

Extraction and analysis methods of microplastics in bivalves

Bibliography

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Background

Melissa B. Duhaime, PhD. Assistant Professor, Department of Ecology & Evolutionary Biology, University of Michigan before the Subcommittee on Oceans, Atmosphere, Fisheries, and Coast Guard, U.S. Senate 115th Congress July 25, 2017.

“Aquatic organisms ingest plastic pollutants (refs. 4,5), which results in energetic and fitness costs (refs. 6,7) and other morbid impacts (ref. 8). Microscopic plastic is found in fish and shellfish sold for human consumption at seafood markets around the world, including in Europe (ref 9) and in the U.S. (ref 10). There is a high likelihood that humans are consuming this plastic. The health consequences of this are unknown. In the wake of these discoveries, the United Nations has declared plastic pollution among the most critical emerging environmental issues of our time (ref 11). The scientific consensus is that plastic pollution must be reduced to avoid the risk of irreversible ecosystem harm (ref 12).”

4. Boerger, C. M., Lattin, G. L., Moore, S. L. & Moore, C. J. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. *Mar Pollut Bull* 60, 2275-2278 (2010).
5. Foekema, E. M., De Gruijter, C., Mergia, M. T., van Franeker, J. A., et al. Plastic in north sea fish. *Environ Sci Technol* 47, 8818-8824 (2013).
6. Besseling, E., Wegner, A., Foekema, E. M., Van Den Heuvel-Greve, M. J. & Koelmans, A. A. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). *Environmental science & technology* 47, 593-600 (2012).
7. Wright, S. L., Thompson, R. C. & Galloway, T. S. The physical impacts of microplastics on marine organisms: a review. *Environ Pollut* 178, 483-492 (2013).
8. Rochman, C. M., Hoh, E., Kurobe, T. & Teh, S. J. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Sci Rep* 3, 3263 (2013).
9. Van Cauwenberghe, L. & Janssen, C. R. Microplastics in bivalves cultured for human consumption. *Environ Pollut* 193, 65-70 (2014).
10. Rochman, C. M., Tahir, A., Williams, S. L., Baxa, D. V., et al. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Sci Rep* 5, 14340 (2015).
11. UNEP Marine plastic debris and microplastics – Global lessons and research to inspire action and guide policy change. United Nations Environment Programme, Nairobi (2016).
12. Rochman, C. M., Browne, M. A., Underwood, A. J., Franeker, J. A., et al. The ecological impacts of marine debris: unraveling the demonstrated evidence from what is perceived. *Ecology* 97, 302-312 (2016).

Scope

The NOAA Central library was asked to prepare the following bibliography on methods of extraction and analysis of microplastics in bivalves in order to support future research by the National Centers for Coastal Ocean Science. This bibliography focuses specifically on literature that discusses methods of extraction and analysis of microplastics in bivalves. Peripheral research on impact, other aquatic life, sediment extraction/analysis etc. is not included unless it also meets the scope outlined above.

Sources Reviewed

The following databases were used to identify sources: Clarivate Analytics' Web of Science: Science Citation Index Expanded, ScienceDirect, ProQuest's Science and Technology including ASFA, BioOne, American Fisheries Society Journals and back files, and the Directory of Open Access Journals. Only English language materials were included. There was no date range specification in order to cover any relevant research.

Bibliography

Beyer, J., Green, N. W., Brooks, S., Allan, I. J., Ruus, A., Gomes, T., . . . Schoyen, M. (2017). Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: A review. *Marine Environmental Research*, 130, 338-365. <https://doi.org/10.1016/j.marenvres.2017.07.024>

The blue mussel (*Mytilus* spp.) is widely used as a bioindicator for monitoring of coastal water pollution (mussel watch programs). Herein we provide a review of this study field with emphasis on: the suitability of *Mytilus* spp. as environmental sentinels; uptake and bioaccumulation patterns of key pollutant classes; the use of *Mytilus* spp. in mussel watch programs; recent trends in Norwegian mussel monitoring; environmental quality standards and background concentrations of key contaminants; pollutant effect biomarkers; confounding factors; particulate contaminants (microplastics, engineered nano materials); climate change; harmonization of monitoring procedures; and the use of deployed mussels (transplant caging) in pollution monitoring. Lastly, the overall state of the art of blue mussel pollution monitoring is discussed and some important issues for future research and development are highlighted. (C) 2017 Elsevier Ltd. All rights reserved.

Bonello, G., Varrella, P., & Pane, L. (2018). First Evaluation of Microplastic Content in Benthic Filter-feeders of the Gulf of La Spezia (Ligurian Sea). *Journal of Aquatic Food Product Technology*, 27(3), 284-291. <https://doi.org/10.1080/10498850.2018.1427820>

The ingestion and retention of microplastics of filter-feeder organisms represent a risk for the final consumers and the environment. Biomonitoring is necessary to deal with the effects of plastic material pollution. The selection of the monitored organisms strongly affects the relevance of the results and the understanding of the environmental conditions. The results discussed in this paper highlight the differences in the estimate of microplastic pollution depending on the species subject of study. *Ascidia* spp. specimens retained a value five-fold higher (0.62 MP/g) than bivalve species (*Crassostrea gigas* 0.11 MP/g; *Mytilus galloprovincialis* 0.05 MP/g; *Anomia ephippium* 0.12 MP/g).

Browne, M. A., Dissanayake, A., Galloway, T. S., Lowe, D. M., & Thompson, R. C. (2008). Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environmental Science & Technology*, 42(13), 5026-5031. <https://doi.org/10.1021/es800249a>

Plastics debris is accumulating in the environment and is fragmenting into smaller pieces; as it does, the potential for ingestion by animals increases. The consequences of macroplastic debris for wildlife are well documented, however the impacts of microplastic (< 1 mm) are poorly understood. The mussel, *Mytilus edulis*, was used to investigate ingestion, translocation, and accumulation of this debris. Initial experiments showed that upon ingestion, microplastic accumulated in the gut. Mussels were subsequently exposed to treatments containing seawater and microplastic (3.0 or 9.6 microm). After transfer to clean conditions, microplastic was tracked in the hemolymph. Particles translocated from the gut to the circulatory system within 3 days and persisted for over 48 days. Abundance of microplastic was greatest after 12 days and declined thereafter. Smaller particles were more abundant than larger particles and our data indicate as plastic fragments into smaller particles, the potential for accumulation in the tissues of an organism increases. The short-term pulse exposure used here did not result in significant biological effects. However, plastics are exceedingly durable and so further work using a wider range of organisms, polymers, and periods of exposure will be required to establish the biological consequences of this debris.

Catarino, A. I., Thompson, R., Sanderson, W., & Henry, T. B. (2017). Development and optimization of a standard method for extraction of microplastics in mussels by enzyme digestion of soft tissues. *Environmental Toxicology and Chemistry*, 36(4), 947-951. <https://doi.org/10.1002/etc.3608>

The authors compared procedures for digestion of mussel soft tissues and extraction of microplastics. Complete tissue digestion was achieved with 1M NaOH, 35% HNO₃, and protease at 9.6 UHb/mL (unit hemoglobin per mL); but use of HNO₃ caused unacceptable destruction of some microplastics. Recovery of microplastics spiked into mussels was similar (93 +/- 10%) for NaOH and enzyme digestions. The authors recommend use of industrial enzymes based on digestion efficiency, microplastic recovery, and avoidance of caustic chemicals. (C) 2016 SETAC

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M. B., & Janssen, C. R. (2013). New techniques for the detection of microplastics in sediments and field collected organisms. *Marine Pollution Bulletin*, 70(1-2), 227-233. <https://doi.org/10.1016/j.marpolbul.2013.03.009>

Microplastics have been reported in marine environments worldwide. Accurate assessment of quantity and type is therefore needed. Here, we propose new techniques for extracting microplastics from sediment and invertebrate tissue. The method developed for sediments involves a volume reduction of the sample by elutriation, followed by density separation using a high density NaI solution. Comparison of this methods' efficiency to that of a widely used technique indicated that the new method has a considerably higher extraction efficiency. For fibres and granules an increase of 23% and 39% was noted, extraction efficiency of PVC increased by 100%. The second method aimed at extracting microplastics from animal tissues based on chemical digestion. Extraction of microspheres yielded high efficiencies (94-98%). For fibres, efficiencies were highly variable (0-98%), depending on polymer type. The use of these two techniques will result in a more complete assessment of marine microplastic concentrations.

Courtene-Jones, W., Quinn, B., Gary, S. F., Mogg, A. O. M., & Narayanaswamy, B. E. (2017). Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. *Environmental Pollution*, 231, 271-280. <https://doi.org/10.1016/j.envpol.2017.08.026>

Microplastics are widespread in the natural environment and present numerous ecological threats. While the ultimate fate of marine microplastics are not well known, it is hypothesized that the deep sea is the final sink for this anthropogenic contaminant. This study provides a quantification and characterisation of microplastic pollution ingested by benthic macroinvertebrates with different feeding modes (*Ophiomusium lymani*, *Hymenaster pellucidus* and *Colus jeffreysianus*) and in adjacent deep water > 2200 m, in the Rockall Trough, Northeast Atlantic Ocean. Despite the remote location, microplastic fibres were identified in deep-sea water at a concentration of 70.8 particles m⁻³, comparable to that in surface waters. Of the invertebrates examined (n = 66), 48% ingested microplastics with quantities enumerated comparable to coastal species. The number of ingested microplastics differed significantly between species and generalized linear modelling identified that the number of microplastics ingested for a given tissue mass was related to species and not organism feeding mode or the length or overall weight of the individual. Deep-sea microplastics were visually highly degraded with surface areas more than double that of pristine particles. The identification of synthetic polymers with densities greater and less than seawater along with comparable quantities to the upper ocean indicates processes of vertical re-distribution. This study presents the first snapshot of deep ocean microplastics and the quantification of microplastic pollution in the Rockall Trough. Additional sampling throughout the deep-sea is required to assess levels of microplastic pollution, vertical transportation and sequestration, which have the potential to impact the largest global ecosystem.

Courtene-Jones, W., Quinn, B., Murphy, F., Gary, S. F., & Narayanaswamy, B. E. (2017). Optimisation of enzymatic digestion and validation of specimen preservation methods for the analysis of ingested microplastics. *Analytical Methods*, 9(9), 1437-1445. <https://doi.org/10.1039/c6ay02343f>

Microplastics are considered to be a widespread environmental contaminant. Due to their small size microplastics have the potential to be ingested by a range of aquatic organisms which mistake them for a food source and can suffer adverse impacts as a result. Development of standardised methods is imperative to provide reliable and meaningful data when analysing microplastic ingestion by marine fauna. A range of proteolytic digestive enzymes (trypsin, papain and collagenase) were tested to establish optimum digestion efficacy of biological samples and assess the effects of enzymes on microplastics; additionally the applicability of freezing and formaldehyde followed by ethanol as specimen preservation techniques for microplastic research was investigated. Of the enzymes investigated, trypsin yielded the greatest digestive efficacy based on weight reduction (88% +/- 2.52 S. D.) at the lowest concentration (0.3125%) with no observed impacts on microplastics. Enumeration of microplastics from wild collected *Mytilus edulis* revealed mean numbers of 1.05 +/- 0.66 S. D. (minimum) to 4.44 +/- 3.03 S. D. (maximum) microplastic particles per g wet weight mussel tissue depending on location. There was no significant difference based on preservation method on the quantification of ingested microplastics and no detrimental impacts were observed on the microplastics directly. Enzymatic digestion using trypsin therefore provides a suitable, time and cost effective method to extract microplastics from *M. edulis*. Furthermore the preservation methods did not have detrimental effects on microplastics, serving to highlight the suitability of biological samples preserved either way for future inquiries into ingested microplastics.

Davidson, K., & Dudas, S. E. (2016). Microplastic Ingestion by Wild and Cultured Manila Clams (*Venerupis philippinarum*) from Baynes Sound, British Columbia. *Archives of Environmental Contamination and Toxicology*, 71(2), 147-156. <https://doi.org/10.1007/s00244-016-0286-4>

Microplastics, plastic particles <5 mm, are an emerging concern in aquatic ecosystems. Because microplastics are small, they are available to many filter-feeding organisms, which can then be consumed by higher trophic level organisms, including humans. This study documents the quantity of microplastics present in wild and cultured Manila clams (*Venerupis philippinarum*). Three active shellfish farms and three reference beaches (i.e., non-shellfish farm sites) in Baynes Sound, British Columbia were chosen to examine the microplastic concentrations in wild and cultured Manila clams. Microplastics were isolated using a nitric acid digestion technique and enumerated from 54 clams (27 farmed and 27 non-farmed). Qualitative attributes, such as colour and microplastic type (fiber, fragment, or film) also were recorded. There was no significant difference ($F = 1.29$; $df = 1,4$; $P = 0.289$) between microplastic concentrations in cultured and wild clams. Microplastic concentrations ranged from 0.07 to 5.47 particles/g (from reference beach and shellfish farm clams, respectively). Fibers were the dominant microplastic (90 %); colourless and dark gray fibers were the most common colours observed (36 and 26 %, respectively). Although this indicates that microplastics are definitely present in seafood consumed by humans, shellfish aquaculture operations do not appear to be increasing microplastic concentrations in farmed clams in this region.

De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., & Robbens, J. (2014). Quality assessment of the blue mussel (*Mytilus edulis*): Comparison between commercial and wild types. *Marine Pollution Bulletin*, 85(1), 146-155. <https://doi.org/10.1016/j.marpolbul.2014.06.006>

This study compared species identity, microplastics, chemical and microbial contamination between consumption mussels and wild type mussels, collected at Belgian department stores and Belgian groynes and quaysides, respectively. Species identification based on genetic analysis showed a high number of *Mytilus* (*M.*) *edulis* compared to *M. galloprovincialis* and *M. edulis/galloprovincialis* hybrid mussels. The number of total microplastics varied from 2.6 to 5.1 fibres/10g of mussel. A higher prevalence of orange fibres at quaysides is related to fisheries activities. Chemical contamination of polycyclic aromatic hydrocarbons and polychlorobiphenyls could be related to industrial activities and water turbidity, with maximum concentrations at the quayside of port Zeebrugge. The inverse was noted for *Escherichia coli* contamination, which was relatively low at Zeebrugge quayside with a total count of 3.9×10^2 CFU/100g tissue, due to limited agricultural effluents. Results of this complementary analysis stress the importance of integrated monitoring and quality assessment.

Dehaut, A., Cassone, A. L., Frere, L., Hermabessiere, L., Himber, C., Rinnert, E., . . . Paul-Pont, I. (2016). Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution*, 215, 223-233. <https://doi.org/10.1016/j.envpol.2016.05.018>

Pollution of the oceans by microplastics (<5 mm) represents a major environmental problem. To date, a limited number of studies have investigated the level of contamination of marine organisms collected in situ. For extraction and characterization of microplastics in biological samples, the crucial step is the identification of solvent(s) or chemical(s) that efficiently dissolve organic matter without degrading plastic polymers for their identification in a time and cost effective way. Most published papers, as well as OSPAR recommendations for the development of a common monitoring protocol for plastic particles in fish and shellfish at the European level, use protocols containing nitric acid to digest the biological tissues, despite reports of polyamide degradation with this chemical. In the present study, six existing approaches were tested and their effects were compared on up to 15 different plastic polymers, as well as their efficiency in digesting biological matrices. Plastic integrity was evaluated through microscopic inspection, weighing, pyrolysis coupled with gas chromatography and mass spectrometry, and Raman spectrometry before and after digestion. Tissues from mussels, crabs and fish were digested before being filtered on glass fibre filters. Digestion efficiency was evaluated through microscopical inspection of the filters and determination of the relative removal of organic matter content after digestion. Five out of the six tested protocols led to significant degradation of plastic particles and/or insufficient tissue digestion. The protocol using a KOH 10% solution and incubation at 60 degrees C during a 24 h period led to an efficient digestion of biological tissues with no significant degradation on all tested polymers, except for cellulose acetate. This protocol appeared to be the best compromise for extraction and later identification of microplastics in biological samples and should be implemented in further monitoring studies to ensure relevance and comparison of environmental and seafood product quality studies.

Doyle, J. J., Ward, J. E., & Mason, R. (2015). An examination of the ingestion, bioaccumulation, and depuration of titanium dioxide nanoparticles by the blue mussel (*Mytilus edulis*) and the eastern oyster (*Crassostrea virginica*). *Marine Environmental Research*, 110, 45-52. <https://doi.org/10.1016/j.marenvres.2015.07.020>

The production rates of titanium dioxide (TiO₂) nanoparticles for consumer products far exceed the pace at which research can determine the effects of these particles in the natural environment. Sedentary organisms such as suspension-feeding bivalves are particularly vulnerable to anthropogenic contaminants, such as nanoparticles, that enter coastal environments. The purpose of this work was to examine the ingestion, bioaccumulation, and depuration rates of TiO₂ nanoparticles by two species of suspension-feeding bivalves, the blue mussel (*Mytilus edulis*) and the eastern oyster (*Crassostrea virginica*). Two representative TiO₂ nanoparticles, UV-Titan M212 (Titan) and Aeroxide P25 (P25), were delivered to the animals either incorporated into marine snow or added directly to seawater at a concentration of 1.0 mg/L for exposure periods of 2 and 6 h. After feeding, the animals were transferred to filtered-seawater and allowed to depurate. Feces and tissues were collected at 0, 12, 24, 72, and 120 h, post-exposure, and analyzed for concentrations of titanium by inductively coupled plasma-mass spectrometry. Results indicated that the capture and ingestion (i.e., transfer to the gut) of TiO₂ nanoparticles by both mussels and oysters was not dependent on the presence of marine snow, and weight-standardized clearance rates of bivalves exposed to TiO₂ nanoparticles were not significantly different than those of unexposed control animals. Both species ingested about half of the nanoparticles to which they were exposed, and >90% of the nanoparticles were egested in feces within 12 h, post-exposure. The findings of this study demonstrate that mussels and oysters can readily ingest both Titan and P25 nanoparticles regardless of the form in which they are encountered, but depurate these materials over a short period of time. Importantly, bioaccumulation of Titan and P25 nanoparticles does not occur in mussels and oysters following exposures of up to 6 h.

Enders, K., Lenz, R., Beer, S., Stedmon, C. A., & Handling editor: Howard, B. (2017). Extraction of microplastic from biota: recommended acidic digestion destroys common plastic polymers. *ICES Journal of Marine Science*, 74(1), 326-331. <https://doi.org/10.1093/icesjms/fsw173>

The chemical digestion of tissue from marine biota for microplastic analysis is currently conducted following a variety of protocols published in scientific literature. Often there is a lack of information on whether and to which degree the applied chemicals are destructive to microplastic particles of various polymer types. In the present study we report that a digestion protocol recently recommended by ICES using nitric and perchloric acid has strong detrimental effects on several common plastic polymers, in particular polyamide and polyurethane and to a lesser degree acrylonitrile butadiene styrene, polymethyl methacrylate and polyvinylchloride. Raman spectroscopic measurements revealed changes in peak occurrence and intensity for several polymers that did not otherwise show visual macroscopic changes. We developed and tested an alkaline digestion protocol in order to preserve small microplastic particles while removing organic tissue material. We recommend this method for the development of guidelines for plastic microplastic monitoring in biota.

European Food Safety Authority (2016). Presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA Journal*, 14(6), e04501. <https://doi.org/10.2903/j.efsa.2016.4501>

Following a request from the German Federal Institute for Risk Assessment (BfR), the EFSA Panel for Contaminants in the Food Chain was asked to deliver a statement on the presence of microplastics and nanoplastics in food, with particular focus on seafood. Primary microplastics are plastics originally manufactured to be that size, while secondary microplastics originate from fragmentation. Nanoplastics

can originate from engineered material or can be produced during fragmentation of microplastic debris. Microplastics range from 0.1 to 5,000 μm and nanoplastics from approximately 1 to 100 nm (0.001-0.1 μm). There is no legislation for microplastics and nanoplastics as contaminants in food. Methods are available for identification and quantification of microplastics in food, including seafood. Occurrence data are limited. In contrast to microplastics no methods or occurrence data in food are available for nanoplastics. Microplastics can contain on average 4% of additives and the plastics can adsorb contaminants. Both additives and contaminants can be of organic as well of inorganic nature. Based on a conservative estimate the presence of microplastics in seafood would have a small effect on the overall exposure to additives or contaminants. Toxicity and toxicokinetic data are lacking for both microplastics and nanoplastics for a human risk assessment. It is recommended that analytical methods should be further developed for microplastics and developed for nanoplastics and standardised, in order to assess their presence, identity and to quantify their amount in food. Furthermore, quality assurance should be in place and demonstrated. For microplastics and nanoplastics, occurrence data in food, including effects of food processing, in particular, for the smaller sized particles ($< 150 \mu\text{m}$) should be generated. Research on the toxicokinetics and toxicity, including studies on local effects in the gastrointestinal (GI) tract, are needed as is research on the degradation of microplastics and potential formation of nanoplastics in the human GI tract.

Goldstein, M. C., & Goodwin, D. S. (2013). Gooseneck barnacles (*Lepas* spp.) ingest microplastic debris in the North Pacific Subtropical Gyre. *PeerJ*, 1, e184. <https://doi.org/10.7717/peerj.184>

Substantial quantities of small plastic particles, termed “microplastic,” have been found in many areas of the world ocean, and have accumulated in particularly high densities on the surface of the subtropical gyres. While plastic debris has been documented on the surface of the North Pacific Subtropical Gyre (NPSG) since the early 1970s, the ecological implications remain poorly understood. Organisms associated with floating objects, termed the “rafting assemblage,” are an important component of the NPSG ecosystem. These objects are often dominated by abundant and fast-growing gooseneck barnacles (*Lepas* spp.), which predate on plankton and larval fishes at the sea surface. To assess the potential effects of microplastic on the rafting community, we examined the gastrointestinal tracts of 385 barnacles collected from the NPSG for evidence of plastic ingestion. We found that 33.5% of the barnacles had plastic particles present in their gastrointestinal tract, ranging from one plastic particle to a maximum of 30 particles. Particle ingestion was positively correlated to capitulum length, and no blockage of the stomach or intestines was observed. The majority of ingested plastic was polyethylene, with polypropylene and polystyrene also present. Our results suggest that barnacle ingestion of microplastic is relatively common, with unknown trophic impacts on the rafting community and the NPSG ecosystem.

Goncalves, C., Martins, M., Costa, M. H., & Costa, P. M. (2018). Development of a method for the detection of polystyrene microplastics in paraffin-embedded histological sections. *Histochemistry and Cell Biology*, 149(2), 187-191. <https://doi.org/10.1007/s00418-017-1613-1>

The concerns about the presence of microplastics (MPs) in marine ecosystems have widely increased in the past years. This is reflected in a growing number of studies addressing the effects of exposure to these materials in indigenous, farmed and even laboratory marine animals subjected to toxicity-oriented bioassays. There have been, however, many constraints in the detection of MPs in biological tissues, as routine histological techniques tend to degrade these materials, which are especially sensitive to organic

solvents. This issue hinders the application of standard histopathological procedures based on convenient paraffin wax-embedding protocols, with consequences for biomonitoring and bioassay procedures. The method described here was developed and validated for the detection of polystyrene microplastics in biological tissue processed for paraffin-based histology. The strategy was developed and tested from whole-soft body sections of marine mussels that internalised the MPs following dedicated bioassays. The protocol is based on the replacement of xylenes with isopropanol for the purpose of intermediate infiltration and deparaffinization. Special modifications for staining, mounting and archiving are needed and are detailed as well. The protocol is shown to be a highly cost- and time-effective procedure compatible with formalin-based fixatives plus standard sectioning and staining, yielding complete preservation of MPs and optimal tissue conditioning. The method also produced excellent results with pre-stained MPs, with fluorochromes included, altogether providing excellent localisation of polystyrene MPs in paraffin-processed biological tissue.

Guilhermino, L., Vieira, L. R., Ribeiro, D., Tavares, A. S., Cardoso, V., Alves, A., & Almeida, J. M. (2018). Uptake and effects of the antimicrobial florfenicol, microplastics and their mixtures on freshwater exotic invasive bivalve *Corbicula fluminea*. *Science of the Total Environment*, 622-623, 1131-1142. <https://doi.org/10.1016/j.scitotenv.2017.12.020>

Microplastics and antimicrobials are widely spread environmental contaminants and more research on their toxicity is needed. The uptake and effects of the antimicrobial florfenicol, microplastics, and their mixtures on *Corbicula fluminea* were investigated. Bivalves were exposed for 96 h to florfenicol (1.8 and 7.1 mg/l), microplastics (0.2 and 0.7 mg/l), or mixtures of the two substances. After 96 h, all bivalves exposed to antimicrobial treatments had florfenicol in their body (e.g. $2 \pm 1 \mu\text{g/g}$). Microplastics were found in the gut, lumen of the digestive gland, connective tissue, hemolymphatic sinuses, and gills surface of animals. Florfenicol caused a significant inhibition of cholinesterase (ChE) activity ($\sim 32\%$). Animals exposed to 0.2 mg/l of microplastics showed ChE activity inhibition (31%), and no other significant alterations. Mixtures caused feeding inhibition (57–83%), significant ChE inhibition (44–57%) and of isocitrate dehydrogenase activity, and increased anti-oxidant enzymes activity and lipid peroxidation levels. Overall, the results indicate that *C. fluminea* take up florfenicol and microplastics from the water and accumulated or at least retained it in their body for some time; both florfenicol (low ppm range) and microplastics (ppb range) were toxic to *C. fluminea*, with mixtures containing florfenicol and microplastics being more toxic. Thus, the risk of exposure and toxic effects of florfenicol to *C. fluminea* and other bivalves, and its predators increase in ecosystems contaminated with the antimicrobial and microplastics, as well as to humans consuming contaminated species from these ecosystems.

Karlsson, T. M., Vethaak, A. D., Almroth, B. C., Ariese, F., van Velzen, M., Hasselov, M., & Leslie, H. A. (2017). Screening for microplastics in sediment, water, marine invertebrates and fish: Method development and microplastic accumulation. *Marine Pollution Bulletin*, 122(1-2), 403-408. <https://doi.org/10.1016/j.marpolbul.2017.06.081>

Measurements of microplastics in biota and abiotic matrices are key elements of exposure and risk assessments for this emerging environmental pollutant. We investigated the abundance of microplastics in field-collected biota, sediment and water. An improved sediment extraction method, based on density separation was developed. For analysis of microplastics in biota we found that an adapted enzymatic digestion protocol using proteinase K performed best, with a 97% recovery of spiked plastic

particles and no observed degradation effects on the plastics in subsequent Raman analysis. Field analysis revealed that 8 of 9 tested invertebrate species from the North Sea and 68% of analyzed individuals of brown trout (*Salmo trutta*) from the Swedish West Coast had microplastics in them. Based on the number of plastic particles per kg d.w. the microplastic concentrations found in mussels were approximately a thousand-fold higher compared to those in sediment and surface water samples from the same location.

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? *Marine Environmental Research*, 66(1), 12-14. <https://doi.org/10.1016/j.marenvres.2008.02.009>

Every day new extraordinary properties of nanoparticles (a billionth of a meter) are discovered and worldwide millions are invested into nanotechnology and nanomaterials. Risks to marine organisms are still not fully understood and biomarkers to detect health effects are not implemented, yet. We used the filter feeding blue mussel as a model to analyse uptake and effects of nanoparticles from glass wool, a new absorbent material suggested for use in floating oil spill barriers. In both, gills and hepatopancreas we analysed uptake of nanomaterials by transmission electron microscopy (TEM) in unstained ultrathin sections over a period of up to 16 days. Lysosomal stability and lipofuscin content as general indicators of cellular pathology and oxidative stress were also measured. As portals of uptake, diffusion and endocytosis were identified resulting in nanoparticle accumulation in endocytotic vesicles, lysosomes, mitochondria and nuclei. Dramatic decrease of lysosomal membrane stability occurred after 12h of exposure. Lysosomal damage was followed by excessive lipofuscin accumulation indicative of severe oxidative stress. Increased phagocytosis by granulocytes, autophagy and finally apoptosis of epithelial cells of gills and primary and secondary digestive tubules epithelial cells indicated progressive cell death. These pathological responses are regarded as general indices of toxic cell injury and oxidative stress. By the combinational use of biomarkers with the ultrastructural localisation of nanoparticle deposition, final evidence of cause-effect relationships is delivered.

Kolandhasamy, P., Su, L., Li, J. N., Qu, X. Y., Jabeen, K., & Shi, H. H. (2018). Adherence of microplastics to soft tissue of mussels: A novel way to uptake microplastics beyond ingestion. *Science of the Total Environment*, 610, 635-640. <https://doi.org/10.1016/j.scitotenv.2017.08.053>

Microplastic pollution is recognized as an emerging threat to aquatic ecosystems. One of the main environmental risks associated with microplastics is their bioavailability to marine organisms. Up to date, ingestion has been widely accepted as the sole way for the animals to uptake microplastics. Nevertheless, microplastics have also been found in some organs which are not involved in the process of ingestion. We hypothesize that the animal might uptake microplastics through adherence in addition to ingestion. To test this hypothesis, we collected mussels from the fishery farms, conducted exposure/clearance experiments and analyzed the accumulation of microplastics in specific organ of mussels. Our studies clearly showed the uptake of microplastic in multiple organs of mussels. In the field investigations, we found that the abundance of microplastic by weight but not by individual showed significant difference among organs, and the intestine contained the highest level of microplastics (9.2 items/g). In the uptake and clearance experiment, the accumulation and retention of microfibers could also be observed in all tested organs of mussels including foot and mantle. Our results strongly suggest that adherence rather than ingestion led to the accumulation of microplastics in those organs which are not involved in ingestion process. To our best knowledge, it is the first time to propose that adherence is

a novel way for animals to uptake microplastics beyond ingestion. This new finding makes us rethink about the bioavailability, accumulation and toxicity of microplastics to aquatic animals. (C) 2017 Elsevier B.V. All rights reserved.

Leslie, H. A., Brandsma, S. H., van Velzen, M. J. M., & Vethaak, A. D. (2017). Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. *Environment International*, 101, 133-142.
<https://doi.org/10.1016/j.envint.2017.01.018>

Environmental contamination by plastic particles, also known as 'microplastics', brings synthetic materials that are non-degradable and biologically incompatible into contact with ecosystems. In this paper we present Concentration data for this emerging contaminant in wastewater treatment plants (W\NWs) and freshwater and marine systems, reflecting the routes via which these particles can travel and the ecosystems they potentially impact along their path. Raw sewage influents, effluents and sewage sludge from seven municipal WWTTPs in the Netherlands contained mean particle concentrations of 68-910 L⁻¹, 51-81 L⁻¹ and 510-760 kg⁽⁻¹⁾, wet weight (ww), respectively (particle sizes between 10 and 5000 pm). Even after treatment, wastewater constitutes a source of microplastic pollution of surface waters, and via biosolids applications in farming and forestry, plastic retained in sewage sludge can be transferred to terrestrial environments. The WWTTPs investigated here had a mean microplastics retention efficiency of 72% (s.d. 61%) in the sewage sludge. In the receiving waters of treated and untreated wastewaters, we detected high microplastic levels in riverine suspended particulate matter (1400-4900 kg⁽⁻¹⁾ dry weight (dW)) from the Rhine and Meuse rivers. Amsterdam canal water sampled at different urban locations contained microplastic concentrations (48-187 L⁻¹), similar to those observed in wastewater that is emitted from sewage treatment facilities in the area. At least partial settling of the particles occurs in freshwater as well, as indicated by microplastics in urban canal sediments (< 68 to 10,500 particles kg⁽⁻¹⁾ dw). Microplastics in suspension in the water column have the potential to be discharged into the sea with other riverine suspended particulates. We report microplastic concentrations from 100 up to 3600 particles kg⁽⁻¹⁾ dry sediment collected at 15 locations along the Dutch North Sea coast. The high microplastic enrichment in marine sediments compared to most literature data for seawater at the surface supports the hypothesis of a seabed sink for these materials. Marine species are heavily exposed to plastic particles. Body residues between 10 and 100 particles g⁽⁻¹⁾ dw were measured in benthic macroinvertebrate species inhabiting the Dutch North Sea coast: filter-feeding mussels and oysters (species for human consumption) as well as other consumers in the marine food chain.

Li, H.-X., Ma, L.-S., Lin, L., Ni, Z.-X., Xu, X.-R., Shi, H.-H., . . . Rittschof, D. (2018). Microplastics in oysters *Saccostrea cucullata* along the Pearl River Estuary, China. *Environmental Pollution*, 236, 619-625.
<https://doi.org/10.1016/j.envpol.2018.01.083>

As a transitional zone between riverine and marine environments, an estuary plays an important role for the sources, accumulation and transport of microplastics. Although estuarine environments are hotspots of microplastic pollution, the correlation between microplastic pollution and aquatic organisms is less known. Here we investigated microplastic pollution in wild oysters *Saccostrea cucullata* from 11 sampling sites along the Pearl River Estuary in South China. The microplastic abundances in oysters ranged from 1.4 to 7.0 items per individual or from 1.5 to 7.2 items per gram tissue wet weight, which were positively related to those in surrounding waters. The oysters near urban areas contained

significantly more microplastics than those near rural areas. Fibers accounted for 69.4% of the total microplastics in oysters. Microplastic sizes varied from 20 to 5000 μm and 83.9% of which were less than 100 μm . Light color microplastics were significantly more common than dark color ones. Based on the results, oysters are recommended as a biomonitor for the microplastic pollution in estuaries.

Li, J., Yang, D., Li, L., Jabeen, K., & Shi, H. (2015). Microplastics in commercial bivalves from China. *Environmental Pollution*, 207, 190-195. <https://doi.org/10.1016/j.envpol.2015.09.018>

We investigated microplastic pollution in 9 commercial bivalves from a fishery market in China. Multiple types of microplastics, including fibers, fragments and pellets, occurred in the tissue of all bivalves. The number of total microplastics varied from 2.1 to 10.5 items/g and from 4.3 to 57.2 items/individual for bivalves. *Scapharca subcrenata* contained on average 10.5 items/g and exhibited the highest levels of microplastics by weight. Fibers were the most common microplastics and consisted of more than half of the total microplastics in each of the 8 species. In *Alectryonella plicatula*, pellets accounted for 60% of the total microplastics. The most common size class was less than 250 μm and accounted for 33.84% of the total microplastics calculated by species. Our results suggest that microplastic pollution was widespread and exhibited a relatively high level in commercial bivalves from China. More intensive investigations on microplastics should be conducted in seafood.

Li, J. N., Qu, X. Y., Su, L., Zhang, W. W., Yang, D. Q., Kolandhasamy, P., . . . Shi, H. H. (2016). Microplastics in mussels along the coastal waters of China. *Environmental Pollution*, 214, 177-184. <https://doi.org/10.1016/j.envpol.2016.04.012>

Microplastic has been confirmed as an emerging pollutant in marine environments. One of the primary environmental risks of microplastics is their bioavailability for aquatic organisms. Bivalves are of particular interest because their extensive filter-feeding activity exposes them directly to microplastics present in the water column. In the present study, we investigated microplastic pollution in mussels (*Mytilus edulis*) from 22 sites along 12,400 mile coastlines of China in 2015. The number of total microplastics varied from 0.9 to 4.6 items/g and from 1.5 to 7.6 items/individual. *M. edulis* contained more microplastics (2.7 items/g) in wild groups than that (1.6 items/g) in farmed groups. The abundance of microplastics was 3.3 items/g in mussels from the areas with intensive human activities and significantly higher than that (1.6 items/g) with less human activities. The most common microplastics were fibers, followed by fragments. The proportion of microplastics less than 250 μm in size arranged from 17% to 79% of the total microplastics. Diatom was distinguished from microplastics in mussels for the first time using Scanning Electron Microscope. Our results suggested that the numbers of microplastic kept within a relatively narrow range in mussels and were closely related to the contamination of the environments. We proposed that mussels could be used as a potential bioindicator of microplastic pollution of the coastal environment.

Löder, M. G. J., & Gerdtz, G. (2015). Methodology Used for the Detection and Identification of Microplastics—A Critical Appraisal. In *Marine Anthropogenic Litter* (pp. 201-227).

Microplastics in aquatic ecosystems and especially in the marine environment represent a pollution of increasing scientific and societal concern, thus, recently a substantial number of studies on microplastics were published. Although first steps towards a standardization of methodologies used for the detection

and identification of microplastics in environmental samples are made, the comparability of data on microplastics is currently hampered by a huge variety of different methodologies, which result in the generation of data of extremely different quality and resolution. This chapter reviews the methodology presently used for assessing the concentration of microplastics in the marine environment with a focus on the most convenient techniques and approaches. After an overview of non-selective sampling approaches, sample processing and treatment in the laboratory, the reader is introduced to the currently applied techniques for the identification and quantification of microplastics. The subsequent case study on microplastics in sediment samples from the North Sea measured with focal plane array (FPA)-based micro-Fourier transform infrared (micro-FTIR) spectroscopy shows that only 1.4 % of the particles visually resembling microplastics were of synthetic polymer origin. This finding emphasizes the importance of verifying the synthetic polymer origin of potential microplastics. Thus, a burning issue concerning current microplastic research is the generation of standards that allow for the assessment of reliable data on concentrations of microscopic plastic particles and the involved polymers with analytical laboratory techniques such as micro-FTIR or micro-Raman spectroscopy.

Lusher, A. L., Welden, N. A., Sobral, P., & Cole, M. (2017). Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Analytical Methods*, 9(9), 1346-1360.
<https://doi.org/10.1039/c6ay02415g>

Microplastic debris (< 5 mm) is a prolific environmental pollutant, found worldwide in marine, freshwater and terrestrial ecosystems. Interactions between biota and microplastics are prevalent, and there is growing evidence that microplastics can incite significant health effects in exposed organisms. To date, the methods used to quantify such interactions have varied greatly between studies. Here, we critically review methods for sampling, isolating and identifying microplastics ingested by environmentally and laboratory exposed fish and invertebrates. We aim to draw attention to the strengths and weaknesses of the suite of published microplastic extraction and enumeration techniques. Firstly, we highlight the risk of microplastic losses and accumulation during biotic sampling and storage, and suggest protocols for mitigating contamination in the field and laboratory. We evaluate a suite of methods for extracting microplastics ingested by biota, including dissection, depuration, digestion and density separation. Lastly, we consider the applicability of visual identification and chemical analyses in categorising microplastics. We discuss the urgent need for the standardisation of protocols to promote consistency in data collection and analysis. Harmonized methods will allow for more accurate assessment of the impacts and risks microplastics pose to biota and increase comparability between studies.

Mathalon, A., & Hill, P. (2014). Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Marine Pollution Bulletin*, 81(1), 69-79.
<https://doi.org/10.1016/j.marpolbul.2014.02.018>

Humans continue to increase the use and disposal of plastics by producing over 240 million tonnes per year, polluting the oceans with persistent waste. The majority of plastic in the oceans are microplastics (<5mm). In this study, the contamination of microplastic fibers was quantified in sediments from the intertidal zones of one exposed beach and two protected beaches along Nova Scotia's Eastern Shore. From the two protected beaches, polychaete worm fecal casts and live blue mussels (*Mytilus edulis*) were analyzed for microplastic content. Store-bought mussels from an aquaculture site were also analyzed. The average microplastic abundance observed from 10g sediment subsamples was between

20 and 80 fibers, with higher concentrations at the high tide line from the exposed beach and at the low tide line from the protected beaches. Microplastic concentrations from polychaete fecal casts resembled concentrations quantified from low tide sediments. In two separate mussel analyses, significantly more microplastics were enumerated in farmed mussels compared to wild ones.

Naji, A., Nuri, M., & Vethaak, A. D. (2018). Microplastics contamination in molluscs from the northern part of the Persian Gulf. *Environmental Pollution*, 235, 113-120.
<https://doi.org/10.1016/j.envpol.2017.12.046>

Microplastics (MPs) are well-known emerging contaminants in the marine environment. A key route by which MPs can directly affect marine life is through ingestion. The objective of the present study was to evaluate the occurrence of MPs in marine life and seafood for human consumption in the Persian Gulf. We conducted a whole body analysis of MP (between 10 and 5000 µm in diameter) abundance in five species of molluscs with different feeding strategies, including both gastropods and bivalves from the littoral zone of the Iranian coast of the Persian Gulf. The mean number of total encountered MPs in all species ranged from 0.2 to 21.0 particles per g of soft tissue (wet weight) and from 3.7 to 17.7 particles per individual. Overall, microfibrils followed by fragments were the most common type of MP isolated in each species (respectively > 50% and 26%). Film (14%) and pellets (2%) were less commonly observed. The observed MPs were classified into three size groups (ca. 10-25 µm, 25-250 µm and 250-5000 µm), and 58% of MPs fell into the smallest size group. Fourier transform infrared (FT-IR) analysis confirmed the presence of polyethylene (PE), polyethylene terephthalate (PET), and nylon (PA). Our results indicated that molluscan shellfish from the Persian Gulf contain MPs, with higher concentrations in a predatory species, suggesting trophic transfer of MPs in the food web. The consumption of edible species may be a source of human microplastic intake. We compared our results with those previously reported for other regions of the world and identified the need for further studies in the Persian Gulf.

Paul-Pont, I., Lacroix, C., Fernandez, C. G., Hegaret, H., Lambert, C., Le Goic, N., . . . Soudant, P. (2016). Exposure of marine mussels *Mytilus* spp. to polystyrene microplastics: Toxicity and influence on fluoranthene bioaccumulation. *Environmental Pollution*, 216, 724-737.
<https://doi.org/10.1016/j.envpol.2016.06.039>

The effects of polystyrene microbeads (micro-PS; mix of 2 and 6 µm; final concentration: 32 µg L⁻¹) alone or in combination with fluoranthene (30 µg L⁻¹) on marine mussels *Mytilus* spp. were investigated after 7 days of exposure and 7 days of depuration under controlled laboratory conditions. Overall, fluoranthene was mostly associated to algae *Chaetoceros muelleri* (partition coefficient Log K_p = 4.8) used as a food source for mussels during the experiment. When micro-PS were added in the system, a fraction of FLU transferred from the algae to the microbeads as suggested by the higher partition coefficient of micro-PS (Log K_p = 6.6), which confirmed a high affinity of fluoranthene for polystyrene microparticles. However, this did not lead to a modification of fluoranthene bioaccumulation in exposed individuals, suggesting that micro-PS had a minor role in transferring fluoranthene to mussels tissues in comparison with waterborne and foodborne exposures. After depuration, a higher fluoranthene concentration was detected in mussels exposed to micro-PS and fluoranthene, as compared to mussels exposed to fluoranthene alone. This may be related to direct effect of micro-PS on detoxification mechanisms, as suggested by a down regulation of a P-glycoprotein involved in pollutant

excretion, but other factors such as an impairment of the filtration activity or presence of remaining beads in the gut cannot be excluded. Micro-PS alone led to an increase in hemocyte mortality and triggered substantial modulation of cellular oxidative balance: increase in reactive oxygen species production in hemocytes and enhancement of anti-oxidant and glutathione-related enzymes in mussel tissues. Highest histopathological damages and levels of anti-oxidant markers were observed in mussels exposed to micro-PS together with fluoranthene. Overall these results suggest that under the experimental conditions of our study micro-PS led to direct toxic effects at tissue, cellular and molecular levels, and modulated fluoranthene kinetics and toxicity in marine mussels.

Phuong, N. N., Poirier, L., Pham, Q. T., Lagarde, F., & Zalouk-Vergnoux, A. (2017). Factors influencing the microplastic contamination of bivalves from the French Atlantic coast: Location, season and/or mode of life? *Marine Pollution Bulletin*. <https://doi.org/10.1016/j.marpolbul.2017.10.054>

Monitoring the presence of microplastics (MP) in marine organisms is currently of high importance. This paper presents the qualitative and quantitative MP contamination of two bivalves from the French Atlantic coasts: the blue mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas*). Three factors potentially influencing the contamination were investigated by collecting at different sampling sites and different seasons, organisms both wild and cultivated. Inter- and intra-species comparisons were also achieved. MP quantity in organisms was evaluated at 0.61 ± 0.56 and 2.1 ± 1.7 MP per individual respectively for mussels and oysters. Eight different polymers were identified. Most of the MPs were fragments; about a half of MPs were grey colored and a half with a size ranging from 50 to 100 μm for both studied species. Some inter-specific differences were found but no evidence for sampling site, season or mode of life effect was highlighted.

Phuong, N. N., Zalouk-Vergnoux, A., Kamari, A., Mouneyrac, C., Amiard, F., Poirier, L., & Lagarde, F. (2018). Quantification and characterization of microplastics in blue mussels (*Mytilus edulis*): protocol setup and preliminary data on the contamination of the French Atlantic coast. *Environmental Science and Pollution Research*, 25(7), 6135-6144. <https://doi.org/10.1007/s11356-017-8862-3>

Microplastics (MPs) constitute a main environmental issue due to their threat to marine organisms and so far to humans. The lack of a fast standard protocol in MP isolation and identification from living organisms bring to challenge for the science. In this paper, an optimized protocol using potassium hydroxide 10%(KOH 10%; m/v) for digestion of mussel soft tissues (*Mytilus edulis*) and multi-steps of sedimentation has been developed. Efficiency higher than 99.9% of organic and mineral matter elimination was shown by application on mussels sampled on the French Atlantic coast. The identification of MPs was performed by FTIR microscopy straight on the filter and the whole analysis can be compatible with a routine goal. Fourteen MPs of four different chemical natures were found and identified in 5 pools of 3 sampled mussels. Their size ranged from 30 to 200 μm . Further investigations are now needed to evaluate the potential risk of such particles within this marine bivalve species and other filter feeders.

Picot-Groz, M., Fenet, H., Bueno, M. J. M., Rosain, D., & Gomez, E. (2018). Diurnal variations in personal care products in seawater and mussels at three Mediterranean coastal sites. *Environmental Science and Pollution Research*, 25(9), 9051-9059. <https://doi.org/10.1007/s11356-017-1100-1>

The presence of personal care products (PCPs) in the marine environment is of major concern. PCPs, UV filters, and musks can enter the marine environment indirectly through wastewater or directly via recreational activities. We conducted this study to document patterns in the occurrence of seven PCPs at three coastal sites impacted by recreational activities during 1 day. The study focused on diurnal variations in these seven PCPs in seawater and indigenous mussels. In seawater, UV filters showed diurnal variations that mirrored variations in recreational activities at the sites. Ethylhexyl methoxycinnamate (EHMC) and octocrylene (OC) water concentrations increased from under the limit of quantification in the morning to 106 and 369 ng/L, respectively, when recreational activities were the highest. In mussels, diurnal variations in OC were observed, with the lowest concentrations recorded in the morning and then increasing throughout the day. As *Mytilus* spp. are widely used as sentinels in coastal pollution monitoring programs (mussel watch), our findings on diurnal variations could enhance sampling recommendations for recreational sites impacted by PCPs.

Qu, X., Su, L., Li, H., Liang, M., & Shi, H. (2018). Assessing the relationship between the abundance and properties of microplastics in water and in mussels. *Science of the Total Environment*, 621, 679-686. <https://doi.org/10.1016/j.scitotenv.2017.11.284>

Microplastic pollution is increasingly becoming a great environmental concern worldwide. Microplastics have been found in many marine organisms as a result of increasing plastic pollution within marine environments. However, the relationship between microplastics in organisms and their living environment is still relatively poorly understood. In the present study, we investigated microplastic pollution in the water and the mussels (*Mytilus edulis*, *Perna viridis*) at 25 sites along the coastal waters of China. We also, for the first time, conducted an exposure experiment in parallel on the same site using *M. edulis* in the laboratory. A strong positive linear relationship was found between microplastic levels in the water and in the mussels. Fibers were the dominant microplastics. The sizes of microplastics in the mussels were smaller than those in the water. During exposure experiments, the abundance of microbeads was significantly higher than that of fibers, even though the nominal abundance of fibers was eight times that of microbeads. In general, our results supported positive and quantitative correlations of microplastics in mussels and in their surrounding waters and that mussels were more likely to ingest smaller microplastics. Laboratory exposure experiment is a good way to understand the relative impacts of microplastics ingested by marine organisms. However, significant differences in the results between exposure experiments and field investigations indicated that further efforts are needed to simulate the diverse environmentally relevant properties of microplastics.

Ribeiro, F., Garcia, A. R., Pereira, B. P., Fonseca, M., Mestre, N. C., Fonseca, T. G., . . . Bebianno, M. J. (2017). Microplastics effects in *Scrobicularia plana*. *Marine Pollution Bulletin*, 122(1-2), 379-391. <https://doi.org/10.1016/j.marpolbul.2017.06.078>

One of the most common plastics in the marine environment is polystyrene (PS) that can be broken down to micro sized particles. Marine organisms are vulnerable to the exposure to microplastics. This study assesses the effects of PS microplastics in tissues of the clam *Scrobicularia plana*. Clams were exposed to 1 mg L⁻¹(20 µm) for 14 days, followed by 7 days of depuration. A qualitative analysis by

infrared. spectroscopy in diffuse reflectance mode period detected the presence of microplastics in clam tissues upon exposure, which were not eliminated after depuration. The effects of microplastics were assessed by a battery of biomarkers and results revealed that microplastics induce effects on antioxidant capacity, DNA damage, neurotoxicity and oxidative damage. S. plan is a significant target to assess the environmental risk of PS microplastics.

Rocha-Santos, T., & Duarte, A. C. (2015). A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. *TrAC Trends in Analytical Chemistry*, 65, 47-53. <https://doi.org/https://doi.org/10.1016/j.trac.2014.10.011>

Plastics can be found in food packaging, shopping bags, and household items, such as toothbrushes and pens, and facial cleansers. Due to the high disposability and low recovery of discharged materials, plastics materials have become debris accumulating in the environment. Microplastics have a dimension <5mm and possess physico-chemical properties (e.g., size, density, color and chemical composition) that are key contributors to their bioavailability to organisms. This review addresses the analytical approaches to characterization and quantification of microplastics in the environment and discusses recent studies on their occurrence, fate, and behavior. This critical overview includes a general assessment of sampling and sample handling, and compares methods for morphological and physical classification, and methodologies for chemical characterization and quantification of the microplastics. Finally, this review addresses the advantages and the disadvantages of these techniques, and comments on future applications and potential research interest within this field.

Santana, M. F. M., Ascer, L. G., Custodio, M. R., Moreira, F. T., & Turra, A. (2016). Microplastic contamination in natural mussel beds from a Brazilian urbanized coastal region: Rapid evaluation through bioassessment. *Marine Pollution Bulletin*, 106(1-2), 183-189. <https://doi.org/10.1016/j.marpolbul.2016.02.074>

Microplastic pollution (particles <5 mm) is a widespread marine threat and a trigger for biological effects, especially if ingested. The mussel *Perna perna*, an important food resource, was used as bioindicator to investigate the presence of microplastic pollution on Santos estuary, the most urbanized area of the coast of Sao Paulo State, Brazil. A simple and rapid assessment showed that 75% of sampled mussels had ingested microplastics, an issue of human and environmental concern. All sampling points had contaminated mussels and this contamination had no clear pattern of distribution along the estuary. This was the first time that microplastic bioavailability was assessed in nature for the southern hemisphere and that wild *P. perna* was found contaminated with this pollutant. This is an important issue that should be better assessed due to an increase in seafood consumption and culture in Brazil and worldwide.

Setälä, O., Norkko, J., & Lehtiniemi, M. (2016). Feeding type affects microplastic ingestion in a coastal invertebrate community. *Marine Pollution Bulletin*, 102(1), 95-101. <https://doi.org/https://doi.org/10.1016/j.marpolbul.2015.11.053>

Marine litter is one of the problems marine ecosystems face at present, coastal habitats and food webs being the most vulnerable as they are closest to the sources of litter. A range of animals (bivalves, free swimming crustaceans and benthic, deposit-feeding animals), of a coastal community of the northern

Baltic Sea were exposed to relatively low concentrations of 10µm microbeads. The experiment was carried out as a small scale mesocosm study to mimic natural habitat. The beads were ingested by all animals in all experimental concentrations (5, 50 and 250beadsmL⁻¹). Bivalves (*Mytilus trossulus*, *Macoma balthica*) contained significantly higher amounts of beads compared with the other groups. Free-swimming crustaceans ingested more beads compared with the benthic animals that were feeding only on the sediment surface. Ingestion of the beads was concluded to be the result of particle concentration, feeding mode and the encounter rate in a patchy environment.

Su, L., Cai, H. W., Kolandhasamy, P., Wu, C. X., Rochman, C. M., & Shi, H. H. (2018). Using the Asian clam as an indicator of microplastic pollution in freshwater ecosystems. *Environmental Pollution*, 234, 347-355. <https://doi.org/10.1016/j.envpol.2017.11.075>

Bioindicators play an important role in understanding pollution levels, bioavailability and the ecological risks of contaminants. Several bioindicators have been suggested for understanding microplastic in the marine environment. A bioindicator for microplastics in the freshwater environment does not exist. In our previous studies, we found a high frequency of microplastic pollution in the Asian clam (*Corbicula fluminea*) in Taihu Lake, China. In the present study, we conducted a large-scale survey of microplastic pollution in Asian clams, water and sediment from 21 sites in the Middle-Lower Yangtze River Basin from August to October of 2016. The Asian clam was available in all sites, which included diverse freshwater systems such as lakes, rivers and estuaries. Microplastics were found at concentrations ranging from 0.34-9 items/g (or 0.4-5.0 items/individual) in clams, 0.5-3.1 items/L in water and 15-160 items/kg in sediment. Microfibers were the most dominant types of microplastics found, accounting for 60-100% in clams across all sampling sites. The size of microplastics ranged from 0.021-4.83 mm, and microplastics in the range of 0.25-1 mm were dominant. The abundance, size distribution and color patterns of microplastics in clams more closely resembled those in sediment than in water. Because microplastic pollution in the Asian clam reflected the variability of microplastic pollution in the freshwater environments, we demonstrated the Asian clam as an bioindicator of microplastic pollution in freshwater systems, particularly for sediments.

Taylor, M. L., Gwinnett, C., Robinson, L. F., & Woodall, L. C. (2016). Plastic microfibre ingestion by deep-sea organisms. *Scientific Reports*, 6. <https://doi.org/10.1038/srep33997>

Plastic waste is a distinctive indicator of the world-wide impact of anthropogenic activities. Both macroand micro-plastics are found in the ocean, but as yet little is known about their ultimate fate and their impact on marine ecosystems. In this study we present the first evidence that microplastics are already becoming integrated into deep-water organisms. By examining organisms that live on the deep-sea floor we show that plastic microfibres are ingested and internalised by members of at least three major phyla with different feeding mechanisms. These results demonstrate that, despite its remote location, the deep sea and its fragile habitats are already being exposed to human waste to the extent that diverse organisms are ingesting microplastics.

Thushari, G. G. N., Senevirathna, J. D. M., Yakupitiyage, A., & Chavanich, S. (2017). Effects of microplastics on sessile invertebrates in the eastern coast of Thailand: An approach to coastal zone conservation. *Marine Pollution Bulletin*, 124(1), 349-355. <https://doi.org/10.1016/j.marpolbul.2017.06.010>

This study assessed the microplastic contamination of 3 most abundant sessile and intertidal invertebrates (Rock Oyster: *Saccostrea forskalii*, Striped Barnacle: *Balanus amphitrite*, Periwinkle: *Littoraria* sp.) in 3 beaches of the eastern coasts of Thailand. The results showed a significant accumulation of microplastics in the invertebrates at rates of 0.2-0.6 counts/g indicating higher pollution levels along the coastline. Filter feeding organisms showed comparatively higher accumulation rates of microplastics. Thus, contaminated bivalves pose potential health risks for seafood consumers. The plastic pollutant prevalence in sessile and intertidal communities was corresponded with pollution characteristics of contaminated beach habitats where they live. Thus, bivalves, gastropods and barnacles can be used as indicators for contamination of microplastics in the areas. This study also demonstrated the need for controlling plastic pollution in Thai coastal areas.

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M. B., & Janssen, C. R. (2015). Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. *Environmental Pollution*, 199, 10-17. <https://doi.org/10.1016/j.envpol.2015.01.008>

We studied the uptake of microplastics under field conditions. At six locations along the French Belgian - Dutch coastline we collected two species of marine invertebrates representing different feeding strategies: the blue mussel *Mytilus edulis* (filter feeder) and the lugworm *Arenicola marina* (deposit feeder). Additional laboratory experiments were performed to assess possible (adverse) effects of ingestion and translocation of microplastics on the energy metabolism (cellular energy allocation) of these species. Microplastics were present in all organisms collected in the field: on average 0.2 +/- 0.3 microplastics g(-1) (*M. edulis*) and 1.2 +/- 2.8 particles g(-1) (*A. marina*). In a proof of principle laboratory experiment, mussels and lugworms exposed to high concentrations of polystyrene microspheres (110 particles mL(-1) seawater and 110 particles g(-1) sediment, respectively) showed no significant adverse effect on the organisms' overall energy budget. The results are discussed in the context of possible risks as a result of the possible transfer of adsorbed contaminants.

Van Cauwenberghe, L., & Janssen, C. R. (2014). Microplastics in bivalves cultured for human consumption. *Environmental Pollution*, 193, 65-70. <https://doi.org/10.1016/j.envpol.2014.06.010>

Microplastics are present throughout the marine environment and ingestion of these plastic particles (<1 mm) has been demonstrated in a laboratory setting for a wide array of marine organisms. Here, we investigate the presence of microplastics in two species of commercially grown bivalves: *Mytilus edulis* and *Crassostrea gigas*. Microplastics were recovered from the soft tissues of both species. At time of human consumption, *M. edulis* contains on average 0.36 +/- 0.07 particles g(-1) (wet weight), while a plastic load of 0.47 +/- 0.16 particles g(-1) ww was detected in *C. gigas*. As a result, the annual dietary exposure for European shellfish consumers can amount to 11,000 microplastics per year. The presence of marine microplastics in seafood could pose a threat to food safety, however, due to the complexity of estimating microplastic toxicity, estimations of the potential risks for human health posed by microplastics in food stuffs is not (yet) possible.

Vandermeersch, G., Van Cauwenberghe, L., Janssen, C. R., Marques, A., Granby, K., Fait, G., . . . Devriese, L. (2015). A critical view on microplastic quantification in aquatic organisms. *Environmental Research*, 143, 46-55. <https://doi.org/10.1016/j.envres.2015.07.016>

Microplastics, plastic particles and fragments smaller than 5 mm, are ubiquitous in the marine environment. Ingestion and accumulation of microplastics have previously been demonstrated for diverse marine species ranging from zooplankton to bivalves and fish, implying the potential for microplastics to accumulate in the marine food web. In this way, microplastics can potentially impact food safety and human health. Although a few methods to quantify microplastics in biota have been described, no comparison and/or intercalibration of these techniques have been performed. Here we conducted a literature review on all available extraction and quantification methods. Two of these methods, involving wet acid destruction, were used to evaluate the presence of microplastics in field-collected mussels (*Mytilus galloprovincialis*) from three different "hotspot" locations in Europe (Po estuary, Italy; Tagus estuary, Portugal; Ebro estuary, Spain). An average of 0.18 +/- 0.14 total microplastics g(-1) w.w. for the Acid mix Method and 0.12 +/- 0.04 total microplastics g(-1) w.w. for the Nitric acid Method was established. Additionally, in a pilot study an average load of 0.13 +/- 0.14 total microplastics g(-1) w.w. was recorded in commercial mussels (*Mytilus edulis* and *M. galloprovincialis*) from five European countries (France, Italy, Denmark, Spain and The Netherlands). A detailed analysis and comparison of methods indicated the need for further research to develop a standardised operating protocol for microplastic quantification and monitoring.

von Moos, N., Burkhardt-Holm, P., & Kohler, A. (2012). Uptake and Effects of Microplastics on Cells and Tissue of the Blue Mussel *Mytilus edulis* L. after an Experimental Exposure. *Environmental Science & Technology*, 46(20), 11327-11335. <https://doi.org/10.1021/es302332w>

In this study, we investigated if industrial high-density polyethylene (HDPE) particles, a model microplastic free of additives, ranging > 0-80 µm are ingested and taken up into the cells and tissue of the blue mussel *Mytilus edulis* L. The effects of exposure (up to 96 h) and plastic ingestion were observed at the cellular and subcellular level. Microplastic uptake into the gills and digestive gland was analyzed by a new method using polarized light microscopy. Mussel health status was investigated incorporating histological assessment and cytochemical biomarkers of toxic effects and early warning. In addition to being drawn into the gills, HDPE particles were taken up into the stomach and transported into the digestive gland where they accumulated in the lysosomal system after 3 h of exposure. Our results show notable histological changes upon uptake and a strong inflammatory response demonstrated by the formation of granulocytomas after 6 h and lysosomal membrane destabilization, which significantly increased with longer exposure times. We provide proof of principle that microplastics are taken up into cells and cause significant effects on the tissue and cellular level, which can be assessed with standard cytochemical biomarkers and polarized light microscopy for microplastic tracking in tissue.

Wang, L., Sun, H., Yang, L., He, C., Wu, W., & Sun, S. (2010). Liquid chromatography/mass spectrometry analysis of perfluoroalkyl carboxylic acids and perfluorooctanesulfonate in bivalve shells: Extraction method optimization. *Journal of Chromatography A*, 1217(4), 436-442. <https://doi.org/10.1016/j.chroma.2009.11.074>

Different extraction methods, including extraction by organic solvents with and without acetic acid digestion, and mixed inorganic acid digestion coupled with solid phase extraction (SPE), were developed for the analysis of perfluorinated carboxylic acids (PFCAs) and perfluorooctanesulfonate (PFOS) in bivalve shells. The extracts were separated, identified and quantified by liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS). The method utilizing mixed acid digestion coupled with SPE performed more efficiently than other extraction methods. Matrix recoveries of the optimized methods ranged from 92% to 104%, with limits of detection of 0.05–0.43 ng/g. The optimized method was successfully applied to the analysis of PFCAs and PFOS in shell samples of two bivalves from Bohai Bay, China. PFCAs and PFOS concentrations in the shells ranged from 0.3 ng/g to 4.1 ng/g, 1–50 times lower than those in the soft tissues of bivalves for most target analytes. No relationship between PFCAs and PFOS in shells and in soft tissues was found; this is explained by the different contaminant uptake mechanism of shells and soft tissues.

Wang, L., Zhang, J. J., Hou, S. G., & Sun, H. W. (2017). A Simple Method for Quantifying Polycarbonate and Polyethylene Terephthalate Microplastics in Environmental Samples by Liquid Chromatography-Tandem Mass Spectrometry. *Environmental Science & Technology Letters*, 4(12), 530-534. <https://doi.org/10.1021/acs.estlett.7b00454>

Microplastics (MPs) have frequently been found in the environment. However, studies of the quantification methods for MPs are still needed. Plastics are polymers with different degrees of polymerization. In this study, alkali-assisted thermal hydrolysis was applied to depolymerize two plastics containing ester groups, polycarbonate (PC) and polyethylene terephthalate (PET), in a pentanol or butanol system. By determining the concentrations of the depolymerized building block compounds, i.e., bisphenol A and p-phthalic acid, we quantified the amounts of PC and PET MPs in environmental samples. Recoveries of 87.2–97.1% were obtained for the PC and PET plastic particles spiked in the landfill sludge. The method was successfully applied to determine the occurrence of PC and PET MPs in samples of sludge, marine sediments, indoor dust, digestive residues in mussel and clam, and sea salt and rock salt. High concentrations of 246 and 430 mg/kg were determined for PC and PET type MPs, respectively, in an indoor dust. In addition, concentrations of 63.7 mg/kg for PC and 127 mg/kg for PET were detected in the digestive residues of a clam.

Ward, J. E., & Kach, D. J. (2009). Marine aggregates facilitate ingestion of nanoparticles by suspension-feeding bivalves. *Marine Environmental Research*, 68(3), 137-142. <https://doi.org/10.1016/j.marenvres.2009.05.002>

As the application of nanomaterials to science and technology grows, the need to understand any ecotoxicological effects becomes increasingly important. Recent studies on a few species of fishes and invertebrates have provided data which suggest that harmful effects are possible. The way in which nanoparticles are taken up by aquatic organisms, however, has been little studied. We examined uptake of nanoparticles by two species of suspension-feeding bivalves (mussels, *Mytilus edulis*; oysters, *Crassostrea virginica*), which capture individual particles <1µm with a retention efficiency of <15%. Given this limitation, it would appear that nanoparticles could not be ingested in large numbers. During certain times of the year, however, >70% of suspended particles are incorporated within aggregates that are >100µm in size. Therefore, we delivered bivalves fluorescently labeled, 100-nm polystyrene beads that were either (1) dispersed or (2) embedded within aggregates generated in the laboratory. Results indicate that aggregates significantly enhance the uptake of 100-nm particles. Nanoparticles had a

longer gut retention time than 10- μ m polystyrene beads suggesting that nanoparticles were transported to the digestive gland. Our data suggest a mechanism for significant nanoparticle ingestion, and have implications for toxicological effects and transfer of nanomaterials to higher trophic levels.

Wegner, A., Besseling, E., Foekema, E. M., Kamermans, P., & Koelmans, A. A. (2012). Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). *Environmental Toxicology and Chemistry*, 31(11), 2490-2497. <https://doi.org/10.1002/etc.1984>

As the industrial production of nanoplastic and the degradation of microplastic into smaller particles at sea increase, the potential amount of nanoplastics in the marine environment rises. It has been reported that mussels uptake 100-nm polystyrene (PS) beads; to date, however, the effects of this uptake on the organism are unknown. In the present study, the authors investigated the effects of 30-nm PS on the feeding behavior of the blue mussel (*Mytilus edulis*) by exposing the organism to different nano PS and different algae (*Pavlova lutheri*) concentrations. The state of nano PS aggregation in the exposure medium was assessed using dynamic light scattering. In all treatments that contained nano PS, *M. edulis* produced pseudofeces. The total weight of the feces and pseudofeces increased with increasing nano PS and increasing algae concentration. Furthermore, *M. edulis* reduced its filtering activity when nano PS was present but still caused a decrease in the apparent nano PS concentration in the water. The presence of nano PS around the foot of *M. edulis* after the bioassay confirmed that the organism removed nano PS from the water. Chronic effect studies are therefore needed to investigate the effects of nanoplastics in *M. edulis* and possible consequences for its predators, including humans.