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Research article

Piscivore food presentation in BIAZA collections: exploring future options to limit risk of ingestion of microplastics

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Abstract
The impacts of marine plastic debris on wildlife have largely been considered by zoos as an in situ conservation issue rather than having a direct impact on zoo animals. However, marine plastic debris has been found in the gastrointestinal tracts of fish species that are used by zoos as animal feed. Microplastics (<5 mm) have been observed in scats of captive pinnipeds fed wild-caught fish. Safeguarding fish-eating species kept in captivity, particularly those involved in the European Association of Zoos and Aquaria's (EAZA) Ex-situ Programmes (EEPs), from potential long-term negative effects of plastic marine pollution is important. To explore options to reduce risks of plastic ingestion that complement current husbandry decisions, this study aimed to identify priorities for presenting whole or chopped fish in BIAZA collections as well as identify key Taxon Advisory Groups (TAGs) that may be most affected. The Penguin and Small Carnivore TAGs recorded the most common presentations of whole fish items. The Small Carnivore TAG and Fish and Aquatic Invertebrate TAG were routinely presented chopped or processed fish in the highest number of collections. Contaminant removal was low priority for chopped fish items, suggesting that mitigation of microplastic ingestion has not been widely considered. Options to reduce risk of plastic ingestion from prey stomach contents include: evisceration of fish for collections already presenting chopped fish, with stomach flushing of prey potentially more suitable for those wishing to present whole, intact feed items. Screening of prey items presents an opportunity to all collections to monitor risk and intervene where necessary.

Introduction

The impacts of marine plastic debris on wildlife have to date largely been considered by the zoological community as an in situ conservation issue rather than having a direct impact on conservation breeding in zoos. In recent years, the conservation education messages of zoos, safari parks and aquariums have increasingly focused on encouraging ecologically sustainable behaviours from their visitors with a particular emphasis on reducing single-use plastic usage to limit the amount of these items entering the marine environment (Baechler et al. 2020). However, direct connections between wild food stocks and captive zoo animals have been less publicly discussed. Limited literature is available on the ingestion of plastic fragments by zoo animals as a result of feeding wild-caught fish which have been previously part of a polluted marine trophic ecosystem. A systematic search of all published European Association of Zoos and Aquaria (EAZA) Best Practice Guidelines (eaza.net/BPG) available at the time of the survey using the term 'plastic' showed no reference to risk of plastic or microplastic ingestion. This suggests that the risk of plastic ingestion by zoo piscivores is not currently a priority and that opportunities exist across the EAZA region for further investigation and knowledge sharing on risk reduction.

The issue of microplastics is of increasing concern across all regions supported by zoo member-based associations, with plastics having been detected in a range of marine habitat types globally (Sarma et al. 2022). Microplastics refer to any plastic fragment measuring under 5 mm which can be introduced to an aquatic environment as industrial pellets or can occur as a result of the breakdown of larger plastic fragments due to sun, weather or physical damage (Alimi et al. 2022). The differing depth profiles of various microplastic types in an aquatic environment can lead to them being deposited in sediment or ingested by aquatic organisms (Setälä et al. 2014). Microplastics have been observed within the digestive tracts of invertebrates (Joyce et al. 2022), fish (Tanaka and Takada 2016), birds (Sühring et al. 2022), reptiles (Beigzadeh et al. 2022) and marine mammals, with species accumulating ingested particles from a number of prey individuals (Lusher et al. 2015). Critchell and Hoogenboom (2018) found that that as particles are further broken down to smaller sizes, retention rates within fish gastrointestinal tracts (GITs) can become higher.

Whole fish are often presented to captive piscivores to replicate natural diets and promote naturalistic feeding behaviours, as well as for other husbandry and logistics-based reasons (Gili et al. 2018). Microplastics have previously been detected in the digestive tracts and surrounding tissues of marine fish (Adeogun et al. 2020) and bivalve species (Van Cauwenberghe and Janssen 2014) as well as fish from freshwater (Curtean-Bănăduc et al. 2023) and inland aquaculture environments (Vieira Dantas Filho et al. 2023). As several food species harvested for human consumption are from similar commercial pathways to animal feed suppliers, this too exposes captive zoo animals to the potential ingestion of microplastics.

Repeated ingestion of microplastics over time may lead to particles gathering within the digestive system, as has been seen in some marine invertebrates such as bivalves, crustaceans and gastropods (Thushari et al. 2017) as well as fish species (Mistri et al. 2022). Microplastics have also been found to accumulate in marine mammals such as fur seals *Arctocephalus* spp. (Eriksson and Burton 2003), though literature on the effects of this in larger vertebrates is limited. In marine invertebrates, the presence of microplastics can affect reproductive success by restricting energy uptake, as seen in the Pacific oyster *Crassostrea gigas* (Sussarellu et al. 2016). In zebrafish *Danio rerio,* microscopic intestinal injuries have been recorded as a result of exposure to microplastics (Lei et al. 2018). Exposure to microplastics has also been reported to adversely affect brain and liver activity in freshwater species such as red tilapia *Oreochromis niloticus*, a commonly used zoo food species (Ding et al. 2018).

Though further research is needed, potential interactions between microplastics and other toxins present in the marine environment such as heavy metals are also increasingly being recorded (Wen et al. 2018), posing additional long-term threats to individuals that present evidence of accumulation of microplastics within their digestive tracts.

Microplastics may also act as carriers for microbial populations (Wright et al. 2020; Yang et al. 2020), posing potential infection risks if unfiltered seawater is used within captive enclosures (Cheung et al. 2018). However, increased uptake of microplastics appears to be as a result of ingesting contaminated prey rather than non-contaminated prey in contaminated water (Hasegawa and Nakaoka 2021). Although if living in contaminated water containing nanoplastics (less than 1 µm) at high levels, this may pose long-term toxicological risks resulting in potential issues such as liver lesions or damage to gill tissue (Sun et al. 2024). This study focuses on mitigation of microplastics ingestion as they are generally less likely to migrate through tissue (Curtean-Bănăduc et al. 2023) than nanoplastics and mitigation may be more focused on diet management.

Evidence of microplastic accumulation in larger bodied vertebrates in captivity (Nelms et al. 2018) suggests other species currently held in zoos and aquariums may be at risk of yet unknown longer-term effects of ingestion of contaminated prey/diet items. If reproductive efforts are negatively affected in larger bodied organisms as a result of microplastic accumulation, this may have significant repercussions on conservation breeding programmes, such as EAZA Ex situ Programmes (EEPs). Currently there are approximately 13 Taxon Advisory Groups (TAGs) representing piscivorous species within EAZA. This suggests a potential region-wide issue that requires further investigation.

To investigate key factors influencing the presentation of fish to EEP piscivores held in British and Irish Association of Zoos and Aquaria (BIAZA) member collections and identify further pathways of research on reducing risks of microplastic ingestion, this study aimed to: 1) determine which EAZA TAGs represent species that are presented whole and/or chopped fish within BIAZA collections; 2) identify prioritised reasons for presentation of whole and/or chopped fish in BIAZA collections; 3) identify fish species most commonly fed to EEP piscivores within BIAZA collections and 4) identify existing options to mitigate the risk of microplastic ingestion that complement current husbandry decisions within responding BIAZA collections.

Methods

A survey consisting of 11 questions was generated using Microsoft forms (forms.office.com) and distributed to 110 BIAZA member collections (biaza.org.uk/members/all). Contact details were gathered for the researchers at each institute using the respective online website for each BIAZA member collection. The survey was open to responses from September 2019 until March 2020. Respondents were asked to provide a collection name and department title in order to differentiate between multiple respondents from the same collection should this occur. No collections were named as a result of this study. A copy of the survey is presented in the appendices of this report.

Survey questions aimed to identify whether piscivorous species held in BIAZA collections were fed whole or chopped fish as part of their routine diet, with a particular focus on species involved in conservation breeding programmes. For the purposes of this study, involvement in a conservation breeding programme was defined as a species being managed within an EAZA EEP. Species were categorised into their relevant EAZA TAGs and were reported at this level of classification by responding collections.

Respondents were asked which TAG represented species that were presented with whole and/or chopped fish within their collection. Six reasons were offered for providing whole fish and seven reasons offered for providing chopped fish and respondents were asked to rank them in order of importance. Chopped fish refers to any fish that were altered in any way prior to feeding, including chopping into numerous segments or evisceration/removal of GITs.

Respondents were also asked to name three fish species most commonly used as food within their respective collections and rank them in order of amounts used from most to least used.

Fish species used as food were recorded both in terms of number of institutions they were used in as well as scores generated to assess for prioritisation within the BIAZA collective. Prioritised use of fish species was also calculated by allocating scores for the first (three points), second (two points) and third (one point) most utilised fish types for each collection. This was done in order to give an overall rank based on usage within the wider BIAZA collective. Scores for each fish species were totalled across all responding collections and averaged to give a collective score. Average scores for each reason for the collective were then ranked to give an overall representation. Each respondent was also asked if plastic debris had been observed within faeces of any species held within their collection within the 12 months prior to the study.

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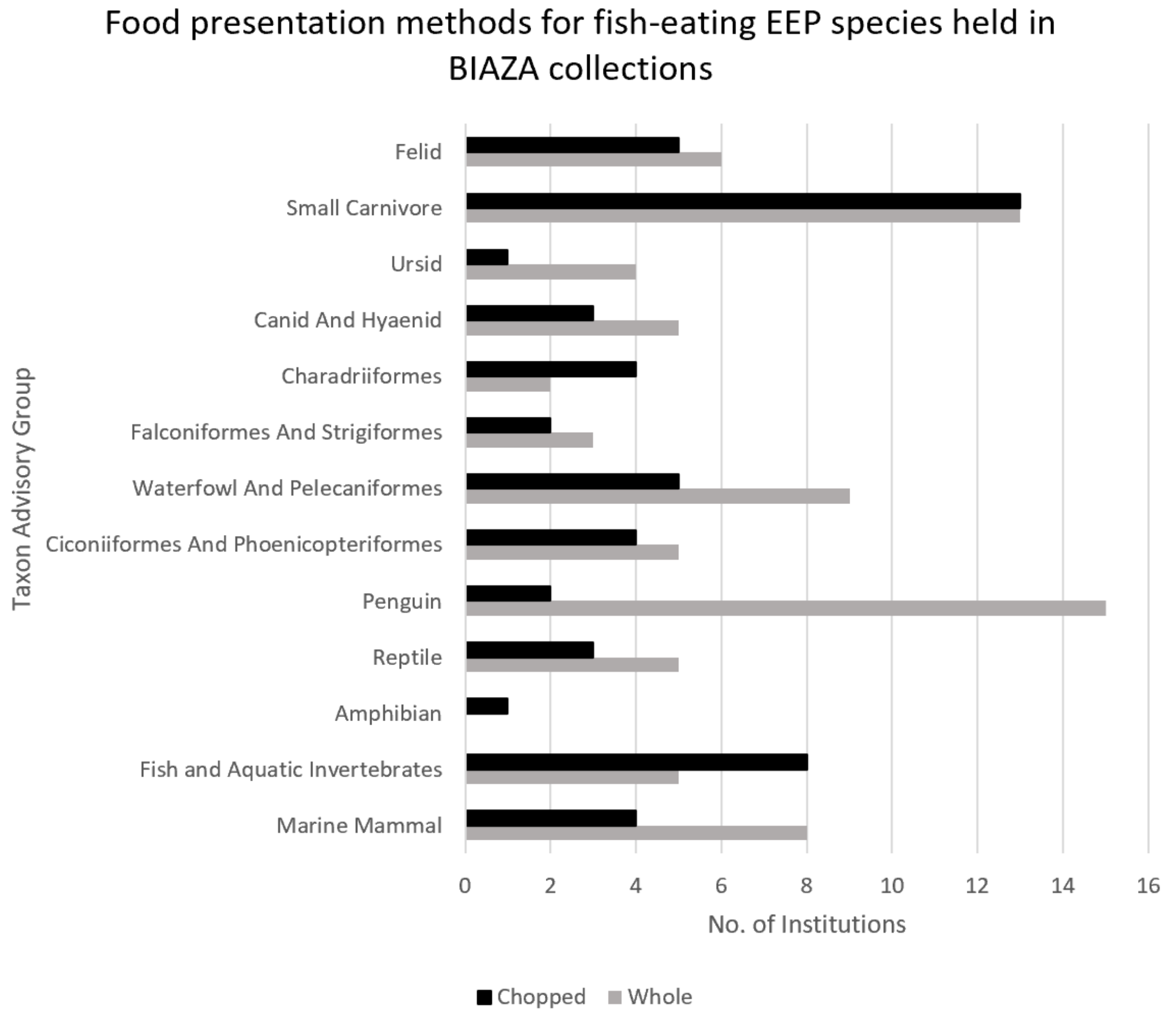

Figure 1. Number of responding BIAZA institutions (n=39) recording of use of whole/chopped fish as part of routine food presentation methods for species represented within each Taxon Advisory Group

Data analysis
Questionnaire responses were entered into a Microsoft Excel spreadsheet and categorised using institutional names. Mean ranks were used to give an overall representation of reasons prioritised for each food presentation method. Using R version 4.3.1 (R Core Team 2015), a chi-square test for independence was used to assess prioritisation of reasons for providing whole or chopped food within routine diets. A chi-square test for independence was also used to assess prioritisation of fish species used as food within each responding institution. Pairwise comparisons were completed for the prioritised reasons for providing whole and chopped fish by responding collections to assess for preferences of individual reasons over others within the dataset. Food species richness (Chao 2006) was documented to produce a species accumulation curve (Ugland et al. 2003) in order to determine the relevance of the sample to the wider BIAZA collective.

Results

Survey responses
Of 110 zoos contacted, 39 BIAZA member collections completed the questionnaire (35%). One collection provided multiple responses to the survey (n=2) from sections housing differing taxa. At the time of data collection, 79% of responding institutions housed piscivorous species involved in EAZA conservation breeding programmes (n=31).

Fish presentation methods
Within conservation breeding programmes, species within the Penguin TAG (n=15) and Small Carnivore TAG (n=13) were recorded as being presented with whole fish by the highest number of responding BIAZA collections (Figure 1). Species within the Waterfowl and Pelecaniformes TAG and Marine Mammal TAG

Table 1. Pre-determined reasons prioritised by responding BIAZA collections for feeding whole fish as part of a routine captive diet.

Reason Stated	Rank (most to least important)	Mean rank
Nutritional composition of whole (intact) fish	1	2.49
Preference of captive individuals towards whole fish over chopped fish	2	3.00
Maintain sustainable expression of predatory feeding behaviours	3	3.07
Small fish used, multiple in one feeding session	4	3.99
Low time cost of preparation	5	4.12
Use of whole fish as rewards in positive reinforcement training	6	4.36

were recorded as being fed whole fish by nine and eight institutions respectively. No responses stated that whole fish were given to species in the Amphibian TAG. Representatives from all other TAG groups were recorded as being fed whole fish in between two and six BIAZA collections.

Within conservation breeding programmes, representatives of the Small Carnivore TAG and Fish and Aquatic Invertebrate TAG were most commonly fed chopped food items with 13 and eight institutes respectively recording this practice. All other TAGs were recorded as feeding chopped fish with accounts ranging from between one and five institutions. Additionally, five responding collections fed whole fish and 15 fed chopped fish to piscivorous species in their care that were not involved in a conservation breeding programme.

Prioritisation of reasons for food presentation methods

Mean ranks for reasons prioritised for providing whole fish were not significantly different from expected values (χ^2=0.79675, df=5, P=0.9772). Nutritional composition ranked overall as the most important reason to present whole fish (mean rank=2.49) with 11 collections stating this as the most important reason and 14 collections stating this as the second most important reason (Table 1). The use of whole fish as rewards in positive reinforcement training was scored overall as the least important reason within the options given for providing whole fish within the diet with only one collection presenting this as the most important reason (mean rank=4.36, Table 2).

Mean ranks for reasons prioritised by respondents for providing chopped fish were not significantly different from expected values (χ^2=2.5963, df=6, P=0.8575). "Available fish species are too large when presented whole" was stated as the most important reason (mean rank=2.73) for presenting chopped fish within the diet with 14 collections stating this as the most important reason and six collections stating it as the second most important reason (Table 3). Twenty responding collections (51%) scored "Gastrointestinal tract is removed to prevent ingestion of contaminants" as the least important reason for presenting chopped fish within the diet (mean rank=5.99) with only two respondents stating this as the most important answer. Pairwise comparisons show that 61% (n=24) of collections prioritised the use of species that would be too large for use in day to day feeding practices if left whole over the balancing of nutrient requirements among a group (Table 4). Two collections prioritised the removal of contaminants over the balancing of nutrient requirements.

Choice of fish used as food within responding BIAZA collections

Common names were reported by all respondents to the question "Which three fish species are most used as food species within your section/department?" Sprat *Sprattus sprattus* was reported as being used as food within 69% of responding collections (n=27) with herring *Clupea* spp. used in 44% (n=17) (Table 5). Roach *Rutilus rutilus* and mackerel *Scomber scombrus* were both reported as being used as food within 33% of responding collections (n=13). Scores used to assess the prioritisation of fish type used resulted in identical rankings to those based on number of collections using each fish type.

One responding collection recorded visible plastic debris as present within the faeces of whiting, a fish type used as food.

Table 2. Pairwise Comparison matrix with respect to routine presentation of whole fish within BIAZA piscivore diets. Table shows number of collections prioritising reasons in top row over left column. Reasons are identified as rank numbers given in Table 3.

Reasons (Ranked)	1	2	3	4	5	6
1		17	15	11	8	7
2	22		20	15	10	11
3	24	19		12	15	11
4	28	24	27		16	14
5	31	29	24	23		20
6	32	28	28	19	25	

Table 3. Pre-determined reasons prioritised by responding BIAZA collections for feeding chopped fish as part of a routine captive diet.

Reason Stated	Rank (most to least important)	Mean rank
Available fish species are too large when presented whole	1	2.74
Keeping staff are able to balance nutritional requirements of individuals within a group setting more effectively	2	2.87
Chopped fish pieces are used as smaller rewards within positive reinforcement setting	3	3.03
Chopped fish pieces are used for behavioural management of competition within a group setting	4	3.61
Keeping staff are able to use feed more effectively as part of behavioural enrichment efforts	5	4.26
Gastrointestinal tract is removed to prevent spoiling of food item	6	5.55
Gastrointestinal tract is removed to prevent ingestion of contaminants	7	5.99

Discussion

Though all piscivores within zoos are at risk of trophic uptake of microplastics, those that maintain a diet of whole prey are more likely to consume potentially contaminated GITs (Nelms et al. 2018). This study highlights four TAGs in particular (Penguin, Small Carnivore, Waterfowl and Pelecaniformes and Marine Mammal) containing species that are potentially at higher risk of exposure to microplastics through ingesting whole fish with intact GITs. All other TAGs included in the study (apart from the Amphibian TAG) represent species involved in conservation breeding programmes that may ingest microplastics as a result of ingestion of potentially contaminated GITs within whole fish, though at lower response rates. Therefore these TAGs are recommended to discuss mitigation measures on an individual species basis where risks or potential long term costs are deemed higher.

All TAGs included within the study except the Amphibian TAG represented species that were fed chopped fish within routine captive diets, with the Small Carnivore TAG and Fish and Aquatic Invertebrate TAGs reported as being fed chopped fish in the highest number of collections. The effects of processing food items on food intake and acceptance have been reported in several non-piscivorious avian species. Food intake was higher in blue and gold macaws *Ara ararauna* when presented with whole foods, though differences were not reported as significant (James et al. 2021). However, food intake was not affected by presentation of whole or chopped food items in two species of turacos (*Tauraco* spp.) (Griffin and Brereton 2022). Yet this may not be an accurate representation of food processing effects on acceptance in piscivores as literature on predatory species food acceptance is limited. As food items are already altered from their original state, this presentation method offers opportunities for mitigation methods to reduce the potential risk of plastic ingestion that are not constrained by the need for whole/intact prey recognition as prioritised by responding collections.

Nutritional composition was considered the most important reason for providing whole intact prey to EEP piscivores in responding collections. Although provision of a whole prey item would generally retain the nutritional composition and appearance of a live-caught prey item prior to storage, this method still requires supplementation of key nutrients such as thiamine to counter the effects of freezing (Mazzaro et al. 2016), which potentially may alter taste/scent during preparation for some piscivorous species if powder-based supplements are used instead of tablets.

Individual animals' preference for whole fish over chopped fish was stated as the second most important reason for inclusion of whole fish in the diet. This suggests that both whole and chopped fish have been presented in the past and potential issues with limited acceptance of processed food items may have been encountered. This may have implications for future efforts to mitigate the risk of plastic ingestion through the removal of digestive tracts, which would result in alteration of the food item and may risk refusal.

Dietary preference testing is a familiar concept to many collections (Brox et al. 2021; Hansell et al. 2020) and may indicate whether key individuals within conservation breeding programmes may be receptive to processed prey items. However, information gathered from these studies should be used in conjunction

Table 4. Pairwise comparison matrix with respect to routine presentation of chopped fish within BIAZA piscivore diets. Table shows number of collections prioritising reasons in top row over left column. Reasons are identified as rank numbers given in Table 3.

Reasons (ranked)	1	2	3	4	5	6	7
1		15	14	16	12	5	5
2	24		22	11	10	2	2
3	25	17		12	9	7	9
4	23	28	27		11	6	6
5	27	29	30	28		6	7
6	34	37	32	33	33		10
7	34	37	30	33	32	29	

Table 5. Fish type most commonly used as food items in responding BIAZA collections

Rank (most to least used)	Common name	No of respondents reporting use of fish type
1	Sprat	27
2	Herring	17
3	Roach	13
3	Mackerel	13
5	Trout	11
6	Sardine	7
7	Whiting	3
8	Sand eel	3
9	Whitebait	2
10	Capelin	2
10	Tilapia	1
12	Conger eel	1
12	Perch	1
12	Saury	1

with nutritional requirement models as diets informed solely by preference tests may lead to deficiencies, with factors such as taste or appearance potentially prioritised by some animals (Woods et al. 2022).

Though nine responding collections stated nutritional composition as the most important reason for providing whole fish within the captive diet, only three collections stated that nutritional considerations were the most important reason for providing chopped fish. As more than half of responding collections prioritised the use of species too large for use in day to day feeding practices if left whole over the balancing of nutrient requirements among a group, this emphasises the relevance of logistics with zoo animal food sourcing. This also presents opportunities for mitigation, suggesting nutritional requirements are already managed as part of a processed diet and that some collections may be open to further processing of fish feed items to remove contaminants.

Limited focus on removal of contaminants from prey species in responses in this study suggests this is currently not an area of concern for many zoological collections. Multidimensional scaling of the reasons prioritised for providing chopped fish showed that removal of GITs to prevent ingestion of contaminants was not closely related to most other husbandry reasons, apart from prevention of spoiling. Though removal of GITs does occur within industry, results from this study show that there appears to be focus on a reduction in risk of the prey item spoiling rather than removal of potential contaminants. Pairwise comparisons revealed that removal of the GIT was prioritised to prevent spoiling over the removal of contaminants by 75% of responding collections (n=29), suggesting that trophic uptake of marine plastic debris has not been widely considered.

Results highlighted several fish species that are commonly used as food within BIAZA collections. Plastic fragments have been recorded as having been ingested by each of the three marine fish species most commonly used by BIAZA collections, sprat, herring and mackerel (Kühn et al. 2020). Presence of polypropylene (PP) and polyethylene terephthalate (PET) fragments have also been detected in sprat prepared commercially for human consumption (Karami et al. 2018). Roach was found to be the most utilised freshwater or brackish water species as food by responding BIAZA collections and has been found to ingest microplastics in coastal habitats (Sainio et al. 2021). This suggests that there is a clear risk of ingestion of microplastics by captive piscivores and that mitigation should be considered by TAGs based on long term conservation and captive breeding goals.

Potential mitigations against trophic uptake of microplastics

Though it is not feasible to manage a trophic web in order to

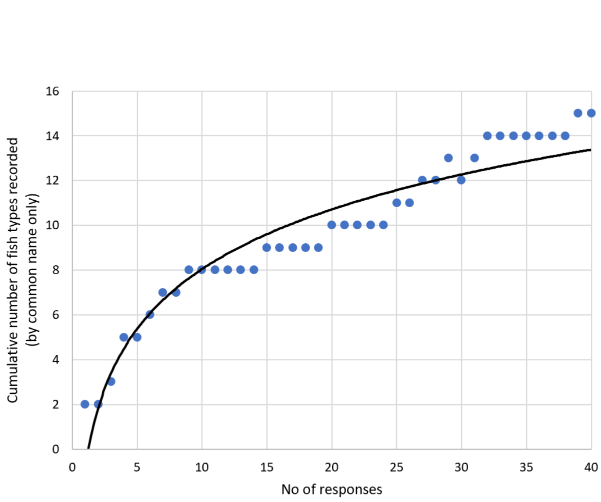

Figure 2. Accumulation curve of recorded fish type (by common name) used as food by responding BIAZA collections

prevent the ingestion of microplastics within captive species, several methods of mitigation may be considered to reduce this risk and the potential long-term effects of plastic ingestion including periodic stomach content screening, evisceration or stomach flushing of prey items.

Periodic screening

Periodic screening of prey GITs may be regarded as a preventative healthcare measure as is often used in anti-helminthic routines with faecal samples being screened at pre-arranged intervals prior to any healthcare intervention (Barrows et al. 2017). At periodic intervals, commonly used commercial species may be screened in order to assess the current risk level of trophic transfer from a particular species or batch of food items. Should microplastics be present within GITs of food items then guidance should be provided on whether further mitigation (such as evisceration or stomach flushing of prey item) is needed following screening, TAGs may advise on whether a zero-tolerance versus threshold approach is taken upon detection of microplastics as is practised when screening food packaged for human consumption (Van Raamsdonk et al. 2011).

Removal of gastrointestinal tracts

Evisceration or removal of the GIT may remove the risk of ingesting microplastics harboured within the digestive system. Assessments carried out on the common nase *Chondrostoma nasus* found that greater amounts of microplastics were found within the contents of the GIT than surrounding GIT tissue (Curtean-Bănăduc et al. 2023). This suggests that the removal of the GIT may be beneficial as an initial measure of reducing potential trophic transfer of microplastics. However, where GITs are removed to reduce the risk of trophic uptake of plastic debris the nutritional composition of a food item would be altered (Owens et al. 2021) and further mitigations necessary. Depending on the rationale for GIT removal and the level of training of those carrying out the process, secondary digestive organs such as the liver and kidneys may also be removed during this process, further altering the nutritional composition of the food item. As these secondary organs are a recognised source of vitamins and trace minerals (Dierenfeld et al. 1991) the use of a supplement should be considered to replace any nutrients lost though the evisceration process. Should GIT removal be adopted as a mitigation against trophic uptake of microplastics, guidance and training may need to be provided to ensure a consistent approach across all collections to avoid the omission of key nutrients with diet plans. Nutritional supplementation is already commonly used in zoos, safari parks and aquariums in the preparation of food for piscivores to replace nutrients lost from thawing processes (Gili et al. 2018) and would have limited impact on current husbandry practices as a mitigation measure. Supplements used to replace key nutrients lost from the removal of GITs and surrounding organs are already available commercially, though additional financial costs would be incurred for collections new to adopting this practice. As with many species held in captivity, supplements may currently be generalised. There may be some benefit from tailoring supplements to species or species groups, but this may also be financially costly. Where fish are chopped before feeding, the additional removal of GITs of prey items may increase the time cost of food preparation though may not affect behavioural management (Griffin and Brereton 2022) unless GITs are used for a particular purpose within group feeding or as differing value rewards within a positive reinforcement training programme. Once eviscerated, fish may need to be either fed shortly after processing or stored in a sealed environment, as exposed tissues can be at higher risk of trapping environmental contaminants than whole, intact fish (Rukmangada et al. 2023). Evisceration, though potentially effective at reducing trophic uptake of microplastics, would likely be unsuitable for smaller species that are often consumed whole, transferring the entirety of stomach contents to the consumer (Mistri et al. 2022). All TAGs included within the study except the Amphibian TAG represented species that were fed chopped fish within routine captive diets, with the Small Carnivore TAG and Fish and Aquatic Invertebrate TAGs being reported as being fed chopped fish in the highest number of collections. As prey items are already altered or processed, these TAGs may consider evisceration and supplementation as a potentially viable mitigation method for priority species.

Flushing/stomach pumping of GITs

Stomach flushing or gastric lavage involves flushing water into the oesophagus and stomach of a fish in order to evacuate the contents into a container for analysis. A range of these techniques are used to assess fish diet/prey within ecological studies with the fish remaining intact and alive throughout the process (Bailey and Moore 2020). These may be effective at removing microplastics from GITs. Specialist mechanised equipment can be employed to carry out this process at a large scale and may be more suitable for collections using high volumes of larger fish species. Smaller fish species can have stomach contents effectively evacuated using syringes with short tubes attached (Kamler and Pope 2001).

Less complex stomach suction pumps based on hand bulb or pipette style operation are also commercially available and are often used in hobbyist angling to assess diet/prey of target species. Both syringes and suction pump equipment would require minimal financial expense and minimal training, potentially providing a cost-effective solution for collections to reduce the amount of microplastics in stomachs of food items. Flushing of GITs with filtered water would largely maintain nutrition composition and potentially reduce the risk of trophic transmission of microplastics, though may increase the time cost for food preparation. Where whole prey is provided and behavioural enrichment or the expression of predatory behaviours is prioritised, stomach flushing or pumping of prey items may be considered as a potentially viable mitigation option in support of husbandry goals.

Alternative sourcing of fish

Additional considerations may be given to acquiring fish from alternative sources. Inland aquaculture facilities are geographically isolated from marine environments preventing direct ingestion of contaminated prey. However, feed used in aquaculture settings may contain marine fish that are not selected for other commercial uses and other agriculture byproducts. Muhib and Rahman (2023) found that finisher-type feeds given to adult life stages of farmed species such as tilapia *Oreochromis niloticus* contained on average 9,150 microplastic particles per kilogram of feed.

Further research

This paper presents several avenues for further research with opportunities to investigate. If it is deemed necessary to remove the GITs of fish used as food to mitigate the risk of ingestion of microplastics, it would also be necessary to investigate the nutritional deficit resulting from this in each key food species. Preference/acceptance tests in priority EEP species may inform whether eviscerated prey items can be realistically used alongside nutritional supplementation to reduce the risk of marine plastic debris ingestion. Further testing of a range of cost-effective stomach flushing equipment would provide insights into which methods and equipment sizes are most effective for each prey species. Further investigation into potential mitigation against nanoplastic uptake may provide additional pathways of safeguarding zoo animals against toxicological effects of plastic ingestion.

Study limitations

As TAGs were used as categories to highlight focal areas for further research, species-specific studies would need to be undertaken in order to assess the potential areas of concern for microplastic ingestion within each TAG.

Although a species accumulation curve generated for fish types used as food began an approach to asymptote, it appears to be still increasing somewhat. This suggests that the majority of fish types used as food in BIAZA collections are present within the given sample, though an increase in sample size and fuller documentation of species used in each collection would be needed to gain a complete understanding of all fish types used as food within BIAZA collections.

Fish types used as food were reported using common names rather than scientific names and so could not always be defined as a single species.

Conclusions

Four TAGs (Penguin, Small Carnivore, Waterfowl and Pelecaniformes and Marine Mammal) recorded the most common presentations of whole fish items. The Small Carnivore and Fish and Aquatic Invertebrate TAGs were recorded as being routinely presented chopped fish in the highest number of collections. No significant differences in ranks of reasons prioritised for presenting both whole and/or chopped fish suggests differing goals across institutions and opportunities for further communication and alignment of intentions within TAGs.

The removal of contaminants was a low priority for presenting chopped fish items, suggesting that mitigation of ingestion of microplastics has not been widely considered. Evisceration of prey items may be suitable for collections already presenting chopped fish, whereas stomach flushing or pumping may be more suitable for those wishing to present whole, intact prey items. Screening of prey items presents a mitigation opportunity to all collections, regardless of presentation method. Sprat, herring and mackerel were used most commonly as food items.

The issue of trophic uptake of microplastics through ingestion of wild-caught fish by zoo animals remains an under-investigated research area and may pose long-term threats to the success of conservation breeding programmes if left unmitigated. This study presents an opportunity for EAZA TAGs containing piscivores to collaborate in order to identify common goals and disseminate guidance on future mitigation procedures against the unknown long-term health effects of ingestion of marine plastics.

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