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Plastics in scene: a review of the effect of plastics in aquatic crustaceans

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ABSTRACT

Plastic pollution in aquatic environments is present in all compartments from surface water to benthic sediment, becoming a topic of emerging concern due to the internalization, retention time, and its effects on aquatic biota. Crustacea with nearly 70000 species, broad distribution and different roles in the trophic webs is a significant target of the increasing plastic pollution. At least 98 publications in the last 10 years report the impact of plastics in crustaceans, all suggesting that this taxon is at high risk for ecosystem disadvantage by plastic contamination loads. This review compiles the current knowledge on physiological effects (endpoints) by plastic contamination analyzed in crustaceans in the last 10 years, highlighting their use as model species for ecotoxicological tests, sentinels species and bioindicators. Plastic contamination analyzed in this review includes macroplastic, microplastic, and nanoplastic, in a wide variety of types. The studies were focused on 38 marine species with an economic interest in fisheries and aquaculture; 14 freshwater with a higher frequency in standard test species and 4 estuarial and 3 mangrove species with ecological interest. The publications reviewed were divided into studies describing plastic presence in crustaceans without reporting toxic effects and those with analysis of plastic toxicity. Publications describing the plastic presence in the organisms show that the ingestion in individual effects and food-web transfer in ecological effects were the most frequent endpoints. The publications that analyzed plastic toxicity through survival, nutrition-metabolismassimilation, and reproduction in individual effects, and bioaccumulation in ecological effects were the most frequent endpoints. This review gathers the available information on the use of crustaceans as model species in environmental impact for toxicity screening and hazard assessment. Besides, identifying knowledge gaps will let us propose some future directions in research and the effects on target fisheries species which involves a possible effect on human health.

Keywords: Plastic pollution, Crustacea, aquatic environments, physiological endpoints, model species, bioindicators

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1 INTRODUCTION

2 Plastic pollution around the world is growing at overwhelming paces, which enter the ecosystem from various sources, accumulated in landfills, transported by wind, floating, 3 biofouling, among others (Geyer et al., 2017; Eriksen et al., 2020). It has become a symbol 4 of emerging concern since most of the plastics are non-biodegradable, hence they may 5 persist in the environment for centuries (Bergmann et al., 2015; Frias and Nash, 2019; 6 7 Wagner and Lambert, 2018; Zeng, 2018). A study published by Geyer et al. (2017) stated 8 that from 1951-2015 only 9% of the plastic produced was recycled, 12% was safely incinerated, and the remaining 79% was either openly dumped, landfilled, or littered in 9 the environment, which is alarming. Besides this, plastic production almost reached 370 10 million tons in 2019, and there are over 150 million tons of plastic waste in the ocean 11 today (Kosior and Mitchell, 2020; Plastics Europe, 2020). Major transport of plastic trash 12 from land to sea is done by rivers which serve as conduits for plastic waste to oceans from 13 major cities of the world (Schmidt et al., 2017; Singh and Devi, 2019). Between 70 and 14 80% of the plastics in marine environments are produced on land (Alimi et al., 2018) and 15 90% are carried up to the benthic compartments (Haegerbaeumer et al., 2019; Ramirez-16 17 Llodra et al., 2013; Chen et al, 2021). Although microplastics (MPs) were first documented in the Sargasso Sea by Carpenter and Smith (1972), the output of such reports 18 has resulted in an exponential increase of plastics literature in the last 15 years. 19

20 Plastics are produced through the biochemical process of polymerization or polycondensation with physical and chemical properties required, like the size, shape, 21 surface, and composition for commercial purposes (color, mechanical properties, and 22 resistance to solar irradiation, bacterial or fungal attacks) (Evode et al., 2021). Plastic 23 debris is an abandoned plastic object in the environment. This debris can break down into 24 different size fractions. MPs have been the most studied size category of plastic debris 25 for several decades (Andrady, 2011; Wright et al., 2013). Nevertheless, no universal 26 definition exists. According to Frias and Nash (2019) MPs are any synthetic solid particle 27 or polymeric matrix, with regular or irregular shape and a size ranging from 1 μ m to 5 28 29 mm, of either primary or secondary manufacturing origin, which are insoluble in water. Both small plastic fragments (MPs) produced by the degradation of larger plastic waste 30 (secondary MPs - SMPs) and microscopic plastic spheres used in cosmetic products and 31 32 industry (primary MPs - PMPs) are ubiquitously present in the environment (Guzzetti et al., 2018; Strungaru et al., 2018; Alimba and Faggio, 2019; Prokić et al., 2021). The 33 34 ingestion and toxic effects of plastics on aquatic life, especially for filter feeders, are a 35 cause of concern given their ubiquitous nature and their similar size as food sources 36 (Browne et al., 2008; Rochman, 2018; Frias and Nash, 2019; Burgos Aceves et al., 2021a,b). In addition, particles can also accumulate in sediment (Thompson et al., 2004), 37 38 suggesting that these would be available to many benthic species (Farrell and Nelson, 2013; Haegerbaeumer et al., 2019). However, the effects of ingested MPs on invertebrates 39 are not consistent and predictable because many aquatic taxa are adapted to ingest 40 nonfood particles such as sediment grains, spicules, or diatom frustules or exhibit diverse 41 mechanisms to select, dispose, or pass indigestible materials unimpaired (Buffan-Dubau 42 and Carman, 2000; Fauchald and Jumars, 1979; Hämer et al., 2014; Lopez and Levinton, 43 44 1987). Therefore, a reliable interpretation and risk assessment of the biological effects of MPs in marine ecosystems is essential. 45

The degradation of plastic objects, the break-down of aged-microplastics, the
manufacturing process, or even during the use of the object could result in nanoplastics
(NPs) (Bouwmeester et al., 2015). According to Gigault et al. (2018), the NPs are particles

unintentionally produced (i.e. from the degradation and the manufacturing of the plastic 49 objects) and present a colloidal behavior, within the size range from 1 to 1000 nm. The 50 51 evaluation of the impacts of these smallest fractions of plastic debris constitutes one of the last unexplored areas to fully understand the importance of this emerging threat for 52 the marine environment (Bergami et al., 2017; Hartmann et al., 2015). Moreover, the 53 54 behavior and fate of plastics are also impacted by an array of physical variables (currents, wave action, ocean turbulence, winds, and upwelling), and the type, shape, age, and 55 density of the polymer, weathering, pH, temperature, irradiation, and position within the 56 water column (Fotopoulou and Karapanagioti, 2017). 57

58 Invertebrates represent a fundamental primary component of marine, brackish, and freshwater biodiversity, and they are the main "group" of Metazoa. Crustacea is the major 59 taxon of aquatic Arthropoda with more than 70000 (Ahyong et al., 2011; Brusca et al., 60 2016) known species inhabiting shallow and deep, cold and warm waters with planktonic, 61 62 pelagic, and benthonic species around the world. Their eggs, larvae, and postlarvae are 63 also relevant components of aquatic trophic webs that include many other invertebrates and vertebrates. Although primary marine aquatic organisms, they have also adapted and 64 colonized brackish, freshwater, and terrestrial environments through many diverse 65 66 physiological strategies and high plasticity. Based on their broad distribution and roles in the trophic web, they are a significant target of increasing plastic pollution (Zeng et al., 67 2020; Sorrentino and Senna, 2021). Filter-feeding, detritivores species, and many larval 68 stages are especially exposed to MPs and NPs particles, while predator species are a 69 particular target for bioaccumulation within their tissues (Haegerbaeumer et al., 2019). 70

71 In the last 10 years (2011 to 2021), at least 98 publications have reported the impact of plastics in crustaceans, all suggesting that this taxon is at high risk for ecosystem 72 73 disadvantage by plastic contamination loads. There are also three recent revisions of MPs 74 and NPs toxicity on aquatic life and environments, including other aquatic taxa (Dioses-75 Salinas et al., 2019; Kögel et al., 2020; Xu et al., 2020). Besides, one review provides an overview of analytical methods used to determine the presence of MPs in crustaceans 76 (Sorrentino and Senna, 2021). In this context, the main aim of this review was to compile 77 78 the endpoints analyzed in crustaceans exposed to plastic contamination, including Macroplastic (MaPs), MPs and NPs, in the last 10 years, highlighting their use as model 79 organisms for ecotoxicology tests, sentinels species, and bioindicators. 80

81 <u>1.1 Literature search and screening process</u>

The review was focalized in the published information about the impact of plastics in 82 crustaceans from marine, brackish and freshwater environments, considering the 83 following topics: the chronological and geographical aspects of the studies, the type and 84 particle size of plastic detected, the species and habitats studied, the type of study 85 (analyzes in field or laboratory exposure), and mainly the endpoints both, at individual 86 and population levels. This review gathers the available information on the use of 87 crustaceans as model species in environmental impact for toxicity screening and hazard 88 assessment. 89

Scientific literature published between 2010 and 2021 was systematically searched through Web of Knowledge and Google Scholar using combinations of the following search terms: plastic pollution, MPs, NPs, plastic debris, marine debris, microfibers (MFb), PMPs and SMPs, fibers, beads, polymers, toxicity, chronic toxicity, trophic transfer, bioindicators, ingestion, stomach contents, reproduction, crustacean, zooplankton, accumulation, among others. All studies found in online databases until July 2021 were compiled in a database summarizing: the year of the publication, authors, title, keywords, geographical point of the study, size, shape, and composition of the plastic
analyzed, crustacean taxon, categorization of studies in function if describe the presence
of plastic or analyze toxicity, endpoint categorization, and type of analysis (field or
laboratory research).

101 CHRONOLOGICAL AND GEOGRAPHICAL ANALYSIS

This review includes 98 published publications from 2011 to July 2021.. The analysis showed a higher frequency of studies in 2018 (Fig. 1). Geographic coordinates utilized in this study were noted as cited in the paper. If the study site was not clearly mentioned in the publication, its location was defined by the laboratory research of the first author.

Most of the publications were performed in Europe (59% publications), 106 represented by 17 countries. The others were done in 4 Asian countries (16 % 107 publications; 2013-2020), in 3 South American countries (10% publications; 2011-2020), 108 in the United States (7% publications; 2015-2020), and Australia (4% publications; 2016-109 2020). There are no reports in Africa and only one report in congress from Costa Rica 110 (2021) (Fig. 1). Two of them cover large areas, one in six deep ocean trenches from 111 112 around the Pacific Rim (Japan, Izu-Bonin, Mariana, Kermadec, New Hebrides, and the 113 Peru-Chile trenches) (Jamieson et al., 2019) and others in the North Pacific Subtropical Gyre (Goldstein and Goodwin, 2013). 114

115 **KEYWORDS ANALYSIS**

In the 98 publications, a total of 392 keywords were used (222 without repetitions). However, the use of keywords was very varied; only 24 keywords were chosen in more than 3 publications. Keywords with the highest frequency were MPs (43 publications), followed by *Daphnia* (12 publications), ingestion (11 publications), and pollution (8 publications) (Fig. 2).

121 The use of keywords in the studies analyzed was divided into categories as: plastic 122 size, plastic composition, plastic as pollutants, crustacean as plastic target and trophic 123 impact, geographical place, and endpoints (Table 1).

124 TYPES OF PLASTICS

125 Plastic contamination analyzed in the publications reviewed differs mainly by the size of the plastic as: MaPs, MPs and NPs (Fig. 3). Over 98 publications reviewed, 6 analyzed 126 MaPs described as: fragments of fishing gears, marine debris, plastic carrier bags or 127 128 rubber pieces, plastic debris (Costa et al., 2018; De Rezende et al., 2011; Hodgson et al., 2018; Lavers et al., 2020; Potocka et al., 2019; Tutman et al., 2017) and only two of them 129 described their composition: polyethylene (PE) (Hodgson et al., 2018) and nylon (Cole et 130 al., 2019) (Fig. 3A). The publications that analyzed MPs were 72 which 65 analyzed the 131 132 compositions of the MPs (Fig. 3B), particularly 8 of them discriminated between PMPs and SMPs (3 publications), microbeads (MBs) (3 publications), and MFb (2 publications). 133 The total of publications that analyzed NPs characterizes the polymer (15 publications, 134 Fig. 3C), all were bioassays polystyrene (PS) and 14 also analyzed its toxicity. 135

In the 98 publications reviewed, 19 polymers were studied being the more frequent the
PS (Fig. 3). In general, in each research, it was analyzed the effect of one plastic-type, but
publications combined mixtures of plastics to evaluate the effects on Crustacea.
However, even though most environmental MPs consist of weathered plastic debris with

irregular shape and broad size distribution (SMPs), experimental studies of organism
 responses to MPs exposure have largely used uniformly sized spherical PMPs.

142 CRUSTACEAN TAXA ANALYSIS

The subphylum Crustacea includes six classes, Cephalocarida (Sanders, 1955), Remipedia (Yager, 1981), Ostracoda (Latreille, 1802), Branchiopoda (Latreille, 1817), Maxillopoda (Dahl, 1956), and Malacostraca (Latreille, 1802) according to Martin and Davis (2001). In the last 10 years, impacts of plastics in the life-cycle of 59 species of crustacean species were reported (Fig. 4). None of them belongs to Cephalocarida, Remipedia, or Ostracoda.

Branchiopoda was represented mainly with freshwater species of cladoceran group as *Daphnia* sp. (three species) and *Ceriodaphnia dubia*, frequently used as standard test species with a well-known life cycle for laboratory studies (Fig. 4, 5). This class was also represented by marine species of the genus *Artemia sp.* (Anostraca) (two species), a standard test species with ecological interest due to their role in trophic webs and economic interest due to use as food in aquaculture (Fig. 4, 5).

Maxillipoda was represented only by marine species, through six species of copepods of ecological interest due to their abundance in zooplankton and three species of Cirripedia; one research analyzed the plastic effects in the adult stage and the others in the larval stage (Fig. 4, 5).

Malacostraca was the most represented group with marine, estuary, mangrove, and 159 freshwater species (Fig. 4). In 48 publications in Malacostraca, 44 species were analyzed, 160 the marine krill Euphausia superba, with high ecological interest due to its role in the 161 162 food chain, and marine and freshwater Peracarida species with ecological interest due to indicator species with a recognized role in trophic webs mainly as zooplankton 163 components (11 Amphipoda and 2 Isopoda). In Decapoda, 29 shallow water species and 164 165 two deep-sea species Nephrops norvegicus and hermit crab were analyzed. Eight publications included four estuarine species (Watts et al., 2014; Watts et al., 2015; Watts 166 167 et al., 2016; Wójcik-Fudalewska et al., 2016; Waite et al., 2018; McGoran et al., 2020; 168 Villagran et al., 2020; Ardusso et al. 2021) and one was a comparative study (Not et al., 2020) with three mangrove species (Fig. 4, 5). 169

170 Decapoda was represented mainly by Bachyura species with ecological interest as Carcinus maenas, Libinia ferreirae, Liocarcinus navigator, Maja squinado, 171 Metopograpsus frontalis, Neohelice granulata Ocypode quadrata, Panopeus herbstii, 172 173 Paraleptuca splendida, Planes minutus, Thalamita crenata, Uca rapax, and particularly 174 with economic interest for its commercialization Callinectes ornatus, Eriocheir sinensis 175 and Portunus pelagicus (Fig. 4, 5). Also, marine shrimps with economic and ecological interest as: Astacidea: Homarus americanus and Nephrops norvegicus; Caridea: Crangon 176 allmanni, Crangon crangon, Neocaridina palmate, Neocaridina davidi, Palaemonetes 177 178 pugio, Palaemonetes varians and Plesionika narval; Penaeoidea: the deep-sea shrimp Aristaeopsis edwardsiana, Aristeus antennatus and Pleoticus muelleri. Besides, four 179 180 marine Anomura, Coenobita perlatus, Emerita analogue and an unidentified hermit crab with ecological interest, and Lithodes santolla with economic interest. 181

Freshwater species selected for the studies are with more frequency standard test species (25/35) and marine species are often species selected for an ecological interest (36/49) or economic interest for fishing or their use in aquaculture (12/49) (Fig. 5,6). The majority (approximately 68%, n=65) of the publications on plastic is based on laboratory studies (Fig. 6). These laboratory studies have been encompassed principally by standard test species (Fig. 7), and only a small percentage (approximately 29%, n=31) of publications are concerned with the uptake of MPs in wild crustaceans. Only two publications use a two-fold approach, whereby they have an interest in demonstrating the MPs-NPs contamination in animals from nature and in experiments (Cole et al., 2013; Murray and Cowie, 2011).

192 ANALYZED PARAMETERS

The analyzes of the parameters investigated were divided into two categories: 1st) 193 Studies describing the presence of plastic in crustaceans without reporting toxic effects; 194 195 2nd) Studies with analysis of plastic toxicity in crustaceans. In the 98 publications reviewed, the first publications were about the presence of plastic in the organism without 196 reporting toxic effects. From 2015, publications of studies analyzing plastic toxicity in 197 198 the organism have become more frequent, and from 2020, the majority of the publications were about the presence of plastic, principally in the digestive tract or gill chamber (Fig. 199 200 7).

201 ENDPOINTS ANALYSIS

The effects caused by plastic particles (endpoints), evaluated in the 98 publications,
were grouped in main physiological processes inside two categories: effects at individual
or ecological level (Fig. 8).

- 205 <u>7.1 Effects at individual level</u>
- 206 <u>Survival</u>: Include survival/mortality and LC₅₀% analysis.
- 207 <u>Ingestion</u>: Include the presence of plastic in the digestive tract and the quantity and
 208 diversity of MPs found.
- 209 <u>Nutrition-Metabolism-Assimilation</u>: Include effects on feeding capacity, feeding rates,
 210 food consumption, and energy balance, food uptake, assimilation efficiency, body mass,
- and nutritional state, retention rates as the accumulation of MPs during the gut passage or
- 212 ingests and retains MPs in the body, and metabolic rate as catabolism of stored lipids.
- Through the analysis of food availability, determination of ingestion and egestion, degradation of MPs through ingestion, get rid of ingested MPs, and the interaction of
- 214 degradation of MPs through ingestion, get ri215 plastic with biomolecules.
 - 216 <u>*Reproduction*</u>: Include reproductive output through the time of first brood, size of
 217 different broods, and measure of fecundity as egg production rates, maternal effects on
 218 offspring survival, and feeding.
 - 219 <u>Growth</u>: Include growth inhibition, growth rate, and effect in the molting process in
 adults, embryos, or larvae.
 - 221 <u>Morphological abnormalities:</u> Include effects in the morphology of adults, embryos or
 222 larval in appendages, organs/tissues.
 - 223 <u>Neurotoxicity</u>: Include effects in brain damage and behavioral disorders, swimming speed
 224 alteration, and mobility/immobilization.
 - 225 <u>Osmoregulation-Ventilation</u>: Include presence of plastic in gill chambers and/or effects
 - in branchial function evaluated by osmoregulation, respiration, or ventilation.

- 227 <u>Enzymatic activity</u>: Include alteration in enzyme activity as cholinesterases, catalase
- 228 (CAT), superoxide dismutase (SOD), aspartate transaminase (GOT), glutathione (GSH),
- 229 glutathione peroxidase (GPx), acetylcholinesterase (ACHE) and alanine 230 aminotransferase (ALT).
- 231 *Oxidative stress*: Include alteration in the hepatopancreas.

Genotoxicity: Include alteration in genes expression levels, as: stress defense genes 232 233 (oxidative stress-mediated and heat shock proteins), activated protein kinase (AMPK) (SOD, CAT, GST, GPx, HSP70, and HSP90), expression of target genes (i.e. clap and 234 235 cstb), expression of the gene encoding p38 in the activation of mitogen-activated protein 236 kinase (MAPK) signaling pathway and expressions of genes encoding ERK, AKT, and MEK. MAPK and nuclear factor erythroid 2-related factor 2 (Nrf2), which acts as a 237 downstream transcription factor of MAPKs, was studied along with the reactive oxygen 238 239 species (ROS) levels, to examine whether they are involved in signal transduction pathways, which may lead to the activation of oxidative stress-induced defense system 240

241 <u>7.2 Effects at ecological level</u>

242 <u>Food-web transfer</u>: Include the distribution of the plastic between the trophic transfers
 243 from prey to predator and include complex behaviors such as predator avoidance and
 244 social interactions.

- 245 <u>*Bioaccumulation*</u>: Include analyzes of residence time, MP build-up, and environmental
 246 fate.
- 247 *<u>Transgenerational effects</u>*: Include analyzes of the reproductive output through maternal
- effects on offspring survival and feeding; also include embryonic or larval uptake and analyzed population growth rate.
- 250 <u>Other interactions with plastic:</u> Include analyzes of plastic as entrapment, select and use
 251 as burrows and spread of species by rafting.

252 OVERVIEW AND INTEGRATION OF RELEVANT PLASTIC 253 EFFECTS IN AQUATIC CRUSTACEANS

254 <u>8.1. Presence of plastics in crustaceans without reporting toxic effects</u>

In studies describing the presence of plastic in crustaceans without reporting toxic effects, the most frequent endpoint process was MPs ingestion that has been reported in a wide range of crustaceans, in both field and laboratory studies. Plastic ingestion by aquatic biota can cause deleterious effects to their health through the gut blockage, tissue damage, and false satiation.

260 8.1.1 Marine studies

261 *Plastics ingestion – field studies and laboratory studies*

The presence of MPs in the digestive tract was also reported in deep-sea species such 262 as: shrimp Aristaeopsis edwardsiana registered at depths from 400-900 m (De Rezende 263 et al., 2011), an unidentified hermit crab (Taylor et al., 2016), and Lysianassoidea 264 amphipod populations of six deep ocean trenches from around the Pacific Rim at depths 265 ranging from 7000 m to 10 890 m (Jamieson et al., 2019). Besides, in feeding ecology 266 researches as: spider crab Libinia ferreirae (Gonçalves et al., 2019), a filter-feeding 267 *Emerita analoga* that is a common previtem in the diet of a wide variety of taxa, including 268 fishes and birds (Horn et al., 2019, 2020), and barnacles (Lepas sp.) associated with 269 270 floating objects, termed the "rafting assemblage," an important component of the North 271 Pacific Subtropical Gyre ecosystem (Goldstein and Goodwin, 2013).

Ingestion bioassays studies reported the presence of MPs in the organism and 272 described some ingestion behaviors, as: Hämer et al. (2014)reported that the isopods did 273 274 not distinguish between food with and without MPs, indicated that no accumulation of MPs happens during the gut passage, and did not show effects on mortality, growth, and 275 276 intermolt duration in long-term bioassays (6 weeks). Particularly, Saborowski et al. 277 (2019) suggested that the process of regurgitation attained a new task, i.e. the elimination of anthropogenic filamentous MPs debris from the stomach to avoid harm. Regurgitation 278 behavior is an evolutionary adaptation of particular crustacean species and other 279 280 invertebrates to remove large and indigestible food particles from the stomach. The authors reported that the shrimp Palaemon varians ingested fibers and beads along with 281 the food. Upon ingestion, the beads and the shortest fibers (up to 100 mm) passed from 282 the stomach into the gut and were egested within the fecal strings, but the longer fibers 283 first remained in the stomach and were regurgitated. Also, Emerita analoga exposed to 284 polypropylene (PP) rope increased adult crab mortality and decreased retention of egg 285 clutches, causing variability in embryonic development rates (Horn et al., 2020). 286

287 Plastic ingestion – food web transfer and biodispersion risk

288 Investigating the biological consequences of plastic ingestion is a relevant point because not only could have implications for aquatic organisms' health but also generate 289 bio-transference and "food web transfer" (Wang et al., 2019; Alimba et al., 2021; Prokić 290 291 et al., 2021). This is reflected mainly in those studies that evaluated plastics ingestion in 292 commercial species. For example, Andrade and Ovando (2017) reported the first record of MPs in stomach contents in the southern king crab Lithodes santolla, an economic and 293 294 ecological important species, while Potocka et al. (2019) found rubber pieces (MaPs) in 295 the stomachs of the economic and ecological important American lobsters, Homarus 296 americanus. Besides, a few studies in Nephrops norvegicus, a species of great importance 297 to the UK fishing industry with a non-selective diet, demonstrated the presence of plastics in their stomachs and the effect on gut morphology by MPs retention (Hara et al., 2020; 298 Murray and Cowie, 2011; Welden and Cowie, 2016a, 2016b). Other publications in deep-299 300 sea species suggest that both N. norvegicus (lobster) and Aristeus antennatus (shrimp), easily available in common fishery markets, could be valuable bioindicators and flagship 301 species for plastic contamination in the deep-sea (Cau et al., 2019). This comparative 302 study evaluated feeding strategy and showed significant differences in MPs amount 303 304 found in digestive tract according to non-selective feeding of N. norvegicus (Cau et al., 2019), and reported long periods of MPs retention due to stomach's morphology in 305 Aristeus antennatus (Carreras-Colom et al., 2020, 2018). Studies in Eriocheir sinensis 306 307 and Carcinus maenas invasive species, considered a delicacy by Asian migrants and 308 therefore commercially fished and sold in many countries, reported the presence of MPs in the form of strands and balls in the stomach of both species (McGoran et al., 2020; 309 310 Wójcik-Fudalewska et al., 2016). Passive ingestion of MPs linked with fishing activities 311 in the high commercial shrimp species, *Plesionika narval* was reported in the eastern Mediterranean (Bordbar et al., 2018). 312

313 Welden et al. (2018) were the first to identify MPs contamination in the spider crab Maja squinado and document trophic transfer in the wild in a comparative investigation 314 of Pleuronectes platessa and M. squinado. This study reported different factors 315 influencing the uptake of MPs in these two taxa, as: proximity to land, if more coastal the 316 species more abundance of MPs. The authors identified the impacts on the contaminated 317 organism and considered the impacts of MPs uptake on the commercial value of the 318 species. A research reported the abundance of MPs type in the muscle of commercial 319 shrimp Pleoticus muelleri with peaks of fishing of 144,000 tons recorded in 2015 (FAO, 320

2018; Fernández Severini et al., 2020). In *Crangon crangon*, an ecologically and commercially important shrimp, Devriese et al. (2015) revealed no spatial patterns in plastic ingestion, but temporal differences were reported, and MPs >20 μ m are not able to translocate into the tissues. The authors suggested that shrimps comply with requisites to be a key species to be monitored for MPs contamination being excellent indicators that reflect the quality *status* of their habitats

327 In addition to the ingestion of MPs in natural environments, the ingestion of MPs, their 328 effects, and their influence on food chains have also been observed experimentally. A chronic bioassay study reported that *Nephrops sp.* fed fish seeded with strands of PP rope 329 were found to ingest but not to excrete the strands (Murray and Cowie, 2011). Exposure 330 331 to high levels of contamination by MPs in this species indicated a reduction in feeding rate, body mass, and metabolic rate as well as catabolism of stored lipids in the organism 332 (Welden and Cowie, 2016a). Farrell and Nelson (2013) reported the first 'natural' trophic 333 transfer of PS-MPs, and its translocation to hemolymph and tissues of a crab, in bioassays 334 335 with Mytilus edulis to Carcinus maenas. Santana et al. (2017) evaluated MPs' biotransference and persistence in a food web using an experimental approach and 336 reported the transference of MPs from prey (MPs in the hemolymph of the mussel Perna 337 338 pernato) to predators (the crab Callinectes ornatus and the puffer fish Spheoeroides 339 greeleyi) but without evidence of particle persistence in their tissues after 10 days of 340 exposure.

341 Publications report that juveniles and adults of A. franciscana and A. parthenogenetica were able to survive after exposure to MPs particles at different experimental MPs 342 343 concentrations. These studies observed that MPs affect juveniles feeding behavior and cell of the gut (fewer and disordered microvilli, increased number of mitochondrion and 344 345 the appearance of autophagosome), and in adults the reproductive success and survival of 346 the progeny were significantly impacted (Peixoto et al., 2019; Y. Wang et al., 2019a, 2019b). Bioassays with four different environmentally relevant MPs pollutants which 347 were derived from two facial cleansers (PCCPs), a plastic bag (PB), and PE textile fleece 348 349 in A. franciscana showed an effect on growth of the individual but not on mortality was reported (Kokalj et al., 2018). 350

351 Cole et al. (2013) showed that MPs were ingested and may impact upon by zooplankton, documented by acute bioassays the ingestion, egestion, and adherence of 352 MPs in a range of zooplankton... Beiras et al. (2018) evaluated the toxicity of PE-MPs 353 354 of size ranges similar to their natural food to zooplanktonic organisms representative of 355 the main taxa present in marine plankton. The authors reported that despite documented ingestion of both virgin and BP-3 spiked MPs no acute toxicity was found at loads orders 356 of magnitude above environmentally relevant concentrations on any of the invertebrate 357 358 models. Other publications propose that MPs in zooplankton crustaceans can be 359 accumulated along the food chain, since worldwide many species of invertebrates and vertebrates feed on, for example, the amphipod, along coastal ecosystems (Iannilli et al., 360 361 2018). Publications in field also report MPs ingestion by zooplankton (Amin et al., 2020), particularly in porcellanid and brachyuran larval (Cole et al., 2013; Zeng et al., 2020). 362

In concordance with ingestion, the biodispersion (degradation of MPs through ingestion and dispersion for egestion) of the MPs, some publications report the potential of the species in the biogeochemical cycling and fate of plastic. Dawson et al. (2018) reported turning MPs into NPs through digestive fragmentation by Antarctic krill. On the other hand, Hodgson et al. (2018) examined ingestion and shredding of plastic carrier bags by *Orchestia gammarellus* and reported that amphipods shredded plastic carrier

bags, generating numerous MPs fragments and this substantially accelerated the 369 370 formation of MPs in the environment. In addition, benthic crustacean digestion also can 371 modulate the environmental fate of MPs on the seabed. Cau et al. (2020) provided evidence that *Nephrops norvegicus* is responsible for the fragmentation of MPs already 372 373 accumulated in sediments through its scavenging activity and digestion. These findings 374 highlight the existence of a new peculiar kind of SMPs, introduced in the environment by biological activities, which could represent a significant pathway of plastic degradation 375 (Cau et al., 2020). This would result in the re-emission of smaller and more bioavailable 376 plastic particles that could potentially impact lower trophic levels (Cau et al., 2020). 377

378 *Osmorregulation-ventilation endpoint studies*

379 Publications of plastic presence in the organisms include the plastic presence in the 380 digestive tract (ingestion endpoint) and in the gill chamber (osmorregulation-ventilation 381 endpoint), some of them analyzed both. Not et al. (2020) reported the presence of MPs in cardiac stomachs and gill chambers in four species of mangrove crabs with significant 382 variability in the abundance and types of anthropogenic MPs across sites and species, 383 suggesting that interspecific differences appear to be explained by their particular feeding 384 385 habits, with less selective species ingesting more particles. However, ventilation as a route of uptake of MPs in decapod crustaceans has received little attention. Watts et al. 386 (2014) reported a conceptual model of particle flow for the gills and the gut in Carcinus 387 388 maenas, the MPs can take up through inspiration across the gills as well as ingestion of pre-exposed food al MPs. Gray and Weinstein 2017) suggested that MPs of various sizes 389 and shapes can be ingested and ventilated by adult shrimp, *Palaemonetes pugio* and the 390 391 residence times of these MPs vary and may be influenced by the ability of the individual to remove the particles, resulting in acute toxicity. Studies on Neohelice granulata 392 reported that gills presented higher total abundances of MPs than the digestive tract, 393 394 which suggests that in this case the main uptake of MPs would be by adherence to the gills (Ardusso et al., 2021; Villagran et al., 2020). However, ventilation as a route of 395 uptake of MPs in decapod crustaceans has received little attention. 396

397 *Effects at ecological level*

398 In function of effects at ecological level, Thushari et al. (2017) indicated that the plastic pollutant prevalence in sessile and intertidal communities was correlated with 399 400 contamination characteristics of the habitats, so propose that sessile invertebrates, particularly barnacles can be used as indicators for contamination of plastics in the beach. 401 The problems associated with plastic debris, particularly large plastic items, such as 402 403 carrier bags, on large marine animals are well documented; only in the last decade that 404 the impacts of smaller plastic fragments, particularly on invertebrates, have been considered (Murray and Cowie, 2011). However, some studies reported that from MaPs 405 pollution, for example, the entrapment in plastic debris endangers crabs. Around 61,000 406 407 (2.447 crabs/m2) and 508,000 crabs (1.117 crabs/m2) are estimated to become entrapped 408 in debris and die each year on Henderson Island and the Cocos (Keeling) Islands, 409 respectively (Lavers et al., 2020).

On the other hand, some authors, suggested that behavior may represent a selective advantage to the increasing environmental plastic pollution.. Tutman et al. (2017) suggest that vast quantities of floating debris, comprised primarily of non-biodegradable plastic polymers, probably will augment natural floating substrates in the marine environment, potentially facilitating the spread of invasive species. They reported that the crab *Planes minutus* and *Liocarcinus navigator* were found rafting on plastic macro-litter floating on the open south Adriatic. Another research reported evidence of marine debris usage by

the ghost crab Ocypode quadrata, as burrows with selectivity by some types, such as soft 417 418 plastic, straw, rope and foam (Costa et al., 2018). Tosetto et al. (2016) assessed the 419 impacts of MPs on the ecology of coastal biota using beach hoppers (*Platorchestia smithi*) as model organisms. Exposure tests showed that MPs consumption can affect beach 420 hopper survival and displayed reduced jump height and an increase in weight, however, 421 422 there was no significant difference in time taken to relocate shelter post-disturbance. Ardusso et al. (2021) reported the presence of MPs in shells in females and males of 423 Neohelice granulata due to its close association with the sediment, as a deposit feeder 424 425 and cave builder, and with water, for being a semi-terrestrial species. The presence of MPs in their shells shows that the bioturbation processes can remove and mix the 426 sediment and these contaminants, adhering to their bodies. 427

- 428 8.1.2 Freshwater and Estuarine studies
- 429 *Plastics ingestion field and laboratory studies*

Redondo-Hasselerharm et al. (2018) reported ingestion but no adverse effects on
survival, growth, and feeding rate in a chronic exposure to car tire tread particles on
freshwater benthic macroinvertebrates (amphipods and isopods).

433 *Plastics ingestion – food web transfer laboratory studies*

Regarding freshwater species, several researches indicated the MPs and NPs web 434 435 transfer. The freshwater amphipod Gammarus duebeni can ingest PE-MPs by feeding the contaminated duckweed species Lemna minor (Mateos-Cárdenas et al., 2019). Other 436 studies demonstrated the food web transfer, as, the mechanistic chain from the uptake of 437 NPs particles by algae, through transport up the food chain by Daphnia magna and, 438 finally, effects on the brain physiology and behavior of top consumer (fish) (Mattsson et 439 al., 2017), or food-web transfer of MPs between wild-caught fish and crustaceans in East 440 China Sea (Zhang et al., 2019). 441

Waite et al. (2018) showed that crabs had two orders of magnitude more than oysters when exposed to MPs, indicating the need to investigate bioaccumulation of MPs and biomagnification of associated contaminants at all levels of the food web.

445 <u>8.2 Analysis of plastic toxicity in crustaceans</u>

In the 98 publications reviewed, 53 of them were studies of plastic pollution in crustaceans with analysis of toxicity. Mostly were publications on both acute and chronic toxicity tests in standard test species. In general, the publications of analysis of toxicity combined more than one endpoint.

450 8.2.1 Marine studies

Bioassays in acute toxicity demonstrated that MPs impeded feeding in copepods, which over time could lead to sustained reductions in ingested carbon biomass (Cole et al., 2015). Watts et al. (2015) reported that the ingested food containing PP-MFs generated significantly reduced food consumption and energy available for growth in *Carcinus maenas*.

In acute bioassays it was observed sub-lethal MPs effects on marine crustaceans, in a wide range of MP concentrations, as alterations in swimming and enzyme activities, indicating neurotoxic effects and oxidative stress but without affecting mortality in the larval stages of *Amphibalanus amphitrite* barnacle and of *Artemia franciscana* brine shrimp (Gambardella et al., 2017). Another research assessed the influence of PS-MPs on the feeding behavior and growth rate of a widespread sandy beach amphipod,

462 *Orchestoidea tuberculate*, and reported that the amphipod's absorption efficiency and 463 estimated growth rates were not significantly affected by the concentration of MPs, but 464 high MPs concentrations cause a reduction in the amphipod's consumption rates and, 465 indirectly, may affect the role of this species as the main consumer of stranded seaweeds 466 in sandy beaches ecosystems (Carrasco et al., 2019). In addition, a research suggests that 467 ingesting low concentrations of PS-MPs does not impair the feeding or growth of 468 amphipods during the exposure period (Bruck and Ford, 2018).

469 Regarding enzymatic activity, there is still a lack of information on the effect of MPs 470 on marine organisms at the cellular level. A study provides the first insight into the mode of action in terms of MPs-induced oxidative stress and related signaling pathways in the 471 copepod Paracyclopina nana (Jeong et al., 2017). A recent research analyzed the 472 473 potential and challenges of measuring and relating the changes in the immune response of Neocaridina davidi to MPs exposure (Arias-Andres, 2021). At the moment, two 474 475 research analyzed oxidative stress and genotoxicity by plastics effects. The results of 476 publications on Eriocheir sinensis, an important economic crab found in freshwater 477 fisheries of China demonstrated that MPs can accumulate in the tissues and negatively affect growth; also, exposure to MPs causes damage and induces oxidative stress in the 478 479 hepatopancreas and gets worse with increasing MPs concentration (Yu et al., 2018). Studies about genotoxicity showed genetic damage with PS-MPs in Neocaridina davidi 480 (Berber, 2019). 481

482 *Effects at ecological level*

Transgenerational endpoint analyzed effects at ecological level. Lee et al. (2013) used 483 both in acute and chronic toxicity tests to investigate transgenerational effects through the 484 effects of the sizes of NPs or MPs on the survival, development, and fecundity in copepod 485 486 *Tigriopus japonicas*. This study observed that in the acute toxicity assay, the copepods ingested and egested the MBs and exhibited no selective feeding, the copepods survived 487 488 at all the sizes and the concentrations of MBs tested. However, in chronic toxicity test concentration greater than 0.5- and 6-µm caused a significant decrease in fecundity and 489 larger than 12.5 µg/mL caused the mortality of nauplii and copepodites in the F0 490 491 generation and even triggered mortality at a concentration of 1.25 µg/mL in the next 492 generation.

493 8.2.2 Freshwater and estuary studies

Au et al. (2015) evaluated the effects of PE-MPs and PP-MPs ingestion on freshwater 494 495 amphipods and concluded that in acute bioassays, the PP-MPs were more toxic than PE-496 MPs. However, chronic bioassay (42-d) exposure to PE-MPs particles significantly 497 decreased growth and reproduction. On the other hand, PET-MPs do not negatively affect the survival, development, metabolism, and feeding activity of the freshwater invertebrate 498 499 Gammarus pulex. As a detritivorous shredder, G. pulex is adapted to feed on nondigestible materials and might, therefore, be less sensitive towards exposure to synthetic 500 particles, so authors suggest that the autecology needs to be taken into account and that 501 502 research should focus on identifying traits that render species susceptible to MPs exposure 503 (Weber et al., 2018). In addition, PE-MPs changes in swimming activity of D. magna (De 504 Felice et al., 2019). The freshwater cladoceran, Daphnia sp. is a model species extensively used in environmental monitoring studies and ecotoxicology testing because 505 it is an important environmental indicator species that may be especially sensitive to MPs-506 507 NPs as a result of being filter-feeders. Thus, plastic pollution in freshwater zooplankton was analyzed with more frequency with these species. Particularly, some authors reported 508 for D. magna that the number of ingested beads increased with increasing particle 509

510 concentration and exposure time (Canniff and Hoang, 2018), but Aljaibachi and 511 Callaghan (2018) suggested that *Daphnia sp.* are selectively, avoiding eating plastics, as 512 MPs concentrations increased, intake did not if algae were present. Chae et al. (2018) 513 suggesting trophic transfer indicating that NPs adhered to the surface of the primary 514 producer and were present in the digestive organs of the higher trophic level species, 515 induced histopathological changes in the livers of fish that were directly exposed, and 516 NPs penetrated the embryo walls and were present in the yolk sac of hatched juveniles.

The osmorregulation-ventilation was evaluated in publications that showed that MPs might be inhaled by the organism. Watts et al. (2016) showed that acute exposure to PS-MPs with different surface coatings had significant but transient effects on branchial function as significant dose-dependent effect on oxygen consumption, a significant decrease in hemolymph sodium ions, and an increase in calcium ions.

522 *Effects at ecological level*

Effects at ecological level were reported in a particular study that investigated the effects of NPs at the first two trophic levels of the freshwater aquatic food chain; algae, represented by *Scenedesmus obliquus*, and zooplankton, represented by *D. magna*. They reported that NPs reduced *D. magna* population growth and reduced chlorophyll concentrations in the algae. Exposed *D. magna* showed a reduced body size and severe alterations in reproduction. (Besseling et al., 2014).

529 Other studies were with the transgenerational effects and recovery of MPs exposure in 530 model populations of the freshwater cladoceran D. magna Straus. In these bioassays, the effect criteria were parental mortality, growth, several reproductive parameters, and 531 population growth rate (Martins and Guilhermino, 2018). The authors observed that for 532 533 D. magna recovery from chronic exposure to MPs may take several generations and that the continuous exposure over generations to MPs may cause population extinction 534 (Martins and Guilhermino, 2018). Additionally, Jaikumar et al. (2019) also reported that 535 536 reproductive output of the same species declined in a dose-dependent manner, and also 537 depended of MPs type that is exposed. These results indicate that exposure to MPs can result in significant adverse effects on the population of *D. magna*, including a reduction 538 539 in the number of individuals as well as total biomass (Bosker et al., 2019).

540 <u>8.3 Role of MPs characteristics on biological effects</u>

541 Some scientific papers on ingestion endpoint demonstrated that the shape and chemical 542 profile of MPs can influence its bioavailability and toxicity. Several studies have also 543 shown that the size, shape, and surface physicochemical characteristics of MPs are 544 essential determinants of their biological effects (Lee et al., 2013; Bour et al., 2018; 545 Jaikumar et al., 2019). Bour et al. (2018) reported that the occurrence of MPs in analyzed 546 biota is not influenced by organism habitat or trophic level, while characteristics and 547 typology of polymers might be significantly affected by the feeding mode of organisms.

548 8.3.1 Marine studies

For example, a study reported in an ecologically important cold water copepod *Calanus finmarchicus* that exposure to fibrous or granules of nylon MPs does not alter the same way feeding, impacting lipid accumulation, and the growth and molting (Cole et al., 2019). A research tested in bioassays ingests and retains of MPs for *Uca rapax*, used a particularly MPs pollution, virgin PS pellets submerged at two differently polluted coastal sites for two weeks, to allow the adsorption of organic pollutants as well as the natural colonization of the plastics by bacteria and diatoms, and reported MPs in gills,
stomach, and hepatopancreas, but fragment retention was not influenced by the two
factors that were manipulated (Brennecke et al., 2015). Vroom et al. (2017) reported
another point suggesting that the aging of MPs promotes their ingestion by marine
zooplankton.

560 8.3.2 Freshwater studies

561 Chronic bioassays examined the interactions between the amphipod Gammarus fossarum in combination with two types of MPs (PA-PS). While both tested polymer 562 types are ingested and egested, PA fibers significantly reduced the assimilation efficiency 563 564 of the animals (Blarer and Burkhardt-Holm, 2016). Also, acute bioassays of ingestion and egestion were realized in the model organism D. magna, some of them reported: 565 differences in ingestion depending on the shape (regular and irregular) of the particles, 566 567 egestion of irregular fragments was slower than MPs beads and MPs concentration in the animals was strongly dependent on the initial MPs concentration in the water (Krogh 568 Frydkjær et al., 2017). According to Klein et al. (2021), particle shape does not affect 569 ingestion and egestion of MPs by the freshwater shrimp Neocaridina palmate but 570 571 demonstrate that the ingestion of beads and fragments were concentration- and sizedependent and indicated that *Neocaridina* is not very selective regarding food properties. 572 which might be linked to its omnivorous feeding behavior. In freshwater species, some 573 574 publications report no significant effects in the function of the aged of MPs. A chronic 575 bioassay (45 d) on freshwater amphipod G. fossarum reported that the presence of the plastic foils independent of their age had no significant effects on survival, behavior, and 576 577 feeding (Gerhardt, 2020).

Ziajahromi et al., (2017) analyzed the adverse effects of waste water-derived MPs in 578 579 the survival, growth, and reproduction of Ceriodaphnia dubia, another freshwater 580 standard test species used for bioassays. Binary mixture of MBs and MFb acute exposure showed a dose-dependent effect on survival. Chronic exposure (8d) to lower 581 582 concentrations did not significantly affect survival but had a dose-dependent effect on growth and reproduction. Fibers showed greater adverse effects than PE beads, being 583 observed carapace and antenna deformities after exposure to fibers. . Bioassays with four 584 585 different environmentally relevant MPs pollutants, which were derived from two PCCPs. a PB, and PE textile fleece, in *D. magna* showed a clear exponential correlation between 586 MPs uptake in the gut and the size of the MPs was identified (Kokalj et al., 2018). 587 588 Bioassays in D. pulex indicate that age affects the sensitivity of its individuals to pollution 589 from these NPs, primarily via alterations to vital physiological and biochemical processes, such as cellular energy homeostasis and oxidation, which were demonstrated 590 in vivo (Liu et al., 2018). 591

592 8.3.3 Microplastic fibers

593 On the other hand, MFb from textile weathering and washing are increasingly being recognized as freshwater environmental pollutants, so several studies focus on this type 594 of plastic in PMPs and SMPs conditions (Jaikumar et al., 2019; Jemec et al., 2016; Ma et 595 al., 2016; Ogonowski et al., 2016; Rehse et al., 2016). Booth et al. (2016) evaluated the 596 uptake and toxicity of 2 poly(methylmethacrylate) nanoplastic (P-NPs) with different 597 surface chemistries (medium and hydrophobic) in qualitative uptake and excretion 598 599 endpoints in D. magna. After exposure to these MPs, D. magna did not exhibit any observable toxicity. However, the analyzes of Daphnia juveniles revealed a variety of 600 601 small and rather subtle responses of morphological traits (body length, width, and tailspin length) and for adult alterations in the expression of genes related to stress responses (i.e. 602

HSP60, HSP70 & GST) as well as of other genes involved in body function and body
composition (i.e. SERCA) them of 48h after exposure to MPs (Imhof et al., 2017). In
addition, some publications reported that MPs fibers are more toxic than MPs particles
for crustacean species due to longer residence times of the fibers in organisms gut,
suggesting that the difference in residence time might have affected the ability to process
food, resulting in energy loss, that reflected in sublethal endpoints as growth,
reproduction and carapace and antenna deformities (Ziajahromi et al., 2017).

610 8.3.4 *Microplastic and climate change*

Kratina et al. (2019) studied the impact of combined different types of MPs exposure 611 612 and warming on metabolism and feeding, and the injection of particles in the hemolymph in G. pulex. Organisms were exposed to experimental MPs concentrations (0.52, 26.12, 613 and 104.48 cm-2) at each of three experimental temperatures (9, 15, and 19 °C). The 614 615 results demonstrated that temperature modified the metabolism (respiration) and the feeding rate and showed that in warmer conditions, the effects of ingested MPs were 616 greater than in colder temperatures. Although the physiological response processes 617 618 require additional study, this first work demonstrates that climate change may enhance 619 the negative impacts of MPs in organisms.

620 <u>8.4 NPs- long-term endpoint</u>

As far as the dimensions of the MPs decrease, the negative effects increase. The use of long-term endpoint has been identified as a valuable tool for assessing the impact of NPs on marine planktonic species, being more predictable of real exposure scenarios for risk assessment purposes

625 *8.4.1 Marine studies*

Bergami et al. (2016) and Bergami et al. (2017) provided the first insight into longterm toxicity (14d) of NPs to marine plankton, underlining the role of the surface chemistry in determining the behavior and effects of PS NPs, in terms of adsorption, growth inhibition, accumulation, gene modulation and mortality in the brine shrimp *Artemia franciscana*. In both studies was reported that PS-NPs affects feeding, behavior and physiology of brine shrimp *A. franciscana* larvae.

In addition, Bergami et al. (2016) reported an accumulation of NPs-PS within the gut during the 48h of exposure in bioassays in acute toxicity, indicating a continuous bioavailability of NPs-PS for planktonic species as well as a potential transfer along with the trophic web. Therefore, NPs-PS might be able to impair food uptake (feeding), behavior (motility), and physiology (multiple molting) of *A. franciscana* larvae with consequences not only at organism and population level but on the overall ecosystem based on the key role of zooplankton to non-marine food webs (Bergami et al., 2016).

639 8.4.2 Freshwater studies

Also, publications in freshwater reported in D. galeata exposed to NPs a reduction in 640 survival, reproduction, and growth (Besseling et al., 2014; Cui et al., 2017). In addition, 641 Nasser and Lynch (2016) demonstrated for the first time that proteins released by D. 642 magna create an eco-corona around PS-NPs which causes heightened uptake of the NPs 643 644 and consequently increases toxicity. Rist et al. (2017) demonstrated that the lower egestion and decreased feeding rates, caused by the NPs, could indicate that particles in 645 646 the nanometer size range are potentially more hazardous to D. magna compared to larger 647 particle sizes.

Cole et al. (2015) reported that PS-NPs might be able to impair food uptake (feeding),
behavior (motility), and physiology (multiple molting) of brine shrimp larvae with
consequences not only at organism and population level but also on the overall ecosystem
based on the key role of zooplankton to non-marine food webs.

52 Studies with NPs, reported that NPs can be ingested by *D. pulex* and affect its growth 53 and reproduction as well as induce stress defense (Liu et al., 2019). Given the importance 54 of *Daphnia sp.* in freshwater food webs, both as a grazer as well as a food source, this 55 can potentially impact the functioning of the ecosystem.

656 **CONCLUSIONS AND FUTURE DIRECTION**

Although Crustacea is a diverse taxon with many different ecological and morpho-657 functional adaptations, most studies in emergent pollutants and specifically in MPs and 658 NPs, are recent and focused on a (very) low number of species/genera (59). Nowadays, 659 most of the studies are reported in shallow marine and freshwater species with a small 660 percentage in deep-waters and estuaries. In particular, brackish waters are stressful 661 physiological habitats, so the impact of the pollutants may increase their biological effect 662 663 since crustaceans need an extra energy demand to cover this habitats stressful. Besides, 664 these areas receive the drainage of terrestrial ecosystems, thus increasing the amount of plastics available for aquatic species. Moreover, wetlands, mangrove swamps, and reefs 665 666 are recognized as nursery areas for breeding and early development of several invertebrate species and fishes, most of them valuable commercial species in addition to 667 668 their ecological roles.

We believe it is very important to plan and focus future analysis (both field and 669 670 laboratory assays) on more crustacean species to highlight a particular effect or a more 671 generalized one. Native species are also an important target of pollutants and in most cases they are not studied or analyzed because they are not properly known in their basic 672 biology. Also, species complexes (e.g. symbiotic/commensal species) are not usually 673 674 analyzed in ecotoxicological analysis, and they represent eco-morpho-functional associations that need to be studied together. Crustaceans also represent (mainly those 675 from zooplankton and larvae of many taxa) key species in complex marine, salt marsh, 676 and freshwater trophic webs, so the impact through biomagnification warrants more 677 678 studies. "Higher" crustaceans (e.g. shrimps, lobsters, crabs) have a high economic value and they support pricey and important worldwide fisheries. This implies both an 679 economic impact and a possible effect on human health by ingestion. Thus, studies 680 681 targeting multi-species assemblages need to be a priority for the future.

We also consider the relevance of pointing out general analysis trends, including many 682 levels of approach as cellular, metabolic, tissue damage, functional and behavioral effects 683 684 besides mortality. This would let us compare many biological processes as a whole when analyzing the effect of these emergent pollutants in different species/taxa. Monitoring 685 686 programs involving a broad range of synergetic bioindicators in crustaceans are still missing. Besides, another more general physiological process in crustaceans, as 687 Ecdysozoa, offers the unique possibility of analyzing other complexes hormonally and 688 689 environmentally regulated events, the ecdysis, scarcely studied with NPs and MPs. Crustaceans offer wonderful models of embryo development including complex 690 691 segmentation patterns, egg dormancy, different embryo morphogenesis, and complex 692 patterns of larval and postlarval development and later metamorphosis, scarcely analyzed 693 as a response variable. All of these biological processes could be reversible or irreversibly 694 impaired by plastics and have transgenerational effects.

From another point of view, the areas/countries where effects of MPs and NPs in 695 696 crustaceans have been studied are a very small sample of aquatic ecosystems highlighting 697 the urgency to study more extended areas around the world. Artic and Antarctic seas, 698 marine protected areas or marine canyons, including the Marianas Trench among others, 699 are not or are scarcely explored in this aspect, and there are recognized as very vulnerable 700 ecosystems (Rowlands et al., 2021). In addition, the need to further investigate the 701 ecological impacts of MPs on wild nekton, especially commercially important species, and its potential implications for human health. Furthermore, plastic particles could also 702 703 produce dispersal of larvae, juveniles, or adults via rafting events, thus increasing the 704 problem of spreading invasive species.

Finally, another aspect scarcely analyzed is plastic as a carrier/adsorbent surface for other pollutants thus increasing the potential risk of this emergent problem and/or a suitable substratum for pathogenic micro-organisms and parasites to enter in crustaceans increasing their toxic effect. In this latter sense, plastic could be the "troyan horse" that hides other dangerous effects for crustaceans.

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Figure Caption

Figure 1 - Number of analyzed papers about plastic contamination in crustaceans in the last 10 years and their distribution around the world. Bottom left: a chronological representation of the publications reviewed.

Figure 2. Keywords analysis. Keywords mentioned in more than three publications were represented.

Figure 3. Discrimination of plastics analyzed in the reviewed papers based on size (Macroplastic, Microplastic; Nanoplastic) and type. Discrimination based on size: A-Macroplastic. B- Microplastic. C- Nanoplastic. The numbers indicate the number of studies and the percentage of the total studies in that item. The table indicates the full name of the abbreviations for the composition and form of plastics.

Figure 4. Representation of the crustacean taxa studied in the 98 publications reviewed (the 4 reviews were not included). Numbers indicate: the number of studies, the percentage of the total studies in this taxon and the number of species analyzed in this taxon.

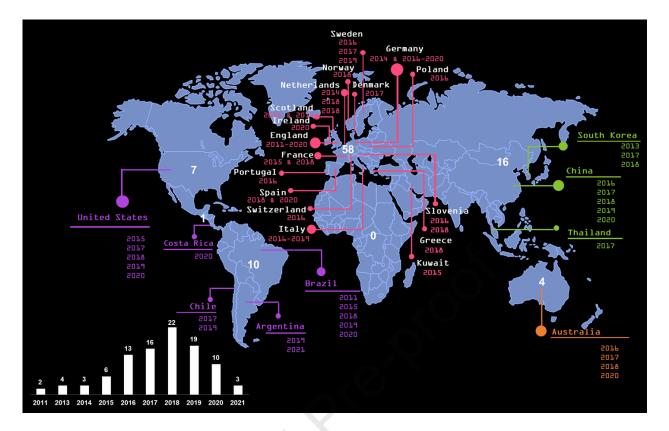
Figure 5. Comparison among the number of publications (at left), the number of species and taxa (at right) and the interest of the species studied (in the center) based on the habitat.

Figure 6. Chronological representation of the 98 publications reviews differentiating the interest of the species study (standard test species, economic species, and ecological species) and the type of studies (field studies or laboratory studies).

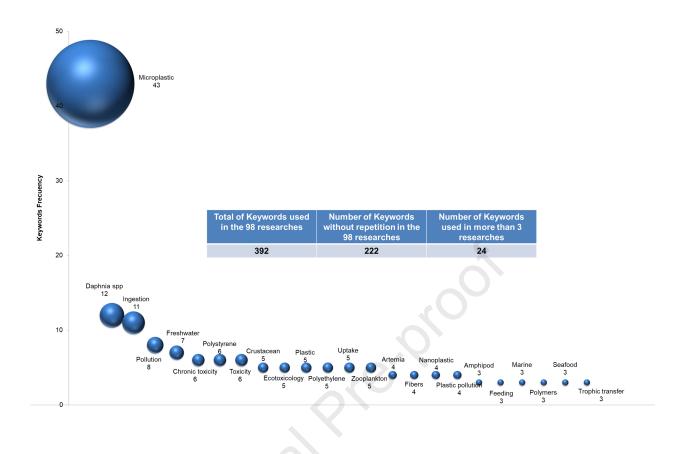
Figure 7. Chronological representation differentiating studies describing the presence of plastics in the crustaceans *versus* those with the analysis of plastic toxicity for the organism. The sectors diagram compares presence studies *versus* toxicity studies over the total of publications reviewed.

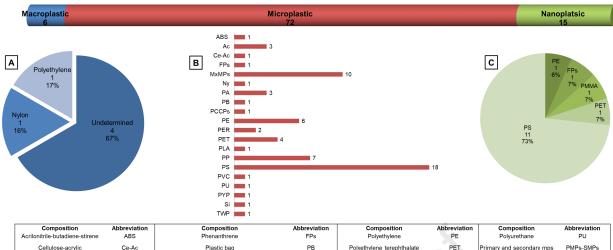
Figure 8. Total number of endpoints analyzed in the reviewed papers discriminated for individual *versus* ecological level effects.

Keywords Categories	Keywords of the Studies
Plastic size as	biofilm, cosmetics, fishing, fishing waste, litter, marine debris, marine
macroplastics	litter, plastic bag, plastic, plastic debris, plastic pollution, plastic wasted,
	single-use carrier-bags, solid waste, textile
Plastic size as	beads, fibers, microbeads, microfibres, microplastic, particles, plastic
microplastics	filaments, primary and secondary microplastics, synthetic fibers
Plastic size as	nanoparticle, nanoplastic, nanosafety
nanoplastics	
	biopolymer, phenanthrene, polyester fibers, polyethylene, polyethylene
Plastic type	beads, polymers, polystyrene, polystyrene beads, pvc, nylon; or with its
	relationship with other contaminants: benzophenone-3, cathepsin l-like
	protease, deltamethrin, insecticide, mercury, nickel
Plastic as pollutants	ecotoxicology, ecotoxicity, toxicology, toxicity, pollution,
	contamination, marine pollution
	Amphipod, Aristaeopsis edwardsiana, Artemia, Aristeus antennatus,
	Carcinus maenas, Ceriodaphnia dubia, Chinese mitten crab, Chlorella vulgaris, Corophium volutator, Crangon crangon, Crustacea, Daphnia
Crustacean as plastic	spp, Decapoda, Eriocheir sinensis, Gammarus fossarum, Homarus
target and trophic	americanus, Neocaridina davidi, Neocaridina palmate, Neohelice
impact	granulata, Nephrops, Dimethoate, Echinogammarus marinus, Emerita
impuet	analoga, Grapsidae, Ocypodidae, Orchestoidea tuberculata, Plesionika
	narval, Polybiidae, Raphidocelis subcapitata, Selenastrum
	capricornutum, seafood, shrimp, and Uca rapax
Crustacean as trophic	bivalve, macroalgae, macroinvertebrates and wild fish.
impact	
	benthic, biofouling, burrow, coastal ecosystem, coastal pollution, deep
Habitat ar lifestyles	sea, estuary, filter feeder, freshwater, host, limnology, marine, marine
Habitat or lifestyles	plankton, marine zooplankton, sandy beach, sand hopper, sessile
	invertebrate, zooplankton
	Adriatic sea, Aegean sea, Bahía Blanca, Baltic sea, Clyde sea, Indian
Geographical place	ocean, Indian river lagoon, Malaysia, Mediterranean sea, Mosquito
	Lagoon Sand eel, South China sea, South Pacific gyre, Tagus estuary,
	and Thames estuary
	absorption, accumulation, assimilation, association, behavior, bioaccumulation, bioindicators, biomonitoring, biotransference, body
	burden, carrying capacity, complex toxicity, defecation, deposit feeder,
	depuration, developmental and reproductive toxicity, detection, deposit reeder,
	digestive tract, elimination, embryo-larval bioassays, entrapment,
	environmental fate, enzyme activity, epithelia, feeding, feeding
	behavior, feeding rate, feeding strategy, fiber ingestion, food safety, food
	web, frequency of occurrence, functional group, gene expression, gills,
	growth, growth inhibition, gut, health condition, hepatopancreas, human
For Joy a Star Am	health warning, indicator organism, ingestion, ingested plastics, joint
Endpoints	toxicity, LC_{50} , life history, marine food chain, marine food web,
	ultrastructure, microplastic accumulation, microplastic ingestion,
	mitogen-activated protein kinases (MAPK), molting, monitoring,
	mortality, nutrition, oxidative stress, percentage points, plastic ingestion,
	population dynamics, protein corona, quantification, rafting,
	regurgitation, reproduction, residence time, retention, stomach contents,
	sub-lethal toxicity, swimming, transfer, transgenerational study, trophic,
	trophic impacts, trophic transfer, uptake, and ventilation. Finally, some
	of them were related to the methodology used: acute effects, acute
	toxicity, chronic toxicity, field study, fluorescent Green, fTIR, low-dose.

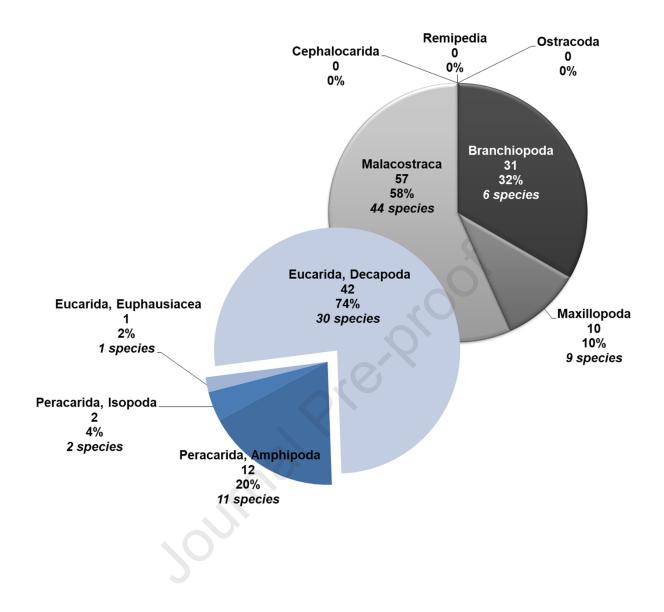


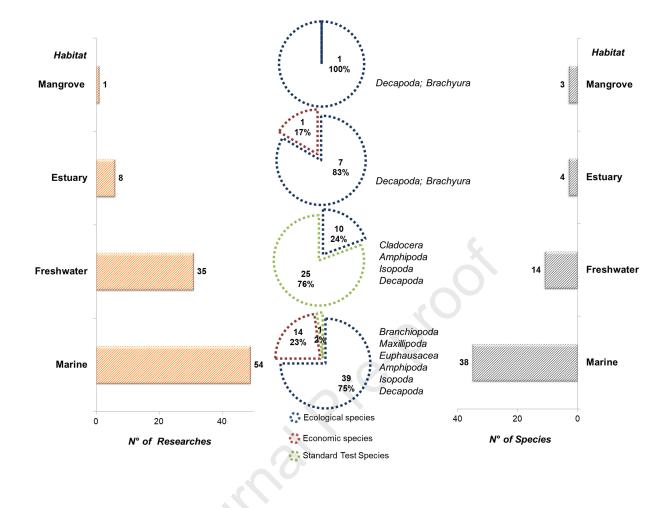
Journal

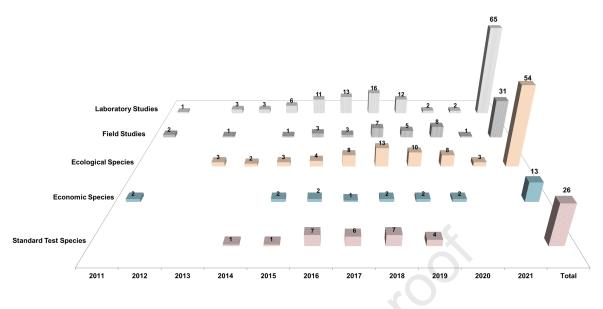




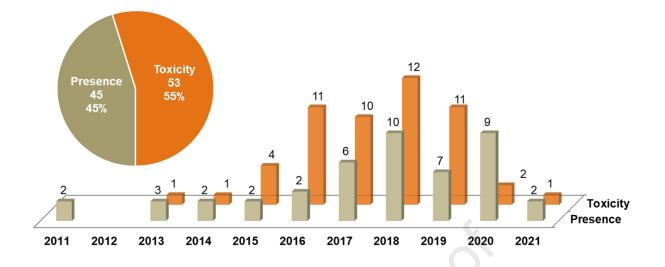
Acrilonitrile-butadiene-stirene	ABS	Phenanthrene	FPs	Polyethylene	PE	Polyurethane	PU	
Cellulose-acrylic	Ce-Ac	Plastic bag	РВ	Polyethylene terephthalate	PET	Primary and secondary mps	PMPs-SMPs	
Microbeads Microfibers	MBs MFb	Polyacrylic - polyacrylate - acrilic particles Polylactic acid	PAc PLA	Polyisoprene Poly(methylmethacrylate)	PYP PMMA	Silicon Tire wear particles	Si TWP	
Microspheres	MPh - PS	Polyamide	PA	Polypropylene	PP	Two facial cleansers (cosmetics products)	PCCPs	
Nanobeads - polystyrene	NBs- PS	Polyester	PER	Polystyrene	PS	Undetermined - mixture of MPs	MxMPs	



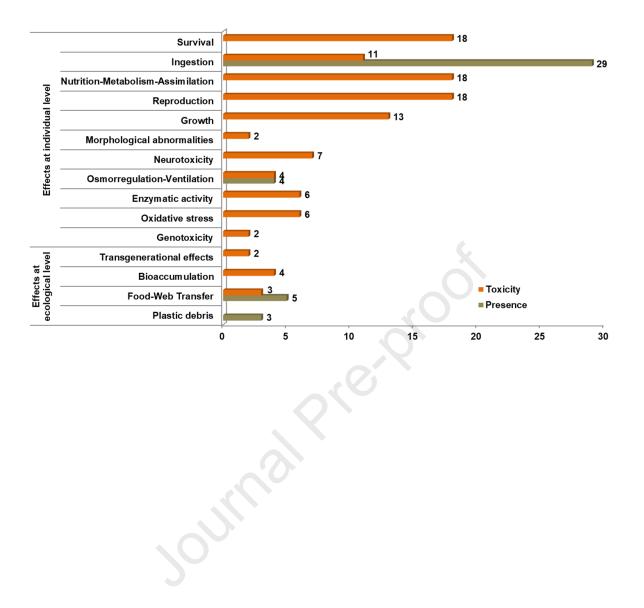




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Declaration of interests

xThe authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

□The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

