

Effects of exposure to microplastics on the development and metamorphosis of *Ciona robusta*

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Microplastics are plastic particles produced to be of small size, as sandblasting media, virgin pellet, cosmetics (Fendall and Sewell, 2009) or that originate from degradation of bigger plastic debris, as polyester fibers, polyethylene plastic bags and polystyrene particles from buoys and floats (Browne et al., 2011; Davidson, 2012; O'Brine and Thompson, 2010). An incredible amount of plastic reaches the marine environment and its ecological impacts are of particular interest. In fact, after ingestion, they may cause damage and block of feeding appendages and digestive system, can migrate from alimentary canal to other body tissues, limit the food intake and transfer pollutants in living organisms (Cole et al., 2013).

We evaluated microplastics effects on development and metamorphosis of the solitary ascidian *Ciona robusta*. We used microbeads with 10 µm diameter made of polystyrene, because it is highly stable with a negligible styrene migration (Cohen et al., 2002). We tested four different concentrations (0.125, 1.25, 12.5 and 25 µg/ml) and analyzed the effects on larval development and survival. Then we exposed attached larvae for five days to test the effects on metamorphosis. Juvenile survival was calculated exposing metamorphosed individuals to microplastics for eight days. We also estimated the ingestion rate as the number of particles ingested in one minute and the expulsion capability measuring the localization of particles in stomach or fecal pellets after 24 hours.

Metamorphosis was the process most affected by the microplastics. In fact, the percentage of individuals that completed metamorphosis was statistically lower in all the exposed groups, compared to control. Feeding individuals were also fed with microalgae. The co-ingestion of algae and beads probably reduced the amount of algae assimilated by the juveniles. Moreover, they might spend energy to expel microplastics thus reducing the energy available for the metamorphosis. After 8 days, juvenile survival was not affected, even if the particles persisted longer in the stomach of individuals exposed to the highest concentration, suggesting that further detrimental effects could be observed after a longer exposure.

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