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Ingestion of Microplastics by Zooplankton in the Northeast Pacific Ocean

Jean-Pierre W. Desforges[1] · Moira Galbraith[2] · Peter S. Ross[1]

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Abstract Microplastics are increasingly recognized as being widespread in the world's oceans, but relatively little is known about ingestion by marine biota. In light of the potential for microplastic fibers and fragments to be taken up by small marine organisms, we examined plastic ingestion by two foundation species near the base of North Pacific marine food webs, the calanoid copepod *Neocalanus cristatus* and the euphausiid *Euphausia pacifia*. We developed an acid digestion method to assess plastic ingestion by individual zooplankton and detected microplastics in both species. Encounter rates resulting from ingestion were 1 particle/every 34 copepods and 1/every 17 euphausiids (euphausiids > copepods; $p = 0.01$). Consistent with differences in the size selection of food between these two zooplankton species, the ingested particle size was greater in euphausiids (816 ± 108 μm) than in copepods (556 ± 149 μm) ($p = 0.014$). The contribution of ingested microplastic fibres to total plastic decreased with distance from shore in euphausiids ($r^2 = 70$, $p = 0.003$), corresponding to patterns in our previous observations of microplastics in seawater samples from the same locations. This first evidence of microplastic ingestion by marine zooplankton indicate that species at lower trophic levels of the marine food web are mistaking plastic for food, which raises fundamental questions about potential risks to higher trophic level species. One concern is risk to salmon: We estimate that consumption of microplastic-containing zooplankton will lead to the ingestion of 2–7 microplastic particles/day by individual juvenile salmon in coastal British Columbia, and ≤91 microplastic particles/day in returning adults.

Microplastics have become an emerging contaminant of concern due to their global abundance and widespread distribution. Microplastics are barely visible microlitter in the form of small fragments, fibres, and granules. These may be deliberately manufactured for application in cosmetics and air-blasting sectors or as virgin pellets for manufacturing; alternatively, they may originate from the breakdown of larger plastic items and debris (Andrady 2011; Barnes et al. 2009; Cole et al. 2011). It has become increasingly evident that the concentration of microplastics in the marine environment increases with decreasing particle size as a result of the progressive breakdown of debris (Andrady 2011; Cózar et al. 2014; Desforges et al. 2014).

Sewage effluent has been identified as a major source of microplastic fibres to the marine environment because it concentrates and delivers particles derived from washing clothes and textiles (Browne et al. 2011). In a study of beach shorelines from sites across six continents, Browne et al. (2011) found plastic abundance to be highest in more densely populated areas. Microplastics are also present in the open water of the world's oceans with major accumulation zones occurring where ocean currents converge into subtropical gyres (Maximenko et al. 2012). Cózar et al. (2014) recently estimated the global ocean load of plastics (the majority being microsized particles) to be on the scale

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✉ Peter S. Ross
peter.ross@vanaqua.org

[1] Ocean Pollution Research Program, Coastal Ocean Research Institute, Vancouver Aquarium, Vancouver, BC V6B 3X8, Canada

[2] Institute of Ocean Sciences, Fisheries and Oceans Canada, Sidney, BC V8L 4B2, Canada

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of tens of thousands of tonnes, which is orders of magnitude lower than expected based on plastic production and input rates. The investigators pointed to a few potential sinks for surface microplastics including shoreline deposition, nano-fragmentation, biofouling and sedimentation, and ingestion (Cózar et al. 2014).

The risks that microplastics pose to the health of marine biota are not clear, but controlled laboratory feeding studies and some studies in the natural environment indicate that a wide range of marine organisms have the capacity to ingest microplastic particles (Cole et al. 2013; Moore 2008; Thompson et al. 2004; Van Cauwenberghe and Janssen 2014). Indiscriminate feeders in the water column maybe at particular risk because they might mistake microplastics for natural food items of the same size. The primary impact related to microplastic ingestion is thought to be physical, whereby particles may entangle feeding appendages and/or block or abrade internal organs resulting in reduced feeding, poor condition, injury, and death (Cole et al. 2013).

Although less understood, another risk arising from the ingestion of plastic relates to its inherent chemical nature and the large surface area-to-volume ratio, which can cause microplastics to leach chemical additives and adsorbed pollutants after ingestion (Wright et al. 2013b). Although some studies report little or no physical or chemical harm to marine biota (Besseling et al. 2013; Barlow et al. 2008; Hamer et al. 2014; Kaposi et al. 2014; Koelmans et al. 2014), others report effects on fitness and reproduction (Besseling et al. 2014; Cole et al. 2013; Wright et al. 2013a) and on immune and endocrine parameters (Koehler et al. 2008; Rochman et al. 2014; von Moos et al. 2012). Uptake of contaminants attributed to leaching from plastics has been documented in some cases (Bakir et al. 2014; Browne et al. 2013; Chua et al. 2014; Tanaka et al. 2013).

Although most studies on microplastic ingestion by marine biota have been performed under carefully controlled conditions in the laboratory, some have examined microplastic ingestion in wild organisms. Few species of marine invertebrates have been found to ingest plastics in their natural environment; 83 % of the decapod crustacean *Nephrops norvegicus* sampled in the Clyde Sea (United Kingdom) accumulated microfilaments that are thought to have been derived from fishing gear (Murray and Cowie 2011). The bivalves *Mytilus edulis* and *Crassostrea gigas* from the German North Sea contained between 0.15 and 0.70 plastic fragments/g of soft tissue (Van Cauwenberghe and Janssen 2014). Wild and farmed *M. edulis* from Nova Scotia (Canada) ingested 116 and 178 microfiber particles/individual, respectively (Mathalon and Hill 2014).

Studies are increasingly documenting the ingestion of plastics by fish. Approximately 37 % of ten demersal and pelagic species examined from the English Channel had plastic particles in their gastrointestinal tract (Lusher et al. 2013). Microplastics were found in 2.6 % of samples and five of seven common fish species in the North Sea (Foekema et al. 2013). Three ontogenetic phases of three ecological important catfish species in a Brazilian estuary were found to have ingested plastics consisting mostly of nylon fibres (18–33 % of individuals) (Possatto et al. 2011). Several studies of the North Pacific Gyre report microplastic in <1–58 % of stomach samples from >27 species of fish (Boerger et al. 2010; Choy and Drazen 2013; Davison and Asch 2011). Last, microplastic fragments have been detected in the scat of fur seals from Macquarie Island likely reflecting food web transfer (Eriksson and Burton 2003). Food web transfer of plastics has also been described experimentally at the base of the food web (Farrell and Nelson 2013; Setälä et al. 2014) and has been suggested in situ in planktivorous/carnivorous fish and marine mammals (Eriksson and Burton 2003; reviewed in Wright et al. 2013b).

In light of the growing concerns about microplastic ingestion by aquatic biota, we examined microplastic ingestion in the Northeast Pacific Ocean using two ecologically important marine zooplankton species, *N. cristatus* and *E. pacifica.*

Materials and Methods

Zooplankton Sampling

Zooplankton samples were collected in August and September 2012 aboard the Canadian Coast Guard Ship (CCGS) John P. Tully during oceanographic cruise of the Line P and the La Perouse Monitoring Programs (Fisheries and Oceans Canada). The zooplankton were collected in vertical net tows from a depth of 250, or 10 m off the seafloor bottom, using Bongo nets (0.5-m mouth diameter, 2.5 m-long sock, and 236-µm mesh; fitted with a TSK flowmeter in the mouth opening on one side of the Bongo). Zooplankton were rinsed from the cod-ends into glass jars made up with 10 % buffered formalin in seawater. After routine counts for species identification and density, samples were archived for long-term storage.

Method Development

We used samples of the euphausiid *Thysanoessa spinifera* to develop a suitable digestion technique because of its large size and its high abundance in one sample. The goal of the digestion technique was to destroy biological materials and then examine remaining (more recalcitrant) materials for microplastic particles. Before digestion, *T. spinifera* samples were passed through a 500-µm sieve and

rinsed several times with deionized water. Each individual was examined under a dissection microscope (Zeiss stereoscope, Discovery V8; Carl Zeiss Canada) to determine whether any microplastics had adhered to the outside of their body. If any particles were found, they were removed with tweezers or a jet of deionized water. After scanning for external plastics, batches of 15 individuals were placed into 20-mL glass scintillation vials, which were then subjected to several test protocols with differing digestion liquids and conditions: 100 % hydrochloric acid (HCl 12.1 M), 1:1 v/v of HCl and nitric acid (HNO_3 15.9 M), 100 % HNO_3, 100 % hydrogen peroxide (H_2O_2, 0.9 M), and 1:1 v/v of HCl and H_2O_2. Each protocol was run in duplicate (i.e., 2 batches consisting of 15 individuals each) at room temperature as well as heated in a water bath to approximately 80 °C. Digestion was evaluated visually after 1 h and again after 3 h. After 3 h of digestion, samples were filtered through 0.45-µm mixed-cellulose ester filter papers (HAWP; Millipore), and the filter paper was examined under a dissection microscope for completeness of digestion as well as presence of microplastics.

Microplastic Analysis in Zooplankton

N. cristatus and *E. pacifica* were selected for this study for their large size (ease of handling), their importance in food webs of the Northeast Pacific Ocean, and because they are filter feeders that are potentially capable of ingesting microplastics (Fig. 2). Another criterion was that these two species are fairly abundant along the shelf and shelf break during summer sampling cruises and would be in the water column at the time of seawater sampling for microplastics.

Zooplankton were taken from archived samples (see previous text) to correspond with the location and timing of seawater samples taken in our previous study (Desforges et al. 2014). Water samples in Desforges et al. (2014) were collected using the saltwater intake of the vessel during routine stops for zooplankton and other oceanographic sampling. Although seawater samples were taken at a standard depth of 4.2 m below the surface, vertical tows of zooplankton were collected from the water column. Despite this difference, the geographical coordinates of the zooplankton tows and the seawater samples coincided precisely for most samples. In certain cases where sampling did not overlap, the zooplankton sample was matched with the nearest water sample using Global Positioning System coordinates. As in our previous study, zooplankton samples were selected to represent four major oceanographic areas of coastal British Columbia: the relatively industrialized Strait of Georgia ($n = 10$), west coast Vancouver Island ($n = 8$), northern Vancouver Island/Haida Gwaii ($n = 13$), and offshore Pacific ($n = 7$) (Fig. 1). The most northern zooplankton samples (Haida Gwaii, $n = 6$; Fig. 1) did not coincide with any water samples and thus were excluded from the comparative analyses with seawater microplastics.

Zooplankton samples from each site were passed through a 500-µm sieve, and individuals were removed with forceps and individually examined for externally adhered microplastics. "Clean" zooplankton individuals were collected and stored in 20-mL glass vials in deionized water until analysis. When possible, 50 individuals were isolated from each site.

The optimized digestion protocol developed using *T. spinifera* was adapted for analysis of single individuals for better statistical resolution. For *N. cristatus*, individuals were placed into single wells of glass-coated polypropylene 96-well plates (7-mm diameter, flat bottom; Thermo Scientific). Larger-sized *E. pacifica* were placed into single wells of a white porcelain spot plate (23-mm diameter, VWR). Nitric acid was added to each well to just cover each individual, and the plates were covered and heated to approximately 80 °C for approximately 30 min (i.e., until all tissue was digested). After digestion, plates were directly examined under the dissection microscope for the presence of microplastics. Because Fourier-transform infrared spectroscopy was not available, plastics were identified according to surface and internal morphological characteristics (e.g., lack of cell structure) as well physical response features (e.g., response to physical stress; microplastics were bendable or soft) as in Desforges et al. (2014). If microplastics were observed, the particles were counted, measured for length, and noted as to colour and shape (i.e., fibre or fragment). The examination of each plate was typically performed in <30 min. Although samples were covered during almost all handling, several blanks (HNO_3 in an empty well) were run on each well plate to correct for potential air-borne particle deposition in the laboratory; no contamination of blanks was observed during the experiments.

Data Analysis

Independent-sample *t* test was used when comparing two variables, such as differences between species or particle shape, whereas analysis of variance, followed by Tukey's Honestly Significant Difference test, was used to compare plastic characteristics between multiple sites. Correlation analysis was performed using linear and nonlinear regression models. All data analysis was performed using SPSS software (SPSS 16; IBM). Maps and contour plots were created using Ocean Data View 4 (available at: http://odv.awi.de).

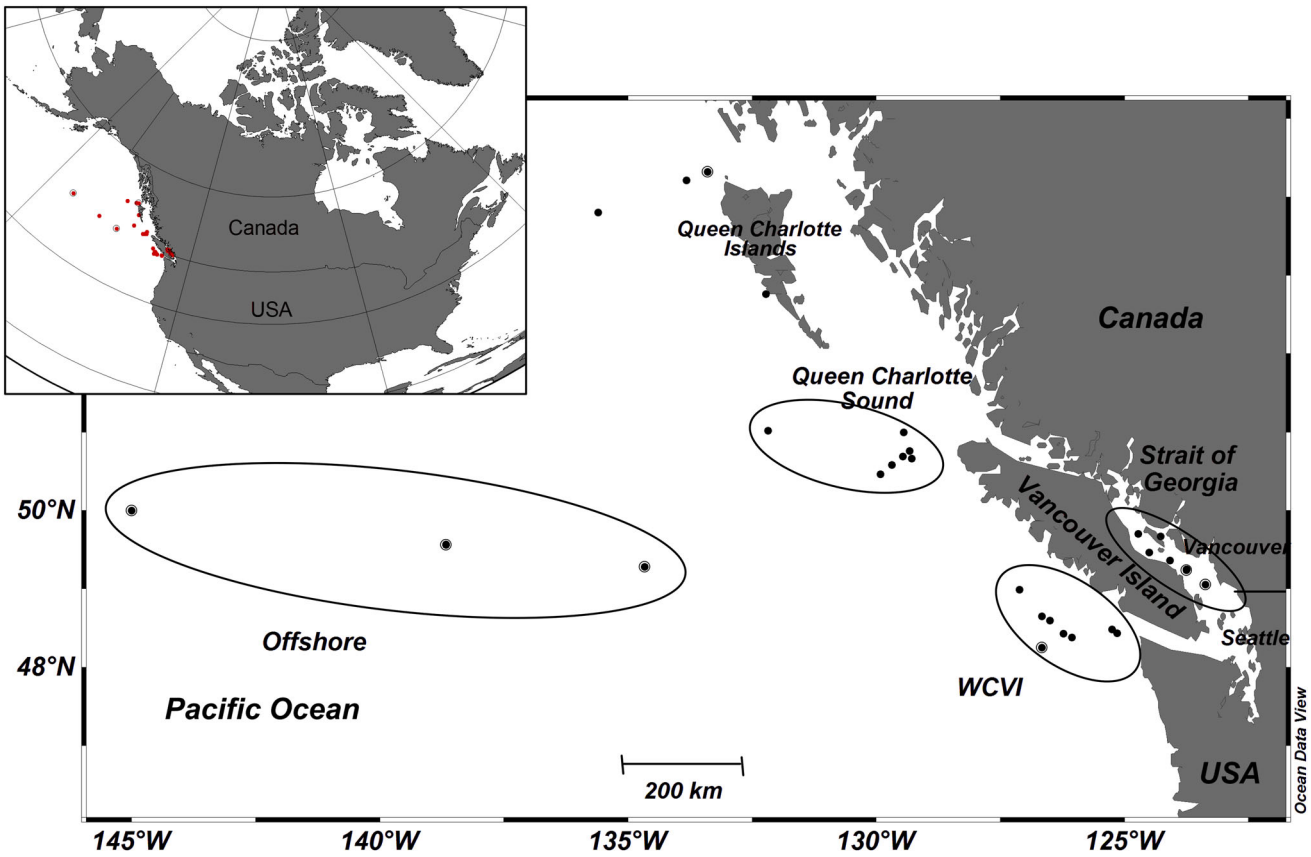

Fig. 1 Zooplankton were collected from four major regions of coastal British Columbia: Strait of Georgia (SoG), Northern Vancouver Island/Queen Charlotte Sound, West coast Vancouver Island (WCVI), and offshore Pacific Ocean. The two plankton species selected for analysis of microplastics were *N. cristatus* and *E. pacifica*

Results

Zooplankton Digestion Protocol

Different digestion conditions resulted in greatly different tissue digestion efficiencies. None of the digestion mixtures completely eliminated zooplankton tissue at room temperature regardless of the time allowed to digest. Only HNO_3 and the mixture of HCl and HNO_3 resulted in the digestion of zooplankton tissue when heated. There were no differences between the 3-h treatment and the 1-h treatment. The treatment using only HNO_3 completely digested the zooplankton tissue and left behind an oily residue, whereas the mixture of HCl and HNO_3 broke down the zooplankton body into smaller organic fragments, which remained in the solution. In both cases, several microplastic fibres were found after digestion.

Microplastics in Pacific Zooplankton

The aim of this study was to evaluate the presence and extent of microplastics in two species of zooplankton in the northeast (NE) Pacific Ocean. Our method, using acid digestion in well plates, allowed the determination of microplastics at the finest possible resolution (i.e., individual level) while retaining the capacity for relatively rapid analysis of a large number of samples. Using this technique, sample visualization is straightforward, and heating times can be adjusted to enable analysis of wet or dry remains. In both cases, particle visualization and isolation is simple, and standard dissection microscope and visualization software can be used for particle measurements and characterization. A Zeiss STEREO Discovery microscope (zoom range 8:1, 0.63 objective with 25×/10 ocular lenses), armed with an AxioCam ICc three basic resolution 2080 × 1540 (3.3 megapixels) camera, was used. This was attached to a Q409 Imaging Computer with imaging documentation (image acquisition, measuring, data handling, and archiving software).

Microplastics were detected in both the copepods and the euphausiids sampled at multiple sites in the NE Pacific Ocean (Table 1). Twenty-five plastic particles were detected after digestion of 960 individual copepods resulting in an encounter rate of approximately one

Table 1 Characteristics of plastics ingested by *N. cristatus* and *E. pacifica* collected from the NE Pacific Ocean near British Columbia, Canada

Characteristics	*N. cristatus*[a]	*E. pacifica*[b]	p[c]
Zooplankton density (no./m^3)	27.9 ± 15.8	1.3 ± 0.6	<0.001
Plastic-encounter rate (no. plankton/plastic particles)	33.5 ± 6.4	16.7 ± 2.8	0.011
Plastic size (μm)	555.5 ± 148.7	816.1 ± 107.7	0.014
% Fibre of total microplastic particles	43.9 ± 12.3	68.3 ± 12.8	0.19

[a] 960 individuals analyzed

[b] 413 individuals analyzed

[c] Results of t test between species

particle/every 34 copepod analysed (or 0.026 ± 0.005 particles/individual zooplankton). For euphausiids, a total of 24 particles were detected from 413 individuals resulting in an encounter rate of one particle/every 17 euphausiids (or 0.058 ± 0.01 particles/zooplankton).

The difference in ingestion between the two zooplankton species was significant (t test, $p = 0.01$) suggesting that euphausiids either ingest more plastics or are less able to eliminate plastics after ingestion than the copepods. Alternatively, the lower accumulation in copepods may result from biodilution in the ocean because their density in our samples was an order of magnitude greater than euphausiids. The average ingested size of microplastic particles was also greater in euphausiids (816 ± 108 μm) than copepods (556 ± 149 μm) ($p = 0.014$, Table 1). Overall, approximately 68 % of particles in euphausiids were fibres and 50 % in copepods (Table 1; Fig. 2). The color composition of ingested particles varied considerably, but it consisted predominantly of black, red, and blue particles (Supplemental Information Table S1). No interspecies differences were found for particle shape (fibre vs. fragment) or color.

The plastic-encounter rates for copepods and euphausiids did not differ among the four oceanographic regions

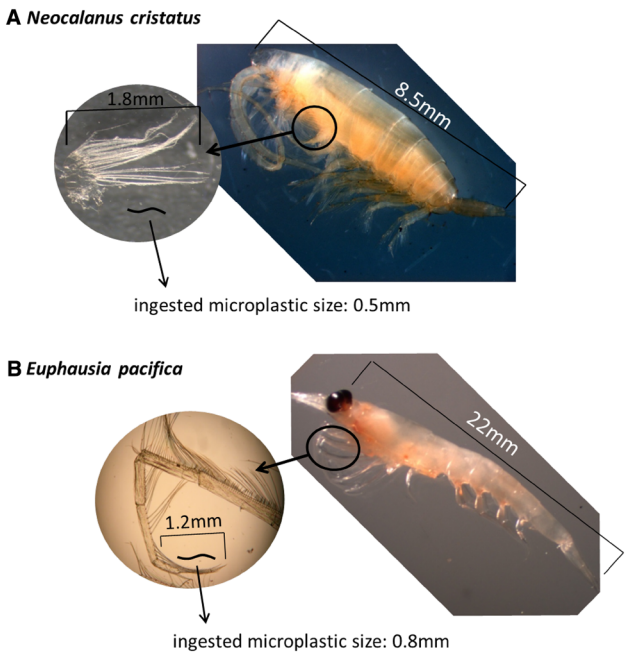

Fig. 2 The feeding appendage anatomy of **a** *N. cristatus* and **b** *E. pacifica* suggest that the sizes of ingested microplastic particles were within the physical limits of mouth gape and handling capacity of setae. The average microplastic particle size detected in this study is shown in relation to the size of setae for both zooplankton species

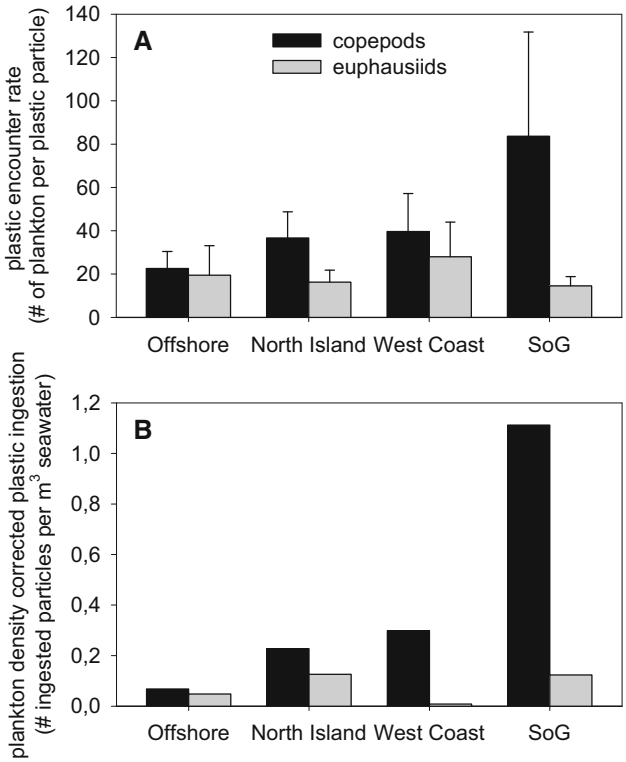

Fig. 3 The concentration of ingested microplastics by *N. cristatus* and *E. pacifica* varied among oceanographic regions of coastal British Columbia. **a** The ingested plastic-encounter rate (no. of plankton analyzed for every plastic particle) is similar between the four major regions. **b** The plankton density-corrected microplastic concentrations (no. of ingested microplastic particles/m^3 of seawater) is greatest for the Strait of Georgia (SoG) due to the high plankton density there. The plankton density-corrected concentration was calculated by multiplying the plankton density (no. of plankton/m^3 seawater) by the ingested microplastic concentration (no. of plastic particles/plankton)

evaluated in this study (Fig. 3a; Table 2). Individual copepods ingested fewer microplastics in the Strait of Georgia, but differences were not significant due to the small number of ingested particles found (Fig. 3a). Because zooplankton density varied widely across sites (not shown), we corrected the plastic-encounter rate at each site with its corresponding zooplankton density to give the number of ingested microplastics per cubic meter of seawater. Zooplankton samples that were collected near the location of the seawater microplastic samples were identified from the Zooplankton Database (Fisheries and Oceans Canada), pooled, and averaged for the two selected species (*E. pacifica* and *N. cristatus*) to obtain an average abundance per cubic meter. This would be the "density" of the particular species in the water at the time of microplastic sampling, thus inferring the potential ingestion of plastics in the water column. Because of the low number of detected ingested plastics, data from all sites were pooled for each region to compare at this broad scale. Using this value, which corrects for biodilution, the ingested plastic concentration was highest in the industrialized Strait of Georgia (both species) and northern Vancouver Island/Haida Gwaii (euphausiids only; Fig. 3b).

The plankton density-adjusted plastic ingestion in zooplankton correlated with microplastic characteristics in seawater (Fig. 4). Total ingested microplastic concentrations correlated with seawater total microplastic concentrations ($r^2 = 0.51$, $p < 0.001$) and particle size ($r^2 = 0.22$, $p = 0.06$) in seawater for *N. cristatus*, whereas only the ingested microplastic fibre concentrations in *E. pacifica* correlated with seawater fibre concentrations ($r^2 = 0.30$, $p < 0.001$) and size ($r^2 = 0.36$, $p = 0.03$).

Discussion

The results from our acid digestion procedure complement the methods developed by Claessens et al. (2013), where nitric acid was used to digest biological samples for microplastic enumeration. The investigators report high extraction efficiencies for polystyrene spheres (>90 %) and nylon fishing line (98 % 100 × 400 μm^2), whereas smaller nylon fibres (30 × 200 μm^2) could not be recovered. Our final protocol applies less harsh conditions than those described in Claessens et al. (2013) including shorter digestion times (30 min vs. overnight) and lower heat (<90 °C). Chemical resistance charts from Plastics International (http://www.plasticsintl.com/plastics_chemical_resistence_chart.html) and Curbell Plastics (http://www.curbellplastics.com/technical-resources/pdf/chemical-resistance-plastics.pdf) show that nylon, polyethylene terephthalate, and biopolymers (e.g., acetal, polyetheretherketone) are moderately or severely affected by concentrated nitric acid. Although the conditions in our method possibly reduce the destruction of vulnerable plastics compared with previous harsher methods, the use of nitric acid at all will likely destroy some fraction of plastics in the samples. The results we present here are thus conservative estimates of microplastic ingestion by zooplankton.

Cole et al. (2014) showed the use of an enzymatic digestion technique that avoids damage to plastic polymers. This method has much potential for the detection of plastics in batch analyses of plankton and other biological samples. However, the technique requires specialized equipment and materials, sample pretreatment (desiccation and grinding), and is more labour-intensive. Our method is

Table 2 Microplastic shape and size characterization for ingested particles by *N. cristatus* and *E. pacifica* at four major regions in coastal British Columbia, Canada

Species	Region	No. of ingested plastic particles	Fibre (%)	Fragment (%)	Fibre size (μm)	Fragment size (μm)
N. cristatus	Offshore ($n = 181$)	8	30 ± 30	70 ± 30	1778 ± 927	168 ± 36
	West Coast ($n = 198$)	5	25 ± 25	75 ± 25	612 ± 20	191 ± 42
	North Island ($n = 330$)	9	36 ± 17	64 ± 17	866 ± 328	213 ± 69
	SoG ($n = 251$)	3	100 ± 0	0 ± 0	461 ± 87	–
	All sites ($n = 960$)	*25*	*44 ± 12*[a]	*56 ± 12*[a]	*951 ± 269*[b]	*196 ± 29*[c]
E. pacifica	Offshore ($n = 39$)	2	0 ± 0	100 ± 0	–	299 ± 8.5
	West Coast ($n = 84$)	3	75 ± 25	25 ± 25	794 ± 394	123 ± 0
	North Island ($n = 130$)	8	44 ± 22	56 ± 22	1561 ± 197	297 ± 106
	SoG ($n = 160$)	11	100 ± 0	0 ± 0	895 ± 101	–
	All sites ($n = 413$)	*24*	*68 ± 13*[a]	*32 ± 13*[a]	*1040 ± 110*[b]	*273 ± 62*[c]

Italicized numbers depict combined results for all four regions for which samples of the two zooplankton species were obtained

SoG Straight of Georgia

[a] Results of *t* test $p = 0.18$

[b] Results of *t* test $p = 0.76$

[c] Results of *t* test $p = 0.13$

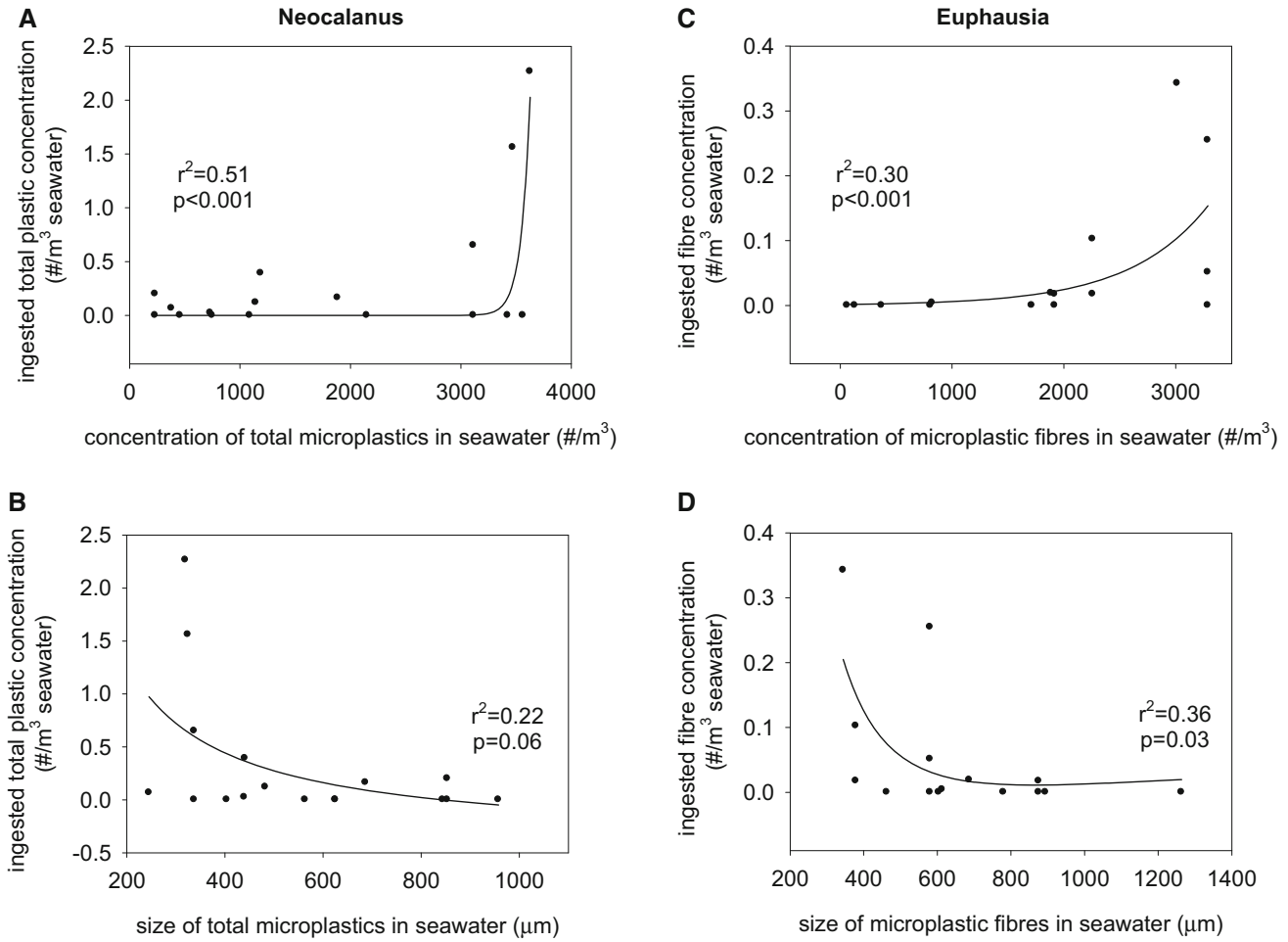

Fig. 4 The concentration of ingested microplastics, adjusted for plankton density, in neocalanus copepods and euphausiid shrimp is associated with the concentration and size of microplastic particles in seawater. The plankton density–adjusted microplastic concentration (no. of ingested microplastic particles/m^3 of seawater) was calculated by multiplying the microplastic concentration by the plankton density. Points represent site-specific averages, and seawater microplastic concentrations and sizes were taken from our previous study (Desforges et al. 2014)

straightforward; it uses material available in most laboratories; and analysis can occur at the individual level. Further work is needed to evaluate the full implication of the impacts of digestion procedure on different plastic polymers.

We show here that two zooplankton species critical to the North Pacific marine food web (neocalanoid copepods and euphausiids) are ingesting microplastics in the open ocean. Our findings provide an ecological context for the results of controlled laboratory feeding experiments with a variety of marine invertebrates including copepods and euphausiids (Cole et al. 2013; Farrell and Nelson 2013; Graham and Thompson 2009; Hamer et al. 2014; Kaposi et al. 2014; Lee et al. 2013; Murray and Cowie 2011; Setälä et al. 2014; Thompson et al. 2004; Watts et al. 2014; Wright et al. 2013b). Furthermore, the majority of laboratory studies expose animals to plastic particles <50 μm; we show that even larger microplastics (≤2000 μm) are also being ingested by zooplankton. In feeding experiments with several zooplankton taxa, Hamer et al. (2014), Kaposi et al. (2014), and Setälä et al. (2014) concluded that the risk of plastic ingestion depends on various factors including particle size, abundance and deposition in the environment (i.e., similarity to prey), as well as the feeding mode and anatomy of feeding/digestive organs of the consumer.

Suspension and filter feeders are predicted to encounter the most microplastics because these feeding modes are used to concentrate food from large volumes of water (Kaposi et al. 2014; Moore 2008). Both *N. cristatus* and *E. pacifica* are suspension filter feeders that use the movement of their external appendages to produce a feeding current, which draws food particles to their feeding basket (Fig. 2). Nonmotile prey, including microplastics, caught in the setae of the feeding basket are then transported to the

mouth and consumed. The size of the prey consumed is determined by the length of the feeding appendage and mouth size (Frost et al. 1983). The combined length of the maxilliped and setae, together comprising the length of the feeding basket, is approximately 4 mm for *N. cristatus* and 6–9 mm for *E. pacifica* (Fig. 2; M. Galbraith, unpublished observations).

The anatomical properties of mouthparts in our two zooplankton species suggest that both are capable to capture and ingest small microplastic particles in the marine environment. Natural food items for *N. cristatus* include phytoplankton, protists, and marine snow/aggregates with preferred sizes of >200 μm. *E. pacifica* feed on large diatoms (often chained), dinoflagellates, ciliates, and marine snow (Frost et al. 1983; Liu et al. 2005; Nakagawa et al. 2001). The microplastic particles detected here are in the same size range as these natural prey items, and the greater size of ingested particles by euphausiids is consistent with its greater body size and accordingly larger size selection of prey. Using an approximate gape size/mouth slit of 750 and 1000 μm for *N. cristatus* and *E. pacifica* (M. Galbraith, unpublished data), the ratio of average microplastic particle size to mouth size is 0.74 and 0.82, respectively. This ratio is likely an overestimation because it considers the length of the plastic particle. In reality, fibres can be folded or twisted on their own or bundled into an aggregate, thus reducing their overall size and potentially increasing their bioavailability. Both crustaceans can feed on chain diatoms, which can reach the lengths of >500 μm with a diameter of 10–100 μm, equivalent to the fibres encountered in this study.

Our results show that zooplankton are ingesting microplastics in the NE Pacific Ocean, but the implications for their health remain unclear. The two major risk outcomes from our study presumably include direct impacts in either the zooplankton themselves or in those species feeding on them. Laboratory-based studies suggest a variety of possible outcomes. A negative influence of nanoplastics and microplastics on survival and mortality has been reported for the marine copepod *Tigriopus japonicus* and the freshwater cladoceran *Daphnia magna* exposed to high levels of polystyrene beads (Besseling et al. 2014; Lee et al. 2013). Various sublethal effects of microplastic ingestion have also been reported. Wright et al. (2013a, b) showed that the polychaete worm (*Arenicola marina*) exposed to environmentally relevant concentrations of microplastics experienced reductions of ≤50 % of energy reserves arising from reduced feeding activity, increased gut residence time of ingested material, and inflammation. Reduced feeding rate has also been observed in the marine copepod *Centropages typicus* (Cole et al. 2013) and in another study of *A. marina* where body weight was also reduced (Besseling et al. 2013). Inflammatory responses and oxidative stress, manifested by the formation of granulocytomas, lysosomal membrane destabilization, increased phagocytic activity, and epithelial cell apoptosis, occurred in the blue mussel *M. edulis* exposed to nanoplastics and microplastics (Koehler et al. 2008; von Moos et al. 2012). Reproductive effects, which may have population level consequences, were apparent in copepods and zooplankton (Besseling et al. 2014; Lee et al. 2013).

Other studies have not detected effects after microplastic ingestion by marine organisms in the laboratory or in models including lugworms (Koelmans et al. 2013), sea urchin larvae (Kaposi et al. 2014), marine isopods (Hamer et al. 2014), and North Sea cod (Koelmans et al. 2014). More controlled feeding experiments with environmentally relevant microplastic concentrations and properties will help to further elucidate the risks that microplastics pose to organisms at the base of marine food webs and the consequent bottom-up implications for higher trophic levels.

The potential impact of food web transfer of microplastics in zooplankton remains largely unanswered. However, zooplankton represent a critical energy source in the world's oceans and are heavily preyed upon by fish and several marine mammal species. Murray and Cowie (2011) first showed that microplastics can be transferred from prey to predator by feeding fish seeded with polypropylene fibres to lobsters. Farrell and Nelson (2013) documented the transfer of polystyrene spheres from contaminated blue mussels fed to common shore crabs (*Carcinus maenas*). Setälä et al. (2014) showed trophic transfer of plastics in the zooplanktonic food web: The intestine of the mysid shrimp (*Mysis* spp.) contained microplastics after feeding on various copepod and polychaete larvae species. Further indirect evidence of food web transfer is suggested by the presence of microplastics in the stomach of planktivorous fish (e.g., Boerger et al. 2010) and scat of piscivorous fur seals (Eriksson and Burton 2003), as well as by the detection of compounds in basking sharks (*Cetorhinus maximus*) and fin whales (*Balaenoptera physalus*), that are thought to have originated in plastic products (Fossi et al. 2014).

These studies highlight the potential for microplastics ingested by zooplankton to be taken up by higher trophic level marine fish and wildlife. In the Northwest coast of North America, salmonids (*Oncorhynchus* spp) are of critical importance to the region's natural and human inhabitants. Most salmon species feed heavily on copepods and euphausiids during their juvenile and/or adult life phases. Salmon have a typical daily food consumption between 1 and 10 % of their body weight (Brodeur 1990). Although adult body sizes for individuals of the largest salmonid [chinook (*Oncorhynchus tshawytscha*)] can attain as much as 50 kg, but most species are smaller ranging from 4 to 15 kg. Because the industrialised Strait of

Georgia in coastal British Columbia is a critical feeding area for out-migrating juvenile salmon and returning adults, we estimated microplastic ingestion based on feeding rates using our data on microplastic-containing zooplankton (Table 3). With juvenile salmon potentially ingesting 2–7 microplastic particles/day, and returning adult salmon ingestion ≤91 particles/day, exposure may be considerable. Although speculative, this exercise provides a sense of possible scale of exposure and raises questions about microplastic risks to populations of ecologically and economically important species. Estimates can also be made for marine mammals that feed heavily on zooplankton. A humpback whale (*Megaptera novaeangliae*) in coastal British Columbia consuming 1.5 % of its body weight in krill and zooplankton daily (Barlow et al. 2008) would ingest >300,000 microplastic particles/day (0.15 % diet × 30,000 kg/whale ÷ 0.00007 kg/plankton × 0.05 plastics/plankton). This estimate does not account for plastics taken up directly from water.

Zooplankton in the present study were collected from locations coinciding with water samples collected as part of our previous study of microplastics in seawater of the NE Pacific Ocean (Desforges et al. 2014). No differences in the mean ingested plastic-encounter rate were found among oceanographic regions for both species (Fig. 3a), but this is likely confounded by the major difference in plankton density between regions. After correcting for plankton density, we see elevated levels of plastic ingestion in the Strait of Georgia and northern Vancouver Island/Queen Charlotte Sound (Fig. 3b) corresponding with the greater density of seawater microplastics reported in these two areas in our previous study (see Desforges et al. 2014). These results suggest that the absolute number of ingested microplastics may not accurately reflect the level of microplastics in seawater as a consequence of possible biodilution. Thus, adjusting the plastic ingestion levels for zooplankton density corrects for the biodilution effect in which microplastics become less available due to competition with a growing number of individuals.

The plastic composition also varied among the four regions examined (Table 2). Zooplankton in the industrialized Strait of Georgia ingested only microplastic fibres and no fragments, whereas offshore zooplankton were found to have ingested almost exclusively microplastic fragments. Indeed, the ingested microplastic composition observed, reported as % fibres of total particles, was correlated negatively with distance from shore with the contribution of fibres decreasing with distance from urbanized coastal areas (Fig. S1). This is consistent with observations from our previous seawater study (Desforges et al. 2014) with collective results suggesting near-shore or land-based sources of microplastics associated with human activities.

Table 3 Estimated microplastic ingestion by Pacific salmon species as a result of food web transfer from two important zooplankton species in the Strait of Georgia, British Columbia

Species	Fish weight (kg)[a]	Daily food ration (% body weight)[b]	Estimated no. of zooplankton consumed per day[d]	Estimated no. of plastic consumed per day[e]
Juveniles				
Pink	0.1 (0.03–0.2)	3.7 (3.2–4.1)	53	2.6
Chum	0.1 (0.03–0.2)	2.5 (1.0–5.0)	36	1.8
Coho	0.3 (0.1–0.5)	3.1 (2.4–3.7)	133	6.6
Sockeye	0.2 (0.1–0.3)	1.8 (1.2–2.3)	51	2.6
Chinook	0.1 (0.05–0.2)	3.2 (2.0–4.3)[c]	46	2.3
Adults				
Pink	1.3 (1.0–1.5)	6.1 (5.8–6.4)	1133	56.6
Chum	3.2 (3.0–3.4)	4.0 (1.0–7.0)	1829	91.4
Coho	2.8 (2.0–3.5)	NA		
Sockeye	2.7 (2.0–3.3)	2.0 (1.6–2.3)	771	38.6
Chinook	10.6 (7.8–13.4)	NA		

NA not analyzed

[a] Taken from (Ishida et al. 1998)

[b] Taken from (Brodeur 1990) unless otherwise stated

[c] Taken from (Benkwitt et al. 2009)

[d] Estimated assuming 100 % daily food ration of zooplankton and average zooplankon weight of 70 mg

[e] Estimated assuming an average plastic encounter rate of 0.05 particles/plankton based on data from this study

Furthermore, when site-specific zooplankton and water data were compared, the concentration of ingested plastics (corrected for plankton density) for the copepods and euphausiids correlated with seawater plastic concentrations and seawater plastic size (Fig. 4). These results suggest that the concentration of ingested plastic is a positive function of available plastic in seawater and is inversely related to plastic size. Taken together, these results point to a strong association between microplastic characteristics in seawater and zooplankton and show heightened microplastic ingestion by zooplankton inhabiting more urbanized coastal areas.

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References

Andrady AL (2011) Microplastics in the marine environment. Mar Pollut Bull 62:1596–1605

Bakir A, Rowland SJ, Thompson RC (2014) Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. Environ Pollut 185:16–23

Barlow J, Kahru M, Mitchell B (2008) Cetacean biomass, prey consumption, and primary production requirements in the California Current ecosystem. Mar Ecol Prog Ser 371:285–295

Barnes D, Galgani F, Thompson R, Barlaz M (2009) Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc Lond B 364:1985–1998

Benkwitt CE, Brodeur RD, Hurst TP, Daly EA (2009) Diel feeding chronology, gastric evacuation, and daily food consumption of juvenile Chinook salmon in Oregon coastal waters. Trans Am Fish Soc 138:111–120

Besseling E, Wegner A, Foekema EM, van den Heuvel-Greve M, Koelmans AA (2013) Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ Sci Technol 47:593–600

Besseling E, Wang B, Lurling M, Koelmans AA (2014) Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. Environ Sci Technol 48:12336–12343

Boerger CM, Lattin GL, Moore SL, Moore CJ (2010) Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar Pollut Bull 60:2275–2278

Brodeur RD (1990) A synthesis of the food habits and feeding ecology of salmonids in marine waters of the North Pacific. FRI-UW-9016. Fisheries Research Institute, University of Washington, Seattle, WA

Browne MA, Crump P, Niven SJ, Teuten E, Tonkin A, Galloway T et al (2011) Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ Sci Technol 45:9175–9179

Browne MA, Niven SJ, Galloway TS, Rowland SJ, Thompson RC (2013) Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr Biol 23:2388–2392

Choy C, Drazen J (2013) Plastic for dinner? Observations of frequent debris ingestion by pelagic predatory fishes from the central North Pacific. Mar Ecol Prog Ser 485:155–163

Chua EM, Shimeta J, Nugegoda D, Morrison PD, Clarke BO (2014) Assimilation of polybrominated diphenyl ethers from microplastics by the marine amphipod, Allorchestes compressa. Environ Sci Technol 48:8127–8134

Claessens M, Van Cauwenberghe L, Vandegehuchte MB, Janssen CR (2013) New techniques for the detection of microplastics in sediments and field collected organisms. Mar Pollut Bull 70:227–233

Cole M, Lindeque P, Halsband C, Galloway TS (2011) Microplastics as contaminants in the marine environment: a review. Mar Pollut Bull 62:2588–2597

Cole M, Lindeque P, Fileman E, Halsband C, Goodhead R, Moger J et al (2013) Microplastic ingestion by zooplankton. Environ Sci Technol 47:6646–6655

Cole M, Webb H, Lindeque PK, Fileman ES, Halsband C, Galloway TS (2014) Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci Rep 4:4528

Cózar A, Echevarría F, González-Gordillo JI, Irigoien X, Ubeda B, Hernández-León S et al (2014) Plastic debris in the open ocean. Proc Natl Acad Sci USA 111(28):10239–10244

Davison P, Asch R (2011) Plastic ingestion by mesopelagic fishes in the North Pacific subtropical gyre. Mar Ecol Prog Ser 432:173–180

Desforges J-PW, Galbraith M, Dangerfield N, Ross PS (2014) Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. Mar Pollut Bull 79:94–99

Eriksson C, Burton H (2003) Origins and biological accumulation of small plastic particles in fur seals from Macquarie Island. Ambio 32:380–384

Farrell P, Nelson K (2013) Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ Pollut 177:1–3

Foekema EM, De Gruijter C, Mergia MT, van Franeker JA, Murk AJ, Koelmans AA (2013) Plastic in north sea fish. Environ Sci Technol 47:8818–8824

Fossi MC, Coppola D, Baini M, Giannetti M, Guerranti C, Marsili L et al (2014) Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: the case studies of the Mediterranean basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*). Mar Environ Res 100:17–24

Frost BW, Landry MR, Hassett RP (1983) Feeding behavior of large calanoid copepods *Neocalanus cristatus* and *N. plumchrus* from the subarctic Pacific Ocean. Deep Res 30:1–13

Graham ER, Thompson JT (2009) Deposit- and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. J Exp Mar Biol Ecol 368:22–29

Hamer J, Gutow L, Kohler A, Saborowski R (2014) Fate of microplastics in the marine isopod *Idotea emarginata*. Environ Sci Technol 48:13451–13459

Ishida Y, Ito S, Ueno Y, Sakai J (1998) Seasonal growth pattern of Pacific salmon (*Oncrohynchus* spp) in offshore waters of the North Pacific Ocean. North Pac Anadromous Fish Comm Bull 1:66–80

Kaposi KL, Mos B, Kelaher BP, Dworjanyn SA (2014) Ingestion of microplastic has limited impact on a marine larva. Environ Sci Technol 48(3):1638–1645

Koehler A, Marx U, Broeg K, Bahns S, Bressling J (2008) Effects of nanoparticles in Mytilus edulis gills and hepatopancreas—a new threat to marine life? Mar Environ Res 66:12–14

Koelmans A, Besseling E, Wegner A, Foekema EM (2013) Plastic as a carrier of POPs to aquatic organisms: a model analysis. Environ Sci Technol 47:8992–8993

Koelmans AA, Besseling E, Foekema EM (2014) Leaching of plastic additives to marine organisms. Environ Pollut 187:49–54

Lee K, Shim WJ, Kwon OY, Kang J (2013) Size-dependent effects of micro polystyrene particles in the marine copepod Tigriopus japonicus. Environ Sci Technol 47:11278–11283

Liu H, Dagg MJ, Strom S (2005) Grazing by the calanoid copepod *Neocalanus cristatus* on the microbial food web in the coastal Gulf of Alaska. J Plankton Res 27:647–662

Lusher AL, McHugh M, Thompson RC (2013) Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar Pollut Bull 67:94–99

Mathalon A, Hill P (2014) Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. Mar Pollut Bull 81:69–79

Maximenko N, Hafner J, Niiler P (2012) Pathways of marine debris derived from trajectories of Lagrangian drifters. Mar Pollut Bull 65:51–62

Moore CJ (2008) Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environ Res 108:131–139

Murray F, Cowie PR (2011) Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus 1758). Mar Pollut Bull 62:1207–1217

Nakagawa Y, Endo Y, Taki K (2001) Diet of Euphausia pacifica Hansen in Sanriku waters off northeastern Japan. Plankton Biol Ecol 48:68–77

Possatto FE, Barletta M, Costa MF, do Sul JA, Dantas DV (2011) Plastic debris ingestion by marine catfish: an unexpected fisheries impact. Mar Pollut Bull 62:1098–1102

Rochman CM, Kurobe T, Flores I, Teh SJ (2014) Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Sci Total Environ 493:656–661

Setälä O, Fleming-Lehtinen V, Lehtiniemi M (2014) Ingestion and transfer of microplastics in the planktonic food web. Environ Pollut 185:77–83

Tanaka K, Takada H, Yamashita R, Mizukawa K, Fukuwaka M, Watanuki Y (2013) Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. Mar Pollut Bull 69:219–222

Thompson RC, Olsen Y, Mitchell RP, Davis A, Rowland SJ, John AWG et al (2004) Lost at sea: where is all the plastic? Science 304:838

Van Cauwenberghe L, Janssen CR (2014) Microplastics in bivalves cultured for human consumption. Environ Pollut 193:65–70

Von Moos N, Burkhardt-Holm P, Kohle A (2012) Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ Sci Technol 46:11327–11335

Watts AJR, Lewis C, Goodhead RM, Beckett SJ, Moger J, Tyler CR et al (2014) Uptake and retention of microplastics by the shore crab *Carcinus maenas*. Environ Sci Technol 48:8823–8830

Wright SL, Rowe D, Thompson RC, Galloway TS (2013a) Microplastic ingestion decreases energy reserves in marine worms. Curr Biol 23:R1031–R1033

Wright SL, Thompson RC, Galloway TS (2013b) The physical impacts of microplastics on marine organisms: a review. Environ Pollut 178:483–492