Environmental Science & Technology

Article

pubs.acs.org/est

Ingestion of Plastic Microfibers by the Crab *Carcinus maenas* and Its Effect on Food Consumption and Energy Balance

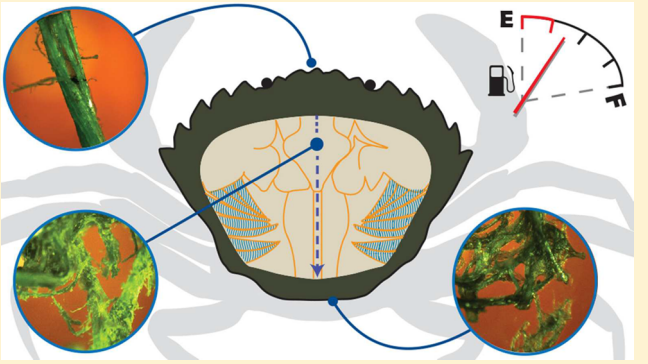
Andrew J. R. Watts,*[,†] Mauricio A. Urbina,[†,‡] Shauna Corr,[†] Ceri Lewis,[†] and Tamara S. Galloway[†]

[†]College of Life and Environmental Sciences: Biosciences, Geoffrey Pope Building, University of Exeter, Stocker Road, Exeter EX4 4QD, United Kingdom

[‡]Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción 4070386, Chile

Supporting Information

ABSTRACT: Microscopic plastic fragments (<5 mm) are a worldwide conservation issue, polluting both coastal and marine environments. Fibers are the most prominent plastic type reported in the guts of marine organisms, but their effects once ingested are unknown. This study investigated the fate of polypropylene rope microfibers (1−5 mm in length) ingested by the crab *Carcinus maenas* and the consequences for the crab's energy budget. In chronic 4 week feeding studies, crabs that ingested food containing microfibers (0.3−1.0% plastic by weight) showed reduced food consumption (from 0.33 to 0.03 g d^{-1}) and a significant reduction in energy available for growth (scope for growth) from 0.59 to −0.31 kJ crab d^{-1} in crabs fed with 1% plastic. The polypropylene microfibers were physically altered by their passage through the foregut and were excreted with a smaller overall size and length and amalgamated into distinctive balls. These results support of the emerging paradigm that a key biological impact of microplastic ingestion is a reduction in energy budgets for the affected marine biota. We also provide novel evidence of the biotransformations that can affect the plastics themselves following ingestion and excretion.



INTRODUCTION

Global plastic production currently exceeds 290 million tonnes annually.[1] Indiscriminate disposal has become a ubiquitous and deleterious problem for the marine environment, with particularly high densities of the pollutant occurring along coastlines and in mid-oceanic gyres.[2,3] Plastics enter the oceans through a variety of processes; terrestrial runoff accounts for 80% alone, with marine aquaculture and fishing estimated to contribute 18%.[4] Approximately 32.2 million tonnes of plastic is released into the marine environment each year,[5] equating with estimates of more than five trillion (5×10^{12}) pieces of plastic in surface waters of the world's oceans.[6] Microplastic (here defined to be particles of <5 mm in size[7]) is formed from the fragmentation of larger items or may be small items manufactured at this size range, such as scrubbers, beads, and nurdles.

Around 54% of manufactured plastics are of greater density than seawater.[8] Plastic debris is also highly susceptible to biofouling growth, sediment retention, and rapid establishment of bacterial colonies, which increase its density.[9,10] Consequently, many plastics sink to the bottom sediments, and it has been suggested that the overlaying 20 cm water layer above the benthos contains a greater abundance of plastic debris than the rest of the water column.[11] Fibers from fishing gear and from washing machine effluent[12] are a growing concern, as they have been reported in many marine samples. Pieces of rope, cord, and fishing line are frequently reported within the top few litter items discovered by beach cleaners.[13] Lusher et al.[14] reported that 95% of all plastic particles collected from surface water samples in the Northeast Atlantic Ocean were fibers. Fibers have also been reported in benthic samples from shorelines worldwide,[12] with concentrations of 31 fibers kg^{-1} in Cornish estuarine sandy sediments, UK,[15] 213 fibers kg^{-1} dry sediment taken from the island of Norderney, Germany,[16] and 200−800 fibers kg^{-1} dry sediment in Nova Scotia, Canada.[17]

Microplastics have been shown to be ingested by over 140 different species of marine animals in the wild,[18] either indiscriminately or by choice.[10,15,19] Microfibers in particular have been identified in the stomach and foregut of many marine organisms. For example, mussels collected from the Belgian coast were found to have between 2.6 and 5.1 fibers (ca. 200 μm length) per 10 g of mussel soft tissue.[20] The marine isopod *Idotea emarginata* has been shown to ingest beads and fragments as well as fibers, with no significant feeding preference shown between food with and without micro-

Received: August 19, 2015
Revised: October 30, 2015
Accepted: November 3, 2015
Published: November 3, 2015

DOI: 10.1021/acs.est.5b04026
Environ. Sci. Technol. 2015, 49, 14597−14604

plastics.[21] A study of the Norway lobster *Nephrops norvegicus* population inhabiting the Clyde Sea, UK, found that 83% of the animals sampled had plastic microfibers present in their guts. These consisted of both single fibers and balls of fibers knitted together through the churning action of the gut.[22] Therefore, it is clear that at particular locations, a high percentage of animals are ingesting plastic fibers at any one time.

The ingestion of microplastic by marine biota has the potential to cause deleterious effects to their health, through gut blockage, tissue damage, and false satiation.[23,24] Laboratory studies using manufactured microspheres have shown reduced energy uptake from the diet and, hence, reduced energy available for growth and/or reproduction as a result of microplastics ingestion in a few species. For example, the ingestion of natural assemblages of algae by planktonic copepods was significantly decreased when they were also exposed to increasing concentrations of 7.3 μm polystyrene microspheres.[25] In chronic feeding studies, the reproductive output of copepods fell, although there was no significant effect on egg production rates, respiration, or survival.[24] Marine worms (*Arenicola marina*) incubated for up to 1 month in sediments contaminated with polyvinyl chloride particles (up to 3% by weight and of a similar diameter to sediment) had significantly reduced lipid stores at the end of the experiment (4 weeks), which was attributed to a reduced food assimilation.[23] The shape and size of plastic particles has potential to influence their passage through the gut and therefore their biological effect; hence, it is important to study the effects of a range of different microplastics, including the most common form found: microplastic fibers.

The abundance, widespread distribution, and indiscriminate forage feeding of the common shore crab (*Carcinus maenas*) makes this organism potentially vulnerable to benthic microplastic contamination. These crabs inhabit estuarine ecosystems across the northern hemisphere and a wide range of coastal habitats, such as intertidal rocky shores, subtidal sediments, salt marshes, and sea grasses. Such environments are prone to high levels of plastic contamination from terrestrial run offs and/or from substantial commercial fishing grounds.[12,26−29] Our previous studies have shown that microplastics (in the form of 10 μm diameter polystyrene microspheres) were readily ingested by crabs, directly and through ingestion of contaminated prey.[30] Bioimaging of the foregut revealed particles retained for up to 2 weeks, adhered to the hair-like setae in the gut. This raises the possibility that fibers, the predominant microplastics type found in crustacean guts in field studies,[22] could be retained in a similar way, with the potential for deleterious effects.

This study investigated the fate of fibrous polypropylene rope microplastics (<5 mm in length) ingested by *C. maenas* and the consequences for the energy budget of the crab. Polypropylene rope was chosen because it is an abundant component of surveys of microplastics in coastal sediments and waters,[27] the effects of which on marine animals have not been evaluated to date.

Two main questions were posed: (1) What is the effect of plastic ingestion on food consumption and energy allocation for growth in the shore crab? (2) Is the size and structure of the plastic altered by the ingestion and egestion processes?

MATERIALS AND METHODS

Experimental Animals and Acclimation. Male crabs were collected from the River Exe estuary, Devon, UK (50°35.2′N, 3°23.59′W), in two batches: October 2013 and November 2014. Intermoult male crabs were used to reduce biological variability associated with reproductive status and moulting cycle. Animals were held communally in 20 L tanks at 15 ± 2 °C, artificial sea water (ASW), 33 PSU, under a light regime of 12:12 light:day.[30] Crabs were held in tanks for a week to acclimatize and empty their digestive system.

Preparation of Test Materials. A three-stranded blue polypropylene rope (University of Exeter stores, Exeter, UK) (diameter of 1 cm) was used to create 500 μm microfibers. These were prepared by cutting several sections from the original rope, snap freezing in liquid nitrogen and further grinding in 3 × 10 s bursts in a commercial coffee grinder. Polymer type was confirmed by Fourier transform infrared spectroscopy (FT-IR). Chemical analysis for contaminants (organochlorines and polychlorinated biphenyls) confirmed trace concentrations to be between 2 and 30 times below the FDA's food tolerance rates;[31] details of these results can be seen in Figure SI.1 of the Supporting Information (SI).

Physiological Effect of Plastic Ingestion: Experimental Design. Forty male crabs (carapace width, 46.05 ± 0.54 mm; weight, 28.34 ± 1.16 g; mean ± SD) were placed in individual tanks in 2 L of ASW (33 PSU, 15 °C, 12:12 light:dark). Feed (ca. 2 g; ∼7% body weight, to allow the crabs to feed ad libitum[32]) was supplied in the form of homogenized mussels mixed with gelatin as used in Watts et al.[30] For full details of the feed see Table SI.1 (SI). Crabs were randomly assigned into four experimental groups differing in the concentrations of plastic microfibers added to the feed [0% (0 mg), 0.3% (0.6 mg), 0.6% (1.2 mg), 1% (2.0 mg) added to 2 g of the feed]. These concentrations were used to reflect the potential concentrations of fibers crabs could be exposed to within their natural benthic environment.[15−17] Crabs were fed between 2 and 3 times a week for 1 month. Water was changed daily. Food consumption was measured at each feed with fecal pellets and samples of water (for ammonia excretion rate) collected for 48 h postfeed and O_2 measured at 48 and 72 h postfeed. Scope for growth of each crab was then determined each week, for 4 weeks.

Scope for Growth. Scope for growth is defined as the amount of energy which is left after all other metabolic costs are subtracted.[33−35] Positive values indicate excess energy to be used in growth and negative values indicate the depletion of internal energy stores. Scope for growth was calculated by the equation

$$\mathrm{SFG} = C - (F + R + U)$$

where SFG = scope for growth; C = energy consumed from food, F = energy excreted as fecal pellets; R = energy lost from respiration (oxygen consumption); U = energy lost from ammonia excretion.

Food Consumption (C). Food consumption was calculated as the difference in weight between the food offered and food left after ingestion (∼1 h). As the food was held together with gelatin, a conversion to allow for water absorption was determined by soaking various sizes of food pellets in tanks without crabs for 1 h. This resulted in the equation $y = 0.9734x - 0.1053$, where x = weight of food pellet with water absorbed and y = actual weight before soaking in water. Once weighed, the pellet was dissolved and passed through a Whatman No. 1 filter paper to recover the plastics. Plastic was rinsed and dried. The plastic microfibers left in the food pellet was therefore extracted from the final weight. Energy ingested was calculated

DOI: 10.1021/acs.est.5b04026
Environ. Sci. Technol. 2015, 49, 14597−14604

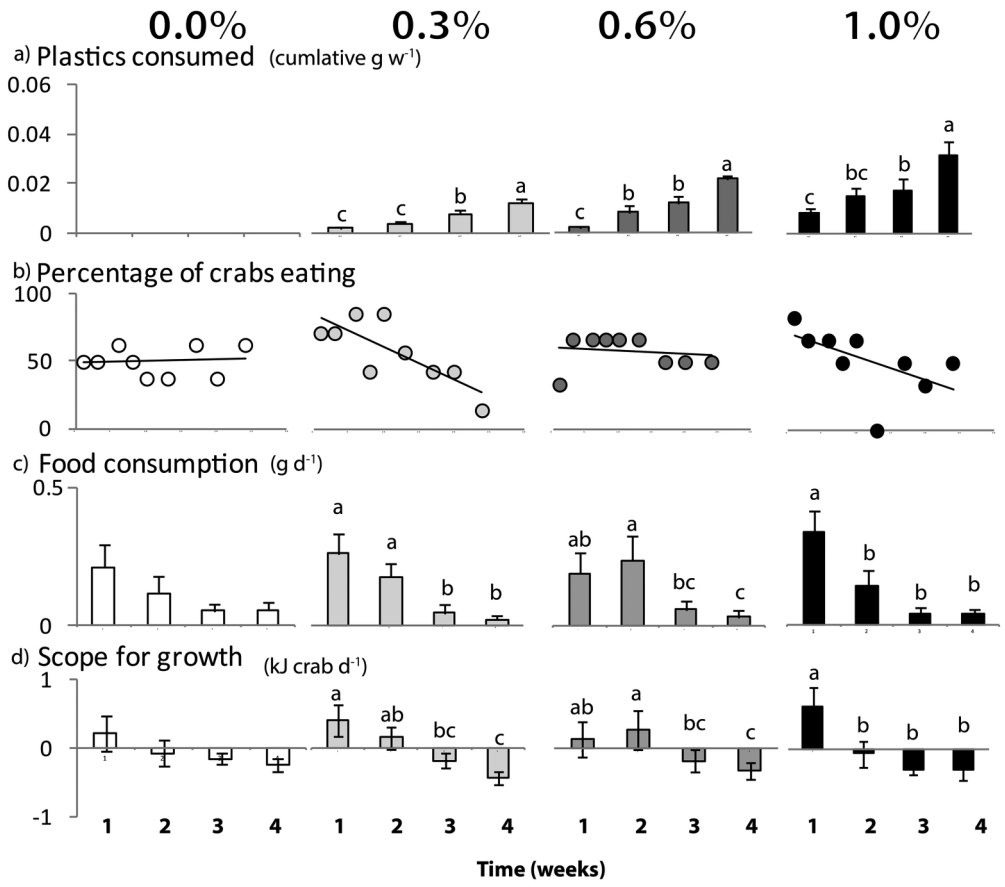


Figure 1. Effect of plastic within the foregut of *C. maenas* on (a) plastics consumed, (b) percentage of crabs eating, (c) food consumption, and (d) scope for growth. White circles and bars, crabs fed no plastic (0%); light gray circles and bars, crabs fed 0.3% plastic by weight of food; dark gray circles and bars, crabs fed 0.6% plastic by weight of food; black circles and bars, crabs fed 1.0% plastic by weight of food. Crabs were fed 2−3 times a week for 4 weeks. Scope for growth was calculated once a week for 4 weeks. Significance determined by repeated measure ANOVA, where means that do not share the same letter are significantly different (Fishers LSD $p < 0.05$).

by taking the average daily food consumption (minus the plastic) weekly and converting it to energy using the conversion 3.414 kJ g^{-1} food (see Table SI.1 of the SI for the calculation of this conversion rate).

Fecal Pellet Organics (F). Fecal pellets were collected daily with a plastic pipet, and feces were quickly but gently rinsed twice in distilled water (DI) to remove potential salts attached to the outside.[35] The organic content was determined gravimetrically, as the difference between the dry weight (dried for 48 h at 60 °C) and the organic-free dry weight of the feces. As polypropylene fibers can be combusted, the organic component of the feces was removed by a 3 day digestion period in 5% (w/v) bleach (sodium hypochlorite). Digested samples were spun down/rinsed with DI three times, dried, and weighed again. Energy excreted via the fecal pellets was estimated by taking the average daily excretion of organic matter in the fecal pellets and converting it to energy using the conversion factor 17.843 kJ g^{-1} pellet (based on the energy content of the food dry weight Table SI.1, SI).

Oxygen Consumption (R). Oxygen consumption in crabs was determined by assessing the reduction of oxygen in the exposure tank from an initial reading (0 h) and a final reading (1 h). Dissolved oxygen was assessed using a needle-type fiber optic sensor (Firesting OXR 230) connected to a FSO2-4 optical oxygen meter. To avoid compensatory responses associated with depleted dissolved oxygen levels, the chamber p_{O_2} values were always in excess of ∼120 mmHg (∼15.5 kPa). ΔO_2 was measured in mmHg and converted into mg of O_2 crab per day. Three chambers lacking crabs were used as controls to account for any potential air−water oxygen diffusion and bacterial influence; in all trials variation in these tanks was minimal (0.34 mmHg, approximately 0.06% of the oxygen consumption measured within the tanks containing crabs). Oxygen electrodes were calibrated daily with fully aerated water (100% oxygen saturation) and a saturated sodium sulfite solution (0% oxygen saturation).[36] Metabolized energy was determined via the conversion of oxygen consumption (mg O_2 crab d^{-1}) into the kJ equivalent via the conversion factor 0.01406 kJ mg^{-1}.[37]

Ammonia Excretion Rate (U). Ammonia excretion rate was determined by assessing the ammonia concentration in water samples (1.5 mL) taken at the beginning and then again at the end of a ∼17 h period. Three chambers lacking crabs were used as controls to account for any potential bacterial influence. Water−ammonia was determined using a colorimetric assay,[38] modified for a plate reader. The ammonia excretion rate (μmol NH_4^+ $crab^{-1}$ h^{-1}) was calculated as the difference in ammonia between the final and initial samples, multiplied by the water volume of the incubation container (L) divided by excretion time (h), following the method of ref 39. Energy excreted via ammonia (μmol NH_4^+ d^{-1}) was converted to energy equivalents via the conversion factor 0.0004477 kJ μmol^{-1}.[40]

DOI: 10.1021/acs.est.5b04026
Environ. Sci. Technol. 2015, 49, 14597−14604

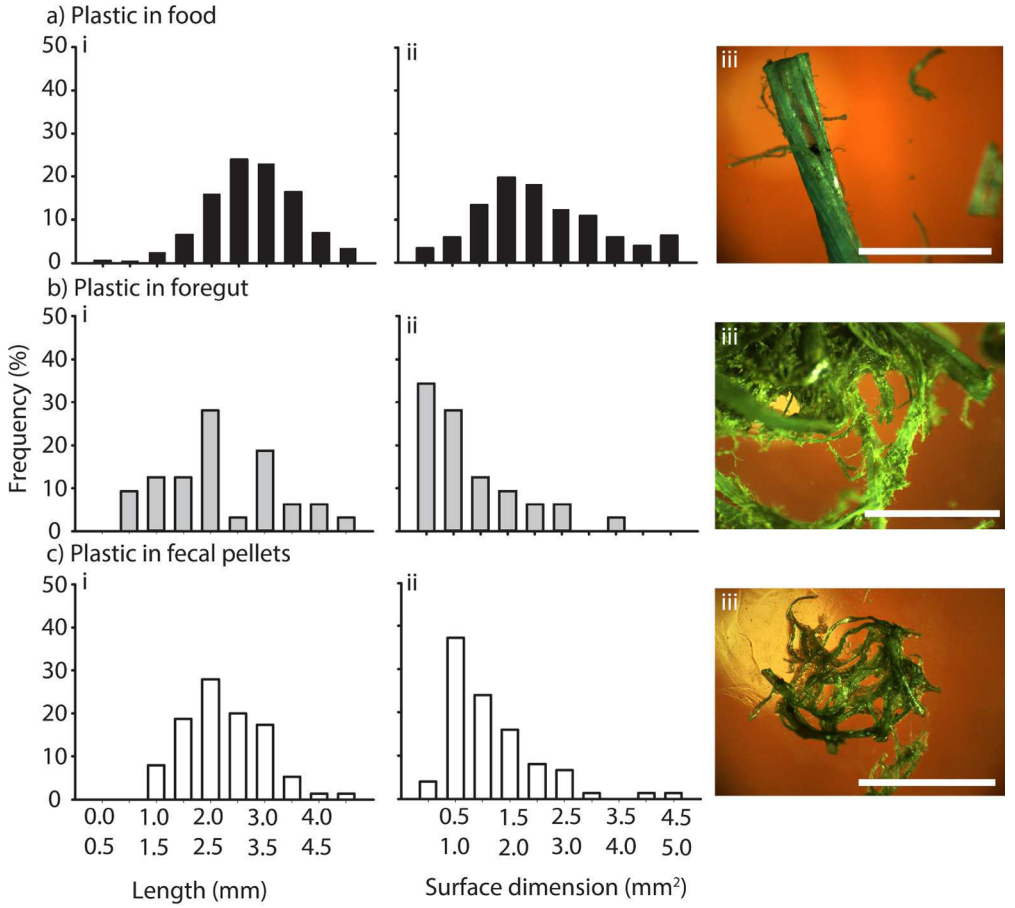


Figure 2. Mechanical breakdown of plastic in the digestive system of *C. maenas*. Plastic at different stages of digestion: (a) preingestion, i.e., in feed, (b) once it is in the foregut, and (c) when it comes out of the crab as a fecal pellet. (ai–ci) The distribution in the length of the plastic at each stage; (aii–cii) the 2D area of the plastic at each stage, and (aiii–ciii) images of how the plastic looks at each stage. White bar represents 2 mm.

Assessing Fiber Breakdown. Ten male intermoult crabs (54.79 ± 1.58 mm carapace width, mean ± SD) were held in similar conditions as above. Crabs where fed homogenized mussels embedded with ~5 mm length test microfibers, prepared as above. An additional 10 crabs were fed with homogenized mussel feed without microfibers. Crabs were fed ca. 2 g of homogenate on days 1, 3 and 5, with food consumption measured as above. Water was changed before and after each feed. After the feed on day 5 all crabs were placed on ice for 20 min and humanely dispatched. Foreguts were dissected and stored in 70% ethanol.

To determine the extent to which plastic fibers were broken down in the digestive system, test microfibers (n = 203 fibers) were measured for both length and surface dimension (2D area: X and Y axis) in a microscope at ×10 magnification. A picture was taken and images were analyzed using ImageJ (http://imagej.nih.gov/ij/). Measurements of fibers located in the foreguts (n = 32 fibers) and within the egested fecal pellets (n = 75 fibers) were also taken. No fibers were found in crabs just fed with homogenized mussels without microfibers. Fibers were grouped according to their length and surface dimensions at 0.5 mm and 0.5 mm^2 intervals, respectively, and the number of fibers in each size class was calculated as a function of all fibers measured in each group (%).

Statistics. Repeated measures ANOVA (Sigma plot) was used to determine if scope for growth or any of its components varied between weeks in the period of study (4 weeks) for each treatment separately. A repeated measures design taking measurements from each individual over four time points was used to account for individual variation in crabs feeding. Normality was assessed via a Kolmogorov–Smirnov normality test and an equal variance test (both built into the analysis). A LSD (least significant difference) Fisher post hoc test was then performed between weeks. Scope for growth values were normalized by transformation to positive values (x + 1). A Friedman repeated measures analysis of variance on ranks was used when normality was not met, followed by a Tukey post hoc test. In all tests $p < 0.05$ was considered as significant.

RESULTS

Food Consumption and Scope for Growth. The cumulative amount of plastics consumed by each of the treatment groups within the four week trial increased for each treatment, as shown in Figure 1a. By week 4, crabs in each group had consumed on average 13 ± 2 mg (0.3% group), 24 ± 2 mg (0.6% group), and 34 ± 6 mg (1.0% group). The proportion of crabs eating throughout the trial, decreased in the 0.3% and 1.0% groups over time; this was not observed in the 0.0% or 0.6% group (Figure 1b). In the control group, around 50% of the crabs were constantly feeding throughout the 4 weeks. The amount of food consumed significantly decreased over time in all crabs fed with plastic (0.3%, $F_{3,27}$ = 8.362, p = 0.001; 0.6%, $F_{3,27}$ = 3.750, p = 0.030; 1.0%, $F_{3,27}$ = 13.780, p < 0.001), while there was no significant drop in the food

DOI: 10.1021/acs.est.5b04026
Environ. Sci. Technol. 2015, 49, 14597–14604

consumption of crabs in the control group ($F_{3,31}$ = 2.923, p = 0.058) (Figure 1c). At week 1, crabs fed with 1.0% plastic consumed slightly (but not significantly more) than crabs in other groups. There was a slight reduction in the mean scope for growth in all treatments over time (Figure 1d), this was also seen in the control group but did not reach statistical significance ($F_{3,31}$ = 1.877, p = 0.164). Crabs that had 0.3% (13 mg total) plastic microfibers added to their food showed reduced scope for growth over 4 weeks ($F_{3,27}$ = 10.40, p < 0.001), with significantly lower values at week 3 (−0.175 ± 0.114 kJ crab d^{-1}) compared to week 1 (0.414 ± 0.223 kJ crab d^{-1}). Crabs that had 0.6% (24 mg total) plastic microfibers added to their food showed significantly reduced scope for growth over 4 weeks ($F_{3,15}$ = 3.40, p = 0.045) with measurements at week 3 (−0.175 ± 0.152 kJ crab d^{-1}) significantly lower than that at week 2 (0.272 ± 0.277 kJ crab d^{-1}). Crabs that had 1.0% (34 mg total) plastics added to their food showed significantly reduced scope for growth over 4 weeks ($F_{3,23}$ = 7.44, p = 0.003), with measurements made at week 2 (−0.070 ± 0.182 kJ crab d^{-1}) being significantly lower than those from week 1 (0.597 ± 0.280 kJ crab d^{-1}). The results of the constituent parts to the scope for growth are shown in Table SI.2 (SI).

Only six crabs of the 30 crabs fed with plastic microfibers had detectable microfibers remaining within their foreguts at the end of the trial (2 days since last feed); they had on average 13.96 ± 3.66% of the plastic ingested left in the foregut (two crabs in 0.3%, one in 0.6%, and three in the 1% treatment). There was no significant difference in the amount of food consumed ($F_{1,5}$ = 1.56, p = 0.280) or the scope for growth ($F_{1,5}$ = 0.12, p = 0.746) in crabs with or without plastic within the foregut.

Microfiber Breakdown. Polypropylene microfibers were broken down during passage through the gut (Figure 2). The size (two-dimensional surface area) of the plastic microfibers was reduced between the plastic present in (a) food (black bars); (b) foregut (gray bars); and (c) fecal pellet (white bars). The distribution of the lengths and surface dimension of plastic microfibers used in the experiment are shown in Figure 2a. The modal length category of the fibers reduced slightly between food (2.5−3.0 mm) and foregut (2.0−2.5 mm), but not between foregut and fecal pellets. There was a reduction in surface dimension between food (1.5−2.0 mm^2) and the foregut and fecal pellet (<1 mm^2). There were no plastic fibers found in the control crab treatment. Images of plastic encased in fecal pellets can also be seen in the Figure SI.2 (SI).

DISCUSSION

In this study, crabs were fed with differing amounts of polypropylene rope microfibers over a 4 week period. Those feeding on plastics had a statistically significant decrease in scope for growth, mainly driven by a reduction in food consumption over time. The polypropylene microfibers were significantly altered by their passage through the foregut, being excreted with a lower overall size and length than before ingestion.

We found a statistically significant switch from positive to negative scope for growth in all crabs exposed to plastics by the end of the 4 week exposure period. This was evident after 3 weeks for the 0.3 and 0.6% plastic treatments and after 2 weeks in the 1.0% plastic treatment. The difficulties in replicating conditions of natural habitats for foraging animals is evident in a nonsignificant trend toward a negative scope for growth in the control animals. However, the presence of plastics in the diet clearly produced a stronger effect on scope for growth than in the absence of plastic (shown in both F statistic and p values). This finding is relevant because a negative scope for growth values when fed with plastic-contaminated food indicates that the metabolic costs of oxygen consumption, fecal pellet production, and ammonia excretion outweigh the energy that is taken in by food ingestion. If a crab was chronically exposed to this condition over monthly time scales, it would have to utilize internal reserves to maintain itself. Energy reserves have been shown to decrease in a few marine species when plastic is consumed. The lugworm *Arenicola marina* showed depleted lipid energy reserves by up to 50% over 4 weeks when exposed to unplastisiced polyvinyl chloride (PVC), accompanied by a reduction in feeding and a longer gut residence time.[23] The marine copepod *Calanus helgolandicus* showed a 2-fold decrease in energy stores compared to controls when fed with 20 μm polystyrene microspheres at a concentration of 75 beads per mL. This was accompanied by a reduction in feeding rate (controls 16.0 ± 1.1 and plastic treatment 9.7 ± 1.3 μg carbon $copepod^{-1}$ d^{-1}) and a subsequent reduction in egg-hatching success.[24] The northern fulmar *Fulmarus glacialis* consumes large amounts of plastic, with 92.5% of birds showing evidence of plastic ingestion. This is believed to be a major cause of mortality in this bird, leading to gastrointestinal blockage, lacerations, and reduced feeding.[41] The chicks of *F. glacialis* are also susceptible, as adults regurgitate plastic along with food when feeding their young.[42]

In the current study, *C. maenas* reduced its food consumption when ingesting plastic-contaminated food, although the impact on energy balance was less pronounced than in the previous examples in other organisms. These differences can be explained by species-specific differences in metabolism. Crabs and other decapods are known to be able to survive long periods of time without food.[43−46] In fact, *C. maenas* can survive for over 2 months without food with little mortally and for 3 months with 50% mortality.[44]

The average food consumption was slightly, but not significantly, higher in the plastic-fed animals compared to the control during the first 2 weeks of the exposure. Individual crabs have highly variable feeding rates, so this may just be an artifact driven by this high variability. This may also be a real compensatory response to the presence of non-nutritious plastic content in the food causing the crabs to initially consume more. This could suggest that ingestion of plastic-contaminated food in *C. maenas* decreases the satiation signal, rather than increases it, as has been suggested in other organisms.[22−24] The further reduction in food consumption and scope for growth at week 3 and 4 could likely be a behavioral response to refrain from eating suboptimal food. Although this study did not investigate the food preference or decision making of *C. maenas* feeding on plastic-contaminated food, this could be an interesting avenue for future studies. Regarding the failure to feed seen toward the end of the study, there is evidence that shore crabs can choose to ingest more favorable food items over others. *C. maenas* display an optimal foraging strategy, trading off the time of handling the prey item with the net rate of energy intake gained from the food.[47] It may be that this is the case when an inert substance such as plastic is detected in the food, since the total calorific value of the food offered to the crabs was not altered across treatments. The presence of rigid fibers may have also proven unpalatable, leading to food avoidance.

DOI: 10.1021/acs.est.5b04026
Environ. Sci. Technol. 2015, 49, 14597−14604

Table 1. Comparison between Three Marine Invertebrate Species, the Planktonic Copepod *C. helgolandicus*, Marine Lugworm *A. marina*, and Common Shore Crab *C. maenas*, in Relation to Energetic Consequences of Feeding on Microplastic[a]

	C. helgolandicus	*A. marina*	*C. maenas*
individual weight (g)	4.522×10^{-4}[b,c]	15[c]	27[53]
food consumption (% body weight per day)	28–85[48]	0.099[54]	0.002 38[55]
growth rate (% body weight per day)		0.133[54] (0.66% food ration)	0.07[56] (1% food ration)
starvation tolerance (days)	3–21[57]		60–90[55]
oxygen consumption (mg O_2 g per day)	6.910[58]	1.129[58]	0.921[58]
effect of ingested plastic	2-fold reduction in energy stores, reduction in egg hatching success[e]	energy reserves depleted by up to 50% over 4 weeks[29]	slight but significant reduction in scope for growth[d]

[a]Comparisons of published values of normal food consumption, growth rate, starvation tolerance, and oxygen consumption are displayed. Superscript numbers refer to the references from which these values were derived (see also the other table footnotes). All studies were conducted at 15 °C. [b]Calculated by length weight ratio.[59] [c]Length determined via Figure 1ii of ref 25. [d]This study. [e]Converted from published values.

As long as the crabs can find uncontaminated food over monthly time scales, they could choose to avoid plastic-contaminated food. Other animals used in microplastic effect studies, however, are not able to modulate their food intake to that extreme. Planktonic copepods (*C. helgolandicus*) and marine worms (*A. marina*), for example, have higher metabolic requirements to maintain day to day function. *C. helgolandicus* requires daily food consumption between 28 and 85% body weight to maintain an oxygen consumption of 6.9 mg O_2 g^{-1} d^{-1}[48] (Table 1). *A. marina* has an oxygen consumption of 1.1 mg O_2 g^{-1} d^{-1}, where it requires a daily food consumption of 0.099% body weight (Table 1). This compares with *C. maenas*, which has an oxygen consumption of 0.9 mg O_2 g^{-1} d^{-1} and requires a food consumption of 0.00283% body weight to maintain itself (Table 1). Daily fluctuations in temperature, salinity, and aerial exposure mean that an adaptive physiology in *C. maenas* is vital for successful homeostatic control.[49] Scope for growth has been shown to be regulated in order to pay higher costs at low salinities in other crab species.[35]

It is important to consider the biology, physiology, and behavior of organisms when predicting the potential for harm of plastics in different species. Species with lower metabolic requirements could be less susceptible than species with higher energy demands. Support for this hypothesis comes from other studies in crabs[50] and fish[39] showing that a low metabolic rate is adaptive for surviving adverse environmental conditions.

Only 6 out of 30 crabs had plastics remaining in their foregut at the end of the trial, and many of these fibers were knotted together into balls.

Balling of plastic fibers was also reported in the lobster *N. norvegicus*.[22] It was suggested that the action of the gastric mill caused the knotting effect, leading to an increased overall size that could not pass into the midgut toward the hindgut. As only 6 of the 30 crabs had these balls of plastic after 4 weeks, this suggests the gastric mill action of the shore crab differs from that in the lobster and that these crabs are better able to excrete microfibers prior to the balling action of the gastric mill. In fact, feces with microfibers were abundant in all treatments fed with plastics.

The polypropylene microfibers were physically altered by their passage through the foregut, emerging with a smaller overall size and length. Due to the species' omnivorous diet, *C. maenas* are prone to the ingestion of nonfood items.[30] The foregut contains a complex gastric mill used for the grinding of carapace shells and animal and plant tissues.[51,52] Although the churning action of this mill has not evolved to break down synthetic flexible polymers, this mechanism combined with the churning mechanism of the cardiac stomach can evidently facilitate breakdown of polypropylene fibers, causing fractures within the filaments. We used fragmented rope fibers to mimic marine debris found in nature. Rope microfibers have been shown to be ingested by bivalve mussels, which are a major food item for shore crabs.[20] It is of interest that the microfibers were excreted encapsulated within fecal pellets and hence were coated in a biological matrix, thus potentially enhancing detection and consumption by other benthic scavengers/detritivors (Figure SI.2, SI).

In conclusion, these results illustrate the impacts of ingesting a diet containing polypropylene rope microfibers, an abundant component of marine debris, in shore crabs, a profuse and important species in northern hemisphere food webs. There was a reduction in the food consumption rates over time in crabs feeding on food containing plastic microfibers, leading to a small but significant reduction in the available energy for growth. This is, however, very unlikely to have any long lasting ecological consequences. The rope fibers were physically altered by their passage through the gut, with a reduction of overall size and a tendency to become balled. These results add to the growing body of evidence detailing the impacts of anthropogenic debris on marine life and highlight the novel effects that biological processes can have on the plastics themselves.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04026.

Figures SI.1 and SI.2 and Tables SI.1 and SI.2 (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +44 (0)1392724677; fax: +44 (0) 1392 263700; e-mail: a.watts.research@gmail.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

A.W., C.L., and T.G. acknowledge funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 308370. The contents of this publication are the responsibility of the CleanSea project and

DOI: 10.1021/acs.est.5b04026
Environ. Sci. Technol. 2015, 49, 14597−14604

can in no way be taken to reflect the views of the European Union. T.G. and C.L. acknowledge additional support from NERC NE/L007010/1. We thank Dorte Herzke from the Norwegian Institute of Air Research for the contaminants analysis.

REFERENCES

(1) *Plastics—The Facts 2013: An Analysis of European Plastics Production, Demand and Waste Data (16.02.14)*; PlasticsEurope: Brussels, Belgium, 2014; http://www.plasticseurope.org/.

(2) Gregory, M. R. Environmental implications of plastic debris in marine settings-entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philos. Trans. R. Soc., B* **2009**, *364* (1526), 2013−2025.

(3) Law, K. L.; Morét-Ferguson, S.; Maximenko, N. A.; Proskurowski, G.; Peacock, E. E.; Hafner, J.; Reddy, C. M. Plastic Accumulation in the North Atlantic Subtropical Gyre. *Science* **2010**, *329* (5996), 1185−1188.

(4) Andrady, A. L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62* (8), 1596−1605.

(5) Jambeck, J. R.; Geyer, R.; Wilcox, C.; Siegler, T. R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K. L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347* (6223), 768−771.

(6) Eriksen, M.; Lebreton, L. C. M.; Carson, H. S.; Thiel, M.; Moore, C. J.; Borerro, J. C.; Galgani, F.; Ryan, P. G.; Reisser, J. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS One* **2014**, *9* (12), e111913.

(7) Arthur, C.; Baker, J.; Bamford, H. In *Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris, NOAA Technical Memorandum NOS-OR&R-30*; NOAA Marine Debris Division: Silver Spring, MD, 2009; p 530.

(8) Stevenson, C. *Plastic Debris in the California Marine Ecosystem: A Summary of Current Research, Solution Strategies and Data Gaps. University of Southern California Sea Grant. Synthetic Report*; California Ocean Science Trust: Oakland, CA, 2011.

(9) Zettler, E. R.; Mincer, T. J.; Amaral-Zettler, L. A. Life in the "Plastisphere": Microbial Communities on Plastic Marine Debris. *Environ. Sci. Technol.* **2013**, *47* (13), 7137−7146.

(10) Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T. S. Microplastics as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* **2011**, *62* (12), 2588−2597.

(11) Lattin, G. L.; Moore, C. J.; Zellers, A. F.; Moore, S. L.; Weisberg, S. B. A comparison of neustonic plastic and zooplankton at different depths near the southern California shore. *Mar. Pollut. Bull.* **2004**, *49* (4), 291−294.

(12) Browne, M. A.; Crump, P.; Niven, S. J.; Teuten, E.; Tonkin, A.; Galloway, T.; Thompson, R. Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. *Environ. Sci. Technol.* **2011**, *45* (21), 9175−9179.

(13) Smith, S. D. A.; Gillies, C. L.; Shortland-Jones, H. Patterns of marine debris distribution on the beaches of Rottnest Island, Western Australia. *Mar. Pollut. Bull.* **2014**, *88* (1−2), 188−193.

(14) Lusher, A. L.; Burke, A.; O'Connor, I.; Officer, R. Microplastic pollution in the Northeast Atlantic Ocean: Validated and opportunistic sampling. *Mar. Pollut. Bull.* **2014**, *88* (1−2), 325−333.

(15) Thompson, R. C.; Olsen, Y.; Mitchell, R. P.; Davis, A.; Rowland, S. J.; John, A. W. G.; McGonigle, D.; Russell, A. E. Lost at sea: Where is all the plastic? *Science* **2004**, *304* (5672), 838−838.

(16) Dekiff, J. H.; Remy, D.; Klasmeier, J.; Fries, E. Occurrence and spatial distribution of microplastics in sediments from Norderney. *Environ. Pollut.* **2014**, *186* (0), 248−256.

(17) Mathalon, A.; Hill, P. Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Mar. Pollut. Bull.* **2014**, *81* (1), 69−79.

(18) Lusher, A. L. Microplastics in the marine environment: Distribution, interactions and effects. In *Marine Anthropogenic Litter*; Bergmann, M., Gutow, L., Klages, M., Eds.; Springer International Publishing: Charm, Switzerland, 2015; pp 245−307.

(19) Derraik, J. G. B. The pollution of the marine environment by plastic debris: a review. *Mar. Pollut. Bull.* **2002**, *44* (9), 842−852.

(20) De Witte, B.; Devriese, L.; Bekaert, K.; Hoffman, S.; Vandermeersch, G.; Cooreman, K.; Robbens, J. Quality assessment of the blue mussel (*Mytilus edulis*): Comparison between commercial and wild types. *Mar. Pollut. Bull.* **2014**, *85* (1), 146−155.

(21) Hämer, J.; Gutow, L.; Köhler, A.; Saborowski, R. Fate of Microplastics in the Marine Isopod *Idotea emarginata*. *Environ. Sci. Technol.* **2014**, *48* (22), 13451−13458.

(22) Murray, F.; Cowie, P. R. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). *Mar. Pollut. Bull.* **2011**, *62* (6), 1207−1217.

(23) Wright, S. L.; Rowe, D.; Thompson, R. C.; Galloway, T. S. Microplastic ingestion decreases energy reserves in marine worms. *Curr. Biol.* **2013**, *23* (23), R1031−R1033.

(24) Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Galloway, T. S. The Impact of Polystyrene Microplastics on Feeding, Function and Fecundity in the Marine Copepod Calanus helgolandicus. *Environ. Sci. Technol.* **2015**, *49* (2), 1130−1137.

(25) Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Goodhead, R.; Moger, J.; Galloway, T. S. Microplastic Ingestion by Zooplankton. *Environ. Sci. Technol.* **2013**, *47* (12), 6646−6655.

(26) Moore, C. J. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. *Environ. Res.* **2008**, *108* (2), 131−139.

(27) Hidalgo-Ruz, V.; Gutow, L.; Thompson, R. C.; Thiel, M. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. *Environ. Sci. Technol.* **2012**, *46* (6), 3060−3075.

(28) Van Cauwenberghe, L.; Janssen, C. R. Microplastics in bivalves cultured for human consumption. *Environ. Pollut.* **2014**, *193* (0), 65−70.

(29) Wright, S. L.; Thompson, R. C.; Galloway, T. S. The physical impacts of microplastics on marine organisms: A review. *Environ. Pollut.* **2013**, *178*, 483−492.

(30) Watts, A. J. R.; Lewis, C.; Goodhead, R. M.; Beckett, S. J.; Moger, J.; Tyler, C. R.; Galloway, T. S. Uptake and Retention of Microplastics by the Shore Crab *Carcinus maenas*. *Environ. Sci. Technol.* **2014**, *48* (15), 8823−8830.

(31) ATSDR. *Case Studies in Environmental Medicine Polychlorinated Biphenyls (PCBs) Toxicity*; U.S. Department of Health and Human Services: Washington, DC, 2014; http://www.atsdr.cdc.gov/csem/pcb/docs/pcb.pdf.

(32) Mente, E. Effect of ration level on individual food consumption, growth and protein synthesis in the shore crab *Carcinus maenas*. In *Nutrition, physiology, and metabolism of crustaceans*; Mente, E., Ed. Science Publishers: Enfield, NH, 2003; pp 53−67.

(33) Wiser, W. *Bioenergetik. Energietransformationen bei Organismen*, 1st ed.; Georg Thieme Verlag: Stuttgart, Germany, 1986.

(34) McGlaun, K. A.; Withers, K. Metabolism, Consumption Rates, and Scope for Growth of Porcelain Crab (*Petrolisthes galathinus*). *Gulf Mex. Sci.* **2012**, *30* (1−2), 1−6.

(35) Urbina, M.; Paschke, K.; Gebauer, P.; Chaparro, O. R. Physiological energetics of the estuarine crab Hemigrapsus crenulatus (Crustacea: Decapoda: Varunidae): responses to different salinity levels. *J. Mar. Biol. Assoc. U. K.* **2010**, *90* (02), 267−273.

(36) Urbina, M. A.; Paschke, K.; Gebauer, P.; Cumillaf, J. P.; Rosas, C. Physiological responses of the southern king crab, Lithodes santolla (Decapoda: Lithodidae), to aerial exposure. *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.* **2013**, *166* (4), 538−45.

(37) Gnaiger, E. Calculation of Energetic and Biochemical Equivalents of Respiratory Oxygen Consumption. In *Polarographic Oxygen Sensors*; Gnaiger, E., Forstner, H., Eds.; Springer: Berlin, 1983; pp 337−345.

(38) Verdouw, H.; Van Echteld, C. J. A.; Dekkers, E. M. J. Ammonia determination based on indophenol formation with sodium salicylate. *Water Res.* **1978**, *12* (6), 399−402.

DOI: 10.1021/acs.est.5b04026
Environ. Sci. Technol. 2015, 49, 14597−14604

(39) Urbina, M. A.; Walsh, P. J.; Hill, J. V.; Glover, C. N. Physiological and biochemical strategies for withstanding emersion in two galaxiid fishes. *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.* **2014**, *176*, 49−58.
(40) Elliott, J. M.; Davison, W. Energy equivalents of oxygen consumption in animal energetics. *Oecologia* **1975**, *19* (3), 195−201.
(41) Avery-Gomm, S.; O'Hara, P. D.; Kleine, L.; Bowes, V.; Wilson, L. K.; Barry, K. L. Northern fulmars as biological monitors of trends of plastic pollution in the eastern North Pacific. *Mar. Pollut. Bull.* **2012**, *64* (9), 1776−1781.
(42) Ryan, P. G. Effects of ingested plastic on seabird feeding: Evidence from chickens. *Mar. Pollut. Bull.* **1988**, *19* (3), 125−128.
(43) Uglow, R. F. Haemolymph protein concentrations in portunid crabs—II. The effects of imposed fasting on *Carcinus maenas*. *Comp. Biochem. Physiol.* **1969**, *31* (6), 959−967.
(44) Wallace, J. C. Feeding, starvation and metabolic rate in the shore crab *Carcinus maenas*. *Mar. Biol.* **1973**, *20* (4), 277−281.
(45) Parslow-Williams, P. J.; Atkinson, R. J. A.; Taylor, A. C. Nucleic acids as indicators of nutritional condition in the Norway lobster *Nephrops norvegicus*. *Mar. Ecol.: Prog. Ser.* **2001**, *211*, 235−243.
(46) Watts, A. J. R.; McGill, R. A. R.; Albalat, A.; Neil, D. M. Biophysical and biochemical changes occur in *Nephrops norvegicus* during starvation. *J. Exp. Mar. Biol. Ecol.* **2014**, *457* (0), 81−89.
(47) Ameyaw-Akumfi, C.; Hughes, R. N. Behaviour of *Carcinus maenas* feeding on large *Mytilus edulis* - How do they assess the optimal diet? *Mar. Ecol.: Prog. Ser.* **1987**, *38* (3), 213−216.
(48) Roman, M. R. Feeding of the copepod *Acartia tonsa* on the diatom *Nitzschia closterium* and brown algae (*Fucus vesiculosus*) detritus. *Mar. Biol.* **1977**, *42* (2), 149−155.
(49) Bamber, S. D.; Depledge, M. H. Responses of shore crabs to physiological challenges following exposure to selected environmental contaminants. *Aquat. Toxicol.* **1997**, *40* (1), 79−92.
(50) Leiva, F. P.; Urbina, M. A.; Cumillaf, J. P.; Gebauer, P.; Paschke, K. Physiological responses of the ghost shrimp Neotrypaea uncinata (Milne Edwards 1837) (Decapoda: Thalassinidea) to oxygen availability and recovery after severe environmental hypoxia. *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.* **2015**, *189*, 30−37.
(51) Ceccaldi, H. J. Anatomy and physiology of digestive tract of crustaceans decapods reared in aquaculture. *Adv. Trop. Aquacult.* **1989**, *9*, 243−259.
(52) Welden, N. A.; Cowie, P. R.; Taylor, A. C. Growth and gut morphology of the lobster *Nephrops norvegicus*. *J. Crustacean Biol.* **2015**, *35* (1), 20−25.
(53) Toulmond, A.; Tchernigovtzeff, C. Ventilation and respiratory gas exchanges of the lugworm *Arenicola marina* (L.) as functions of ambient PO2 (20−700 Torr). *Respir. Physiol.* **1984**, *57* (3), 349−363.
(54) Casado-Martinez, M. C.; Smith, B. D.; Luoma, S. N.; Rainbow, P. S. Bioaccumulation of arsenic from water and sediment by a deposit-feeding polychaete (*Arenicola marina*): A biodynamic modelling approach. *Aquat. Toxicol.* **2010**, *98* (1), 34−43.
(55) Mente, E. Effect of ration level on individual food consumption, growth and protein and synthesis in the shore crab *Carcinus maenas*. In *Nutrition, Physiology and Metabolism of Crustaceans*; Mente, E., Ed.; Science Pub Inc.: Enfield, NH, 2003; pp 53−66.
(56) Barnes, D. K. A.; Galgani, F.; Thompson, R. C.; Barlaz, M. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc., B* **2009**, *364* (1526), 1985−1998.
(57) Borchers, P.; Hutchings, L. Starvation tolerance, development time and egg production of *Calanoides carinatus* in the Southern Benguela Current. *J. Plankton Res.* **1986**, *8* (5), 855−874.
(58) Dagg, M. Some effects of patchy food environments on copepods. *Limnol. Oceanogr.* **1977**, *22* (1), 99−107.
(59) Williams, R.; Robins, D. B. Effects of preservation on wet weight, dry weight, nitrogen and carbon contents of *Calanus helgolandicus* (Crustacea: Copepoda). *Mar. Biol.* **1982**, *71* (3), 271−281.

DOI: 10.1021/acs.est.5b04026
Environ. Sci. Technol. 2015, 49, 14597−14604