounters, shown by their high MPF ingestion, both in mean maximum number counts (~7–120 MPF $larva^{-1}$, Fig. 5b) and uptake rate (up to 24 MPF mL^{-1} d^{-1}, Table 2), when compared to stage I and II larvae (2–100 fold lower).

Crustaceans are able to ingest plastic, either directly from the water or indirectly when feeding on prey (Allsopp et al., 2006; Von Moos et al., 2012). So far, over 250 taxa of marine fauna, including 32 marine invertebrate species, have been shown to accumulate microplastics from the water (e.g., by entanglement or ingestion; Laist, 1997; Goldstein and Goodwin, 2013; Hämer et al., 2014), including zooplankton (Cole et al., 2013, 2015; Setälä et al., 2014), filter-feeding mollusks (Woods et al., 2018) and scavenging decapod crustaceans (Farrell and Nelson, 2013; Watts et al., 2014). It is well known that lobster larvae are obligate carnivores and feed on other crustaceans, including crab larvae, copepods, barnacle nauplii and other lobster larvae (Harding et al., 1987; Juinio and Cobb, 1992), and possibly phytoplankton (Varma, 1977), while post-larvae are deemed omnivorous, opportunistic feeders (Factor, 1995). The transfer of microplastics, including MPF, vertically through the food web provides an additional mechanism for lobster larvae to accumulate plastics in their gut and potentially transfer them up the food chain to their own predators, such as juvenile fish, shrimp and krill.

Lobster larvae, like most zooplankton, are not homogeneously distributed within the water column. Stage I lobster larvae are most frequently caught above 10 m depth while stage II and III larvae are typically found throughout the upper 20 to 30 m of the ocean. In contrast, stage IV planktonic post-larvae are almost exclusively found at the surface in coastal regions (Harding et al., 1987) until they quickly settle to the benthos. Similarly, microplastics are unevenly distributed horizontally and vertically in the water column (Kane and Clare, 2019).

Microplastics concentration decreases exponentially with depth in certain regions (Enders et al., 2015; Reisser et al., 2015) with the highest concentrations at the surface; however, current sampling techniques that average over several meters of water depth may underestimate microplastics concentration by up to 3000% (Kooi et al., 2016). Combined, these findings suggest that lobster larvae may well experience much higher microplastics concentrations than current estimates would suggest; furthermore, stage IV planktonic post-larvae, in particular, may experience concentrations of MPF several orders of magnitude higher than the average ambient load. Based on the large increase in MPF ingestion by stage IV post-larvae in our study, higher microplastics loads in their natural preferred environment could pose a serious threat to their further development. In addition, when stage IV post-larvae settle to the bottom, they may again experience very high levels of MPF (Murray and Cowie, 2011).

While the rates and consequences of microplastic ingestion are being investigated, egestion (or depuration) rates are less commonly measured despite being equally important for individual organisms. Invertebrate organisms apply several approaches to get rid of MPF and other undesirable particles, in addition to molting or ecdysis discussed earlier (Middlemiss et al., 2015; Welden and Cowie, 2016b). They include regurgitation (Saborowski et al., 2019), pseudofaeces (Woods et al., 2018) and fecal pellets, though related species may show different depuration adaptations (Gray and Weinstein, 2017) or be unable to discard microplastics by adult individuals (GESAMP, 2016). Most published reports on microplastics ingestion, accumulation and depuration, as well as their physiological and ecological effects, have focused on adult individuals (e.g., Woods et al., 2018), rather than larval or juvenile stages, which are essential to ensure population recruitment and growth. The release and export of organic and/or organic-coated mineral or plastic particles are especially important for those organisms that recycle and feed on excretions in the water column and ocean floor, and for carbon storage. Given the ubiquity of microplastics in our marine environments, research should start considering population and ecosystem level effects, such as differential age/cohort survival causing demographic shifts, food/prey shifts, taxa-specific vulnerability, etc. Quantitative experiments are needed at concentrations as close to ambient levels as possible, as reported here, for both acute and chronic exposures in the water column (surface and mid-depths) as well as the ocean floor. This is a difficult task in any marine environment, most especially the deep-sea; regardless, it is still an important challenge to undertake.

Rising temperatures in the Gulf of Maine (Mills et al., 2013; NOAA, 2016) have driven the peak of the American lobster population northward by 2° latitude over the last 30 years (Pinsky et al., 2013), causing unparalleled lobster expansion in this region (Hare et al., 2016; Wahle et al., 2015). Sustained warming has not only changed lobster ecology but is also changing lobster physiology in many locations (e.g., timing and size of mature females; Le Bris et al., 2017; Waller et al., 2017), including the Gulf of Maine (Pugh et al., 2013). Despite recent record landings in Maine, the possibility of multiple stressor effects resulting from the combination of enhanced microplastics concentration, increasing temperature and ocean acidification (Salisbury and Jönsson, 2018) make the future of this lobster fishery far from certain.

CRediT authorship contribution statement

Madelyn N. Woods:Conceptualization, Methodology, Investigation, Data curation, Supervision, Writing - review & editing, Funding acquisition.**Theresa J. Hong**: Investigation.**Donaven Baughman**:Investigation.**Grace Andrews**: Investigation.**David M. Fields**:Conceptualization, Methodology, Investigation, Data curation, Supervision, Writing - review & editing, Funding acquisition.**Patricia A. Matrai**:Conceptualization, Methodology, Investigation, Data curation, Supervision, Writing - review & editing, Funding acquisition.

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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Research data for this article

The data presented herein are available from P.A. Matrai and D.M. Fields.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2020.111280.

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