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Fish characteristics and microplastic ingestion: a mediation analysis of fish length and trophic level in Western Mediterranean pelagic demersal fish

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Abstract. *This paper provides valuable insights into the factors that influence microplastic ingestion by Mediterranean fishes. We present the results of a mediation analysis investigating the relationship between fish length and the number of ingested microplastics, with fish trophic level as a mediating variable. Results show a significant positive mediating effect of fish trophic level on the relationship, indicating that higher trophic level fish have a greater impact on microplastic ingestion. However, the overall effect of fish length on microplastic ingestion was found to be non-significant. Overall, our findings suggest that feeding habits and trophic niche descriptors can have a significant impact on the ingestion of plastic particles in fish species. The study highlights the need for continued research and action to reduce microplastic pollution in aquatic ecosystems, as higher trophic level fish may pose a risk to human consumers.*

Keywords. *Microplastics; Stable isotopes; Feeding habits; Mediation analysis; Direct and indirect effects*

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1 Introduction

The impact of humans on the environment has been profound in recent decades, leading to the Anthropocene era, characterized by climate change, biodiversity loss, and various types of pollution. One of the most prevalent forms of pollution is plastic, especially microplastics, which constitute over 92% of all plastic items in the oceans [13]. The Mediterranean basin is a crucial accumulation zone for plastic, likely due to its semi-closed basin nature, high maritime traffic, densely populated coasts, and long water residence time [14].

The accumulation of microplastics in the Mediterranean basin and their ingestion by marine organisms, including fish species, is a growing concern due to its potential impact on the ecosystem and human health. Studies have reported varying rates of plastic ingestion among fish species in the Mediterranean, which may be attributed to their ecological features and foraging areas [2, 3, 4]. However, few studies

have investigated the relationship between trophic niche features and microplastic ingestion.
Trophic niche features, such as trophic level, trophic niche width, and trophic niche diversity, are important ecological parameters that describe an organism's feeding behavior and its role in the food web [5]. Moreover, the trophic levels is influenced by the fish features. Indeed, larger fish are higher up in the food chain and have a higher trophic level, while smaller fish are lower down and have a lower trophic level. This is because larger fish require more food and energy to sustain themselves, and they typically feed on smaller fish or other organisms. Therefore, the length of a fish can be a useful indicator of its position in the food chain and its ecological role within an ecosystem. Understanding the relationship between these features and microplastic ingestion can provide valuable insights into the ecological impacts of microplastics on the ecosystem.
Fish are particularly susceptible, and plastic ingestion rates vary widely depending on the ecological features of the species and the foraging areas. Identifying the primary causes of microplastic ingestion among species is essential due to its harmful impacts on fish species and the potential risks to human health. The study of fish features can provide valuable information as bioindicators of microplastic ingestion, given their availability, easy collection, high commercial and ecological value.
In this study, eight fish species were selected to explore the variation in microplastic ingestion based on habitat and trophic niche features. Four pelagic species, three benthopelagic species, and one demersal species were collected from the North Tyrrhenian Sea. Understanding the effects of microplastics on marine life and ecosystems is essential for identifying effective management and mitigation strategies.

1.1 Data description

This research was conducted in the waters surrounding Elba Island, which is located in the North Tyrrhenian Sea, part of the Geographical Sub-Area 9 (GSA9) designated by the General Fisheries Commission for the Mediterranean (GFCM) of the FAO. This area is recognized as a significant commercial fishing area and is also known for having high levels of floating macro- and microplastics in the Mediterranean Sea [1, 11].
During the summer of 2018, fish specimens were collected from bottom trawl and trammel fishing vessels operating in the North Tyrrhenian Sea. Pelagic trawls were used to catch sardines, anchovies, bogues, mackerel, and horse mackerel in shallow coastal areas. Red mullet, European hake, and blue whiting were captured with a bottom trawl at depths ranging from 50 to 250 meters. All collected fish were adults, and information such as fish biological parameters, deformations, and external body conditions were recorded for each specimen. The gastrointestinal tract of each fish was removed, and muscle tissue was stored for stable isotope analysis. Fultonâs condition factor (K) was calculated for each fish based on its weight and standard length. The chosen sampling window was short-term to minimize potential environmental variables affecting the trophic niches of the selected species. Habitat and trophic habit categories were assigned to each species based on the relevant literature. In our data, Trophic Level (TL) of fish species were estimated at individual level following [8] equation: $TL_f = \frac{\delta^{15}N_f - \delta^{15}N_b}{\Delta_n} + \lambda$, where $\delta^{15}N_f, \delta^{15}N_b$ are the nitrogen isotopic signatures, respectively for the fish and the reference baseline The study area has one of the most urbanized and industrialized coastlines in Italy, which makes it an important site for examining plastic pollution.
Out of the 396 fish analysed, 98 fish (25%) had 139 plastic items in their gastrointestinal tract. The bogue species had the highest occurrence of MMPs with 37% of individuals containing plastic particles, followed by the European sardine with 35%. We observed that MMPs occurrence was influenced by some of the assessed trophic niche metrics. Zooplanktivorous species had a higher number of MMPs per individual than benthivore and piscivorous species. Furthermore, benthopelagic and pelagic species had a higher ingestion rate of plastic particles per individual compared to demersal species, resulting in

lower body condition.

2 Causal mediation analysis

Causal mediation analysis is a statistical technique used to understand the underlying mechanisms through which a treatment or an exposure affects a response variable through one or more mediator variables. The mediator variable(s) helps explain the relationship between the treatment and the exposure by revealing the underlying causal pathway(s) between them.
A widely used framework to define causal relationships is the counterfactual framework introduced by Rubin (1987). A causal effect of a variable X on a response Y can be defined as the difference in the outcome under two scenarios, where X is set to two different values, x and x^*. For example, suppose to be interested in the causal effect of aspirin on fever. If a patient takes an aspirin to lower their temperature and, after some hours, the temperature decreases, is it merit of the aspirin? To answer this causal question, one should compare the patient's temperature in the actual scenario where they took the aspirin, with the unobservable temperature corresponding to the scenario where the patient did not take the aspirin. If there are no unobserved confounders, if the two values of temperature are not significantly different, then taking aspirin had no effect on the subject's fever. On the contrary, if the two values differ, then aspirin had a causal effect on the improvement (or worsening) of the patient conditions.
When there is a mediator M, the effect of X on Y can be either direct and indirect, i.e. transmitted by the mediator. There exist different definitions of causal mediation effects, but the most widely used are the natural effects introduced by (Robins and Greenland, 1992). Considering again two different values of X, x and x^*, the average direct effect (ADE) is the difference in the outcome values corresponding to two scenarios: in both the mediator is fixed to the natural value it would assume under $X = x^*$, while the exposure changes from x^* to x. The average causal mediated effect (ACME) is the average difference in the outcome if X were set to x and the mediator changed from the natural value under x^* to the natural value under x. Thus, natural mediational effects are functions of nested counterfactuals, and strict assumptions about the absence of unobserved confounders are required to estimate them from observed data, see [7].
In our analysis, we explore the relationship between fish length and microplastic ingestion and evaluate if it is mediated by fish trophic level. The underlying idea is that fish length can influence its trophic level, which is a proxy of the fish role in the food chain, and it is therefore connected to fish's feeding habits. In turn, this can affect the number of microplastics ingested by the fish (Fig.1).

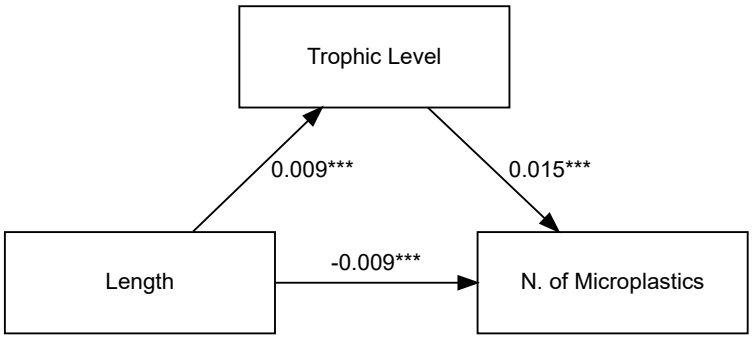

Figure 1: Mediation model for the relationship between fish length and the number of ingested microplastics, with fish trophic level as the mediator.

3 Results

Results of the mediation analysis are shown in Tab.3. The ACME is 4.169, which is statistically significant with a p-value very close to 0, indicating a positive and significant mediating effect of the fish trophic level in the relationship between fish length and the number of ingested microplastics.
In contrast, the ADE is -0.330 , and it is highly statistically significant at the 5% significance level, indicating that the fish length also directly affect the number of ingested microplastics in a negative way.
Although the total effect of fish length on the number of ingested microplastics, including both the direct and mediating effects, is estimated to be 3.839, our analysis indicates that this value is not statistically significant (p = 0.418). One possible explanation for this lack of significance is the small number of observations in our dataset. The analisys was performed using the `R` package `mediation` [12].

	Estimate	95% CI Lower	95% CI Upper	p-value
ACME	4.169	0.151	7.714	0.002**
ADE	-0.330	-0.523	-0.187	<2e-16***
Total Effect	3.839	-0.212	7.348	0.418

Table 1: Estimates and confidence intervals for the Average Causal Mediation Effect (ACME), Average Direct Effect (ADE), and Total Effect of fish length on the number of ingested microplastics, with the mediating variable of fish trophic level.

4 Conclusions

In summary, the study found that the trophic level of fish mediates the effect of fish length on the number of ingested microplastics, with higher trophic level fish having a greater impact. The direct effect of fish length on the number of ingested microplastics was negative and statistically significant. However, the lack of significance of the overall effect may be due to the complex relationship between fish physical traits and the number of microplastics ingested, as well as the small sample size.
These results have important implications for managing microplastic pollution and human health, as higher trophic level fish may pose a greater risk to human consumers by transferring microplastics up the food chain. While the study suggests a positive indirect relationship between fish length and microplastic ingestion, more research with larger sample sizes is needed to confirm these findings.
Overall, this study highlights the need for continued research and action to address the emerging concern of microplastic pollution and its potential impact on the environment and human health.

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