Trophic transfer of microplastics and an associated legacy pollutant from microzooplankton to their predators.

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Abstract

Microplastics are becoming more abundant in estuarine systems. The surface of plastic attracts lipophillic compounds, such as the pollutant DDT, that can leach into the tissues of marine organisms upon plastic ingestion. This study used larval inland silversides, *Menidia beryllina*, as predators, and tintinnid ciliates, *Favella* sp., as prey. LDPE microspheres treated with DDT and virgin microspheres were used to determine whether the presence of plastic-associated pollutants affects the feeding preference of larval fish and their prey.

Silversides (*Menidia species*), are common fishes found in along the East, West, and Gulf coasts.

After one two-hour feeding period, trophic transfer treatment groups ingested a significantly higher number of microplastics than direct ingestion treatment groups, suggesting ingestion of contaminated prey could be an important route for microplastic exposure. Larvae also ingested significantly more prey exposed to DDT-laden plastics than prey exposed to virgin plastics.

DDT seems to play a role in the prey preference of larval fish, and potentially affects the predator avoidance behavior of *Favella*. Microplastic gut retention time was not significantly different between DDT-laden and untreated microplastics. The rate of excretion of microplastics was 0.15 particles hour^{-1}. This was the first study to investigate gut retention time in larval fish and trophic transfer of contaminated microplastics in estuarine systems.

Hypotheses

Hypothesis 1: The test organisms do not feed differentially on untreated plastics vs. DDT-treated plastics or prey items contaminated with untreated plastics or DDT-treated plastics.
Hypothesis 2: Trophic transfer is a significant route of microplastic exposure for larval *M. beryllina*.
Hypothesis 3: There is no difference in gut retention time of untreated plastics vs. DDT-treated plastics.

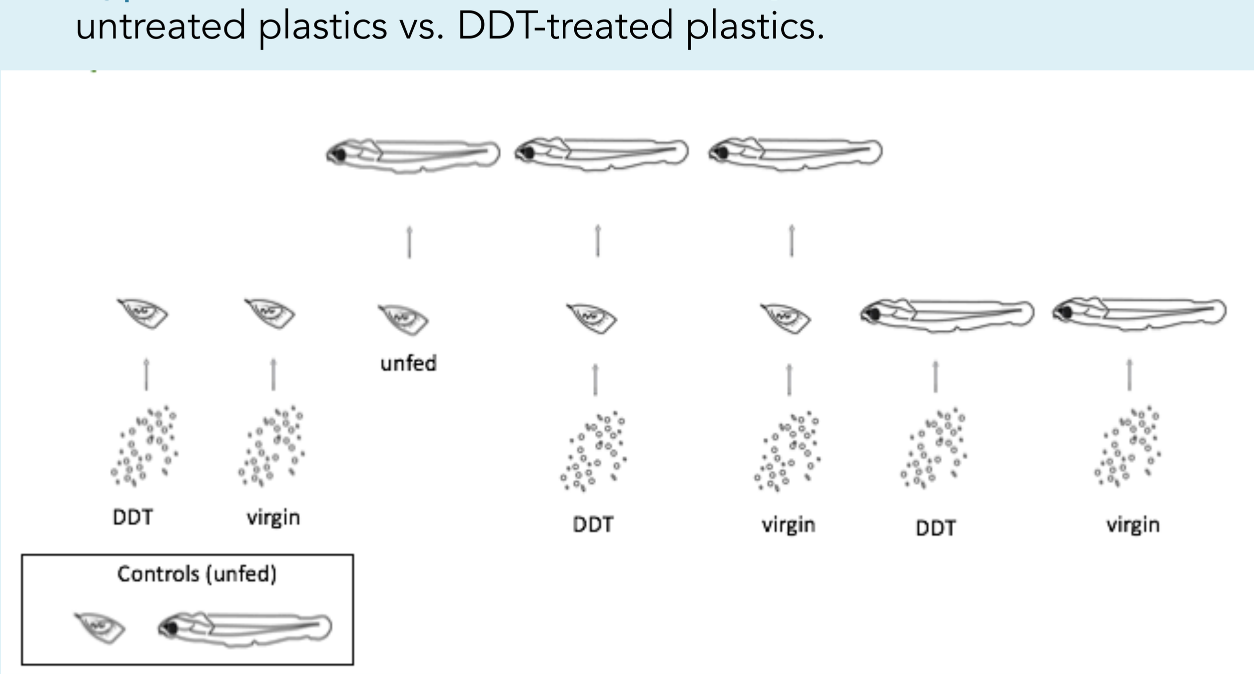


Figure 1. Outline for feeding experiments, including the three treatment groups (untreated microspheres, DDT treated microspheres, and unfed organisms.

Methods

Culturing and spawning of silversides, ciliates and zooplankton
- Ciliates were maintained at 16°C (12:12 light/dark)
- Silverside eggs were incubated at 22-25°C until hatching and then acclimated to 16°C for feeding experiment. Experiments were conducted at 4 days post hatch.

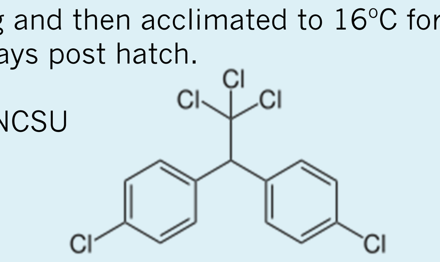
Microsphere preparation
- LDPE microspheres treated with DDT (2150 ng/mg) at NCSU
- Concentration of microspheres ~$5.3x10^5$

Ciliate feeding (1 h)

Larval silverside feeding (2 h)
- 5 larvae per replicate (3-4 replicate beakers)

Retention time
- Individual larvae were sampled every 30 minutes for 2.5-6 h after feeding, as well as 24, 48 and 72 h after feeding.

Analysis
- Brightfield microscopy to observe particles in ciliates and larvae (Olympus BX-60 microscope)



Dichlorodiphenyltrichloroethane (DDT)

Results

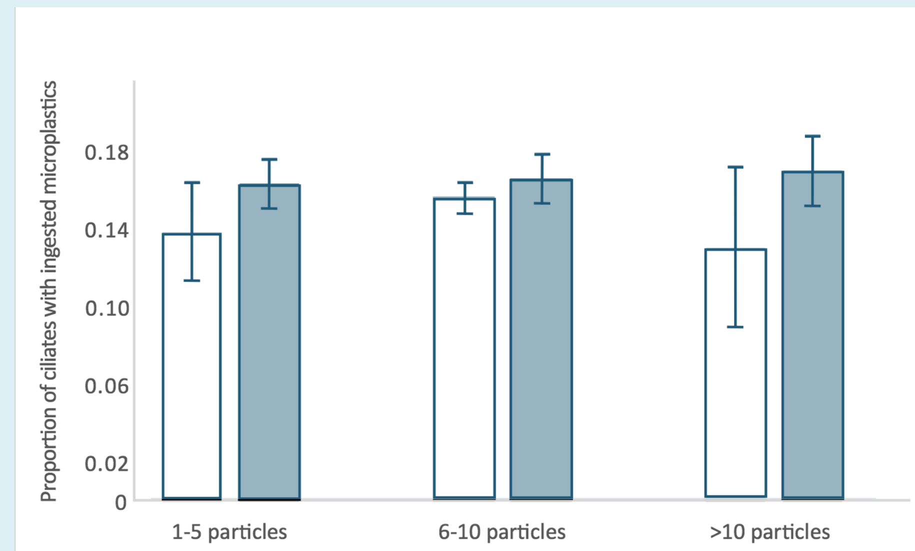


Figure 2. Favella **feeding experiment.** *Favella* prey consume equal amounts of virgin (white, 45% of ciliates consumed plastics) and DDT-laden plastics (blue, 42% of ciliates consumed plastics) microspheres. (P > 0.05; student t-test). No plastics were detected in the control groups.

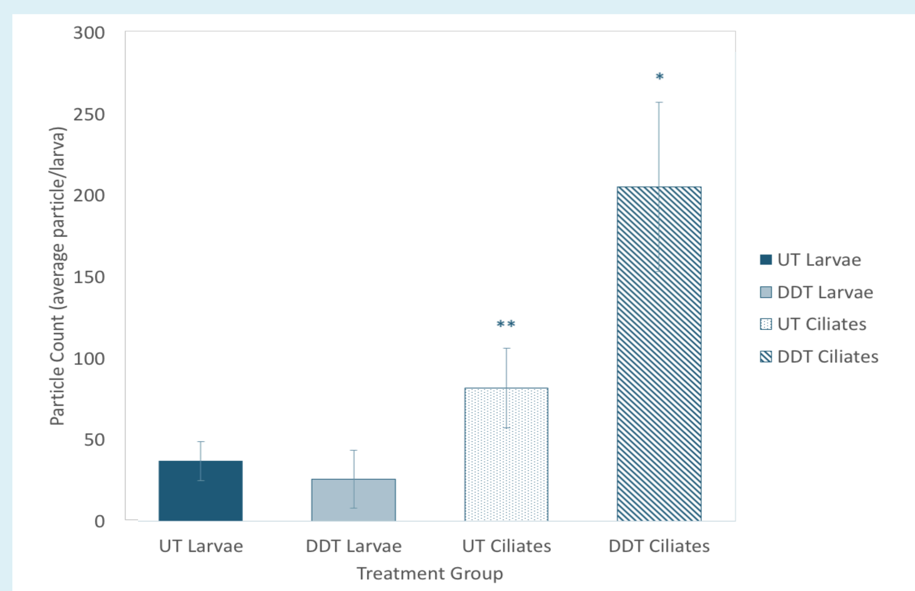


Figure 3. Menidia **trophic transfer and direct ingestion.** Larvae **in** direct ingestion treatments ingested a significantly lower number of particles than trophic transfer treatment groups (ANOVA ($P<0.05$); Schefe's test ($S>S_{critical}$)).

There was a significant difference in particles ingested between DDT and UT trophic transfer groups and no significant difference between direct ingestion treatment groups (Tukey HSD test; $q>q_{critical}$).

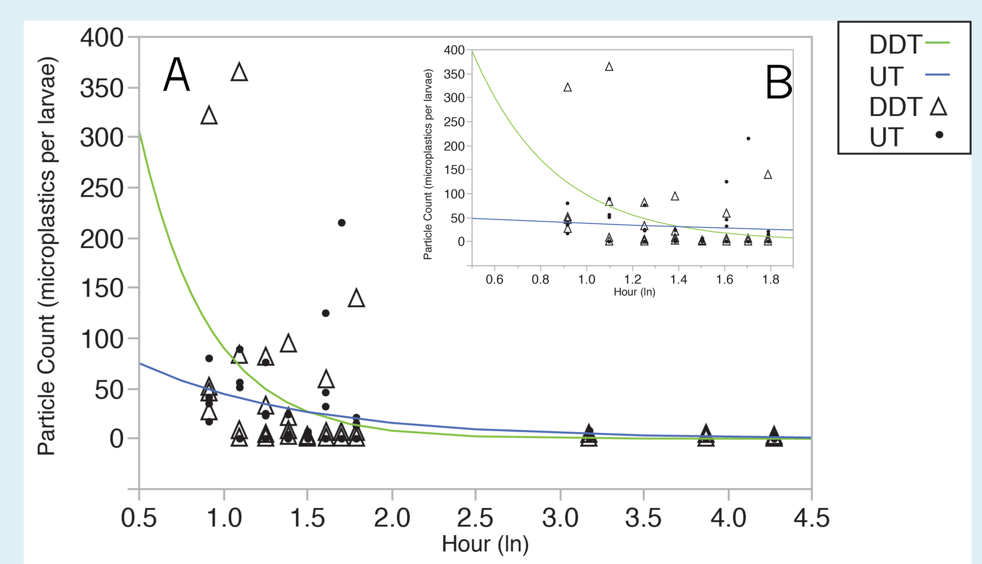


Figure 4. **Gut retention time of microplastics.** There was no significant difference in the rate of excretion between DDT-laden and untreated microplastics. Most particles were excreted within the first 24 h after ingestion. Rate of excretion for 2.5 – 6 h was 0.4 particles hr^{-1} (*Figure 5B*). The excretion rate integrated over the entire sampling period (2.5 – 72 h) is 0.15 particles h^{-1} (*Figure 5A*).

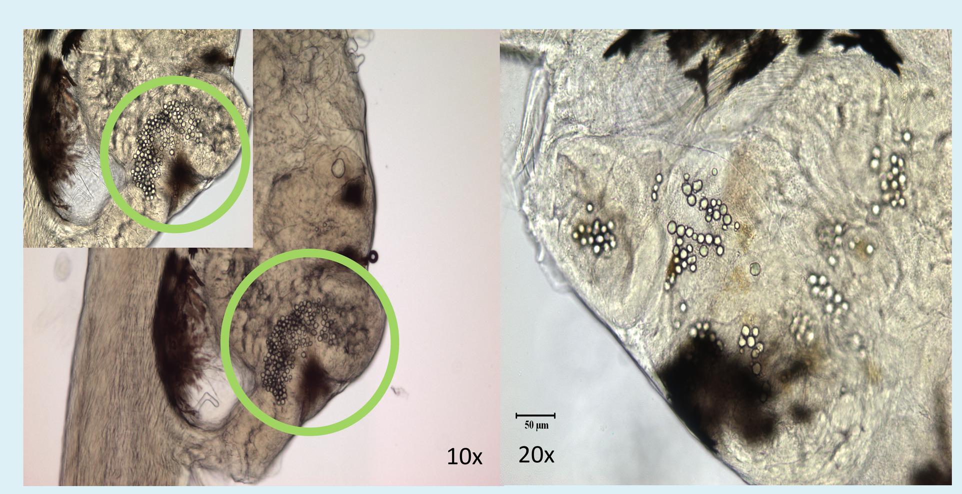


Figure 5. **Examples of microscopic analysis of** ***Menidia sp.*** **Left:** Brightfield image of larvae exposed to DDT-treated microspheres directly. This specimen contained 273 microspheres in its gut after a 2 h feeding period. Image captured using the BX-60 microscope. **Right:** Brightfield image of larvae exposed to virgin microspheres via contaminated prey. This specimen contained 71 microspheres in its gut after a 2 h feeding period.

Summary

- *Favella* and larval silversides do not feed differentially on clean microplastics vs. those contaminated with the harmful pollutant DDT.
- Larval silversides ingest more microplastics from prey than they ingest directly from the water.
- Larval silversides feed differentially on prey that have ingested DDT-contaminated microplastics. It may be possible that DDT inhibits the prey's predator avoidance response and warrants further study.

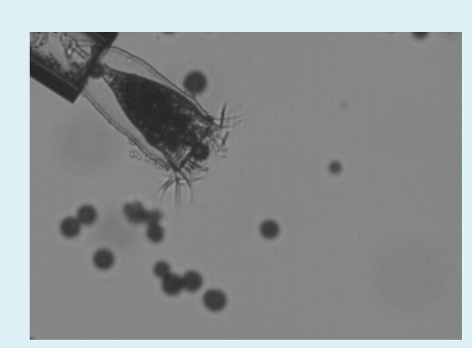


Image of a tethered ciliate *Favella* ingesting 10-20µm microspheres.

- Microplastic gut retention time was not significantly different between DDT-laden and untreated microplastics. This was expected since all particles were the same shape and size range.
- The rate of excretion of microplastics was 0.15 particles h^{-1}. Most plastics were excreted within the first 24 h after ingestion.

Discussion

This was the first study to investigate the role of a persistent organic pollutant on feeding preferences of larval fish and zooplankton as well as the role of trophic transfer. It was also the first to determine gut retention time of microplastics in a larval fish species. Our results suggest that trophic transfer could be a significant route of microplastic exposure. Our findings also show that prey exposed to DDT-laden microplastics are being eaten significantly more than prey exposed to untreated microplastics, suggesting DDT may interfere with the prey avoidance response in *Favella*.

Silversides are important forage fish in southeastern US estuaries.

Microplastics ingestion are an issue of high concern for marine organisms.

Larval silversides and their prey do not feed differentially on microplastics when plastics are contaminated with a harmful pollutant. This has major implications for the food web as DDT is well known to bioaccumulate within upper trophic levels. Trophic transfer of microplastics in estuarine systems is not well studied and is a major concern as many commercially valuable seafood species spend all or part of their life cycle within these habitats.

Further studies on microplastics in estuarine systems will be essential for understanding the local impacts of microplastics on estuaries, developing cleanup strategies, and monitoring their release into the marine environment.

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