



UNIVERSITÀ DI SIENA 1240

Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente

**Dottorato in Scienze e tecnologie ambientali,
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37° Ciclo

Coordinatrice: Prof.ssa Letizia Marsili

Polystyrene nanoparticles (PS NPs) as proxy for nanoplastics
for studying uptake, biodisposition, morphological and
biological effects on *Ciona robusta* developmental
stages

Settore scientifico disciplinare: BIO/07

Candidata

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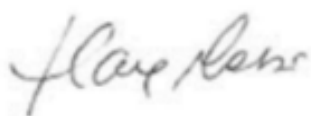
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Abstract

Plastic pollution in marine environments is one of the most pressing ecological challenges of the 21st century. With over 14 million tons of plastic waste entering the oceans annually, the fragmentation of plastic debris into smaller particles, including microplastics and nanoplastics ($<1\mu\text{m}$), has become a significant concern. Due to their small size and high dispersal capacity, nanoplastics can accumulate in surface waters, where they may interact with planktonic species and early life stages of benthic invertebrates, enhancing their bioavailability. However, the ecological risks posed by nanoplastics—particularly in terms of bioaccumulation and biological responses—remain poorly understood. Synthetic polystyrene nanoparticles (PS NPs), which are the most used as a proxy for nanoplastics, have been widely employed in ecotoxicological studies. These particles, depending on their surface charge and functionalization, can cause developmental and immune toxicity, particularly during the early life stages of marine invertebrates. The embryotoxic effects of PS NPs have been well-documented across various marine species, including fish, crustaceans, mollusks, and more recently ascidian as *Ciona robusta*. The transparency and simplicity of *C. robusta* during early developmental stages, combined with its rapid life cycle, fully sequenced genome and ease of laboratory culture, make it an ideal model organism for studies in developmental biology, evolutionary research, and, more recently, for examining the toxicological effects of environmental contaminants including nanoplastics. Furthermore, *C. robusta* is an ecologically significant marine invertebrate, playing a vital role in marine ecosystems, especially as a filter feeder from early development stages. This feeding behavior increases its exposure to plastics and associated contaminants, making it a relevant species for studying the impact of environmental pollutants. Exposure to nanoplastics can interfere with cellular processes, gene expression, and developmental mechanisms, leading to malformations, delays in growth, and reduced survival. However, nanoplastics do not act in isolation in marine ecosystems, their uptake and ecotoxicity may be influenced by the presence of other environmental pollutants. Among emerging contaminants, plastic additives, particularly Bisphenol A (BPA), have attracted significant attention. These chemicals, often leached into marine environments alongside plastics can adsorb to the surface of nanoplastics, altering their bioavailability and enhancing their toxicity. BPA, a widely used industrial chemical in plastic production, is a well-known endocrine disruptor with a broad range of biological effects. It has been shown to interfere with reproductive development and endocrine system function in both aquatic and terrestrial organisms. Its widespread presence in marine environments, combined with its potential for bioaccumulation, raises significant concerns about its effects on marine species, particularly during the most vulnerable early developmental stages. The interaction between BPA and nanoplastics could potentially magnify the adverse effects on marine organisms, highlighting the importance of studying their combined impacts.

While significant research has been conducted on the individual effects of nanoplastics and Bisphenol A (BPA), a critical gap remains in understanding the combined effects of these contaminants. Studies exploring the co-exposure of nanoplastics and emerging contaminants like BPA are essential for fully assessing the ecological risks. Additionally, while the biological impacts of nanoplastics have been widely studied, significant challenges remain in tracking their localization and accumulation within biological matrices. Radiolabeling NPs with isotopes, such as ^{14}C , provides a promising method for investigating their biodistribution and bioaccumulation of NPs in organisms. This approach enhances the sensitivity and accuracy of *in vivo* tracking, enabling the observation of how NPs accumulate in various tissues over time. However, the process of radiolabeling requires careful optimization to ensure stable and efficient labeling, as well as maintaining the biological relevance of the nanoparticles. This thesis investigates the exposure of *C. robusta* to nanoplastic using PS NPs and BPA, both alone and in combination, during larval and juvenile stages. It further explores the uptake and potential transgenerational impacts in adults exposed to radiolabeled ^{14}C PS NPs. Through a series of *in vitro* and *in vivo* experiments, the study evaluates the acute and chronic effects of PS NPs and BPA, emphasizing gene modulation linked to oxidative stress, detoxification mechanisms, and potential neurotoxicity by analyzing acetylcholinesterase (AChE) activities. The results indicate that BPA exposure *in vitro* inhibited AChE activity, while PS NPs did not affect this enzyme. Both contaminants, alone and combined, triggered significant changes in the expression of genes associated with oxidative stress and detoxification. Chronic exposure to PS NPs resulted in morphological alterations in juveniles and increased oxidative stress. Additionally, exposure to ^{14}C -labeled PS NPs led to their accumulation in adult internal organs, implying potential transgenerational effects. These findings enhance our understanding of the combined impact of PS NPs and BPA on marine organisms, underscoring the broader implications for marine ecosystem health and the management of emerging contaminants. This research provides new insights into the adaptive and resistance mechanisms of marine invertebrates, addressing critical gaps in knowledge regarding the effects of nanoplastics and chemical pollutants on marine life.