

The relationship between Ascidians and plastic pollution: a systematic review of interaction mechanisms, biological impacts, and ecological roles

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Abstract. Microplastic (MP) and nanoplastic (NP) pollution are global threats that permeate marine ecosystems. As sessile filter-feeders, ascidians interact heavily with these pollutants. This systematic review synthesizes the literature regarding the interaction between ascidians and plastics, focusing on their role as bioindicators, physiological impacts, status as model organisms, and potential in vertical pollutant transport. Sixteen primary studies indicate that various ascidian species consistently accumulate MP, highlighting their strong potential as bioindicators. Significant sublethal impacts, including disturbances in reproduction, development, immune response, and oxidative stress, have been widely reported. Laboratory studies confirm that ascidians, particularly from the genera *Ciona* and *Phallusia*, are valid model organisms for tracking particle pathways, toxicity, and underlying molecular mechanisms. Moreover, evidence suggests that ascidians play a role in the vertical transport of MP from the water column to sediments through fecal pellets, underscoring their dual ecological function. In conclusion, ascidians are not merely passive victims but serve as a unique and multifaceted model system for plastic research. Further research is needed to standardize bio-monitoring protocols and validate their bioremediation potential in the field.

1 Introduction

Marine debris has become an escalating global crisis, threatening marine life, ecosystems, and human health. Marine debris refers to solid, persistent materials created by humans, either directly or indirectly, intentionally or unintentionally discarded or left in the marine environment. About 80% of marine debris is plastic. Since its production began in the 1950s,

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the amount of plastic waste has reached 368 million tons, with approximately 11 million tons ending up in the oceans. 90% of this waste originates from land-based activities, such as household, industrial, and agricultural waste, while the remaining 10% comes from coastal and marine activities, including fisheries, shipping, and beach recreation. This waste enters the oceans through rivers, surface runoff, ocean currents, and wind. Approximately 30 million tons of plastic waste are projected to end up in the oceans by 2040.

The durability of plastic, which takes a long time to degrade, causes its accumulation in marine environments. This substance can be found in almost all marine environments, from the coastline to the surface, water column, and seafloor. Its presence poses a serious threat to aquatic organisms. Some of the impacts caused by plastic waste include entanglement, ingestion, serving as a carrier for invasive species, and accumulating toxic compounds. Small plastic particles (microplastics 1 - 5 mm and nanoplastics < 1 µm), when ingested, can cause oxidative stress, digestive, reproductive, growth, immune system disorders, and even genetic damage. This makes it a real threat to organisms living in the oceans.

Ascidians, also known as “sea squirts,” are marine organisms belonging to the phylum Chordata. They are commonly found attached to hard substrates such as rocks and coral reefs in shallow to deep waters. These organisms exhibit a wide range of sizes and forms, from solitary species to colonial groups that can form large, dense aggregations. Ascidians play a vital role in marine ecosystems, functioning as filter feeders that help process organic matter in the water, and serving as a food source for many other organisms. Their filtration activities contribute to improving water clarity and nutrient cycling, which is essential for the overall health of marine environment. Furthermore, ascidians can act as habitats for other smaller organisms, thus enhancing biodiversity within their ecosystems.

However, the increasing presence of plastic pollution in the oceans has raised concerns about how such contaminants interact with marine organisms, particularly filter feeders like ascidians. In recent years, the relationship between ascidians and plastic pollution has begun to attract scientific attention, given the high potential for interactions between these organisms and various forms of plastic waste entering the oceans. Research has shown that plastics can impact ascidians in several ways, both directly and indirectly. Floating plastics or those that sink to the seafloor may attach to the bodies of ascidians, disrupting their filtration processes and worsening the water quality they manage. Additionally, exposure to micro/nano-plastics on the bodies of ascidians may potentially lead to physiological disturbances and toxicity in these organisms.

Although the impact of plastic pollution on various marine organisms has been widely studied, there is limited research specifically examining the interactions between ascidians and plastic pollution. Understanding the mechanisms of interaction, biological impacts, and ecological roles of ascidians in plastic-contaminated ecosystems remains underexplored. Therefore, a systematic review is needed to further investigate this relationship by reviewing existing literature. This study aims to conduct a systematic literature review and bibliometric analysis of research examining the relationship between ascidians and plastic pollution, exploring interaction mechanisms, biological impacts, and ecological roles. Through this approach, it is expected that a clearer understanding of the effects of plastic pollution on ascidians will be achieved, as well as their contributions to maintaining the balance of marine ecosystems increasingly threatened by plastic pollution.

2 Methodology

This study follows the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta- Analyses) framework, in conjunction with bibliometric analysis, to identify trends in research within a particular sector (Figure 1). This approach allows for the identification of frequently explored topics and areas that have received less attention. The combination of

systematic literature review and bibliometric analysis is widely utilized because it helps researchers uncover research gaps and discover potential topics for future study. For the analysis, several software tools were utilized, including Excel and Biblioshiny through R. These tools are particularly useful for summarizing prior research both qualitatively and quantitatively. Additionally, they assist in the data visualization process, making the collected data easier to interpret and understand. The research articles included in this study were sourced from the Scopus database (<https://www.scopus.com>) and the Web of Science Core Collection database (<https://www.webofscience.com>). The search focused on the following keywords: “Microplastics” OR “micro litter” OR “microplastic*” OR “nanoplastic*” AND “Ascidia” OR “Ascidian” OR “Ascidiacea” OR “Tunicata” OR “Tunicate.” All articles, reviews, and conference proceedings in English from these databases, published from the inception of the database until 2024, were eligible for inclusion. The data were first extracted as Bibliography TeX (BibTeX) files, then processed using the RStudio application with the Biblioshiny (bibliometrix) package. Afterward, the datasets were merged, and duplicate entries were removed to ensure data integrity.

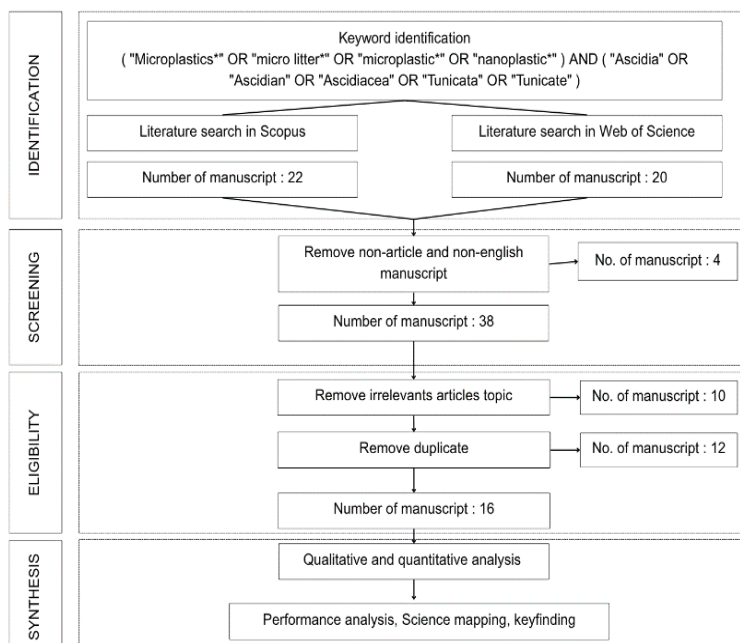


Fig. 1. PRISMA research framework.

3 Result and discussion

3.1 Performance analysis

The first step in this study is to conduct a research performance analysis to identify developments and provide an overview of research on the interaction between ascidians and plastic pollution. This topic has not been extensively studied yet. From 2018 to 2024, there were 16 research publications produced by 64 researchers, sourced from 12 different publishers. Research on the interaction between ascidians and plastic pollution has demonstrated a positive impact, with an average citation rate of 33.19 per document and an annual growth rate of 6.99%. However, despite the high citation impact, the overall number

of publications remains limited, indicating that while the topic attracts significant attention in terms of citations, it has not yet generated a substantial volume of research. However, further investigation is needed to explore current trends and uncover potential research gaps for future studies. A summary of the research performance in the area of ascidian-plastic pollution interaction is provided in Table 1.

Table 1. Descriptive analysis of research performance.

Component	Description	Result
Publication	Total number of research publications	16
Productivity	Annual Growth Rate %	6.99
Source	Total number of journal sources in related fields	12
Total citation	Total citation in all publication	531
Average Citations per Document	Total citation/publication	33.19
Total Author	Total research authors contributing to the field	64
Author's Keywords	Total publication keywords	71

3.2 Annual scientific production

Figure 2 depicts the growth in yearly publications related to the interaction between plastic pollution and ascidians from 2018 to 2024. The dashed line represents the linear regression analysis conducted on the total number of publications. The R^2 (coefficient of determination) is calculated to be 0.54, indicating that the linear trend line accounts for approximately 54% of the increase in publications. The trend line shows a decline in 2019-2020, likely due to the COVID-19 pandemic and lockdowns. However, the number of publications increased again in 2021 and continued to rise, reaching 3 publications in 2022-2024. Despite this increase, the overall volume of research remains relatively low, indicating that the academic and scientific community has not extensively focused on this topic yet.

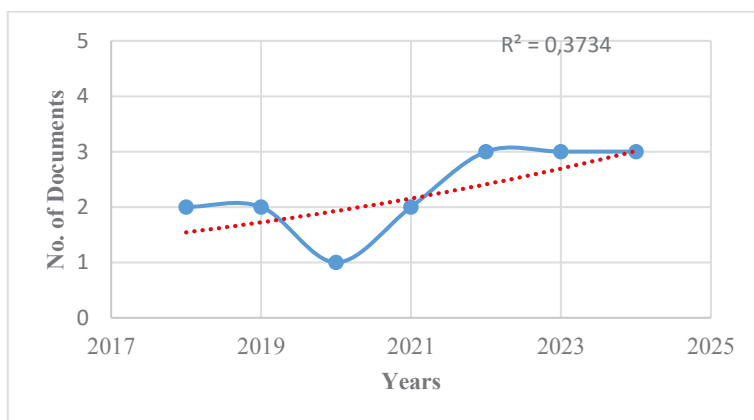


Fig. 2. Annual publication trend from 2018 to 2024.

3.3 Analysis of journals

The bibliometric data obtained highlights the contributions of various sources related to research on the interaction between ascidians and plastic pollution (Table 2 & Figure 3).

Among the listed journals, Environmental Pollution has the highest number of publications (4 articles) and a significant total citation count of 239, with an H-index of 4, indicating a strong influence in this field. The next source, Marine Pollution Bulletin, published 2 articles with a total citation count of 123 and an H-index of 2, showing relevant contributions, albeit fewer than Environmental Pollution. Other sources, such as Biomolecules and Chemosphere, each published 1 article with lower citation counts of 8 and 3, respectively, and an H-index of 1, reflecting a more limited influence. Additionally, European Zoological Journal with 1 article has a total citation count of 28 and an H-index of 1, indicating a moderate contribution in the related field. Other journals with a single publication include Frontiers in Immunology (6 citations), Journal of Aquatic Food Product Technology (51 citations), Journal of Experimental Zoology Part A-Ecological and Integrative Physiology (4 citations), Journal of Xenobiotics (2 citations), and Microplastics and Nanoplastics (28 citations), each with an H-index of 1. Overall, although most sources have published only one article, their citation counts and h-indices demonstrate their influence on the development of research in plastic pollution and its interaction with ascidians.

Table 2. Top ten most productive Journals and their Citations and H-Index.

No	Sources	Articles	Total Citation	H-Index
1	Environmental Pollution	4	239	4
2	Marine Pollution Bulletin	2	123	2
3	Biomolecules	1	8	1
4	Chemosphere	1	3	1
5	European Zoological Journal	1	28	1
6	Frontiers in Immunology	1	6	1
7	Journal of Aquatic Food Product Technology	1	51	1
8	Journal of Experimental Zoology Part A- Ecological and Integrative Physiology	1	4	1
9	Journal of Xenobiotics	1	2	1
10	Microplastics and Nanoplastics	1	28	1

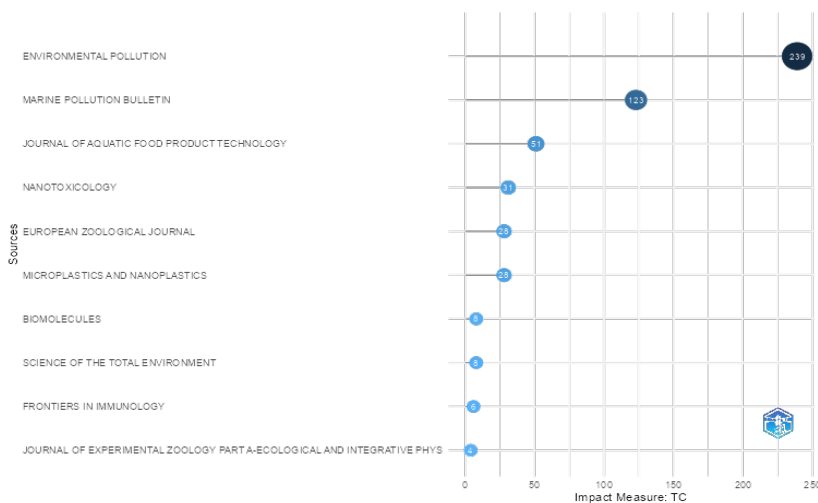


Fig. 3. Top ten most cited journals.

3.4 Most relevant countries

The bibliometric data reveals significant variations in research contributions and collaboration among countries (Table 3). Italy, with 11 articles and 318 citations, leads in both publication volume and citation impact, with notable international collaboration reflected in 3 Multi-Country Publications (MCP). Israel, contributing 4 articles with 205 citations, focuses primarily on national research, as evidenced by the absence of MCP. China, with just 1 article and 8 citations, also has no MCP, indicating a more isolated approach to its research. Overall, the data highlights Italy's strong international engagement, while Israel and China show more self-contained research activities. Research into the interaction between ascidians and plastic particles is a geographically concentrated field, largely driven by the unique environmental conditions of the Mediterranean Sea. This region is a recognized hotspot for both ascidian biodiversity, with approximately 229 species recorded, and for plastic pollution, containing an estimated 7% of the world's MPs. This unfortunate confluence makes it a critical natural laboratory, spurring focused research from bordering nations. Overall, the data illustrates how the severe MP pollution in the Mediterranean and its rich ascidian fauna have positioned regional actors like Italy as highly collaborative international leaders in this field, while the contributions from other nations like Israel and China remain more insular.

Table 3. Highest producing and cited nation.

Country	Articles	Total Citation	SCP	MCP
Italy	11	318	8	3
Israel	4	205	4	0
China	1	8	1	0

3.5 Most frequent and trending keywords

This section investigates the primary author's keywords in the study of asidians and plastic pollution, with a notable trend in the prevalence of MP (Figure 4). Although the broader topic is plastic pollution, the term "microplastic" appears most frequently (6 times), highlighting the increasing focus on MP within the field. This suggests that, while plastic pollution encompasses a wide range of pollutants, MP have garnered more attention in recent research due to their widespread distribution and potential ecological impacts. The term "ascidian," with 5 occurrences, emphasizes the significance of marine organisms in studies of plastic pollution, particularly in terms of their interactions with pollutants like MP. Other terms such as "polystyrene nanoparticles," "bioaccumulation," and "*Ciona robusta*" reflect common areas of research focused on the bioaccumulation of plastic pollutants in marine organisms. This concentration on MP highlights a critical area of concern within plastic pollution research, emphasizing the need for further studies to understand their effects on marine biodiversity and ecosystems.



Fig. 4. Most frequent keywords.

3.6 Report finding on research of ascidian and plastic pollution interaction

1.6.1 Mechanisms of interaction

As non-selective suspension feeders, ascidians are highly vulnerable to the unintentional ingestion of plastic particles. They pump large volumes of water through their oral siphon, capturing food particles such as phytoplankton and organic detritus in mucus webs that line their branchial sac [1]. Since MP and NP often overlap in size with their natural food sources, these particles can easily enter the digestive system of ascidians. The efficiency of plastic capture and retention is not uniform across all ascidian species and is strongly influenced by the morphology of their filtering apparatus. The filtration rates in ascidians are primarily determined by the area of the branchial basket and the length of the ciliary band lining the stigmal openings, as observed in a study of seven ascidian species. Filtration rates were measured by the exponential reduction in algal cell concentration, with no impact from the form of the stigmal opening itself on the filtration efficiency [1]. These findings suggest that simpler filtering structures may be less efficient at sorting and rejecting non-food particles, leading to higher ingestion rates of plastics. This highlights the importance of considering species-specific anatomical traits when assessing susceptibility to MP pollution.

The fate of ingested plastics in ascidians varies depending on their size. Larger MP (e.g., >10 µm) largely remain in the digestive tract and are eventually excreted as fecal pellets [2]. However, smaller particles can cross biological barriers, leading to systemic contamination. Crucial experimental evidence from *Ciona intestinalis* demonstrated that 1 µm polystyrene particles could migrate from the gut cavity into the hemocoel (the circulatory cavity) within just eight days of exposure [2]. This finding fundamentally alters the understanding of plastics from mere gut content to persistent internal contaminants. Once in the circulatory system, these particles may be phagocytosed by immune cells (amoebocytes), which acts as a defense mechanism but also serves as a pathway for further distribution throughout the body tissues [2]. The ability of NP to cross the gut barrier represents a “Trojan Horse” mechanism for cellular toxicity. NP have a high surface-area-to-volume ratio, making them highly effective sorbents for chemical pollutants, including additives released from plastic matrices (e.g., phthalates, BPA) and environmental toxins [3]. When these contaminant-laden nanoparticles are translocated into the hemocoel and taken up by cells, they deliver a

concentrated dose of toxins directly to internal tissues. This delivery mechanism can bypass key detoxification pathways located in the gut, potentially resulting in much higher cellular toxicity compared to exposure to the same chemicals dissolved in water.

Beyond direct ingestion, plastic debris serves as a potent ecological vector. Its high durability and buoyancy make it a new, long-lasting substrate for the transportation of marine organisms over long distances, a process known as “rafting” [4]. This process dramatically increases the dispersal opportunities for many species, including ascidians, allowing them to cross previously insurmountable biogeographical boundaries [4]. Many ascidian species are notorious invasive species that often spread via ship hull fouling and thrive in human-modified environments [4]. Rafting plastics provide an additional dispersal pathway, potentially accelerating the rate and range of biological invasions. This creates a feedback loop between two major global change drivers. First, plastic pollution facilitates the spread of invasive ascidians into new habitats. Second, many of these invasive species are highly tolerant of polluted conditions and often form dense populations in harbors and marinas, the same areas where plastic concentrations tend to be high [4]. Third, these invasive species can be highly efficient accumulators of MP. As such, successful invasive ascidians can become new hubs for the accumulation and processing of MP in the ecosystems they colonize, potentially altering the fate and bioavailability of local plastics for other organisms. This synergy likely exacerbates the ecological impacts of both stressors.

The surface of plastic debris in the ocean is rapidly colonized by unique microbial communities known as the “plastisphere” [5]. This biofilm community, consisting of bacteria, diatoms, and other protists, differs significantly from the microbial communities in the surrounding water column. The plastisphere can harbor pathogenic species (e.g., *Vibrio* spp.), harmful algae, and bacteria carrying antibiotic resistance genes, turning plastic debris into vectors for disease and genetic material [5]. Additionally, this biofilm alters the chemical properties of the plastic surface, potentially increasing its attractiveness to organisms by mimicking the chemical cues of natural food sources.

1.6.2 Biological impacts

Ingestion of MP can significantly disrupt basic physiological functions in tunicates. A study on the pelagic tunicate *Doliolotta gegenbauri* showed that exposure to environmentally relevant concentrations of microbeads caused a sharp decline in feeding rate (58%), growth rate (85%), and oxygen consumption (33%) [6]. These effects are primarily due to false satiety, where the digestive tract fills with indigestible plastic, reducing energy intake and causing a negative energy balance. The diversion of energy from vital functions such as growth and reproduction is a key mechanism of sublethal damage in filter-feeding organisms [6]. Although comparable quantitative data for benthic ascidians is still limited, these physiological principles are likely applicable, suggesting that chronic plastic exposure may harm energy allocation and overall fitness in ascidian populations.

Plastic pollution presents a multifaceted threat to the life cycle of ascidians, affecting development and reproduction through both physical particle effects and chemical toxicity. Physically, exposure to 10 μm polystyrene MP in *C. intestinalis* has been shown to cause significant delays in juvenile growth and metamorphosis [2]. The post-metamorphic juvenile stage appears to be particularly vulnerable, likely due to its high energy requirements for growth and organ reorganization. In contrast, pre-metamorphic larval development appears unaffected, indicating a stage-specific vulnerability to particle-induced stress [2]. Chemically, plastic additives are a significant concern. Phthalates, common plasticizers, have been detected in high concentrations in ascidian tissues from the Mediterranean Sea and the Red Sea, confirming the uptake of these chemicals from the environment [7]. Phthalates are endocrine-disrupting chemicals (EDCs) known to interfere with the hormonal systems

regulating reproduction in various marine organism [8]. While direct studies on endocrine disruption by phthalates in ascidians are lacking, extensive evidence from other marine organisms including disrupted gametogenesis, decreased fecundity, and developmental abnormalities provides a strong framework to hypothesize similar long-term risks for ascidian populations [9]. Studies investigating the combined effects of NP and other additives, such as Bisphenol A (BPA), on *C. robusta* embryos found that while BPA itself caused developmental defects (abnormal pigmentation of sensory organs), NP did not show synergistic effects in seawater [10]. This suggests that the high ionic strength of seawater may alter the surface properties of nanoparticles, limiting their role as vectors for BPA in this context and highlighting the complexity of pollutant interactions in the marine environment [10].

Exposure to plastics triggers complex responses at the molecular and cellular levels in ascidians, beyond observable physiological effects, providing insights into the mechanisms of toxicity. Transcriptomic analysis of *C.robusta* embryos exposed to amino-modified polystyrene NP has revealed widespread changes in gene [3]. Major molecular pathways affected include glutathione metabolism (involved in antioxidant defense), immune defense, nervous system development, and energy metabolism [11]. These findings offer clear molecular evidence that NP induce multi-system stress responses, even during early developmental stages. Additionally, proteomics, the study of the entire set of proteins, has been employed to identify stress biomarkers in ascidians collected from the field. Studies on species such as *M. exasperatus* and *C. robusta* have highlighted proteins that are differentially expressed in response to various environmental stressors [12]. These proteins are often linked to cytoskeletal structure, energy metabolism, and general stress responses (such as heat shock proteins). Changes at the protein level serve as highly sensitive early indicators of stress, occurring well before observable impacts like growth reduction or mortality, thus underscoring the value of “-omic” approaches in biomonitoring. Some of the effects that may result from plastic particles exposure in ascidian organisms are summarized in Table 4.

Table 4. Plastic particles effect in ascidians or related organisms.

Biological Organization Level	Organisms Studied	Plastic Type	Key Findings	References
Molecular/ Cellular	<i>C. robusta</i> , <i>C. intestinalis</i>	PS NPs and MPs	Gene transcription related to glutathione metabolism and immune defense altered; translocation from the gut to the hemocoel within 8 days.	[2]
Physiological	<i>Dolioletta gegenbauri</i> (pelagic tunicate)	Microbeads	Feeding rate reduced by up to 58% and oxygen consumption by up to 33%.	[6]
Developmental	<i>C. robusta</i> , <i>C. intestinalis</i>	PS MPs, BPA	Growth affected at high concentrations; larval development unaffected; BPA causes abnormal pigmentation of sensory organs.	[2]
Behavioral	<i>C. robusta</i>	PS NPs	Disrupted swimming behavior identified as a key event before adverse outcomes at the organism level.	[11]

1.6.3 Ecological roles

Ascidians have many characteristics that make them ideal bioindicator or sentinel organisms for plastic pollution. Their sessile nature ensures that the contamination they accumulate reflects the local environmental conditions. Their high filtration rate effectively concentrates particles from the water column, enabling the detection of pollutants even at low environmental concentrations [1]. Additionally, their global distribution, including the ability of many invasive species to thrive in heavily polluted coastal areas such as ports and marinas, makes them accessible for monitoring programs worldwide [1]. Field studies have successfully used solitary ascidian species such as *H. momus*, *M. exasperatus*, and *S. plicata* to measure MP levels and chemical additives such as phthalates in the Eastern Mediterranean and Red Sea, demonstrating the feasibility of this approach [7]

While the potential is vast, the utilization of ascidians within the framework of formal Environmental Risk Assessment (ERA) remains minimal, with ascidians appearing in less than 0.13% of ERA studies [1]. The major barrier to broader adoption is the lack of standardized methodologies [1]. Currently, there is no universally aligned protocol for ascidian sampling, plastic particle extraction from their tissues, chemical analysis, or data reporting. This methodological inconsistency makes it difficult to compare results between studies and hinders the development of a robust global dataset [13]. Therefore, the lack of standardization is not only a scientific challenge but also a critical policy barrier. Without comparable and reliable data, ascidians cannot be effectively integrated into large-scale monitoring programs, such as the Global Ocean Observing System (GOOS), or used to inform and assess the effectiveness of international agreements aimed at reducing plastic pollution [13]

Ascidians could also act as an important link in the movement of plastics through marine ecosystems. They can facilitate the trophic transfer of MP from lower trophic levels to higher ones. A significant experimental study showed that juvenile *C. intestinalis* accumulated significantly more MP when they consumed mixotrophic flagellates that had previously ingested plastic beads [14]. This provides direct evidence of trophic transfer at the base of the food web, where ascidians act as secondary consumers of plastic by eating contaminated protists. Additionally, ascidians are active “ecological transformers” of plastic pollution, significantly altering its physical properties and fate. Through the digestive process, they repackage small suspended MP into larger, denser fecal pellets [15]. Studies on *S. plicata* show that these fecal pellets containing MP sink much faster than normal feces. This process creates a rapid pathway for transporting MP from the surface water column to the seafloor, a process known as benthic-pelagic coupling [15]. By accelerating this vertical flux, ascidians effectively move plastics from the pelagic environment to the benthic environment, where they become available to deposit-feeding organisms and can be buried in sediments. Furthermore, the digestive process coats the plastic particles with organic material, which may obscure their synthetic nature and potentially make them more attractive to other organism [15].

4 Conclusion

Research on the interaction between ascidians and plastic pollution remains limited, with only 16 publications by 64 researchers between 2018 and 2024. Despite the small number of publications, the research demonstrates significant impact, with an average of 33.19 citations per publication and an annual growth rate of 6.99%. Ascidians, acting as filter feeders, interact with plastic particles by ingesting them, which disrupts their filtration mechanisms. This leads to physiological effects, reduction in feeding rate and decrease in oxygen consumption. Moreover, ascidians accumulate MP, causing biological impacts such as

oxidative stress and developmental delays. Ecologically, they serve as bioindicators of plastic pollution and play a crucial role in the vertical transport of plastics through the production of fecal pellets that move MP from the water column to the seafloor. Looking ahead, standardization of methods for global monitoring, development of molecular biomarkers for early detection of MP exposure, and further exploration of ascidians bioremediation potential are necessary. With these advancements, ascidians could play a broader role as agents of bioremediation and monitoring, helping to mitigate the growing plastic pollution in marine ecosystems.

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