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Effects of polystyrene nanoparticles on the early development and life cycle of *Tisbe battagliai*: role of surface functionalisation

Background

Plastics are prolific environmental contaminants, widely identified in marine and freshwater ecosystems. Progressive fragmentation of larger plastics through UV radiation, mechanical/physical and biological degradation leads to the formation of smaller-sized particles whose further fragmentation will likely result in the formation of plastic particles in the nanoscale (particles with dimensions <100 nm as defined for nanomaterials). Currently there is limited knowledge on the effects of nano-sized plastics, their fate, behaviour and interaction with cellular membranes and organisms, that could differ greatly from the original larger-sized material. Moreover, studies on uptake and long-term effects on marine species are largely lacking.

Aim: The aim of this study is to better understand the behavior, uptake and long-term effects of nano-polystyrene particles and assess the uptake, bioaccumulation and subsequent developmental and life cycle effects on the marine harpacticoid copepod *Tisbe battagliai*.

Approach

- The harpacticoid copepod *T. battagliai* was selected as a relevant marine species and the effects on naupliar development were assessed over a 6 day exposure period
- Effects on further development, female to male ratio and reproduction were assessed after transfer in clean seawater
- Initial investigations focused on polystyrene particles (plain, aminated, carboxylated, nominal primary size of 50 nm, Phosphorex and Sigmal Aldrich)
- Fluorescently labelled counterparts were used to study uptake and localisation
- Characterization techniques to study the particle behaviour in natural seawater during exposure (DLS, nanoparticle tracking analysis) and uptake (confocal microscopy)

Nano-polystyrene particle characterization → Early developmental and life cycle effects

Table 1. Overview of polystyrene particles used in the present study.

		-COOH	-NH_2
Z average (d. nm)	46.6±0.28	49.71±0.06	57.8±0.52
Polydispersity index (PDI)	0.047	0.06	0.123
ζ potential (mV)	- 58.9±0.56	- 36.9±0.62	+ 40.4±3.2
Concentration (particles/L)	1.72E+15	1.15E+15	1.27E+15

- Size distribution in milliQ H_2O and natural seawater over time (DLS, nanoZSP, Malvern)
- ζ potential in milliQ H_2O and natural seawater over time (M3-PALS, nanoZSP, Malvern)
- Particle number (Nanoparticle tracking analysis, NTA, Malvern)

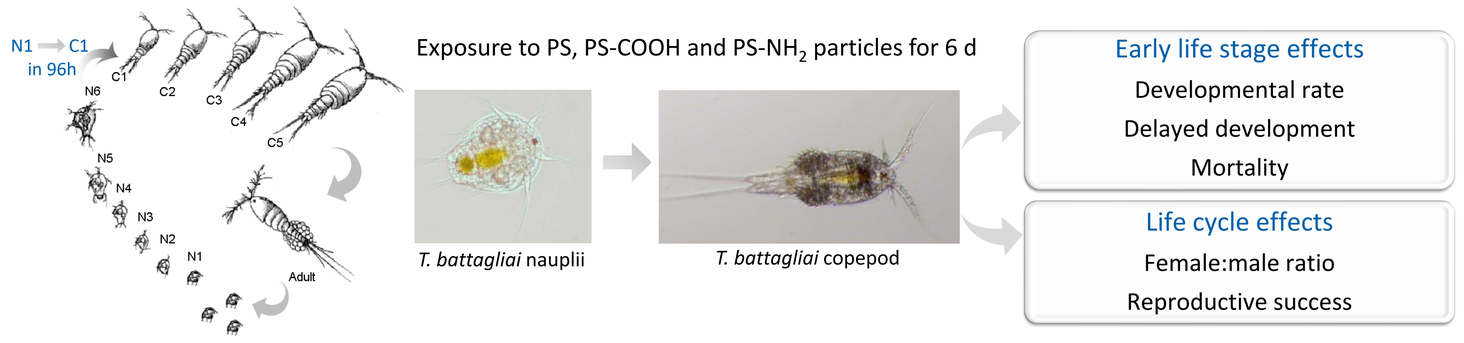

Figure 1. Life cycle of *T. battagliai* (from Macken et al. 2015). The animals at N1 stage were exposed to increasing concentrations of PS-plain, aminated or carboxylated particles (50 nm, 0.25-10 mg/L) and the development was followed for 6 days. The effects on the developmental rate and the development of nauplii to copepods were assessed. Further development, female to male ratio, appearance of gravid females and reproductive success were studied for 21d.

Results

Nano-polystyrene characterization

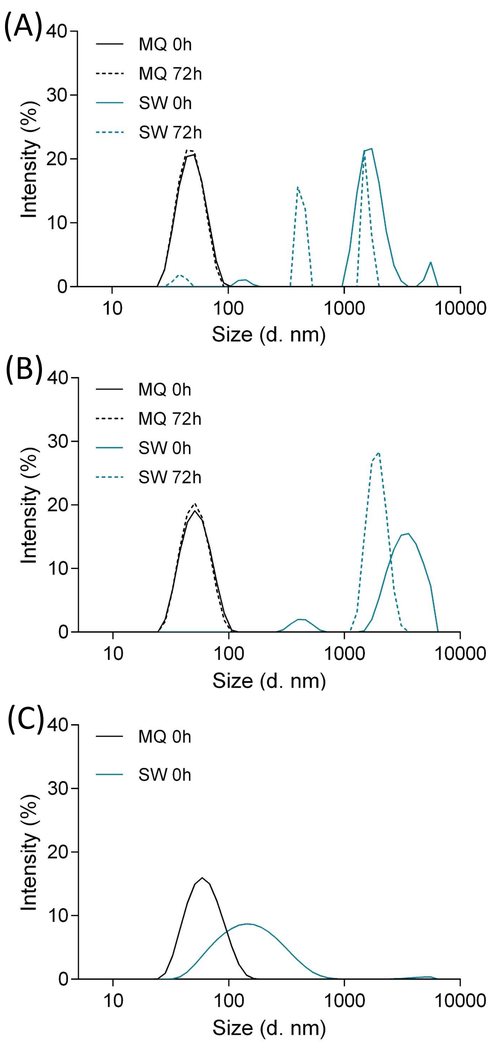

Figure 2. Size distribution of (A) PS, (B) PS-COOH and (C) PS-NH_2 particles (50 mg/L) over 72h in milliQ water and filtered natural seawater as measured by dynamic light scattering (NanoZSP, Malvern, UK).

Early developmental effects

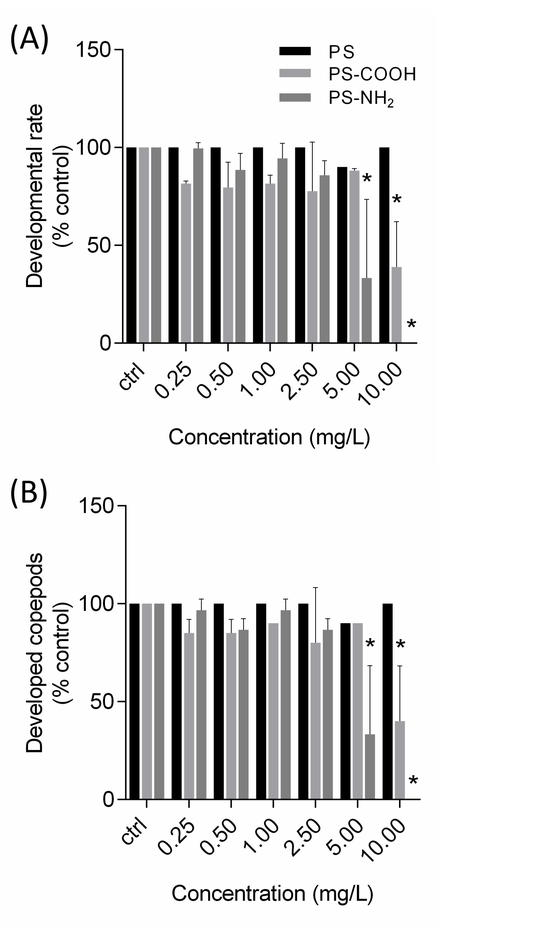

Figure 3. Effects of nano polystyrene particles (50 nm) on the early development of *T. battagliai*. The nauplii were exposed to increasing concentrations of plain PS, PS-COOH and PS-PS-NH_2 for 6 days. The exposure media were renewed at t= 72 h. The impact on (A) the developmental rate and (B) the number of nauplii reaching the copepodid stage after 6d of exposure was assessed.

Fecundity and reproduction

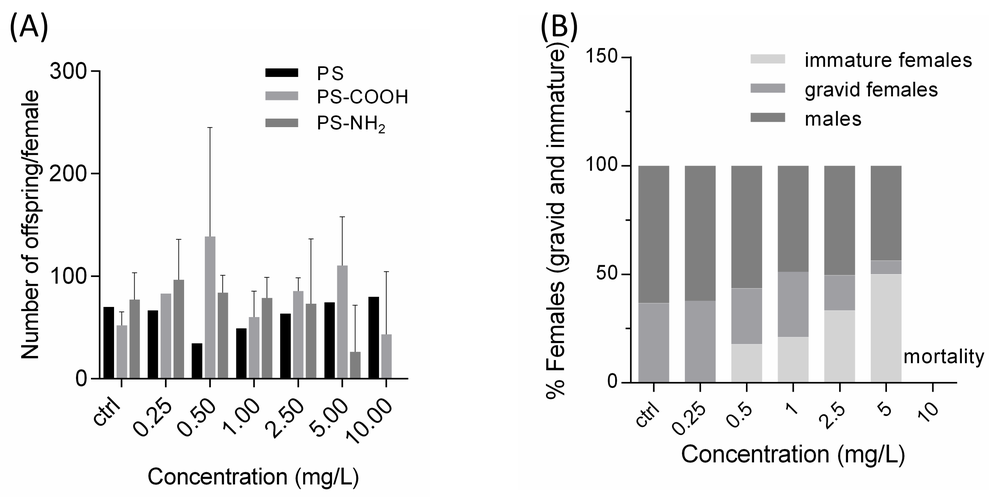

Figure 4. Number of offspring per female (A) after 21 days. Nauplii (<12h) were exposed to PS, PS-COOH and PS-NH_2 for 6 d, transferred in clean seawater and the further development and reproduction was followed until day 21. (B) Female to male ratio and presence of immature females post 6d naupliar exposure to increasing concentrations of PS-NH_2 particles.

Overview

- Increased particle aggregation for PS and PS-COOH while PS-NH_2 more stable
- *T. battagliai* development affected by PS-COOH and PS-NH_2 particles
- PS-NH_2 led to presence of immature females and decreased number of offspring
- Mechanical damage due to aggregates (PS-COOH) vs. charge and presence of nanoscale particles (PS-NH_2)?
- *T. battagliai* whole life cycle is sensitive and relevant bioassay
- Studies needed on environmentally relevant particles (different sizes/shapes occurring during fragmentation)

Ongoing and future plans

- Mechanistic understanding of observed effects
- Uptake and biodistribution evaluation

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