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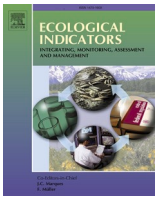

Review

Can natural history collection specimens be used as aquatic microplastic pollution bioindicators?

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ABSTRACT

Microplastic pollution has risen to such a level that concerns are being raised regarding its consequences on the environment, especially the marine environment. Understanding microplastic pollution temporal dynamics is critical but requires time-series. However, concerns about microplastic pollution being recent, long term monitoring programs have only started very recently and remain scarce. Natural History Collections that represent archives from the past can constitute time-series. Although underused, they have evinced their success to study various stressors including pollutants, in particular when using bioindicators. Bioindicator species should be defined with regards to the studied environmental disturbance according to established criteria. Those criteria include occurring frequently, being sensitive to the pollutant, and allowing, via their monitoring, a summary of the pollutant's impacts at molecular, organismal or population levels. However, to analyse bio-indicator species in Natural History Collection time-series, several specificities need to be considered. Starting from a review of articles that utilised such collections to study microplastic evolution in a given ecosystem, and focusing on their methodologies, we emphasise Natural History Collection features that need to be taken into account when choosing the most adequate taxon and extraction techniques. In particular we discuss four collection features: sampling heterogeneity, taxonomic misidentification, past environmental contamination and specimen destruction and provide leads to address these issues. We believe that combining the concept of bio-indicator with valuable samples from Natural History Collections is of particular interest to monitor past microplastic pollution and better predict future trends. This constitutes a necessary step in assessing the basal level and the continuing evolution of this ever-increasing pollution.

1. Introduction

Following the spectacular rise of plastic production since the 1950s (Bergmann et al., 2015; Geyer et al., 2017), plastic now represents the vast majority of total marine litter (Barnes et al., 2009; Pham et al., 2014). The impact of anthropogenic macro-litter on marine life was first studied in Carpenter (1972), yet the field is now increasingly focusing on smaller litter: the *microplastics* (Bergmann et al., 2015). Microplastics are easily ingested by marine fauna (Thompson et al., 2004) and their presence has now been documented in all marine ecosystems.

Limiting plastic pollution requires augmenting recycling capacities and, more importantly, limiting the quantities discharged into the environment (*i.e. Reduce, Reuse, Recycle,* see Thushari and Senevirathna, 2020). As a result, the United Nations agreed in 2022 to edit a legally binding instrument (*i.e.* the *Plastics Treaty*) which should be finalised by 2025. Its goal is to prevent rising levels of environmental pollution (UNEP, 2022).

There are multiple and likely non-reversible consequences of plastic pollution including habitat change, toxicity to biota, etc. (MacLeod et al., 2021). Microplastic pollution encompasses a family of pollutants, as *plastic* particles differ in their chemical composition, shape, colour, size etc…(Koelmans et al., 2022), The physicochemical properties of each individual plastic particle can be altered in different ways, driven by abiotic factors, such as water salinity or ultraviolet radiation, or by biotic factors such as surface alterations by microorganisms (Andrady, 2017; Arp et al., 2021). These weathering processes can trigger the

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release of plastic monomers, but also of additives (Arp et al., 2021; Kwan and Takada, 2016). Therefore, the global toxicity of microplastics and their additives released into the environment is difficult to assess (Bucci and Rochman, 2022).

Since the 1950 s, 8300 million tons of plastic have been introduced into the environment (Sonke et al., 2022), with 4.8 to 12.7 million tons entering the oceans in 2010 alone (Jambeck et al., 2015) and 170 trillion plastic particles currently floating in the oceans (Eriksen et al., 2023). Sonke et al. (2022) estimated that most of the plastic present in the environment now resides on shelf sediments (116 Teragrams (Tg)) and in the deep ocean (82 Tg). It is estimated that the quantity of microplastics in deep sediments will dramatically increase to 350 Tg by the year 3000, even if production were halted in 2025 (Sonke et al., 2022).

In this context, understanding plastic's accumulation and life cycle in the environment is critical (DuBay et al., 2023) and requires repeated sampling over an extended period of time: time-series. Current data suggest that microplastics are becoming a persistent factor in certain ecosystems, therefore temporal reference points, including a reference point pre-dating the apparition of the pollutant, are needed to understand how it impacts the biodiversity structure and functions of these ecosystems. Long term time-series have been invaluable to unravelling how biodiversity is durably impacted by numerous anthropogenic factors such as: climate change (Richardson et al., 2006; Smith et al., 2020; Hartman et al., 2021); pollution (Hawkins et al., 2002; Likens, 2004); and resource exploitation (Edwards et al., 2010). These studies have played a vital role in informing environmental policies (Hughes et al., 2017). However, biodiversity time-series data spanning more than a couple of decades are scarce particularly in tropical and southern temperate localities (Johnson et al., 2011; Magurran et al., 2010; Peters, 2010; Wolfe et al., 1987). Long-term time-series are scarce since they are time-consuming and expensive (Magurran et al., 2010; White, 2019), discouraged by a variety of institutional disincentives (Wolfe et al., 1987), and not appropriate to typical experimental time frames with short-time funding cycles and rapid publication rhythms (Hughes et al., 2017; White, 2019). Some programs have been developed to enhance long time-series biodiversity data acquisition (e.g. LTER program, see Alber et al., 2021) but have not reached the required time scale for significant analysis.

An important reservoir of time-series data can be found in Natural History Collections (NHCs). These collections consist of biological, geological, and anthropological specimens providing physical records of the natural world's biodiversity (Monfils et al., 2017). They can be stored in natural history museums, universities, research centres or in living stock collections (zoos, botanical gardens…) that preserve, curate, catalogue and archive those specimens (Miller et al., 2020; Monfils et al., 2017). NHCs are valuable resources of long-term biodiversity data (Bartomeus et al., 2019; Meineke et al., 2019). They allow us to make comparisons with the recent past and can be used to understand how biota respond to anthropogenic influences (Johnson et al., 2011; Lister, 2011; Rainbow, 2009). NHCs are often made up of billions of specimens and can include material obtained through repeated collecting over several decades at a given location (Johnson et al., 2011). With advances in technology (next generation sequencing, CT scanning, isotope analysis…), substantial data can be derived from these specimens beyond taxonomic and systematic information. This could include analysis of DNA, proteins, metabolic compounds, associated pathogens, microbes, and other biotic and abiotic conditions in which a specimen was collected (Bakker et al., 2020; Lister, 2011; McLean et al., 2016; Meineke et al., 2019; Miller et al., 2020; Sampaio et al., 2019; Suarez and Tsutsui, 2004). Thus, NHCs enable us to answer a large number of scientific questions, some of which have not yet been posed (Bakker et al., 2020). With increasing anthropogenic pressures, NHCs have been used to investigate environmental issues such as diseases (Yates et al., 2002; Schindel and Cook, 2018; Schmitt et al., 2019), pathogens (Lorch et al., 2021; Pinto et al., 2010; Ristaino et al., 2001), climate change (Lang et al., 2019; Lavoie, 2013; Pyke and Ehrlich, 2010; Robbirt et al., 2011), urbanisation (Shultz et al., 2021) and pollution (Cao et al., 2008; DuBay and Fuldner, 2017; Ellegren et al., 1997; Hayes et al., 2002; Hickey and Anderson, 1968; Ratcliffe, 1967; Thompson et al., 1998; Vo et al., 2011). NHC specimens provide a historical baseline against which current levels of pollution can be compared (Suarez and Tsutsui, 2004).

The effectiveness of NHC time-series data for monitoring pollution can be increased if relevant taxa are selected as bioindicators. A bioindicator is a species or group of species that enables the evaluation of their environment (Gerhardt, 2006). Multiple types of bioindicators are defined in the literature including *accumulation bioindicators* that concentrate specific substances in their tissues to levels significantly higher than those in the ambient environment (Beeby, 2001), and *impact bioindicators* that display morphological, histological, behavioural or populational changes in response to a disturbance or substance (Markert et al., 2003). Accumulation bioindicators can incorporate pulses of pollution into their tissues throughout their lifespan. These pulses can be missed by traditional physicochemical measurements (Holt and Miller, 2011) rendering these organisms particularly useful for environmental monitoring purposes. Different bioindicator species should be selected depending on the environmental response required for a specific research or policy objective. For example, an objective could be to determine the environmental load of a pollutant in a section of the ocean, or to assess ecosystem health. Numerous species have been characterised as bioindicators (Chowdhury et al., 2023) including the famous canary bird, which can detect small amounts of carbon monoxide in mines (Holt and Miller, 2011). Communities can also be used as bioindicators such as tiger beetles whose assemblage variations are a proxy of the degree of alteration of Venezuelan forests (Rodríguez et al., 1998). Lastly, bioindicators can be good instruments for communicating with wider audiences such as the general public or policy makers (Burger, 2006). In this review focusing on plastic pollution, the term *bioindicator* will refer to accumulation bioindicators, the main type of bioindicator found in the plastic monitoring literature.

Combining the use of bioindicator species and time-series has proven to be a powerful tool in monitoring various environmental disturbances, mainly by demonstrating a correlation between the concentration of a pollutant in the environment and in the organism. This has been demonstrated for heavy metals or trace elements in mosses (Blagnytė and Paliulis, 2010; Halleraker et al., 1998), persistent organic pollutants in Antarctic marine biota, and radionuclide pollution in rivers using mussels (Charmasson et al., 1999). At the population scale, time-series have been used to assess herring stocks, where the stomach content of seabirds acted as a predictor of fish spatial distribution (Scopel et al., 2018); atmospheric metal pollution in lichen diversity and physiology (Abas, 2021); and coral cover looking at foraminifera family abundance (Humphreys et al., 2022).

To date, few studies have used NHCs to investigate microplastic pollution (Ilechukwu et al., 2023), and even fewer have used bioindicators in NHCs. However, NHCs are the main source of past data as almost no research was conducted on this pollutant prior to 1980. Questions were beginning to arise at this time, but awareness remained low and there were few means to investigate the impacts of plastics. Only recently have specific techniques been developed given scientists' and society's increasing awareness of plastic pollution (see section 4.1). With little data available prior to the 2000 s, using NHCs is critical for establishing past reference points, constructing temporal trends, and understanding persistence and circulation (DuBay et al., 2023; Yap et al., 2022; Ilechukwu et al., 2023).

In this paper, we build on Ilechukwu et al.'s (2023) literature review exploring the presence of microplastics in specimens conserved in NHCs. Our literature review focuses on how and why specific species and extraction methods were chosen. Next, we incorporate the concept of bioindicators and analyse methodological constraints that must be considered when studying microplastics from NHCs vs. freshly collected material. Sampling heterogeneity and taxon identification status will be discussed as possible impediments in taxon selection. Lastly, we discuss

how past NHC specimen contamination and specimen damage from microplastic extraction methods need to be accounted for and propose some recommendations to manage these issues.

2. Literature review

2.1. Article selection methodology

Literature browsing was performed using Web of Science and Google Scholar using the same approach described in Ilechukwu et al. (2023). Based on a PRISMA approach, we performed a search using keywords: "microplastic pollution" and "natural history collections'' (as of August 2023, 22 results), "microplastic pollution" and "time-series" (as of August 2023, 1090 results), "microplastic pollution" and "museum" (as of August 2023, 815 results) to recover articles using NHC time-series for microplastic studies. We then narrowed down our results to aquatic specimens only. We found 10 studies that matched all the above criteria. Those articles corresponded with those reviewed by Ilechukwu et al. (2023), with two exceptions: (i) we included Soares et al. (2022), which was not published at the time of manuscript submission by Ilechukwu et al. (2023), (ii) we did not include Gül et al. (2022) as we focused strictly on the aquatic and preferably marine component. Although our final list of articles is similar, our reviews are complementary. We analysed the article methods while Ilechukwu et al. (2023) analysed their results. In particular, we analysed their organism selection and microplastic extraction methods and analysis protocols (see Table 1).

2.2. Methods used in selected studies

Overall, ten studies used time-series to investigate microplastic pollution, hereafter referred to as *the selected studies* (Table 1). Seven of these used marine time-series from NHCs to investigate microplastic evolution (Beer et al., 2018; Courtene-Jones et al., 2019; Halbach et al., 2022; Hou et al., 2021; Thompson et al., 2004; Toner and Midway, 2021; Van Der Hal et al., 2018) and three additional studies used specimens from collections without a long time-series analysis (just data from one or two past reference points, see Ehlers et al., 2022; Modica et al., 2020; Soares et al., 2022). Only Thompson et al. (2004) and Hou et al. (2021) used data pre-dating the appearance of microplastics and found an increase of these particles in organisms over time. The studies that used time-series starting after plastic apparition gave contrasting results. Two reported increasing trends (Soares et al., 2022; Van Der Hal et al., 2018). Three reported a constant level of microplastics in organisms over time (Beer et al., 2018; Courtene-Jones et al., 2019; Ehlers et al., 2022). One found an increase in microplastics over time in one study location but constant levels in a second study location (Halbach et al., 2022). One found almost no microplastics in their samples (Toner and Midway, 2021) and one did not draw conclusions of trends over time (Modica et al., 2020).

For each selected study, we analysed several methodological parameters: the organism studied, the type of tissue sampled, whether the specimens were altered to perform the microplastic analysis, whether samples were solely visually inspected, whether environmental contamination of samples by plastic was addressed, whether samples were digested and if so, the filter size used for recovering microplastics. We also reported whether filters were examined visually before being analysed, and the type of polymer identification method used (Fourier-Transformed Infrared (FTIR), Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS), Raman spectroscopy). More details about microplastic extraction and analysis methodologies are given in section 4.1. Finally, we also reported the results in terms of microplastic temporal variation.

2.2.1. Organisms used

The organisms used in the selected studies included plankton (Beer et al., 2018; Thompson et al., 2004), marine fish (Beer et al., 2018; Van Der Hal et al., 2018), freshwater fish (Hou et al., 2021; Toner and Midway, 2021), echinoderms (Courtene-Jones et al., 2019), sponges (Modica et al., 2020; Soares et al., 2022), gastropods (Ehlers et al., 2022), and mussels (Halbach et al., 2022) (Table 1). Nine of the studies explained why they selected a particular organism. Organisms were chosen due to their ecology, such as filter feeding in sponges (Courtene-Jones et al., 2019; Modica et al., 2020; Soares et al., 2022), their position in the food chain, such as being prey or predators (Beer et al., 2018; Hou et al., 2021), the availability of time-series from past and ongoing programs (Courtene-Jones et al., 2019; Thompson et al., 2004), their frequent occurrence (Halbach et al., 2022; Soares et al., 2022; Van Der Hal et al., 2018), or to fill a gap in the literature (Ehlers et al., 2022).

All ten studies investigated plastic pollution at the organismal level by quantifying microplastics found within tissues (Table 1). The selection of organisms was based on hypotheses about their interactions with microplastics potentially present in their environments, thus that they may be considered as potential bioindicators. Yet four out of the ten selected studies did not mention the concept of *bioindicator* (Beer et al., 2018; Courtene-Jones et al., 2019; Thompson et al., 2004; Toner and Midway, 2021) and three of the remaining six studies did not provide justification as to why the selected organisms could be considered bioindicators (Modica et al., 2020; Soares et al., 2022; Van Der Hal et al., 2018). The last three studies detailed the reasons behind their choices (Ehlers et al., 2022; Halbach et al., 2022; Hou et al., 2021). First, Hou et al. (2021) compared the quantity of microplastics found in multiple species of fish and near-by sediments and water. They showed that microplastic quantities found in fish were correlated with those found in sediments but not those found in the surrounding water. The authors acknowledged that this result was unexpected but could be explained by the fact that these fish feed on invertebrates found in the sediments (Hou et al., 2021). The authors conclude that fish reflect the levels of microplastics found in the nearby environment and could be good bioindicators. Second, Halbach et al. (2022) used mussels in their study. They concluded that mussels can be good indicators of water microplastic levels, as they can filter large quantities of water (but see section 3.1 about the choice of mussels as bioindicator). However, the authors acknowledged that filtration rates can be impacted by the age or size of the mussel, or local hydrodynamics. These factors can in turn influence microplastic uptake and weaken mussels' potential as reliable bioindicators (Halbach et al., 2022). Third, Ehlers et al. (2022) used rocky intertidal snails instead of mussels for their study since mussels cannot be found in that habitat. They showed a relationship between the quantity of microplastics in surrounding water and in snails, concluding that this organism is a good bioindicator in rocky intertidal habitats (Ehlers et al., 2022).

We noted that though most authors explained organism selection with regards to microplastics, only three authors described some sort of NHC time-series post-hoc sampling strategy (Hou et al., 2021; Soares et al., 2022; Toner and Midway, 2021). They evoked mainly sampling location and data quantity or quality with regards to their tested hypotheses. Only one author mentioned the taxonomic identification status of the collection as a reason behind their choice (Modica et al., 2020). It may be that the remaining studies also considered these issues but did not explicitly report them.

Though not all studies used the concept of bioindicator, it can be a powerful tool for monitoring purposes, allowing for rapid and reliable assessment of pollution in a given environment (Holt and Miller, 2011). In section 3, we detail the importance of having criteria to classify a species as a bioindicator and provide guidance with regard to NHC sampling heterogeneity and taxonomic misidentification.

2.2.2. Microplastic extraction methods used

The selected studies used a variety of methodologies to extract and analyse microplastics. This is partly due to the rapid evolution of these methodologies both since 2004 and particularly between 2018 and 2023 (see section 4.1). This heterogeneity in methods precludes us from

Table 1
Methodological parameters of the selected studies (n = 10).

Reference	Year of publication	Study organism	Tissue analysed	Specimen alteration	Visual inspection of specimens	Environmental contamination control	Digestion of the sample	Filter mesh size	Visual inspection of filters	Polymer identification method	Microplastic quantity evolution
Thompson *et al.*	2004	Plankton	External	No destruction	Yes	None	–	–	–	–	Increase from 1960s to 1990s
Beer *et al.*	2018	Plankton + Fish	Whole specimen (plankton), digestive tract (fish)	Partial destruction	No	None	Chemical	100 μm	Yes, combined with hot needle test	–	Consistency
Van der Hal *et al.*	2018	Fish	Digestive tract	Partial destruction	No	None	Chemical	125 μm	Yes	–	Increase from 1960s to 2016
Courtene-Jones *et al.*	2019	Brittle Star + Sea Star	Whole internal soft tissue (exoskeleton not analysed)	Destruction	No	Water rinsing of the specimen, only internal soft tissue analysed	Enzymatic	52 μm	Yes	FTIR	Consistency since 1976
Modica *et al.*	2020	Sponge	External	No destruction	Yes	None	–	–	–	–	Not mentioned
Hou *et al.*	2021	Fish	Digestive tract	Partial destruction	No	None	Chemical	0.45 μm	Yes	Raman (subsample)	Increase from 1900 to 2020
Toner & Midway	2021	Fish	Digestive tract	Partial destruction	No	None	Chemical	20 μm	Yes	FTIR	Consistency (only 3 particles found across the study)
Ehlers *et al.*	2022	Snail	Soft tissue	Partial destruction	No	None	Chemical	0.2 μm	Yes	FTIR	Consistency between 2007 and 2020
Halbach *et al.*	2022	Mussel	Fraction of the specimen (soft tissue)	Partial destruction	No	Report the need to have access to controls when sampling but often do not exist. Attempted to use blank-like samples but failed	Enzymatic + Chemical	1 μm	No	Py-GC/MS	Slight increase (North Sea) and consistency (Baltic Sea)
Soares *et al.*	2022	Sponge	Fraction of the specimen (one quarter)	Partial destruction	No	Reporting MP found in internal tissues	–	–	Yes, combined with hot needle test	Raman	Increase from 1981 to 2017

making a global comparison between studies. It is unclear if the variable temporal trends in microplastic pollution found in the different studies are genuine, or due to experiment differences. First, various sample preparation techniques were used. Two studies did not alter the specimens, analysing only external parts. This limited reliability of the results since contamination was not prevented and internal tissues were not examined (Modica et al., 2020; Thompson et al., 2004). The remaining eight studies used all or parts of the specimens for microplastic analysis, resulting in partial or total destruction of the specimens. None of these eight studies explain if measures were taken to minimise such damage and keep records of this collection material such as photographs, DNA, radula, otoliths, spicules, or any other morphological features used for identification. Second, when digestion of the organic matter was performed (see Table 1), it was either chemical (Beer et al., 2018; Ehlers et al., 2022; Halbach et al., 2022; Hou et al., 2021; Toner and Midway, 2021; Van Der Hal et al., 2018) or enzymatic (Courtene-Jones et al., 2019; Halbach et al., 2022). Both can impact downstream identification methods (Dehaut et al., 2016; Santana et al., 2022; Tsangaris et al., 2021). Third, filtration was performed using different filter mesh sizes, ranging from 0.2 to 125 μm (see Table 1). This size determines the minimal size of particle retained, and thus defines the size range of microplastics detected. Finally, seven of the selected studies did not use what is currently considered a reliable polymer identification method (Beer et al., 2018; Hou et al., 2021; Modica et al., 2020; Soares et al., 2022; Thompson et al., 2004; Toner and Midway, 2021; Van Der Hal et al., 2018). They relied on visual inspection of the filters, either alone or combined with a hot needle test (see section 4.1. and Beckingham et al., 2023), or analysed only part of the sample. However, subsampling can be unreliable if the sample is not homogeneous. The other three studies used reliable state-of-the-art methods to characterise the chemical composition of the particles. They used either Fourier Transformed Infrared spectroscopy (FTIR) (Courtene-Jones et al., 2019; Ehlers et al., 2022), or mass spectrometry (Halbach et al., 2022, see section 4.1 for more details on these methods). Note that in the papers using FTIR, the authors did not scan the whole filter but only inspected it to identify particles to analyse. This can introduce a detection bias favouring the more clearly visible microplastics.

Lastly, while most authors reported quality control during sample preparation itself, concerns about past environmental contamination were not reported (*i.e.* contamination of samples from contact with surfaces, air, containers…). Most studies used internal tissues to assess microplastic content, which are unlikely to have been impacted by environmental contamination (see Table 1). Courtene-Jones et al. (2019) acknowledged the possibility of environmental contamination and therefore only analysed inner soft tissue, after careful rinsing of each specimen. Soares et al. (2022) mentioned that they never found particles on the outer surface of specimens, claiming that this demonstrated an absence of environmental contamination. The best attempt to control for environmental contamination was reported by Halbach et al. (2022) who accessed the inner muscle of eelpout, and herring gull intact egg content as controls. These were dissected under clean air conditions and should thus be free from environmental contamination. However, since higher levels of microplastics were present in these control samples than in the mussel samples, the authors concluded that their control methods were unsuitable.

Overall, authors used a range of different methods to extract microplastics and rarely explained why they preferred one method over another. Only a few papers addressed the questions of past environmental contamination and damage caused to the specimens. In section 4, we discuss how to take into consideration these two specificities of NHCs during microplastic extraction.

3. Taxon selection

3.1. NHC bioindicator selection criteria for microplastic studies

Regardless of the pollutant, criteria need to be met in order to consider a species as a bioindicator (Fossi et al., 2018; Holt and Miller, 2011; Kershaw et al., 2019; Savoca et al., 2022). Those criteria can include: being sensitive but tolerant to the disturbance, displaying a response reflective of the ecosystem disturbance (*i.e.* population change, molecular change…), occurring frequently, having a well-understood ecology with a well-resolved taxonomy, being easy to survey, and not being a protected species. However, these criteria are often not assessed when coining the term *bioindicator* (see section 2.2.1), including in the field of microplastics. Here the primary criterion should be that the quantity or types of microplastics found within organisms should reflect those found in their surrounding environment. Most studies that use bioindicators of microplastic pollution investigate the quantity of plastic found within the organism, thus referring to *accumulation* bioindicators sensu Markert et al. (2003) (Bonanno and Orlando-Bonaca, 2018; Kershaw et al., 2019; Multisanti et al., 2022). Multiple vertebrate and invertebrate species have been considered as useful microplastic bioindicators, due to their feeding behaviour (Fossi et al., 2018; Multisanti et al., 2022). For instance, marine bivalves such as mussels have been tested in many studies as bioindicators to monitor microplastic levels in water (Li et al., 2019; Qu et al., 2018). Although mussels are known to selectively uptake plastic particles (Ward et al., 2019), they are already used by Canada and the Republic of Korea as aquatic microplastic bioindicators (Savoca et al., 2022). There does not seem to be a consensus on the use of specific species as microplastic bioindicators in the literature.

Identifying species that can be considered as bioindicators for a given environment could be a powerful way to assess environmental loads of pollutants or disturbances, and thus to reliably inform public policy. Nevertheless, it is important to bear in mind that even if criteria are met, a bioindicator can only be adequate to monitor its own limited habitat or ecosystem (Holt and Miller, 2011). Resorting to such a concept is by essence a simplification that should be adopted with caution. Multiple taxa should be used in order to build a comprehensive understanding of an environmental disturbance using bioindicators (Valente et al., 2022).

3.2. NHC bioindicator selection constraints

When using collection material as bioindicator species, supplementary criteria need to be considered. Not all taxa can be used for both ethical and practical reasons (Johnson et al., 2011). From an ethical point of view, the selected taxa should not be extinct or endangered taxa (Bastos-Silveira and Lister (2007)), originate from primary habitats that have since been degraded, or taxa that cannot be replicated due to economic or political restrictions (Gaubert et al., 2006). Any type or voucher specimens within the time-series of the selected taxa should be excluded from the microplastic analysis as explained in section 4. From a practical point of view, most collections were not originally designed as time-series, leading to various biases. In particular, regardless of the subject (biodiversity assessment, biogeography, climate change…), two main issues can influence taxon selection: (i) heterogeneity of sampling, (ii) taxonomic error or lack of identification. It is therefore necessary to retrospectively establish a sampling plan based on sampling conducted with other objectives. Efforts should be made to ensure a coherent analysis of the entire time-series.

3.2.1. Specificity of NHCs: Sampling heterogeneity

The issue of sampling heterogeneity has multiple facets. Time-series specimens in collections are often heterogeneous in terms of sampling method, sampling gear used, intensity and frequency of collecting, species selection (Were all species kept? If not, was the selection consistent over time? Are there records of the selection process?), availability of metadata records, personal interest of collectors and preservation techniques used (Andreone et al., 2022; Bakker et al., 2020; Bartomeus et al., 2019; Magurran et al., 2010; Peters, 2010; Pyke and Ehrlich, 2010). Such heterogeneity can bias results and lead to false conclusions (Magurran et al., 2010), but does not preclude the use of NHC time-series. A post-hoc sampling plan must be developed that takes into account the structure and biases of the NHC. First, one can pose general questions then conduct an inventory of the collection and analyse the associated metadata. Next, one can reformulate more specific questions that the selected samples can effectively answer. For example, when sampling frequency and intensity are inconsistent in an NHC time-series, it is not possible to yield information about species abundance in general, species absence in particular or patterns of species co-occurrence (Pyke and Ehrlich, 2010). In the case of microplastic (or any other pollutant) time-series studies, frequency and intensity inconsistency are not a predicament when the investigated questions are: have microplastics been present in specimens of a given taxon over time? Has the quantity or types of microplastics found per individual of a given taxon changed over time? If the time-series covers more than one location, do microplastics present in a given taxon change with sampling location? In biological or ecological studies, the number of specimens of the chosen taxon over time must be considered. There must be enough individuals over the time-series to ensure the statistical power of the tests used to investigate the hypotheses.

3.2.2. Specificity of NHCs: Taxonomic non–/mis-identification

The second issue pertaining to taxon selection in NHC time-series is linked to taxonomic identification (Johnson et al., 2011; Sigwart et al., 2023). NHC specimens can either be identified (*i.e.* a species name was given to the specimen) or not. If identified, their identification can be correct (no error), incorrect (misidentification), correct but based on incomplete knowledge (cryptic species, Pfenninger and Schwenk, 2007), or correct but based on outdated knowledge (synonyms, Graham et al., 2004). Therefore, it is recommended to use collection specimens for which there is a detailed knowledge of the taxonomic and systematic history (Peterson and Navarro-Sigüenza, 1999). However, most NHCs contain unidentified and unsorted material (Kemp, 2015; Meineke et al., 2019). In practice, these specimens are likely not catalogued, can be mixed with other taxa, and can be hard to locate (Sampaio et al., 2019). Species level identification often requires great expertise. Observable morphological characters that determine a species may be difficult to find, particularly in little known deep-sea organisms (Henry et al., 2014; Sampaio et al., 2019). Significant taxonomic work may be required before using time-series specimens for a study investigating microplastic pollution.

When specimens are not well identified, taxonomic issues are not necessarily a deterrent but should be considered early on as extra means may be required (time, expertise and money) to coherently analyse the taxon time-series. It is essential to establish a clear taxonomic reference for the targeted taxon and define an operational identification key. As taxonomic work is notoriously long, we recommend a two-step process. The first step should be a short-term simpler identification process to quickly determine the different taxa in a collection enabling the selection of specimens for the microplastic study. Second, a comprehensive and standardised taxonomic approach that enables the delineation, identification, and, if necessary, naming of newly discovered species should be implemented. A plan could be: (i) to compile photographs and genetic barcodes for all specimens. It may require individualising each specimen (giving it a unique identifier), photographing it and taking external tissue samples for integrative taxonomy (COI barcoding and morphological analyses). The use of external tissue for this stage is strongly recommended (see section 4), (ii) then to use a turbo taxonomy approach (Butcher, 2012; Fernandez-Triana, 2022; Riedel et al., 2013). Molecularly delineated species (Molecular Operational Taxonomic Units (MOTUs), see Floyd et al., 2002) can be identified at the lowest possible taxonomic level using available genetic data for the group. Next, study more thoroughly the remaining MOTUs (those not molecularly identifiable to a species or genus or for which the status of a new species is not confirmed) through longer time scale collaboration with taxonomists and/or the acquisition of additional data. Projects studying time-series from NHCs should focus on species that can be identified using standardised molecular or morphological tools, rather than those that require further study to address taxonomic issues. It is necessary to implement a comprehensive and standardised taxonomic approach that enables the delineation, identification, and, if necessary, naming of newly discovered species.

4. Extraction and analysis

4.1. Microplastic extraction and analysis considerations

There is currently no overall standardised method for monitoring microplastic pollution (Cowger et al., 2020) neither in terms of sample type (water, soil, sediments, biota), sample collecting equipment, treatment of the sample, type of analysis performed to quantify microplastics (see below), nor the type of reporting (units). Here we focus on the post sampling processes, since sampling of NHC material has already occurred.

The first stage in analysing the level of microplastics in marine biota is sample preparation. The preparation protocol should be adapted depending on the sample type and the objective of the study. Each step of the protocol will first require testing, which can be time-consuming (Courtene-Jones et al., 2017; Karami et al., 2017). Researchers should establish whether the whole organism should be analysed or only parts of it. The contact of the sample with a non-controlled environment that could trigger microplastic environmental contamination of the sample or the presence of impeding biological elements (bones, spicules, high fat content…) can be elements to take into account. These biological elements can indeed interfere with downstream analytical methods.

Direct observation of microplastics is sometimes possible, for example by direct observation of stomach contents. This can be unreliable, as microplastic particles are frequently difficult to observe with the naked eye. Thus, tissues (*i.e.* organic matter) should be removed and microplastics isolated through digestion of the tissues. This can be accomplished using basic or acidic solvents such as HNO_3, KOH, NaOH or enzymes. Evaluation and comparison of the various digestive agents show that the method chosen (Dehaut et al., 2016; Santana et al., 2022; Tsangaris et al., 2021) is always a trade-off between good digestion of the organic matter (often referred to as *matrix clarification*) and preservation of the plastic particles (in terms of recovery rates, shape, size, and/or chemical properties). Based on a comparison study of different solvents, Santana et al. (2022) advise the use of HNO_3 arguing that KOH does not provide efficient matrix clarification. However, other authors recommend the use of KOH as it preserves the particle integrity of most polymers (Dehaut et al., 2016; Tsangaris et al., 2021). In addition, solvent cost, and digestion time should be considered in the design of the study (Tsangaris et al., 2021). The choice of solvent ultimately depends on the downstream analytical method, as well as the type and size of polymers analysed. Tests should also be performed to decipher whether samples should be freeze-dried before digestion depending on the efficacy of the solvent.

Third, following the digestion of organic matter, filtration is necessary to retain only solid residues. This step requires choosing an appropriate mesh size that can vary between less than a micron to several hundreds of microns. With increasing mesh size, fewer particles are retained leading to less plastic to be included in the analysis. If the

study objective is not limited to a specific microplastic size, a smaller mesh size will provide more accurate results. However, filters with small mesh size tend to clog. Several mesh sizes should be assessed to find the best compromise between particle size retained and prevention of filter clogging, a process that can be time-consuming.

Finally, solid residues trapped on a filter need to be assessed for microplastics. Quick identification of filter microplastic content can be achieved using basic light microscopy, with identification based on the colour of the particle. This is based on the inference that colours classically not found in the environment such as bright pink, orange or blue correspond to plastic. A low-cost approach to ensure plastic presence consists of applying a hot needle on suspected plastic particles that should melt upon contact (Beckingham et al., 2023). These methods are easy to set up but their accuracy remains limited. For this reason, more reliable methods have been developed and are now widespread within laboratories: spectroscopic methods (Fourier transformed infrared (FTIR) or Raman spectroscopy) or thermal-analytical methods (mass spectrometry, Py-GC/MS (Pyrolysis-Gas Chromatography/Mass Spectrometry)) (for a detailed comparison, see Dierkes et al., 2021). The choice between these methods depends on the scientific question and the type of information required. Both spectroscopy and thermal-analytical methods can reliably identify the chemical composition of a particle (*i.e.* polymer type) ensuring plastic detection. They are complementary in that spectroscopic methods provide information on the size and the number of particles, whilst thermal-analytical methods give information on the mass of polymers.

4.2. Microplastics extraction and analysis constraints in NHC specimens

Methods used to analyse microplastics from NHCs do not differ much from those used to analyse microplastics from fresh biota samples. However, such methods have to be adapted to two NHC specificities: (i) controlling for and characterising possible sources of environmental contamination after sampling of the specimens (Gwinnett and Miller, 2021), (ii) minimising damage to or destruction of the specimens required for the digestion step. Both call for supplementary tests to be conducted.

4.2.1. Specificity of NHCs: Uncontrolled past environmental contamination of specimens

Prevention of plastic environmental contamination is usually not considered while collecting specimens nor during the long-term storage of collections. Environmental contamination during the collecting event can come from several sources including the atmosphere or from sampling gear (clothes, ropes, containers, etc.). Which material is used during fieldwork is not routinely recorded, and may not exist anymore, preventing analysis of its plastic composition. Environmental contamination during storage can occur when specimens are preserved in fluids such as ethanol or formaldehyde. These are sold in plastic containers and are likely not filtered before use, potentially introducing microplastics. Specimens themselves are often stored in plastic containers which could further leach microplastics. Testing storage fluids for plastic content could indicate all the environmental plastic contamination encountered by a specimen since its sampling. However, these fluids are often replaced over time, a routine task in specimen curation (Miller et al., 2020) limiting the relevance of this test. Therefore, there is no certifiable means to identify and characterise all environmental plastic contamination. To help control for past environmental contamination, we recommend: (i) testing the preservation fluid of specimens for its microplastic content. The microplastic types found in specimens should be compared to microplastics found in the preservation fluids, (ii) working only with specimens whose external structure is undamaged and only using internal organs that have remained insusceptible to environmental contamination. Dissection of internal organs should be conducted with strict measures to prevent microplastic environmental contamination, such as working under a clean-air hood (Halbach et al., 2022).

Note that the preservation fluid in itself has proven to be unproblematic with regards to microplastic extraction (Courtene-Jones et al., 2017) although the study has not yet been extended to longer exposure time, or to non-pristine microplastics weathered in the environment. Environmental factors such as ultraviolet radiation, salinity or microorganisms can cause the fragmentation of microplastics, and alter their surface, and their physicochemical and spectral properties (Andrady, 2017; Dong et al., 2020; Ter Halle et al., 2017). As particles found in NHCs are weathered, such differences could make them more prone to be altered by the preservation fluid.

4.2.2. Specificity of NHCs: Damage or destruction of specimens

The methods presently available to characterise microplastics from organisms involve damaging the specimens (see section 4.1), through removal of specific tissues (Jamieson et al., 2019; Lusher et al., 2017) or use of whole specimens (Halbach et al., 2022). A balance must be found between the use of collections by scientists and their preservation by curators. In theory, NHCs are scientific collections therefore scientific interest can justify the destruction of a specimen. But curators must also manage rules related to national regulations and the potential heritage status of samples deposited in collection., Some museums forbid any destruction of specimens. The curator's conundrum is often to know whether to allow sampling with existing technologies rather than waiting for the development of less destructive approaches (Freedman et al., 2018). Using only a part of the specimen should be prioritised whenever possible and degradation of the specimen should be limited. In many studies presently using specimens for various purposes, the discussion is often dominated by instructions on how to limit degradation of the to specimens (e.g. Freedman et al., 2018; Gilbert et al., 2007; Raxworthy and Smith, 2021; Tin et al., 2014; Wisely et al., 2004). We are not yet aware of the questions these specimens could be used for, enabled by yet to be discovered technologies. Specimens are records of both the organism itself and of their environmental conditions and both need to be preserved (Bakker et al., 2020; Li et al., 2023). With the constant advances in technology, the use of NHCs will continue to rise in the future, leading to an ever pressing need to improve the use of collections. For Freedman et al. (2018), a key issue is the lack of accessible, clear, published guidelines for museum curators faced with destructive sampling requests. Best practices for using collection specimens should be defined in a collaborative effort made by museum professionals and researchers.

Another point to bear in mind when considering damaging or destroying NHC organisms is the status of the individual specimen. Collections are comprised of three broad categories of specimens (Andreone et al., 2022): (i) type specimens such as holotypes, paratypes, syntypes, lectotypes and neotypes (ii) voucher specimens which are cited in publications (Funk et al., 2005) or published in public databases such as the *Barcoding of Life Data Database*, BOLD (see Ratnasingham and Hebert, 2007), (iii) specimens not cited either in the literature or in public databases. Type specimens are permanent references for a new species and the definitive authority for applying the rules of taxonomic nomenclature in species descriptions or taxonomic revisions (Sluys, 2021). Their status is dictated by the International Code of Zoological Nomenclature (ICZN) which states that the name of a species is fixed by its name-bearing type specimen. Type specimens must be subject to re-examination for future systematic research (e.g. Mutanen et al., 2015; Zuccon et al., 2020). Destruction of types should be avoided at all costs and damage kept to a minimum to permit taxonomic revisions (for example when using new sets of morphological or genetic characters to revise taxonomy, it can be essential to damage the specimen to determine of the state of such characters in the type specimens). Vouchers are similar to type specimens because they are often deposited for the monitoring of taxonomic identification. In the case of BOLD, vouchers allow the verification of the identification and, if necessary, revision. Therefore, vouchers should be preserved whenever possible for future

use by taxonomists.

This leaves us with the third specimen category - specimens that are neither cited in the literature nor in databases. To decide if these specimens can be altered or destroyed, one should determine: (i) what motivated their collection, (ii) what potential uses they may serve, (iii) when they were identified and by whom and (iv) if the specimens are duplicates. Detailed taxonomic work may be required before knowing if a collection can be used (see section 3.2.2). When there are numerous specimens of a well-known and easily identifiable species, destruction is less detrimental (Johnson et al., 2011). Johnson et al. (2011) pointed out that although collections of common material are of value for specific research questions, they have typically been perceived as a low priority for acquisition, taxonomic revision and curatorial effort and have even been identified as prime candidates for disposal. Therefore, it may be difficult to find such collections.

Overall, clear rules are lacking when using collection materials. Until procedures are established, one should take the necessary measures to ensure that a proper characterisation of the specimens is possible even retrospectively. In the context of microplastic studies, we recommend: (i) preferentially using a species commonly found in the collection, (ii) using only a portion of the specimen. To avoid past environmental contamination issues, internal tissues should be targeted, favouring tissues not required for taxonomic identification. Note that internal organs should preferentially be taken from individuals whose external structure was not damaged during the sampling, storage, and curation processes to limit environmental contamination from microplastics in the preservation fluids. Keeping parts of the specimens also ensures it can be partially re-examined in the future, (iii) creating a digital twin of the specimen before damaging it by thoroughly documenting its morphology prior to destruction, keeping a portion of the specimen or tissue fragments for subsequent analyses, especially to reassess its identification if taxonomic knowledge evolves. Ideally, each specimen should be computerised, recording metadata on sampling and location, photographs of the external morphology as well as the COI gene sequence, photographs of the internal organs before some are removed for microplastic analysis. These digital data will be a complement to the external tissues that can be kept for future use, (iv) using contemporary specimens specifically sampled nearby presumed microplastic polluted areas to perform tests.

4.2.3. *Perspectives on future NHC use in microplastic studies*

The future of NHCs for microplastic studies might reside in using non-invasive techniques that do not alter the integrity of the specimens and could even enhance the understanding of the location of microplastics within tissues. Classical histology or fluorescence microscopy methods could be of interest but remain invasive. Additionally, microplastic histology results are often misinterpreted by authors. Artefacts created during sample preparation can be interpreted as tissue alteration, and dye leaching from fluorescent particles can lead authors to misinterpret the location of microplastic within tissues (De Sales-Ribeiro et al., 2020; Schür et al., 2019).

Legitimately non-invasive methods could be used. Non-invasive methods frequently enable visualising the tissues in three-dimensions. Tomography methods seem promising. They consist of mathematically reconstructing a 3D volume from 2D slice images. Computed Tomography (CTscan) and Neutron Tomography are based on X-ray, and neutron attenuation of the sample, respectively. Different objects can be detected based on the differences of attenuation when exposed to a source. The combination of these two methods has been used to detect large microplastics (1 mm) from sediment carrots (Tötzke et al., 2021). Such a combination is necessary as microplastics are to some extent transparent to X-rays but not to neutrons. The authors successfully managed to detect microplastics, but they were of rather large dimensions, and of known polymer composition (polyethylene).

Higher resolution tomography techniques could include Optical Coherence Tomography, as described by Barroso et al.'s (2019) pioneering work with microplastics. This technique is widely used in ophthalmology to visualise retina layers, and offers a micrometric resolution (Drexler and Fujimoto, 2008). Studies showed that visualisation of microplastic ingestion could be attainable using this technique (Asani et al., 2023; Barroso et al., 2019). However, the depth of imaging cannot exceed 500 to 1000 μm, which is lower than CTscans.

The aforementioned studies show success using microplastics of known size and composition, information that is not normally available when studying samples collected in the field such as NHC samples. In addition, the employed techniques require specialised knowledge and cannot be easily adopted by biologists or chemists and thus would require in-depth development and multidisciplinary collaboration. Finally, results obtained through these methods are not equivalent to those provided by FTIR or Py-GC/MS in terms of the precision of quantification, and remain semi-quantitative. Therefore, using non-invasive methods seems promising but still requires methodological development before it can be routinely used with NHCs.

5. Conclusion

This paper presents important aspects to consider when studying microplastics in NHCs using a bioindicator approach. The use of historical specimens requires additional steps to be taken, in particular with regards to the choice of the taxon and of microplastic extraction technique. For this we suggest the following guidelines:

- In terms of taxon selection, we recommend finding a species meeting the bioindicator features in the time-series and whose sampling heterogeneity and bias can be accounted for in a post-hoc sampling plan to define the research questions that can be effectively answered. Additionally, according to the state of the NHC time-series (specimens recently identified or not, catalogued or not, sorted or not), an important integrative taxonomic work may have to be established. Thus, any microplastic study wanting to use time-series NHCs should take into consideration the extra time, money and expertise required to use the collection. A possible way to quickly obtain a clear taxonomic reference for a targeted taxon may be to use turbo-taxonomy approaches.
- In terms of microplastic extraction methods, we recommend that the microplastic environmental contamination from sampling and storage is investigated (taking samples of collecting gear if available, assessing the microplastics present in the preservation fluids). A technique often employed to limit past environmental contamination is to work with internal tissues assuming the physical integrity of specimens was preserved, and tests have been conducted to show that microplastics can indeed be found in such tissues. Also, because microplastic extraction requires damaging the specimens, at least in part, we recommend (i) targeting common species, avoiding vouchers and type specimens, (ii) using only some internal tissues, and before using any NHC specimen, creating its digital twin (database with metadata, photographs of specimens and of any important morphological features helpful for description, tissue samples, barcoding sequences, (iii) conducting tests with fresh specimens to avoid wasting historical material, (iv) exploring the use of non-invasive techniques.

Beyond NHC holders, it may not be obvious why collecting specimens matters nowadays and this practice is tending to decline worldwide (Andreone et al., 2022; Gardner et al., 2008; Ilechukwu et al., 2023; Lavoie, 2013; McLean et al., 2016; Miller et al., 2020; Pyke and Ehrlich, 2010; Sampaio et al., 2019). Nevertheless, studying microplastic pollution is yet another demonstration of the relevance of collections and working with them in combination with contemporary specimens. NHCs have proved invaluable when looking at climate change impact, pathogens and disease, drug discovery and so on and their use will rise in the future when answering pressing societal

questions. As stated by Suarez & Tsutsui (2004), NHCs provide direct financial and social benefits to society (Suarez and Tsutsui, 2004). Therefore, NHCs should keep growing without neglecting the collection of common taxa often useful for environmental monitoring and a feature of bioindicator species.

Overall, we believe that combining NHC specimens with a bioindicator approach is of interest when monitoring microplastic pollution, furthering our understanding of its prevalence and distribution both in the past and the future.

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Declaration of competing interest

The 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.

Data availability

Data will be made available on request.

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