**Accumulation of marine microplastics along a trophic gradient as determined by an Agent-Based Model**

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**Abstract**

Microplastics are ubiquitous in the marine environment and are now consistently found in almost all marine animals. This study examines the rate of accumulation in a modelled filter feeder (mussels) both from direct uptake of microplastics and from direct uptake in addition to trophic uptake (via consuming plankton which have consumed microplastic themselves). We show that trophic uptake plays an important role in increasing plastic present in filter feeders, especially when consumption of the plastic does not reduce its overall abundance in the water column (e.g. in areas with high water flow such as estuaries). However, we also show that trophic transfer increases microplastic uptake, even if the amount of plastic is limited and depleted, as long as plankton are able to reproduce (for example, as would happen during a plankton bloom). If both plankton and plastic are limited and reduced in concentration by filter feeding, then no increase in microplastic by trophic transfer occurs, but microplastic still enters the filter feeders. The results have important implications for large filter feeders such as baleen whales, basking and whale sharks, as these animals concentrate their feeding on zooplankton blooms and as a result are likely to consume more plastic than previous studies have predicted.

Key words: microplastic; plankton; mussel; filter feeder; trophic ecology; trophic transfer

## 1. Introduction

Plastic, especially microplastics, have become ubiquitous in the marine environment (Eriksen et al. 2014), with recent studies showing their presence in almost all marine animals including those from the deep sea (Taylor et al. 2016). Microplastic ingestion by marine organisms can cause a range of negative effects including endocrine disruption, mutagenicity and carcinogenicity (Rios et al. 2007), which can have repercussions for growth, sexual development, fecundity, morbidity and mortality (reviewed by Cole et al. 2013).

Trophic transfer of microplastics has been demonstrated in laboratory studies, from zooplankton to mysid shrimp (Setälä et al. 2014) and from mussels to crabs (Farrell and Nelson 2013). However, little is known about the accumulation of microplastics through trophic transfer outside of laboratory studies, partially due to the difficulties of tracking microplastics and small organisms such as plankton through space and time.

In this study we present an agent-based modelling approach to investigate the role of trophic transfer of microplastics. We modelled plastic microbeads, plastic thread, zooplankton (three ‘species’ with three different feeding preferences for microbeads and other zooplankton) and mussels as agents in the model. As much research has previously been conducted on zooplankton uptake of microbeads, we assumed in the models that microbeads could be consumed by zooplankton and mussels, where as thread could only be consumed directly by mussels; hence comparing thread to microbead concentration in mussels allowed us to assess the effects of tropic transfer (we are subsequently aware of some research indicating thread can be consumed by zooplankton e.g. Dedman, 2014, but in the model, this was not permitted as it allows for comparisons of trophic transfer on uptake). We examined scenarios where filter feeding by mussels would: 1) not affect the concentration of microplastic and zooplankton in the water (i.e. both were highly abundant, or there was continuous movement of water); 2) not effect the concentration of zooplankton, but would reduce the abundance of microplastics (i.e. ‘clean’ water with little microplastic, but with rapid growth in zooplankton, such as a plankton bloom) and; 3) reduce both the concentration of plastic and of zooplankton as they were consumed.

## 2. Methods

Agent-based models were built in R (R Core Team 2015; see www.rickstafford.com/plastic_models.html for source code) to simulate the actions and interactions of the following six agents; mussels, selective feeding zooplankton (e.g. nauplii and cirripede nauplii), non-selective zooplankton (e.g. gastropods) and predatory feeding zooplankton (e.g. copepod, decapod and worms), and microplastic (both bead and thread) in order to assess the uptake of microplastics by mussels either directly (by examining thread uptake, which did not pass through zooplankton in the model, see introduction), or by direct and trophic transfer uptake (by examining beads, which were consumed by zooplankton as well as directly by mussels). By modelling thread and beads in this manner, it was possible to examine the differences in uptake between only direct uptake, and uptake through trophic transfer.

The model was run in a 100 x 100 grid arena and lasted 100 time-steps. Mussels were non-moving and always present (but positions of mussels were randomly generated on the grid), whereas the zooplankton and microplastic moved around and once ingested, in some simulations, were replaced by new agents in random locations (regeneration). Mussels were programmed to uptake beads, threads, and all 3 types of zooplankton, if in the same grid cell or one of the neighbouring nine grid cells to the mussel. Uptake was stochastic with a certain probability defined for likelihood of consumption if the agent to be consumed was in the specified cells. Selective and non-selective feeding zooplankton were programmed to uptake beads only, if both were in the same grid square, and predatory feeding zooplankton were programmed to uptake beads and both selective and non-selective feeding zooplankton. In all cases, uptake was not guaranteed, but stochastic and based on probability estimates of uptake of zooplankton and microplastic as defined in Cole et al. (2013), see Table 1 for the probability values used in this study.

Zooplankton, beads and threads moved by one grid square per time-step (including diagonal movement), with a heading generated from that of the heading of the previous time-step. Following directionality rules used in previous ecological ABM models (Stafford and Davies 2005) plastic particles could adjust their bearing by up to 90 degrees per time step and plankton by up to 45 degrees per time step. These changes in heading were generated from random numbers drawn from a uniform distribution.

Three plastic scenarios were simulated based on the empirical data results; 1 = equal amounts of thread and beads, 2 = more thread then beads and 3 = more beads than threads. Three different ratios of plastic to zooplankton were also conducted based on the empirical data results; Plastic:Plankton ratio 1 = 75:25, 2 = 50:50 and 3 = 25:75. Four zooplankton community structures were used: 1 = medium to high numbers of most species, 2 = medium to high numbers of copepod and cirripede, 3 = low to medium numbers of most species, and 4 = low to medium numbers of copepod, decapod and gastropod. In all cases, the numbers of mussels remained fixed (see Table 2 for exact numbers used in each simulation).

In total 36 scenarios were run, each scenario was run 3 times and a mean taken (total n = 108 model runs). Model 1 regenerated both microplastic and zooplankton, so once a plastic bead, thread or plankton agent was consumed, and another reappeared in a random location.  Model 2 was run to regenerate zooplankton only (hence microplastic in the water column was depleted over time) and Model 3 was run with no regeneration of either zooplankton or microplastic.

## 3. Results

A number of factors influence microplastic uptake in the models. For model 1, the different input parameters and plastic uptake in each biological agent type are shown in Table 2. Not only does the amount of plastic increase in plankton and mussels with increasing amounts of plastic in the water, but more plankton also result in more plastic accumulating in the mussels.

The three Trophic Interaction Agent-based Models, showed different results in total microplastic uptake based on the different regeneration scenarios (Figure 1). When both microplastic and zooplankton were regenerated there was a large increase in the uptake of microplastic in the presence of zooplankton, with three times as much microplastic ingested at some levels of microplastic concentration compared to no regeneration of either plastic or plankton (Figure 1a). This difference was reduced when there was no regeneration of microplastic. However, there was still a higher uptake of microplastic in the presence of zooplankton, with ~ 50% more microplastic ingested if passing through zooplankton as an additional uptake route (Figure 1b). If there is no regeneration of either microplastic or zooplankton then the amount of uptake is similar between beads (which are consumed by zooplankton) and threads (not consumed by zooplankton) indicating no significant increase in microplastic uptake in mussels was occurring through trophic transfer (Figure 1c). The variability of plastic bead concentration in mussels increased with plastic bead concentration in the water due to the changes in the amount of plankton in the model in different scenarios (as seen in Table 2), so while the overall trend was for increases in plastic beads in mussels as their concentration in the water increased, this was modified by plankton density. This created heteroscedasticity of data making it unsuitable for parametric statistical analysis. However, the difference in gradients between beads and threads in models 1 and 2 are clear and do not require statistical verification.

## 4. Discussion

The results demonstrate that under two of the three studied scenarios, the ingestion of microbeads by zooplankton, and subsequent consumption of zooplankton by mussels increased the amount of plastic found in mussels as compared to routes with no trophic intermediate stage present (as determined by thread uptake in the mussels).

These scenarios where plastic and/or plankton are ‘regenerated’ after consumption are not ecologically unrealistic. Coral reefs, for example, exist in nutrient poor areas, and the basis of the plankton-based food chain is through plankton continuously drifting over the reef (Odum and Odum 1955; Atkinson and Grigg, 1984). Such currents and condition which bring plankton are also likely to carry microplastics. The same is likely to be true of many coastal environments, especially tidal areas such as estuaries, where again, much material is imported with each tidal cycle (Peterson et al. 1985). Both estuaries and coral reefs are also important grounds for commercial fishing and shellfish stocks, meaning that further transfer into humans is then possible.

Equally, ‘regeneration’ of zooplankton would be likely to occur during plankton blooms, as reproduction and growth is normally rapid and opportunistic based on phytoplankton abundance. Hence, even where the amount of plastic of plastic in the water may be limited, high numbers of zooplankton can result in faster rates of uptake than may have been previously thought. This may have implications for plastic uptake in large filter feeders, such as baleen whales and basking or whale sharks, as they are known to selectively target these high abundance patches of zooplankton when feeding (e.g. Sims and Quayle, 1998).

Microplastics are another increasingly important stressor on marine ecosystems, already under stress inflicted by factors such as climate change, overfishing and other pollutants (Halpern et al. 2008). While there are policies and procedures designed to protect against further plastic pollution, e.g. the EU's Good Environmental Status (Galgani et al. 2013; Wright et al. 2013), these policies only consider the effects of plastics directly in the water column. While further work is necessary to fully quantify the magnitude of trophic transfer in situ, this current study demonstrates the potential increase in uptake that could occur in higher trophic level species. Consequently, the role of trophic transfer needs to be given substantial consideration when developing appropriate limits for microplastic in the ocean.

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Table 1. Uptake probabilities (%) used for all scenarios in Model 1, 2 and 3. If random number was ≤ probability when in the same grid cell (or additional 9 neighbouring grid cells for mussels) then the object would be consumed. Zooplankton feeding rate probabilities taken from Cole et al. (2013)

| **Scenario** | **Probability** |
|---|---|
| Selective plankton feeding on bead | 0.8 |
| Non-selective plankton feeding on bead | 0.9 |
| Predatory plankton feeding on bead | 0.8 |
| Predatory plankton feeding on selective plankton | 0.7 |
| Predatory plankton feeding on non-selective plankton | 0.7 |
| Mussel feeding on bead | 0.9 |
| Mussel feeding on selective plankton | 0.9 |
| Mussel feeding on non-selective plankton | 0.9 |
| Mussel feeding on predatory plankton | 0.9 |
| Mussel feeding on thread | 0.9 |

Table 2. The 36 scenarios of different plastic and plankton concentrations used in each model and the mean outputs from three replicate runs for each scenario for model 1.

| **Plastic thread** | **Plastic bead** | **Selective plankton** | **Non-selective plankton** | **Predatory plankton** | **Mussels** | **Plastic in mussels** | **Plastic thread in mussels** | **Plastic in Selective plankton** | **Plastic in non-selective plankton** | **Plastic in predatory plankton** |
|---|---|---|---|---|---|---|---|---|---|---|
| 200 | 200 | 200 | 100 | 200 | 10 | 169 | 56 | 131 | 80 | 378 |
| 400 | 400 | 200 | 100 | 200 | 10 | 357 | 99 | 277 | 154 | 763 |
| 100 | 100 | 200 | 100 | 200 | 10 | 98 | 23 | 74 | 31 | 200 |
| 100 | 300 | 200 | 100 | 200 | 10 | 294 | 25 | 213 | 108 | 570 |
| 200 | 600 | 200 | 100 | 200 | 10 | 546 | 49 | 404 | 247 | 1145 |
| 50 | 150 | 200 | 100 | 200 | 10 | 131 | 9 | 101 | 61 | 277 |
| 300 | 100 | 200 | 100 | 200 | 10 | 81 | 92 | 65 | 36 | 192 |
| 600 | 200 | 200 | 100 | 200 | 10 | 184 | 169 | 134 | 79 | 396 |
| 150 | 50 | 200 | 100 | 200 | 10 | 39 | 36 | 45 | 19 | 101 |
| 200 | 200 | 50 | 50 | 400 | 10 | 172 | 48 | 29 | 27 | 539 |
| 400 | 400 | 50 | 50 | 400 | 10 | 358 | 102 | 55 | 49 | 1119 |
| 100 | 100 | 50 | 50 | 400 | 10 | 88 | 26 | 11 | 12 | 269 |
| 100 | 300 | 50 | 50 | 400 | 10 | 251 | 21 | 35 | 40 | 861 |
| 200 | 600 | 50 | 50 | 400 | 10 | 509 | 65 | 72 | 80 | 1645 |
| 50 | 150 | 50 | 50 | 400 | 10 | 122 | 20 | 20 | 20 | 425 |
| 300 | 100 | 50 | 50 | 400 | 10 | 83 | 73 | 9 | 9 | 271 |
| 600 | 200 | 50 | 50 | 400 | 10 | 171 | 143 | 30 | 30 | 536 |
| 150 | 50 | 50 | 50 | 400 | 10 | 42 | 46 | 6 | 8 | 132 |
| 150 | 150 | 100 | 100 | 100 | 10 | 101 | 40 | 66 | 81 | 128 |
| 400 | 400 | 100 | 100 | 100 | 10 | 263 | 97 | 194 | 205 | 331 |
| 50 | 50 | 100 | 100 | 100 | 10 | 28 | 14 | 21 | 33 | 42 |
| 100 | 200 | 100 | 100 | 100 | 10 | 129 | 27 | 86 | 100 | 159 |
| 200 | 600 | 100 | 100 | 100 | 10 | 359 | 54 | 289 | 301 | 536 |
| 25 | 50 | 100 | 100 | 100 | 10 | 32 | 7 | 21 | 26 | 47 |
| 250 | 100 | 100 | 100 | 100 | 10 | 58 | 59 | 47 | 49 | 84 |
| 600 | 200 | 100 | 100 | 100 | 10 | 116 | 140 | 90 | 108 | 167 |
| 140 | 50 | 100 | 100 | 100 | 10 | 27 | 43 | 22 | 44 | 31 |
| 200 | 200 | 50 | 150 | 200 | 10 | 150 | 50 | 32 | 117 | 345 |
| 400 | 400 | 50 | 150 | 200 | 10 | 327 | 111 | 69 | 232 | 689 |
| 100 | 100 | 50 | 150 | 200 | 10 | 72 | 26 | 19 | 58 | 171 |
| 100 | 300 | 50 | 150 | 200 | 10 | 212 | 22 | 53 | 188 | 551 |
| 200 | 600 | 50 | 150 | 200 | 10 | 421 | 43 | 90 | 362 | 1049 |
| 50 | 150 | 50 | 150 | 200 | 10 | 121 | 13 | 18 | 82 | 244 |
| 300 | 100 | 50 | 150 | 200 | 10 | 86 | 74 | 23 | 53 | 167 |
| 600 | 200 | 50 | 150 | 200 | 10 | 177 | 169 | 36 | 116 | 338 |
| 150 | 50 | 50 | 150 | 200 | 10 | 33 | 40 | 7 | 25 | 80 |

Figure 1. Relationship between amount of plastic thread in the water and uptake by mussels (grey line) compared to the relationship between amount of plastic beads in the water and uptake by mussels (direct uptake and via plankton, black line). (a) Model 1 – regeneration of consumed beads and plankton, (b) Model 2 – regeneration of plankton only, (c) Model 3 – no regeneration