The ecological impacts of marine debris: unraveling the demonstrated evidence from what is perceived.

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Abstract

Anthropogenic debris contaminates marine habitats globally, leading to several perceived ecological impacts. Here, we critically and systematically review the literature regarding impacts of debris from several scientific fields to understand the weight of evidence regarding the ecological impacts of marine debris. We quantified perceived and demonstrated impacts across several levels of biological organization that make up the ecosystem and found 362 perceived threats of debris across all levels. 292 of these perceived threats were tested, 80% of which were demonstrated. The majority (82%) of demonstrated impacts were due to plastic, relative to other materials (e.g., metals, glass) and largely (89%) at suborganismal levels (e.g., molecular, cellular, tissue). The remaining impacts, demonstrated at higher levels of organization (i.e., death to individual organisms, changes in assemblages), were largely due to plastic marine debris (>1 mm; e.g., rope, straws and fragments). Thus, we show evidence of ecological impacts from marine debris, but conclude that the quantity and quality of research requires improvement to allow the risk of ecological impacts of marine debris to be determined with precision. Still, our systematic review suggests that sufficient evidence exists for decision-makers to begin to mitigate problematic plastic debris now, to avoid risk of irreversible harm.

Keywords: Plastic debris, systematic review, biological organization, ecology, population, assemblage

Introduction

Under existing legislation, materials are not considered hazardous unless research demonstrates with certainty that a material harms humans, wildlife and/or the environment (EU Directive 2008/98/EC, EU 52000DC0001, USEPA CERCLA, ILGRA 2002). If classified hazardous, existing laws can be used to help eliminate sources, rehabilitate habitats, find safer alternative products (Rochman et al. 2013a) and/or shift the burden of proof towards the manufacturer to demonstrate safety (EU 52000DC0001 ILGRA 2002). For marine debris, decisions-makers have been unable to use existing laws to mitigate contamination because they are uncertain about the nature and extent of the risk of marine debris to humans and the ecosystem. This lack of understanding is surprising because peer-reviewed literature describing marine debris has grown substantially since the turn of the century. A search of the literature in *Web of Science* for key words “marine debris” and “plastic debris” shows that the number of studies published annually has doubled since the year 2000. In the year 2000, 65 and 85 studies were published respectively and in 2013, 129 and 182 studies were published respectively. This large increase in scientific literature probably reflects a growing concern that marine debris is hazardous and requires appropriate responses.

Contamination of habitats and organisms by marine debris is now globally ubiquitous (Thompson et al. 2009) with no signs that environmental accumulations are decreasing (Thompson et al. 2004, Law et al. 2010). Debris contaminates a diversity of habitats, including shorelines (Browne et al. 2015a), coral reefs (Donohue et al. 2001), shallow bays (Endo et al. 2001, Ashton et al. 2010), estuaries (Browne et al. 2010), the open ocean (Carpenter and Smith 1972, Cozar et al. 2014) and the deep sea (Goldberg, 1997, Galgani et al., 2000). Exposure of organisms to marine debris causes concern, and the quantity, frequency of occurrence, type and size of debris may all determine the consequences to wildlife, and ultimately, the ecosystem. Contamination in the form of entanglement and ingestion is recorded in tens of thousands of individual animals (Gall and Thompson 2015) and at least 558 species, including all known species of sea turtles, 66% of all species of marine mammals and 50% of all species of seabirds (Kühn et al. 2015). In some species, ingestion is reported in over 80% of a population sampled (e.g., Murray and Cowie 2011, van Franeker et al. 2011). Moreover, marine debris hosts microbial assemblages distinct from surrounding seawater through the creation of novel habitat (Zettler et. al 2013).

Scientists, industry and government are in agreement that marine debris is a global environmental issue, contaminating habitats and wildlife from the poles to the equator (Thompson et al. 2009, Sutherland et al. 2010, Browne et al. 2015a, Gall and Thompson 2015). Now, scientists and policy-makers aim to understand the ecological *impacts* of this debris on the biosphere. There can be no doubt that marine debris poses several potential threats. It may be hazardous to wildlife physically, by entanglement and ingestion, or via alteration of habitat and/or transport of non-native and potentially pathogenic species (i.e., acting as potential fomites; Gregory 2009, Zettler et al. 2013). It can also be hazardous to wildlife chemically, if chemical constituents of the debris itself (i.e., incorporated during manufacture) or environmental chemicals (i.e., organic and metal pollutants sorbed from the surrounding environment) are transferred to the tissues of organisms upon direct ingestion of plastic or via the foodweb (Browne et al. 2013, Rochman et al. 2013b, Tanaka et al. 2013). It may even be hazardous to humans because small particles of debris, demonstrated to bioaccumulate in tissues of animals (Browne et al. 2008), are present in a range of commercially important fish and shellfish (Choy et al. 2013, van Cauwenberghe and Janssen 2014). Although there is considerable evidence of harmful effects on individual organisms and seemingly many perceived threats to populations, assemblages and species, there is currently little knowledge or agreement regarding whether such potential threats are demonstrated ecologically relevant impacts, affecting wildlife at higher levels of biological organization.

Here, we aim to understand the weight of the evidence regarding perceived and demonstrated impacts and whether existing published data demonstrates ecological effects of marine debris. For several environmental stressors, including marine debris, effects are shown at one or several different lower levels of organization (e.g., molecular, cellular, organism; Underwood and Peterson 1988, Adams et al. 1989). Although ecological impacts are generally considered those relevant to populations, assemblages and species, understanding responses at these lower levels of organization can provide insight into causal relationships between stressors and their effects at ecological levels (Adams et al. 1989, Browne et al. 2015b). As such, we examined the evidence across several levels. Moreover, because other types of debris (e.g., terrestrial, atmospheric and medical) are the same type, shape and size and likely behave similarly to marine debris, we examined the literature for impacts from debris in general to gain further insight into how marine debris may impact marine organisms.

Several narrative reviews provide useful information regarding the historical developments about the extent to which organisms are contaminated with debris. Yet, these provide: (i) limited information to demonstrate biological, and especially ecological, impacts, and (ii) no systematic and critical assessment of the quality, quantity and level of uncertainty of evidence about these impacts. To evaluate the weight of evidence regarding the ecological impacts of marine debris, we systematically and critically reviewed relevant literature regarding effects of debris at several levels of biological organization, spanning the fields of medicine, biological oceanography, conservation biology, toxicology, and ecology, asking two questions: 1) What are the perceived threats? and 2) What are the demonstrated impacts of debris?

Methods

Literature Review

We systematically reviewed the literature regarding contamination (i.e., the presence of debris) and pollution (i.e., a biological response to debris) associated with debris, to determine the perceived, tested, and demonstrated impacts of marine debris to marine life. We searched the literature using Web of Knowledge, Science Direct and Scopus for the keyword terms: “marine debris” and “plastic debris” from all available years for each database (1898, 1823 and 1990 respectively) through 2013. In addition, we examined the journals of *Marine Pollution Bulletin* and *Environmental Science & Technology* for these keywords individually because of their publication record regarding this topic. Our literature search resulted in a collection of literature spanning the fields of medicine, oceanography, conservation and marine biology, toxicology and ecology. We chose to examine studies across a broad range of disciplines to gather knowledge regarding the effects of debris in general. Thus, all papers discussing impacts relevant to marine debris, including studies regarding medical, terrestrial and atmospheric debris, were included in our systematic review.

Data Extraction and Quality Assessment

Two individuals from our group first assessed publications for relevance to our objective based upon the title and abstract, then further reviewed these for relevance to impacts (see Appendix for a list of references). Any discrepancy was discussed among co-authors of this paper. For each relevant publication, we examined perceived and demonstrated effects of debris at 14 levels of biological organization: subatomic particle, atom, small molecule, macromolecule, molecular assemblage, organelle, cell, tissue, organ, organ system, organism, population, species and assemblage. There were no studies discussing impacts to a species, so we only report findings from 13 levels. We then categorized each paper according to the levels of organization discussed and sorted them into the following broader categories: suborganismal,

organismal and ecologically relevant levels (population and assemblage). All publications that were included were assigned to co-authors of this paper for data extraction. Where appropriate, we recorded information regarding the source of the material (i.e., grey literature, conference proceeding, peer-reviewed paper with original data, peer-reviewed paper with no original data), characterization of the affected area (i.e., location of study, type of area studied: habitat, organism, cell, etc.), the pattern and/or perceived threat determined, source of data (i.e., anecdotal, qualitative description, quantitative, correlative, experimental), characteristics of debris (i.e., shape, size, type, whether the type of material was identified using appropriate methods), logic and interpretation (i.e., clarity, closeness of fit to hypotheses), experimental design (i.e., use of controls, environmental relevance of exposures) and statistical analyses (i.e., appropriateness of tests, statistics done appropriately). Those who synthesized the data returned to each paper to confirm the data that was extracted before including it in analysis. Any discrepancy was discussed among co-authors to reach an agreement, which occurred for fewer than ten publications in total. In addition, all data was revisited to assure numbers within the spreadsheet, figures and tables matched. Errors, such as typos or mathematical errors, were fixed and were never found to change our overall results by more than 5%. Figure 1 summarizes this process (see Appendix for the detailed protocol).

Synthesis of data

We only used primary literature and excluded non-original data from review papers. We synthesized perceived (i.e., hypothesized in or described by extrapolation from the data presented within or from other studies), tested and demonstrated impacts of debris across each level of organization in increasing order of ecological relevance using an established framework for pollutants (Adams et al. 1989).

For each study, we recorded the size of debris, the level of biological organization, whether the impact was solely perceived, tested or demonstrated and the nature of the impact. For many papers, impacts were discussed at multiple levels of biological organization and sizes of debris. Each impact, from each size of debris at each level of biological organization, were accounted for individually and plotted on a matrix depicting the magnitude of total impacts at each level of biological organization as a function of the size of debris, ranging from 1 nm (e.g., nanomaterial) to 1 km (e.g., fishing net) (Figure 2). All perceived impacts are depicted in Figure 2a, those that were tested in Figure 2b and impacts that were both tested and demonstrated in Figure 2c.

Several studies made assumptions about how contamination by debris may be harming wildlife or how an effect at one level of organization will affect the organism at a higher level of organization. Such studies do not demonstrate an impact and thus are depicted only in the matrix of perceived impacts (Figure 2a). We did not consider correlative evidence to have demonstrated an impact with the same level of confidence as experimental evidence, because any correlation of an impact with an amount or type of debris could be due to other causes and not the debris itself (Goodsell et al. 2009). As such, where there was correlative evidence, it is depicted with a diamond symbol among the demonstrated experimental evidence (Figure 2c) and was not included in calculations for the quantity of demonstrated impacts caused by debris (Table 1). Some studies were not properly controlled, used environmentally unrealistic or irrelevant exposures and/or lacked proper statistical procedures or interpretations. These could not be considered to have demonstrated an impact where the difficulties of the study compromised or confounded any interpretation and thus were included as tested but not as demonstrated. See Appendix Tables A1, A2 and A3 for lists of studies included in Figure 2 and rationale for inclusion in 2a, 2b and/or 2c respectively and Appendix Table A4 for a list of studies not included in Figure 2 and rationale for not including them).

Because our objective was to evaluate the weight of evidence regarding ecological impacts, we highlighted studies that included impacts from debris at the highest levels of organization (organismal and the ecologically relevant levels of population and assemblage; i.e., in the top three rows of Figure 2). These are depicted in a separate figure (Figure 3), considering an impact undeniably demonstrated when the observed effect could only have been caused by the debris. We classified effects as: (i) organism: an individual organism's death was a direct result of debris, (ii) population: population size changed as a result of debris, (iii) assemblage: there was a change in the structure or composition of assemblages as a direct result of debris. All studies discussing effects at these levels were revisited by two co-authors to determine whether: (i) no effect had been suggested, (ii) an effect had been perceived but not tested and demonstrated, (iii) an effect was tested and the results explicitly did not show any effect or (iv) where an effect had been perceived, tested and demonstrated.

Results

Perceived Impacts

The reviewed literature shows that scientists perceive hundreds of impacts from debris across all levels of organization, from subatomic to assemblage (Figure 2a). In total, there were 362 cases of perceived impacts due to debris composed of several materials. Overall, perceived impacts from plastic marine debris overwhelmed cases due to other types of debris, a trend that is consistent with relative amounts of debris found in marine habitats (Barnes et al. 2009). 87% of described perceived impacts included an association with plastic, 21% with metal, 2% wood and <1% glass. To organize our results, we discuss perceived impacts from debris according to size; debris > 1 mm (hereafter called macrodebris) and debris < 1 mm (hereafter called microdebris) as in Browne et al. (2010).

Overall, 57% (207 of the 362 cases) of perceived impacts were associated with microdebris composed of only two types of materials, 77% plastic and 25% metal (note some studies perceived effects from both metal and plastic). Studies about impacts from microdebris in marine habitats were scarce. Only 13% of perceived impacts for microdebris were about marine debris. The remaining studies were from literature researching the impacts of medical debris (debris originating from implanted medical devices; 72%), nanomaterials (6%, although sometimes involving their impacts in aquatic habitats) and atmospheric debris (6%), all relevant to marine debris according to size, type and route of exposure. Moreover, for microdebris, the majority of perceived impacts (93%) were suborganismal, with many suggesting changes in the structure and functions of macromolecules and cells from medical debris and inhalation/ingestion of small particles. See Table 1 for a list of perceived impacts within each level of organization.

The remaining 43% (155 of 362 cases) of all perceived impacts were about macrodebris. Perceived impacts from macrodebris were related to four types of materials: 99% of studies discussed plastic, 15% metal, 5% woody debris and 1% glass. In contrast to microdebris, the majority (87%) of concerns about macrodebris were about marine debris. Macrodebris in marine habitats has been studied for decades (Laist 1987). The remainder was from literature discussing the impacts of medical debris (5%) and terrestrial debris (8%). Unlike microdebris, the majority of concerns (58%) for macrodebris were potential impacts to individual organisms (i.e., death) and/or ecological impacts to populations and assemblages. These perceived impacts were relatively evenly distributed across these higher levels of organization and were generally due to ingestion, entanglement and the transport of non-native species. See Table 1 for a breakdown of perceived impacts within each level of organization.

Demonstrated Impacts

Of the 362 perceived impacts, 292 (81%) were from studies that tested hypotheses. The remaining were impacts extrapolated or theorized within the discussion of the manuscript regarding how their findings might lead to harmful effects to organisms or how an impact at one level of organization may lead to an effect at a higher level of organization. Of the perceived impacts that were tested, 235 (80%) were demonstrated using non-correlative experimental evidence and 15 (5%) were demonstrated via correlative evidence (shown as diamonds in Figure 2c). It is noteworthy that none of the remaining 15% found that debris did *not* cause an effect. Rather, these remaining studies claimed to demonstrate an impact, but we could not accept that the impact had been demonstrated unambiguously because the studies lacked appropriate controls, used inappropriate statistical methods or misinterpreted their results. For example, some experiments did not include negative controls and thus could not determine if the impact was from the debris or from some other factor in their design. Other experiments used inappropriate statistical tests (e.g., a 1-factor analysis when the study was clearly multifactorial). Some studies simply claimed to show an impact when there were no data to support this view.

Overall, the majority (89%) of demonstrated impacts were at suborganismal levels of organization and the majority (83%) were due to plastic debris. Of the demonstrated impacts at suborganismal levels of organization, 77% were due to microdebris and were solely caused by plastic (74%) and metal (28%) (note some studies considered effects from plastic and metal). For microdebris, only 12% of the studies were related to marine debris while 70% were related to medical debris, 7% to nanomaterials (sometimes regarding their impacts in aquatic habitats) and 11% to atmospheric particulates. Impacts from microplastic at suborganismal levels were generally demonstrated via laboratory experiments and due to inhalation/ingestion or to wear debris from surgical materials. The remaining 23% of demonstrated impacts at suborganismal levels were caused by macrodebris; all due to plastic. Some studies included multiple types of debris and thus some of these impacts were also caused by metal (24%), glass (3%), and wood (3%). In contrast to microdebris, 72% of demonstrated impacts from macrodebris were related to marine debris and the other 28% to terrestrial debris (e.g., plastic debris ingested by goats). Demonstrated effects from macrodebris at suborganismal levels were all due to entanglement and ingestion. See Table 1 for the biological levels at which suborganismal impacts were due to micro- and macro-debris. Such impacts at suborganismal levels are specific, related to a particular physiological mechanism and are considered less ecologically relevant (Adams et al. 1989).

At organismal and ecological (population and assemblage) levels of organization, evidence of demonstrated impacts relative to perceived threats was extremely sparse (Figure 3). At these higher levels of biological organization, we found 26 examples of non-correlative demonstrated effects in 17 published studies (Figure 3). The majority of these effects were at the organismal level (92%), demonstrating deaths of individuals due to debris. In fact, all demonstrated deaths of individual organisms were due to marine debris and demonstrated at nearly all sizes of debris examined (Figure 3). The remaining 8% were impacts at levels considered ecologically relevant and were solely demonstrated for assemblages and due to marine debris.

In fact, all of the evidence for impacts at higher levels of organization (i.e., organism and above) came from studies testing hypotheses regarding the effects of *marine debris*, 85% of which examined effects of macrodebris. All of the impacts were due to plastic debris, and only 2 include impacts from metallic debris and 2 from glass debris. The most common items of marine debris reported to cause demonstrated effects at the organism or ecological levels were lost fishing gear (e.g., nets) and other items of plastic debris such as rope, bags, straws and degraded fragments.

Of these demonstrated impacts on organisms, 63% of deaths were caused by ingestion, 29% by entanglement and 8% by smothering. Demonstrated organismal effects from ingestion were reported for 2 species of marine mammals, 1 species of sea turtle, 1 species of seabird and 2 species of marine invertebrates. Demonstrated organismal effects from entanglement have been reported for 27 species of fish, 10 species of marine mammals, 49 species of seabirds, 1 species of sea snake and 75 species of marine invertebrates. Demonstrated organismal effects due to smothering were reported in one species of cord-grass, *Spartina alterniflora*, including the complete loss of vegetation in some cases (Uhrin and Schellinger 2011). Because many other species are associated with *Spartina alterniflora*, there may well be effects to the associated assemblage, but this was not examined.

We found 2 examples of demonstrated impacts to assemblages. One demonstrated the negative ecological impact of derelict fishing-gear smothering a coral assemblage and causing the mortality of several species of corals and associated sessile fauna (Moore et al. 2009). The second study demonstrated an ecological effect whereby adding plastic bottles and glass jars to a soft sediment benthic habitat altered the assemblage of soft-bottom benthic organisms (Katsanevakis et al. 2007). They found more organisms and species where debris was added, possibly explained by the debris providing extra hard-substratum for some species (including one species each of gastropod, ascidian and sponge) and acting as a refuge for others (including one species of hermit crab; Katsanevakis et al. 2007).

Discussion

Our systematic review confirmed that there are many perceptions about how marine debris can cause harm in marine habitats (i.e., many cases of perceived impacts) across all levels of biological organization. Here, we show that many of these perceptions have been tested, and that in every case where an effect was properly tested an impact was demonstrated. Thus, we found substantial evidence of impacts caused by debris, including marine debris. Overall, we found numerous impacts at suborganismal levels, several at the organismal level demonstrating clear evidence that marine debris can be the cause of death in individual organisms and little at the ecological levels demonstrating that marine debris can alter assemblages.

While we found most evidence at suborganismal levels, it is not a foregone conclusion that sublethal effects and/or increased mortality due to debris will cause an ecological impact (the evidence of deaths of individuals observed here may suggest a hazard to substantial numbers of individuals, and therefore possibly to the population and/or assemblage). Furthermore, it is noteworthy that we narrowed the definition of an organismal effect to death, ignoring the fact that demonstrated sublethal impacts (e.g., reduction in weight, changes in behavior) on many individuals is often inferred to affect populations. To be sure that such an ecological response exists, requires a stronger weight of evidence at ecological levels or the establishment of clear linkages between impacts caused by debris at lower levels to ecological impacts (Browne et al., 2015b).

Thus, our findings do demonstrate impacts from marine debris, but also demonstrate that the quantity and quality of current research regarding *ecological* impacts of marine debris requires improvement before any clear general ecological conclusions could be reached. Due to the large amount of literature reviewed, it is not possible to provide details describing every scenario where impacts were demonstrated or not (See Appendix Tables A1, A2 and A3 for detailed information regarding all studies included in our systematic review). Instead, below we selected examples to illustrate the state of the present knowledge represented in the literature.

Several studies investigated environmental contamination caused by marine debris and discussed perceived ecological impacts, but did not measure any. For example, Carson et al. (2011) measured the permeability and thermal properties of the sand on beaches in experimental areas where they mixed sediments with specified amounts of plastic (< 10 mm in size). Experimental sediments increased water-flow and warmed more slowly than did natural sediments (although these effects were only significant for plastics in large amounts, i.e., treatments with 10 – 20 times more plastic than found on average in the field). Thus, debris in large amounts can clearly alter physical attributes of sediments, which may, as pointed out by Carson et al. (2011) cause alterations to populations and assemblages or to reproduction and survival of individual animals in the sediments, but these were not examined.

Our systematic review found that for some studies (9 in total—2 about marine debris and 7 about medical debris), the perceived impact was not tested using well-designed experiments. For example, some studies simply did not test the hypotheses regarding effects that were discussed. Others used inappropriate designs or contained statistical errors and thus results were not interpreted correctly. Some failed to include a negative or procedural control making it impossible to determine if the observed effects were due to the debris or some other experimental factor. In such cases, the data were not sufficiently convincing for us to accept that an effect had been demonstrated.

In other cases, effects (including at the population and assemblage levels) were accepted as demonstrated, but with less confidence because experiments were correlative and thus difficult to interpret as conclusive. For example, Ödzilek et al. (2006) found a negative correlation between amounts of debris on different parts of the Turkish coastline and the success of hatchling turtles (*Chelonia mydas*) reaching the sea. The authors attributed this to larger numbers of predatory ghost-crabs where there was more debris, but also noted the limitations of their study in that the turtles and crabs could well have been affected by numerous environmental variables other than marine debris.

Several studies used experimental comparisons and demonstrated clear evidence of impacts, including at the higher levels of organism and/or assemblage. Uhrin and Schellinger (2011) tethered wire crab-pots and, separately, tires in areas of saltmarsh, keeping areas with no attached debris as controls. After 9 or 13 weeks, there was a sustained decrease (56% due to crab-pots and 54% due to tires) in amounts of cordgrass, *Spartina alterniflora*, a species that forms habitat for many other organisms. While this study demonstrated organism-level effects, it did not demonstrate assemblage-level effects because no other organisms were sampled. Katsanevakis et al. (2007) demonstrated assemblage-level impacts by placing debris (12 plastic bottles and 4 glass jars in each plot, which was in the upper part of the range of amounts of litter found in the field) into 10 X 10 m experimental plots of sediment at 16 – 20 m depth in coves on a Greek coast. Over one year, the numbers of species of benthic animals increased in plots with debris, compared with plots with no added debris, clearly demonstrating alterations of the composition of benthic assemblages due to marine debris.

Overall, we conclude that there is a pressing need for robust, quantitative information to predict ecological impacts to species of wildlife that are considerably contaminated with marine debris. The presence, sizes, frequencies and nature of ecological impacts are currently largely unknown. There may be large-scale impacts that we are missing simply due to a failure to examine them. Testing hypotheses regarding ecological impacts has been sparse to date, especially in relation to microdebris in the marine environment. We found that there were not yet sufficient data to include a meta-analysis or risk assessment as part of our systematic review. Thus, we chose to quantify the weight of the evidence regarding perceived and demonstrated impacts caused by marine debris by reviewing the literature regarding impacts from debris in general. To assess the scale, magnitude and frequency of realized impacts due to marine debris, research investigating specific ecological questions is warranted. Future studies must use more experimental work where possible and better modeling of effects of mortality of individuals on the size of the population.

While we call for more conclusive evidence regarding ecological impacts from marine debris, it should be recognized that, for some species (particularly for megafauna) and/or scenarios, our lack of knowledge is not attributable to problems in experimental design or interpretation of results from published papers. Instead, the problem is attributed to logistics in sampling and/or a lack of knowledge of how the damage to or deaths of individuals might actually affect populations. For some marine mammals and seabirds, there are plenty of data to demonstrate that the addition of debris to their habitats causes contamination of marine life via ingestion or entanglement. Still, there is little evidence for this contamination being the cause of any *ecological* harm. Ingestion of plastic has been reported in as many as 95% of samples of some species of seabirds (van Franeker et al. 2011). Also, entanglement and ingestion have been reported in 66% of all species of marine mammals (Kühn et al. 2015). Even though we know from studies that plastic debris can perforate the gut and/or obstruct the passage of food, which may lead to sublethal (e.g., weight-loss, reduced growth) and lethal effects (Beck and Barros 1991, Jacobsen et al. 2010, Brandao et al. 2011), it is often difficult to determine whether the plastic in a stranded animal actually caused such impacts.

In other cases, such as "ghost fishing" (the continued catching of organisms by nets and traps that have been lost or abandoned by the fishing industry), many ghost-nets remain active for long periods and are the cause of death of thousands of individuals from many taxa, including invertebrates and vertebrates, some rare and/or endangered (Laist 1987, Good et al. 2010, Gall and Thompson 2015). Nevertheless, it is still not demonstrated that the deaths of these individuals actually cause identifiable ecological impacts (i.e., altered the population or assemblage). Establishing ecological (as opposed to individual) impacts would require that the amounts of mortality due to ghost fishing alone be estimated in relation to the sizes and rates of change in populations.

To determine ecological impacts of debris, it may be difficult to obtain necessary information in many scenarios and for many marine species due to the logistics of sampling and obtaining permits for experimentation (e.g., mammals). Without the appropriate experiments and/or modeling it will be difficult to link the presence of debris to ecological impacts. This problem calls into question the role of certain species of birds and mammals in existing programs of ecological monitoring. Because of the difficulties of experimentation, some of these programs measure contamination rather than ecological impact. Still, for sea birds, some types of manipulative experiments are possible. For marine mammals, laboratory experiments with cell-cultures (using the same debris and cell-types we have reviewed from the medical literature) may be linked to population models. For ghost fishing data, modeling may be used to determine how the populations might be affected.

Moreover, limitations to experimental design can make it difficult to determine whether marine debris is the cause of ecological impact in the presence of other environmental stressors (e.g., chemical pollutants, overfishing, climatic change). As such, decisions by policy-makers will have to be based upon the best available evidence. In some cases, demonstrated impacts at all levels of organization can be used to provide the links to determine how a stressor may disrupt the ecology of the organisms. As has been the case with other forms of contamination leading to pollution, it is important to consider responses across several levels of biological organization to evaluate, interpret and/or predict reliably the net effect of contaminants on wildlife (Underwood and Peterson 1988; Adams et al. 1989; Browne et al., 2015b). Using existing methods, such as ‘adverse outcome pathways’ (Ankley et al. 2010, Kramer et al. 2011), suborganismal impacts from debris can be translated to lethal and sublethal effects on individuals (many of which have been demonstrated) to a quantified effect on the population, species and assemblages to underpin ecological risk assessment and management. Our systematic review synthesizes the existing demonstrated impacts across a wide range of sizes and types of debris and biological levels of organization (e.g., molecular, cellular, organism and population), providing a useful structure to organize the existing data to be used in such future analyses and identify key uncertainties and priorities for research.

Systematic and critical reviews increase the accessibility of the best available evidence, but also provide a more efficient and less biased platform for decision-making (Pullin and Stewart 2006, Mayer-Pinto et al. 2010). Global industries are requesting comprehensive science-based policies and enforcement of existing laws to prevent marine debris (GPA 2012). Despite clear legal guidelines on what evidence is required, some government agencies and industries (e.g., American Chemistry Council, The Coca-Cola Company, UNEP, USEPA) have formed a Global Partnership on Marine Litter and are requesting additional evidence of ecological harm by marine debris to build effective policies for managing waste (UNEP/NOAA, 2011). While we agree that better quality evidence is needed to fill in research gaps at the higher levels of organization to assess the ecological risk and impacts of marine debris, our systematic review found 235 lines of evidence demonstrating valid concerns regarding adverse effects of marine debris and that this persistent and bio-accumulative material causes impacts across 13 levels of organization, including at ecological levels.

Thus, despite the problems and uncertainties in the literature, there appears to be enough evidence for policy-makers to recognize the hazards and take a precautionary and/or anti-catastrophe approach (UNEP 1992, ILGRA 2002, Sunstein 2005), by beginning to mitigate the problem now before there is any irreversible harm from such pervasive materials. For example, many impacts were associated with plastic debris in the form of lost fishing gear or single-use plastic items such as bags and straws. Policy-makers can use existing laws designed for responses to similar persistent and bioaccumulative pollutants (i.e., EU Directive 2008/98/EC, USEPA CERCLA 1980) to help ameliorate problems caused by marine debris.

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Ecological Archives Material. See Appendix for a list of all references gathered in the literature search and used for analysis and synthesis, the protocol used for the literature search and four tables displaying the data that was extracted and a rationale for studies to be included or not included in Figure 2.

Table 1. Breakdown of perceived and demonstrated impacts from micro- and macro-debris at each level of biological organization. Demonstrated impacts do not include those where evidence was correlative.

Perceived and demonstrated impacts due to debris				
Type of study	Perceived		Demonstrated	
No. of cases	362		235	
Size of debris	Micro	Macro	Micro	Macro
Size (mm)	(<1)	(>1)	(<1)	(>1)
%	57	43	70	30
No. of cases	207	155	165	70
No. of cases at each level of biological organization				
Suborganismal				
Subatomic (e.g., oxidative stress)	7	1	7	0
Atomic (e.g., greater concentrations of intracellular Calcium)	2	0	2	0
Small Molecules (e.g., toxic metabolites)	4	0	4	0
Macromolecules (e.g., protein, DNA damage)	67	3	60	2
Molecular assemblies (e.g., formation of protein-chains)	7	2	6	0
Organelles (e.g., more micronuclei)	12	4	7	2
Cells (e.g., necrosis, less viable cells)	54	5	45	3
Tissues (e.g., inflammation, lacerations observed)	25	25	29	22
Organs (e.g., change in size, lesions)	8	5	6	3

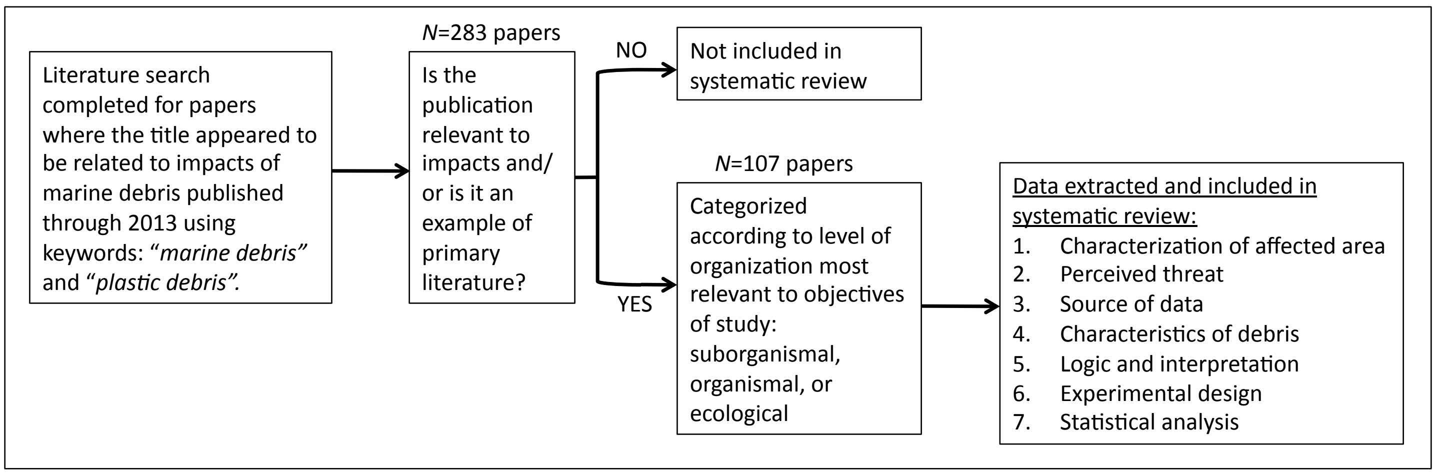
Organ System (e.g., poorly functioning digestive system)	7	20	5	16
Organismal				
Organism (i.e., death to an individual)	11	34	4	20
Ecological				
Populations (e.g., increase or decrease in size of population)	1	29	0	0
Assemblages (e.g., change in abundance or diversity of biota)	2	27	0	2

Figure 1. A schematic representation of our literature selection and a decision-making tree for extraction of data for this review.

Figure 2. Perceived, tested and demonstrated impacts of debris. Rows in each matrix represent different levels of biological organization from subatomic particles, atoms, small molecules, macromolecules, molecular assemblies, organelle, cell, tissue, organ, organ system, organism, population to assemblage. Columns represent order-of-magnitude sizes of debris from smallest (left) to largest (right). Shading in the individual cells of the matrix represent the magnitude of a) perceived b) tested and c) demonstrated impacts of debris in peer-reviewed literature identified using the search terms: *plastic debris* and *marine debris*. White represents 0, light grey 1 – 5, grey 6 – 10, dark grey 11 – 20 and black > 21 impacts. Diamonds in matrix 2c correspond to cells where at least one impact has been demonstrated by correlative evidence. All impacts described at multiple size ranges and levels of biological organization are represented such that there are more impacts than there are papers.

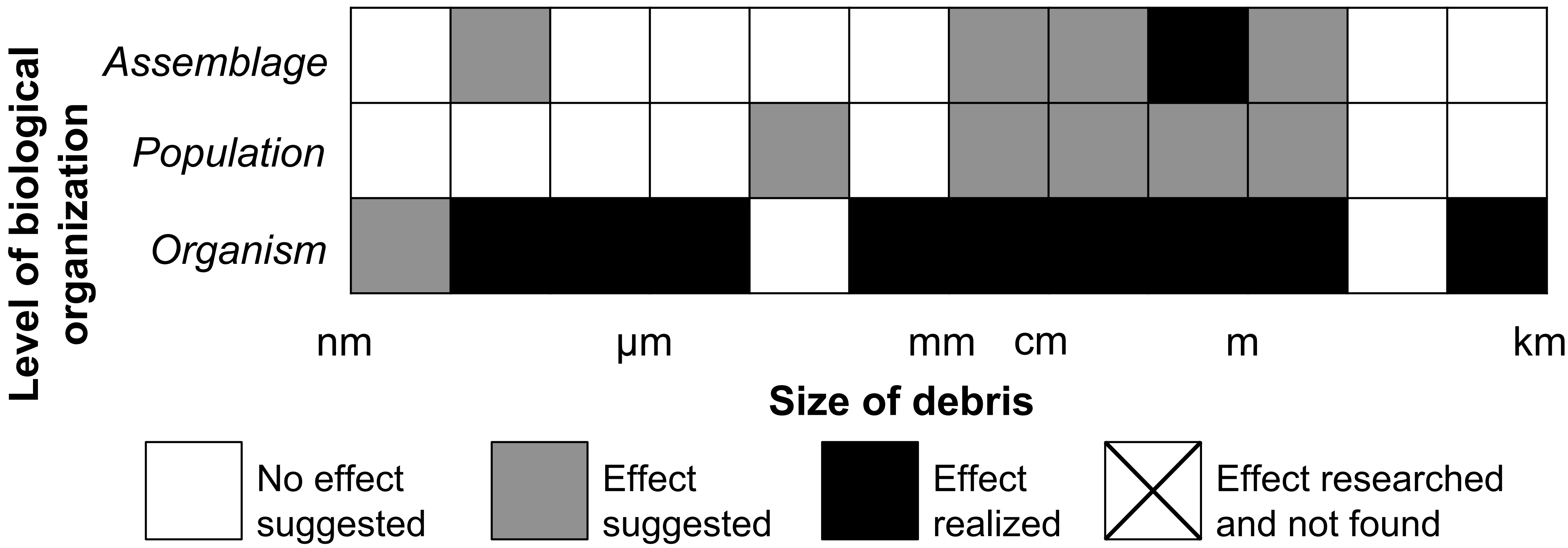
Figure 3. Demonstrated impacts of marine debris across higher levels of biological organization. a) Rows represent levels of higher biological organization (organism, population and assemblage). Columns represent order-of-magnitude sizes of debris from smallest (left) to largest (right). White boxes represent where no perceived effect was suggested. Grey boxes represent where literature suggested an effect but it has not been demonstrated. Black boxes represent where literature reported a demonstrated effect (demonstrated effects do not include those where evidence was correlative). If an example had been found, a crossed-box would represent a study where a perceived effect was suggested, but could not be demonstrated. b) References where demonstrated impacts were found, i.e., that were used to fill the boxes in

black, are provided with the level of biological organization, the size-range of the debris and the cause of the impact.


Literature search completed for papers where the title appeared to be related to impacts of marine debris published through 2013 using keywords: "marine debris" and "plastic debris".
N=283 papers
Is the publication relevant to impacts and/or is it an example of primary literature?
NO
Not included in systematic review
YES
N=107 papers
Categorized according to level of organization most relevant to objectives of study: suborganismal, organismal, or ecological
Data extracted and included in systematic review:
1. Characterization of affected area
2. Perceived threat
3. Source of data
4. Characteristics of debris
5. Logic and interpretation
6. Experimental design
7. Statistical analysis

a
Perceived Threats
b
Tested Impacts
c
Demonstrated Impacts
Level of biological organization
Assemblage
Population
Organism
Organ System
Organ
Tissue
Cell
Organelle
Molecular Assemblies
Macromolecules
Small molecules
Atoms
Subatomic Particles
nm
µm
mm
cm
m
km
Size of debris
0
1 - 5
6 - 10
11 - 20
> 21

a



b

Reference Cited	Level of Organization	Size Range	Cause of Impact
Beck and Barros *Mar. Pollut. Bull.* **1991**, *22*, 508-510.	Organism	100 mm – 10 m	ingestion
Bjorndal et. al. *Mar. Pollut. Bull.* **1994**, *28*, 154-158.	Organism	1 mm – 1 m	ingestion
Brandao et al. *Mar. Pollut. Bull.* **2011**, *62*, 2246-2249.	Organism	10 mm – 1 m	ingestion
Browne et al. *Curr. Biol.* **2013**, *23*, 2388-2392.	Organism	100 μm – 1 mm	ingestion
Bugoni et al. *Mar. Pollut. Bull.* **2001**, *42*, 1330-1334.	Organism	10 mm – 100 mm	ingestion
de Stephanis et al. *Mar. Pollut. Bull.* **2013**, *69*, 206-214.	Organism	100 mm – 1 m	ingestion
Fowler et al. *Mar. Pollut. Bull.* **1987**, *18*, 326-335.	Organism	1 mm – 1 m	entanglement
Gilardi et al. *Mar. Pollut. Bull.* **2009**, *60*, 376-382.	Organism	1 m – 10 m	entanglement
Good et al. *Mar. Pollut. Bull.* **2010**, *60*, 39-50.	Organism	100 m – 1 km	entanglement
Jacobsen et al. *Mar. Pollut. Bull.* **2010**, *60*, 765-767.	Organism	1 mm – 1 m	ingestion
Lee et al. *Environ. Sci. Technol.* **2013**, *47*, 11278-11283.	Organism	10 nm – 10 μm	ingestion
Moore et al. *Mar. Pollut. Bull.* **2009**, *58*, 1045-1051.	Organism	1 mm – 1 m	entanglement
Udyawer et al. *Mar. Pollut. Bull.* **2013**, *73*, 336-338.	Organism	10 mm – 100 mm	entanglement
Uhrin and Schellinger *Mar. Pollut. Bull.* **2011**, *62*, 2605-2610.	Organism	100 mm –1 m	smothering
Vélez-Rubio et al. *Mar. Biol.* **2013**, *160*, 2797-2811.	Organism	10 mm – 100 mm	ingestion
Katsanevakis et al. *Mar. Pollut. Bull.* **2007**, *54*, 771-778.	Assemblage	100 mm – 1 m	addition of habitat
Lewis et al. *New Zeal J. Mar. Fresh.* **2009**, *43*, 271-282.	Assemblage	100 mm – 1 m	smothering