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NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS

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## OCEANOGRAPHY AND MANAGEMENT OF THE MARINE ENVIRONMENT

# Analyzing Microplastics in the Stomach Contents of Deep-Sea Fish and Assessing Their Diet Composition.

**MASTER THESIS**

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ΔΙΙΔΡΥΜΑΤΙΚΟ ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ ΕΚΠΑ - ΕΛΚΕΘΕ

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# «Διερεύνηση για μικροπλαστικά στο στομαχικό περιεχόμενο ψαριών της βαθιάς θάλασσα και αναγνώριση του στομαχικού περιεχομένου με σκοπό την ανασκόπηση της διατροφής τους.»

## ΜΕΤΑΠΤΥΧΙΑΚΗ ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

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# Abstract

This thesis investigates the feeding habits of the deep-water fish species *Chlorophthalmus agassizi, Gadiculus argenteus*, and the examination of microplastic occurrence on the stomach contents of the deep-water fish species *Chlorophthalmus agassizi, Gadiculus argenteus*, *Peristedion cataphractum*, and *Nettastoma melanura*. A total of 444 specimens were collected from various locations in the eastern Ionian Sea, and stomach contents were examined for microplastic occurrence and the prey composition. Microplastics were detected in the 9.4% of all the individuals examined. Fibers were the most common microplastic shape among all species. The feeding habits of *C. agassizi* consisted mainly of Fishes and especially on the mesopelagic fishes of the family Myctophidae and on crustacean, especially Euphausiacea. Fish (Osteichthyes), followed by crustacea were the principal and most favorite prey. In addition, the analysis of the diet composition of *Gadiculus argenteus* showed that the species fed mainly on crustacean, especially Euphausiacea identified.

Keywords: deep-water fish, microplastics, diet, Mediterranean Sea

Λέξεις-κλειδιά: ψάρια βαθέων υδάτων, μικροπλαστικά, διατροφή, Μεσόγειος Θάλασσα

# Περίληψη

Η παρούσα διπλωματική εργασία ερευνά τις διατροφικές συνήθειες δύο ειδών ψαριών βαθέων υδάτων των *Chlorophthalmus agassizi* και *Gadiculus argenteus*, καθώς και την παρουσία μικροπλαστικών στο στομάχι τεσσάρων ειδών των *Chlorophthalmus agassizi*, *Gadiculus argenteus*, *Peristedion cataphractum* και

*Nettastoma melanura*. Συνολικά συλλέχθηκαν 444 δείγματα από διάφορες τοποθεσίες στο ανατολικό Ιόνιο Πέλαγος, και έγινε ανάλυση των περιεχομένων του στομάχου για την παρουσία μικροπλαστικών και τη σύνθεση της διατροφής τους. Μικροπλαστικά ανιχνεύθηκαν στο 9,4% των εξετασθέντων ατόμων. Οι ίνες αναδείχθηκαν ως το πιο κοινό σχήμα μικροπλαστικών που εντοπίστηκαν σε όλα τα είδη. Οι διατροφικές συνήθειες του *Chlorophthalmus agassizi* περιλάμβαναν κυρίως ψάρια, με έμφαση στα μεσοπελαγικά ψάρια της οικογένειας Myctophidae, καθώς και καρκινοειδή, ιδιαίτερα του τάγματος Euphausiacea. Τα ψάρια (Osteichthyes), ακολουθούμενα από τα καρκινοειδή, αποτελούσαν τη βασική και προτιμώμενη λεία. Επιπλέον, η ανάλυση της διατροφικής σύστασης του *Gadiculus argenteus* έδειξε ότι το είδος τρεφόταν κυρίως με καρκινοειδή, και ιδιαίτερα με Euphausiacea, τα οποία ταυτοποιήθηκαν ως κυρίαρχη τροφή.

# 1. Introduction

Microplastics are small plastic particles less than 5 mm in size, originating from various sources like the breakdown of larger plastic debris, microbeads in personal care products, and synthetic fibers. These particles have become ubiquitous pollutants in marine environments, being found in coastal waters, surface waters, and even the deep ocean. Their persistence in the environment and potential to adsorb toxic chemicals make them a significant concern for marine life and ecosystems (Espinosa *et al*., 2016).

In the deep-water environment, microplastics pose a particularly severe threat. Unlike surface waters where they can be transported by currents, microplastics in deep waters tend to accumulate due to processes like sedimentation and aggregation with organic matter. The widespread presence of microplastics in the marine environment, combined with their resemblance to natural prey and other factors, contributes to their ingestion by deep-water fish and other organisms, either directly or through the food web. Studies have documented the presence of microplastics in the stomachs of deep-sea fish, indicating that these pollutants are not confined to surface layers but permeate the entire marine ecosystem (Anastasopoulou *et al*., 2013; Wootton *et al*., 2021).

The impact of microplastics on marine organisms, especially deep-water fish, includes physical and chemical risks. Microplastics have been found to cause endocrine disruption, which affects the hormonal systems in mammals, including humans. These particles can carry harmful chemicals like bisphenols and phthalates, which are known endocrine-disrupting compounds (EDCs). Once in the body, microplastics and the chemicals they carry interfere with hormone receptors, causing disruptions in processes controlled by glands like the thyroid, testes, and ovaries. This can lead to reproductive toxicity, altered hormone levels and potential long-term effects on fertility, pregnancy and child health (Enyoh *et al*., 2023; Ullah *et al*., 2023). Furthermore, microplastics can act as vectors for harmful chemicals, including persistent organic pollutants (POPs) and heavy metals, which can leach into the tissues of marine organisms, potentially causing toxicological effects (Espinosa *et al*., 2016). Moreover, the deep-sea fish in the Mediterranean have been found to have microplastics in their digestive systems, with some species showing signs of oxidate stress due to the ingestion of these particles (Cantasano, 2022). The accumulation of microplastics in deep-sea environments raises concerns about the long-term health of marine ecosystems and the potential for these pollutants to enter human food chains through seafood consumption (Torresi *et al*., 2024).

Microplastic pollution in the Mediterranean Sea is a growing concern, particularly in its deep waters. Studies estimate that around 229,000 tons of plastic enter the Mediterranean annually, with more than 1 million tons of plastic currently present in the basin (Boucher, 2020). The Mediterranean exhibits a concentration of microplastics four times higher than the global average, posing a significant threat to marine ecosystems and human health. Microplastics in the region mainly originate from coastal activities, with Egypt, Turkey, and Italy being the primary contributors. These microplastics infiltrate the marine food web, affecting various marine organisms, including deep-sea fish, and potentially accumulating in higher trophic levels. This accumulation poses health risks to marine life and humans who consume seafood.

*Chlorophthalmus agassizi*, commonly known as the shortnose greeneye (Figure 1a), is widely distributed across the Atlantic Ocean, particularly in the eastern Atlantic, from the Mediterranean Sea and off the coast of West Africa to the Azores and Madeira Islands (Anastasopoulou *et al*., 2006; Froese & Pauly, 2024; D'Onghia *et al*., 2006). *C. agassizi* typically inhabits in depths between 200 and 1000 meters (D'Onghia *et al*., 2006). It feeds primarily on benthic and benthopelagic organisms, with its diet mainly consisting of small crustaceans, polychaete worms, and other invertebrates, along with small fish, playing an important role in the deep-sea benthic and bathyal communities (Anastasopoulou & Kapiris, 2008).

*Gadiculus argenteus*, commonly known as the silvery pout (Figure 1b) is widely distributed in the northeastern Atlantic Ocean, primarily from the Bay of Biscay in the south, along the western coasts of the British Isles, up to Norway and the Faroe Islands (Magnussen, 2007; Froese & Pauly, 2024). The species is also present in parts of the Mediterranean, especially in the deeper, cooler waters of the western basin (Gaemers & Poulsen, 2017). *G. argenteus* is typically found at depths ranging from 50 to 500 meters (Moranta *et al.*, 1998). Ecologically, *Gadiculus argenteus* plays a key role as both predator and prey within the marine food web. It primarily feeds on small crustaceans, such as copepods and amphipods, as well as other zooplankton (Kabasakal, 1999).

*Peristedion cataphractum* commonly known as African armoured searobin (Figure 1c), is a demersal species widely distributed across the eastern Atlantic Ocean and the Mediterranean Sea. Its range extends from the southern coasts of Norway and Iceland, down to the waters off West Africa, as well as throughout the Mediterranean and parts of the Black Sea. The species primarily inhabits continental shelf and upper slope areas, typically found at depths between 50 and 500 meters, although it has been recorded at depths as deep as 800 meters (Bottari *et al.*, 2010; Moranta *et al.*, 1998). Ecologically, *Peristedion cataphractum* is a benthic predator that feeds on small invertebrates such as crustaceans, polychaete worms, and occasionally small fish (Froese & Pauly, 2024). This specialized feeding behavior plays a role in structuring benthic communities, as it preys on a variety of species that dwell within the substrate (Whitehead *et al.*, 1986).

*Nettastoma melanura*, commonly known as the blackfin sorcerer (Figure 1d), is a species of fish found primarily in the eastern Atlantic Ocean. Its distribution extends from the coastal waters of Norway and Iceland southward to the Azores, Madeira, and the Canary Islands (Froese & Pauly, 2024). This species inhabits a range of depths, typically found between 200 and 1,200 meters (Porcu *et al.,* 2021). Ecologically, *Nettastoma melanura* is a predator of small benthic organisms. Its diet consists mainly of fish and invertebrates that inhabit the deep-sea floor.

Published literature concerning feeding habits of the examined species of *C. agassizi* and *G. argenteus* is very limited. More specifically, the feeding habits of *C. agassizi* have been studied by Anastasopoulou & Kapiris (2008) in the Eastern Ionian Sea and Kabasakal (1999) in the North-Eastern Aegean Sea. Similarly, the diet of *G. argenteus* has been reported by Albert (2012) in the Norwegian Deep and Mattson (2011) in the West-Norwegian fjord. The only published work in the Mediterranean Sea is given by Kabasakal (1999) for the North-Eastern Aegean Sea.

The only published literature on microplastic presence in *C. agassizi* has been reported by Sciutteri *et al.* (2023) in Southern Tyrrhenian Sea. For microplastic ingestion for the species *Nettastoma melanura* has been reported also by Sciutteru *et al*. (2023) in Southern Tyrrhenian Sea and Cartes *et al*. (2016) in Western Mediterranean Sea. There is no published information on the microplastic ingestion for the species *G. argenteus* and *P. cataphractum*. The objectives of the present study were a) to examine the feeding habits of the species *C. agassizi* and *G. argenteus*, b) to detect the presence of microplastics in the guts of the species *C. agassizi, G. argenteus*, *P. cataphractum* and lastly *N. melanura* and c) to improve the existing knowledge on feeding habits of the examined species as well as on the presence of microplastics in their guts, comparing our results with findings from other areas.

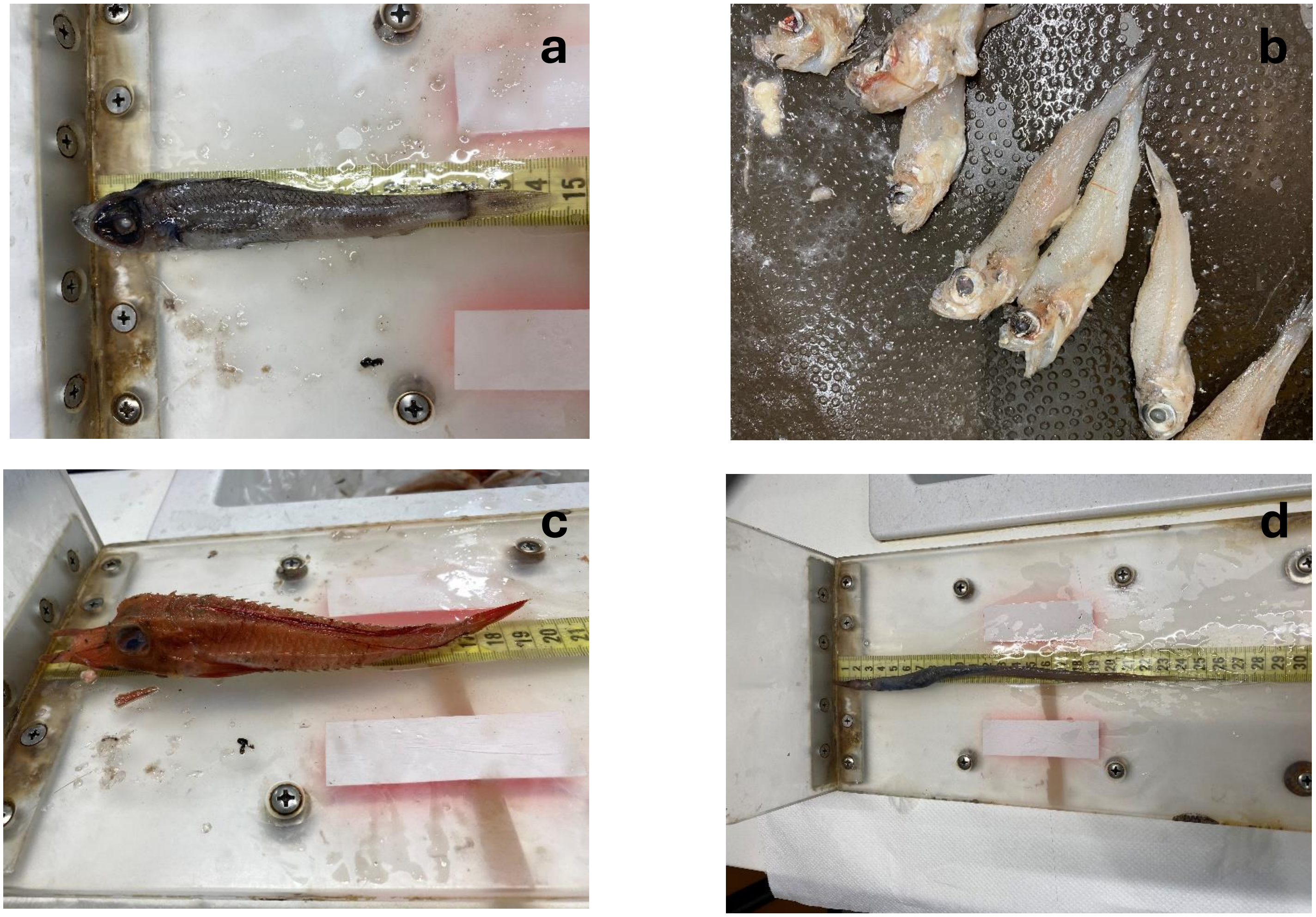

*Figure 1: The species used in this study: a) Chlorophthalmus agassizi b) Gadiculus argenteus c) Peristedion cataphractum d) Nettastoma melanura*

# 2. Materials and Methods

## 1) Study Area

The sampling area was carried out in the eastern Ionian Sea, between South of Leukada and South of Zakynthos Islands and the Peloponnisos peninsula in the framework of the 'International bottom trawl survey in the Mediterranean' (MEDITS 2022) (Figure 1). The experimental trawl survey cruises took place on July 2022.

## 2) Sampling stations

The sampling was based on the MEDITS standardized sampling protocol. The samples were collected from 9 stations at depths ranging from 200m to 500m during daylight hours in July 2022. In total, 444 individuals were sampled, 200 individuals of *Chlorophthalmus agassizi* (shortnose greeneye), 200 individuals of *Gadiculus argenteus* (silvery pout), 24 individuals of *Peristedion cataphractum* (african armoured searobin) and 20 individuals of *Nettastoma melanura* (blackfin sorcerer). The samples were frozen immediately after capture and analyzed in the laboratory.

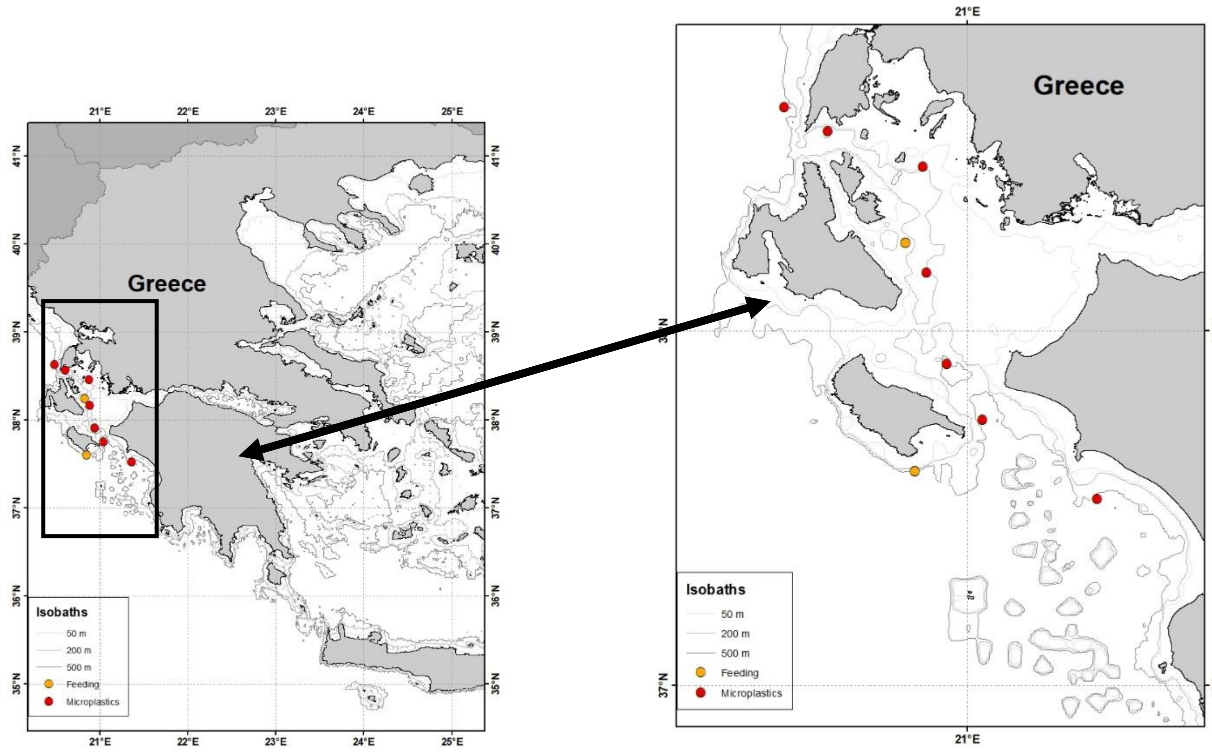

*Figure 2: Map of the study area and location of sampling stations (dots) in the Eastern Ionian Sea, used for the collection of the individuals for the study of microplastics and feeding (isobaths at 50, 200 and 500m).*

## 3) Laboratory experiment

In the laboratory, each specimen was measured (total length, TL, in mm), and weighted to the nearest 0.1 g s. Then, fishes dissected, and the stomachs were removed, weighted, placed in numbered sealed bags and frozen until the next proceeding steps.

### i. Microplastic analysis

A total of 244 individuals were used for the microplastic analysis. Samples were treated with KOH for microplastic extraction, according to Tsangaris *et al*. (2021) with minor modifications. Samples were covered with lids in glass vials and left at room temperature until the digestion of all organic matter was completed (Tsangaris *et al*., 2021). After the digestion of the organic matter, samples were then filtered using two sieves of 250 and 80 μm mesh size in sequence (Torre *et al*., 2016). Then the samples were filtered under vacuum on fiberglass filters (Whatman GF/C, pore size 1.2 or 1.6 μm) (Figure 10). Each filter was placed in Petri dishes and dried at room temperature. All filtering procedures took place inside a laminar flow cabinet. Filters were examined under a stereomicroscope using a video camera (Sony

Exwave HAD, Digital color video-camera) and the images were stored and analyzed with the Image pro PLUS Vs. 4.5.29, 98/NT/2000 for Windows. Microplastic items were photographed, counted, and categorized according to maximum length, color, and type (fragment, fiber, sphere), following the classification criteria of the MSFD technical group on marine litter.

To reduce air contamination, working surfaces were cleaned with ethanol before every use, the equipment was carefully cleaned and the samples were covered during all processing stages e.g. digestion, observation under the stereomicroscope. During all the processing steps, control blanks, consisting of dumped papers in Petri dishes, were exposed to the air to monitor airborne contamination by microfibers (Hermsen *et al.*, 2017; Hermsen *et al.*, 2018; Torre *et al.*, 2016; Torres-Agullo *et al.*, 2022). Lastly cotton lab coats were used during all laboratory procedures and the presence of staff in the lab was kept to a minimum.

### ii. Diet analysis

A total of 200 individuals were used for the diet analysis of *Chlorophthalmus agassizi* and *Gadiculus argenteus (*100 individuals from each species). The stomach contents were analyzed under a stereomicroscope to identify prey items, which were counted and weighted. Prey items were identified to the lowest taxonomic level when possible. Partially and totally digested fishes were identified by their otoliths. Unidentified crustaceans and fishes are referred to separately. Empty guts with unidentifiable (digested) material were excluded from the analysis (Anastasopoulou *et al.*, 2013; Anastasopoulou *et al.*, 2018; Anastasopoulou & Kapiris, 2008).

## 4) Data analysis

### i. Microplastic analysis

The proportion of fish with ingested microplastics was calculated in relation to the total number of fish examined by species.

For the ingested microplastics 2 indices were used in this study, (1) the percentage frequency of litter occurrence (F%) = the number of stomachs containing a given microplastic item/ total number of non-empty stomachs examined x 100. (2) The percentage numerical litter abundance (N%) = the number of litter items of a given litter category to the total number of litter items of all categories in all guts (*100) (Anastasopoulou *et al.*, 2018).

### ii. Diet analysis

The vacuity index (VI) of both species was calculated, by using the following:

The vacuity is calculated as = Number of samples with empty stomachs / Total number of samples

Three standard indices (Hyslop, 1980) were calculated to describe the prey composition in the individuals examined. The indices used were:

(1) relative abundance (N%) of prey to the total number of prey items, (2) percentage mass (W%) of prey item in relation to the total mass of prey items and (3) frequency of prey occurrence (F%). The importance of each prey in the diet of *C. agassizi* and *G. argenteus* was studied by two indices: the alimentary coefficient Q (Q=F%*W%) and the index of relative importance (IRI%) as modified by (Hacunda, 1981) [IRI=(N%+W%) * F%] and expressed as a percentage. Using the Q coefficient, the preys were separated into three categories (Hureau, 1970): principal for Q>200, secondary for 20<Q<200 and accidental for Q<20.

The feeding strategy was determined using the method of Amundsen *et al.*, (1996) where prey-specifying abundance (pi) is plotted against the frequency of occurrence (%F). Expressed as a percentage, prey-specific abundance is a given prey taxon proportion in relation to all prey items observed in only those predators' stomachs that contained the given prey taxon:

pi = 100 x ∑Si X ∑Sti$^{-1}$ , where ∑Si is the sum of the stomachs comprising prey i, ∑Sti is the sum of all prey items found in only those predator stomachs that contained prey i.

# 3. Results

## Microplastic particles

### i) Microplastic ingestion

In total, 100 fish of the species of *Chlorophthalmus agassizi*, 100 fish of the species of *Gadiculus argenteus*, 24 fish of the species of *Peristedion cataphractum* and 20 fish of *Nettastoma melanura* were sampled from the eastern Ionian Sea and examined for microplastic ingestion. The min, max and mean length (± S.D.) of the samples is given in Table 1. Similarly, their min, max and mean weight (± S.D.) is presented in Table 2.

*Table 1: Fish species examined for microplastic ingestion. N: number of individuals, min, max and mean total length TL(cm) are shown. SD: standard deviation*

| SPECIES | N | TL Min | TL Max | TL Mean | SD TL Mean |
|---|---|---|---|---|---|
| *Chlorophthalmus agassizi* | 100 | 102 | 177 | 131.51 | 16.09 |
| *Gadiculus argenteus* | 100 | 62 | 108 | 89.79 | 10.59 |
| *Nettastoma melanura* | 20 | 175 | 382 | 272.85 | 66.11 |
| *Peristedion cataphractum* | 24 | 85 | 200 | 149.08 | 34.15 |
| **Grand Total** | **244** | **62** | **382** | **127.73** | **54.59** |

*Table 2: Total weight TW of Fish species examined for microplastic ingestion. Min, max and mean values (gr) are shown SD: standard deviation*

| SPECIES | N | TW Min | TW Max | TW Mean | SD TW Mean |
|---|---|---|---|---|---|
| *Chlorophthalmus agassizi* | 100 | 6 | 30 | 14.00 | 5.86 |
| *Gadiculus argenteus* | 100 | 3 | 12 | 8.00 | 2.09 |
| *Nettastoma melanura* | 20 | 3 | 42 | 8.00 | 10.80 |
| *Peristedion cataphractum* | 24 | 3 | 40 | 26.00 | 12.99 |
| **Grand Total** | **244** | **15** | **124** | **56.00** | **31.74** |

### ii) Microplastics detection

Microplastics detected at the stomachs of the fish examined in the eastern Ionian Sea are given in Table 3. Microplastics found in the blanks were excluded from the analysis (i.e. items similar to those found in the blanks excluded from the results). The total number of microplastics found in all the species at all stations was 25 items, corresponding to a 9.4% frequency of occurrence. The highest microplastic abundance (17 microplastics) was found in the stomachs of *Chlorophthalmus agassizi*, whereas the lowest abundance (1 microplastic) was found at the species *Peristedion cataphractum* and *Nettastoma melanura*. The mean number of microplastics ingested/individual was 0.10 ±0.34. Considering only positive samples from all samples, mean microplastic abundance was 1.09±0.42 items/individual (Table 3).

*Table 3: Frequency of occurrence and number (min, max and mean) of microplastics detected in the stomach content of the four examined species in the eastern Ionian Sea. F: frequency of occurrence. F%: frequency of occurrence %. TOT N: total number of m. Mean N(1): Mean number (N) of litter in stomachs where litter was found. Mean N(2): Mean number (N) of litter in all the stomach analyzed.*

| SPECIES | F (Occurrence) | F % | TOT N | Min N | Max N | Mean N (1) | Mean N (1) SD | Mean N (2) | Mean N (2) SD |
|---|---|---|---|---|---|---|---|---|---|
| *Chlorophthalmus agassizi* | 15 | 15 | 17 | 1 | 3 | 1.13 | 0.52 | 0.17 | 0.45 |
| *Gadiculus argenteus* | 6 | 6 | 6 | 1 | 1 | 1.00 | - | 0.06 | 0.24 |
| *Nettastoma melanura* | 1 | 5 | 1 | 1 | 1 | 1.00 | - | 0.05 | 0.22 |
| *Peristedion cataphractum* | 1 | 4.2 | 1 | 1 | 1 | 1.00 | - | 0.04 | 0.20 |
| **ALL SPECIES** | **23** | **9.4** | **25** | **1** | **3** | **1.09** | **0.42** | **0.10** | **0.34** |

### iii) Microplastic characterization (shape, size, color)

Black microplastics were the most prevalent, comprising 24% of the non-identified (NI) items and 12% of the synthetic items. Blue microplastics followed, representing 16% of NI (not identified) items and 8% of synthetic items. Blue-white and green NI items accounted for 8% and 20%, respectively (Table 4). Other colors were less abundant. Fibers were the most common microplastic shape among all species (Table 4). In table 5 there are analytically the sizes of microplastic items (min, max and mean) detected in the guts of the examined species. Microplastics were categorized into three size classes: micro (<5 mm), meso (5-25 mm), and macro (>25 mm), where most microplastics fell within the micro category in this study (Table 6). There are examples of the microplastic items found in the guts of the species in Figure 3.

*Table 4: Colors and shape of microplastic items percentage found in the examined species.*

| **LITTER ITEMS** | | | ***Chlorophthalmus agassizi*** | ***Gadiculus argenteus*** | ***Nettastoma melanura*** | ***Peristedion cataphractum*** | **TOT** |
|---|---|---|---|---|---|---|---|
| **Fiber** | **NI** | **Black** | 18 | 33 | | 100 | 24 |
| | | **Blue** | 12 | 33 | | | 16 |
| | | **Blue-White** | 6 | 17 | | | 8 |
| | **Synthetic** | **Black** | 18 | | | | 12 |
| | | **Blue** | 6 | | | | 4 |
| | | **Blue-Transparent** | 6 | | | | 4 |
| | | **Transparent** | 6 | | | | 4 |
| **Piece** | **Natural** | **Black** | | | 100 | | 4 |
| | **NI** | **Green** | 29 | | | | 20 |
| | **Synthetic** | **Blue** | | 17 | | | 4 |
| **ALL LITTER N** | | | **17** | **6** | **1** | **1** | **25** |

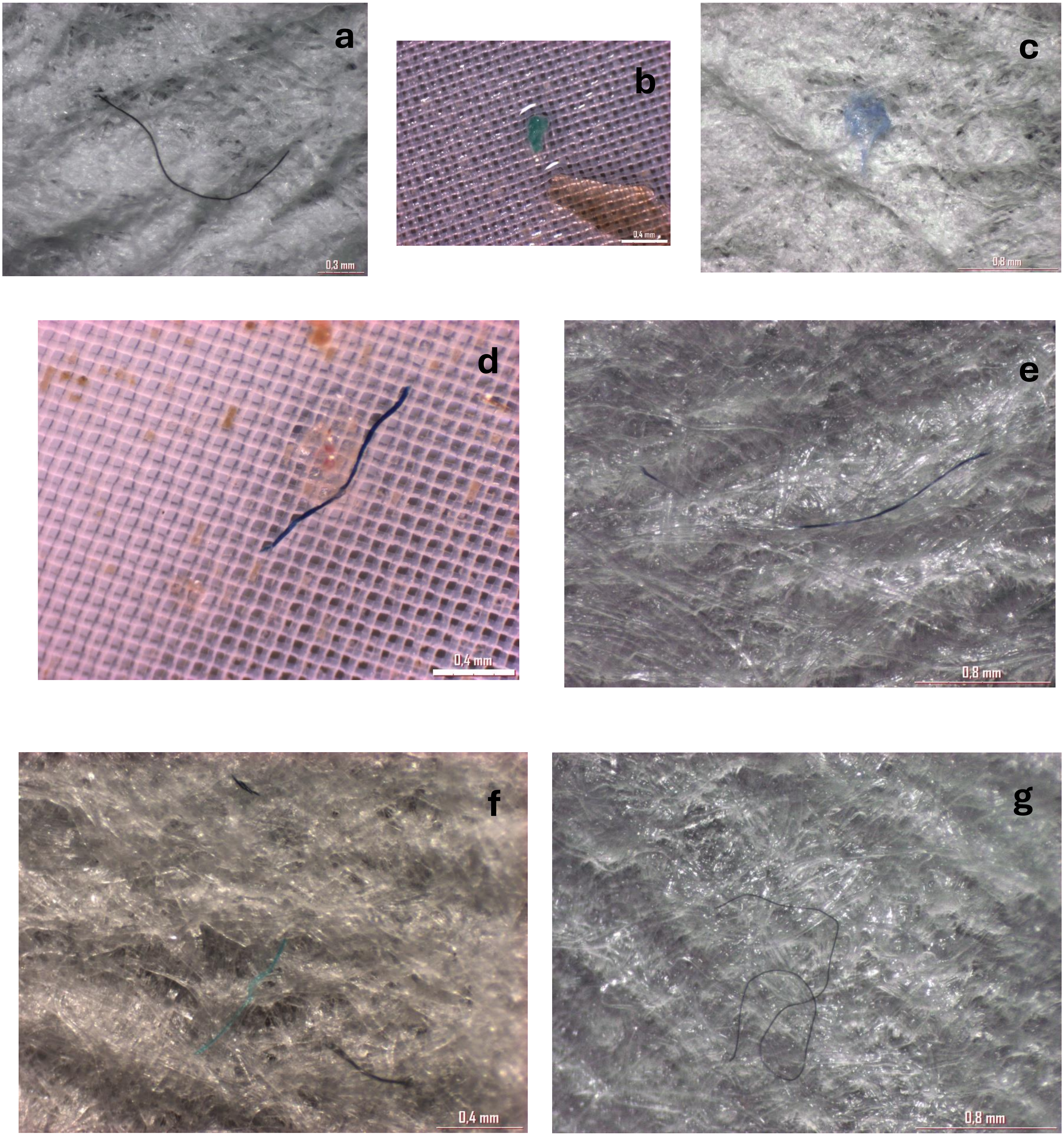

*Figure 3: Microplastics found in the specimen stomachs: a) Black synthetic fiber b) green particle c) blue particle d) blue synthetic fiber e) transparent-blue synthetic fiber f) light blue synthetic fiber g) black synthetic fiber*

*Table 5: Size of microplastic (min, max and mean) detected in the guts of the four examined species in the E. Ionian Sea SD: standard deviation*

| SPECIES | Min Size | Max Size | Mean Size | Mean Size (SD) |
|---|---|---|---|---|
| *Chlorophthalmus agassizi* | 0.15 | 5.61 | 1.36 | 1.40 |
| *Gadiculus argenteus* | 0.55 | 2.36 | 1.41 | 0.79 |
| *Nettastoma melanura* | 0.35 | 0.35 | 0.35 | - |
| *Peristedion cataphractum* | 3.4 | 3.40 | 3.40 | - |
| **ALL SPECIES** | **0.15** | **5.61** | **1.42** | **1.28** |

*Table 6: Total microplastic size percentage of the examined species categorized in micro (<5mm), meso (5mm-25mm) and macro (>25mm) and categorized by length*

| SPECIES | Micro % | Micro Number of microplastics | Meso % | Meso Number of microplastics | Macro % | Macro Number of microplastics |
|---|---|---|---|---|---|---|
| *Chlorophthalmus agassizi* | 94 | 16 | 6 | 1 | 0 | 0 |
| *Gadiculus argenteus* | 100 | 6 | 0 | 0 | 0 | 0 |
| *Nettastoma melanura* | 100 | 1 | 0 | 0 | 0 | 0 |
| *Peristedion cataphractum* | 100 | 1 | 0 | 0 | 0 | 0 |
| **ALL SPECIES** | **96** | 24 | **4** | 1 | **0** | **0** |

## Feeding habits

A total of 100 stomachs of *Chloropththalmus agassizi* were studied. Only 29 stomachs contained prey and there was no indication of regurgitation. The vacuity index was found 40%. In most cases (31 stomachs) the stomachs contained digested food at the advanced stage which was not identifiable and for this reason were excluded from the analysis. The diet of the species consisted of 40 prey items belonging to 15 prey taxa (Table 7). *C. agassizi* fed mainly on Fishes and especially on the mesopelagic fishes of the family Myctophidae and on crustacean, especially Euphausiacea. Fish (Osteichthyes), followed by crustacea were the principal and most favorite prey (Q>200; IRIst: 57,92%) for the species. Fish identified in the stomachs of the species belong to the family Myctophidae, and the species *Stomias boa* and *Argyropelecus hemigymnus*. Several orders of Crustacea taxa were also identified (Table 7) with Euphausiacea to be the most abundant and important prey in its diet. In terms of frequency, unidentified crustacean, unidentified fish and Myctophidae predominated the diet of the species. Moreover, one parasite of Nematoda was found in one stomach of *Chlorophthalmus agassizi*. The feeding strategy of *C. agassizi* (Figure 4) showed that most prey taxa were located on the left side of the diagram with low prey-specific abundances (<20%) and relatively low frequency of occurrence (<40%), indicating that most prey taxa were rare in the diet of the species. Fish unidentified, Myctophidae and crustacea unidentified, which were more frequent, were located below the prey importance axis (50%), showing a kind of generalized feeding behavior towards these prey taxa.

*Table 7: Diet composition of Chlorophthalmus agassizi. (N%: relative abundance; W%: weight percentage; F%: frequency of occurrence; Q: alimentary coefficient; IRI%: index of relative importance)*

| PREY TAXA | N % | W % | F % | Q | IRI % |
|---|---|---|---|---|---|
| CRUSTACEA | | | | | |
| CRUSTACEA Unidentified | 20.00 | 15.63 | 17.14 | 267.94 | 24.93 |
| CALANOIDA Unidentified (juveniles) | 7.50 | 0.40 | 2.86 | 1.15 | 0.92 |
| Paracalanus | 2.50 | 0.04 | 2.86 | 0.12 | 0.30 |
| Ctenocalanus | 5.00 | 0.08 | 2.86 | 0.23 | 0.59 |
| AMPHIPODA Unidentified | 2.50 | 0.16 | 2.86 | 0.46 | 0.31 |
| MYSIDA Unidentified | 5.00 | 2.71 | 5.71 | 15.46 | 1.80 |
| EUPHAUSIACEA unidentified | 12.50 | 8.12 | 14.29 | 115.97 | 12.02 |
| DECAPODA | | | | | |
| BRACHYURA | 2.50 | 4.12 | 2.86 | 11.77 | 0.77 |
| Pashipaeidae | 2.50 | 1.17 | 2.86 | 3.35 | 0.43 |
| OSTEICHTHYES | | | | | |
| FISH Unidentified | 15.00 | 4.40 | 17.14 | 75.47 | 13.58 |
| FISH LARVAE Unidentified | 2.50 | 1.62 | 2.86 | 4.62 | 0.48 |
| MYCTOPHIDAE Unidentified | 15.00 | 43.42 | 17.14 | 744.29 | 40.88 |
| MYCTOPHIDAE LARVAE Unidentified | 2.50 | 1.90 | 2.86 | 5.42 | 0.51 |
| STOMIIDAE | | | | | |
| *Stomias boa* | 2.50 | 10.99 | 2.86 | 31.39 | 1.57 |
| STERNOPTYCHIADAE | | | | | |
| *Argyropelecus hemigymnus* | 2.50 | 5.25 | 2.86 | 15.00 | 0.90 |

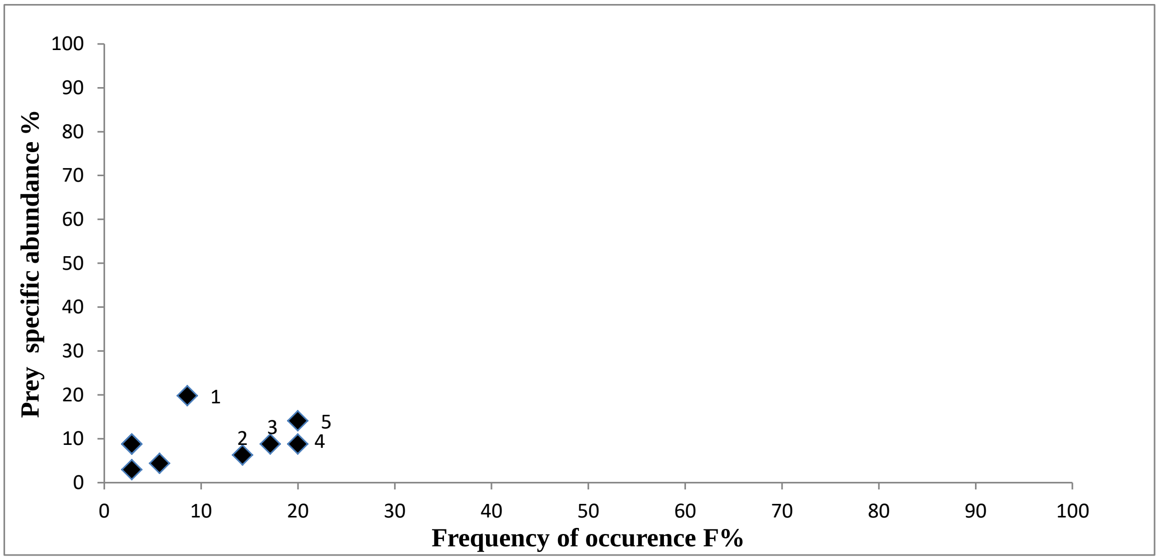

*Figure 4: Feeding strategy plot of C. agassizi. Prey types (as squares) are: 1. Calanoida; 2. Euphausiacea; 3. Crustacea; 4. Myctohidae; 5. Teleostei*

A total of 100 stomachs of *Gadiculus argenteus* were studied. Out of 100 stomachs of *G. argenteus*, 24 stomachs were empty (VI = 14%). The rest 76 stomachs contained food remains. Stomachs with digestive food (n=10) at an advanced stage were not included in the analysis. The diet of the species consisted of 8 prey taxa (Table 8). *G. argenteus* fed mainly on crustacean, especially Euphausiacea identified (Q>200; IRIst: 100,05%). From the taxon Euphausiacea only the species of *Euphausia krohnii* was identified at species level. The heaviest and the most frequent prey taxon was also Euphausiacea identified (W%= 94,43%, F%=79,31%). The study of the feeding strategy of *Gadiculus argenteus* (Figure 5) showed that the species consume more frequently Euphausiacea (high frequency of occurrence), than the other prey categories which are located in the lower left part of the diagram. Crustacea and Calanoida were located below the prey importance axis (50%), showing that the species exhibited a kind of generalized feeding behavior towards these prey taxa.

*Table 8: Diet composition of Gadiculus argenteus. (N%: relative abundance; W%: weight percentage; F%: frequency of occurrence; Q: alimentary coefficient; IRI%: index of relative importance*

| PREY TAXA | N % | W % | F % | Q | IRI % |
|---|---|---|---|---|---|
| CRUSTACEA | | | | | |
| CALANOIDA | | | | | |
| CALANOIDA Unidentified | 6.84 | 0.60 | 2.30 | 1.38 | 0.12 |
| Calanidae | | | | | |
| *Calanus* | 6.32 | 0.55 | 6.90 | 3.81 | 0.34 |
| Paracalanidae | 1.05 | 0.09 | 1.15 | 0.11 | 0.01 |
| Oncaeidae | | | | | |
| *Oncaea* | 1.58 | 0.14 | 3.45 | 0.48 | 0.04 |
| Temoridae | | | | | |
| *Temora* | 0.53 | 0.05 | 1.15 | 0.05 | 0.00 |
| EUPHAUSIACEA unidentified | 81.05 | 94.43 | 79.31 | 7489.41 | 99.36 |
| *Euphausia krohnii* | 0.53 | 0.92 | 1.15 | 1.06 | 0.01 |
| DECAPODA unidentified | 2.11 | 3.22 | 4.60 | 14.81 | 0.17 |

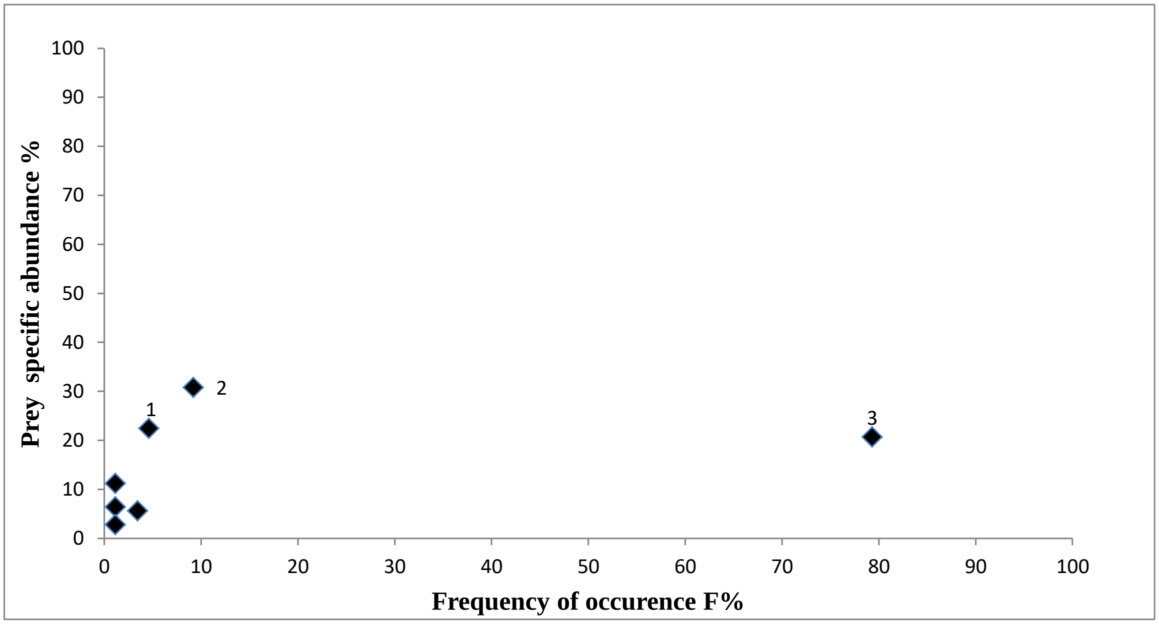

*Figure 5: Feeding strategy plot of Gadiculus argenteus. Prey types (as squares) are: 1. Crustacea unid; 2. Calanoida; 3. Euphausiacea*

# 4. Discussion

Information on the feeding habits and ingested microplastics for the species *C. agassizi*, *G. argenteus*, *P. cataphractum* and *N. melanura* is very limited. This study provides for the first time information on the feeding habits of *G. argenteus* and updated knowledge on the feeding habits of *C. agassizi* from the last reported one (Anastasopoulou & Kapiris, 2008). In addition, this study provides for the first time information on ingested microplastics for the species *C. agassizi, G. argenteus, P. cataphractum* and *N. melanura* in the study area and for the species *Gadiculus argenteus* and *Peristedion cataphractum* for the whole mediterranean.

Microplastic ingestion

Our findings confirm the presence of microplastic in the stomachs of *C. agassizi, G. argenteus, P. cataphractum* and *N. melanura* in the deep water of Eastern Ionian Sea. The higher number of microplastics was observed in the stomach content of *C. agassizi* with 17 microplastic items and the lowest microplastic number in the species of *Peristedion cataphractum* and *Nettastoma melanura*, with 1 microplastic item per species. The occurrence of microplastics in the stomachs of *C. agassizi* in this study was 15%, which is higher than those reported by Sciutteri *et al*. (2023) from the same species collected in the Southern Tyrrhenian Sea (9,1%) but lower that the frequency reported by Panarello *et al*. (2019) (21%) in southern Tyrrhenian Sea and by Panunzi *et al*. (2023) (26,7%) in the North Tyrrhenian Sea. The frequency of occurrence for *Nettastoma melanura* was found in this study 5%; Sciutteri *et al*. (2023) did not detect microplastics in the guts of the same species examined in the southern Tyrrhenian Sea. On the contrary, Cartes *et al*. (2016) found in the stomachs of *Nettastoma melanura* plastic threads with higher frequency of occurrence (9,1%) in the Western Mediterranean Sea. No published information on microplastic ingestion exists for the species *G.argenteus* and *P. cataphractum* and for this reason we cannot compare the results of our study. However, higher values of the frequency of occurrence (28,6%) than the species examined in our study have been reported by Davison & Asch (2013) on other fish species e.g. *Diaphus fulgens* collected from the North Pacific Subtropical Gyre in depths of 200 m and 800 m . Similarly, lower values of the frequency of occurrence (1,7%) were reported from Anastasopoulou *et al.* (2013) for the species of *Pagellus bogaraveo* sampled from the deep water of the Eastern Ionian (300-850 m). Gianni *et al*. (2023) reported occurrence values range from 8%-24% for several deep-water fish sampled from North Tyrrhenian Sea. Frequency of occurrence for the species examined in this work seems to be within the range of the published literature (Table 9). The majority of the ingested plastics were fibers of black color.

*Table 9: Microplastic ingestion reported in the literature for deep water fish in the Mediterranean*

| **Species** | **No Individuals** | **Frequency of occurrence** | **Abundant shape** | **Area** | **Depth** | **References** |
|---|---|---|---|---|---|---|
| *Pagellus bogaraveo* | 60 | 1,7% | Fragment | Eastern Ionian Sea | 300-850 m | Anastasopoulou *et al*., 2013 |
| *Chlorophthalmus agassizi* | 88 | 9,1% | Fragment/ Fiber/ Film | Southern Tyrrhenian Sea | 525-576m | Sciutteri *et al.,* 2023 |
| *Nettastoma melanura* | 14 | 0% | Fragment/ Fiber/ Film | Southern Tyrrhenian Sea | 525-576m | Sciutteri *et al.,* 2023 |
| *Nettastoma melanura* | 11 | 9,1% | Fiber | Western Mediterranean Sea | 996-1352 | Cartes *et al*., 2016 |
| *Chlorophthalmus agassizi* | - | 21% | - | Southern Tyrrhenian Sea | - | Panarello *et al*., 2019 |
| *Chlorophthalmus agassizi* | 30 | 26,7% | - | North Tyrrhenian Sea | - | Panunzi *et al*., 2023 |
| *Mullus barbatus* | 36 | 17% | Fiber | North Tyrrhenian Sea | 50-250m | Giani *et al*., 2023 |
| *Merluccius merluccius* | 36 | 8% | Fiber | North Tyrrhenian Sea | 50-250m | Giani *et al*., 2023 |
| *Micromesistius poutassou* | 25 | 24% | Fiber / Fragment | North Tyrrhenian Sea | 50-250m | Giani *et al*., 2023 |
| *Diaphus anderseni* | 13 | 15,4% | Fragment/ Fiber | North Pacific Subtropical Gyre | 200m, 800m | Davison & Asch, 2013 |
| *Diaphus fulgens* | 7 | 28,6% | Fragment/ Fiber | North Pacific Subtropical Gyre | 200m, 800m | Davison & Asch, 2013 |
| *Myctophum nitidulum* | 25 | 16% | Fragment/ Fiber | North Pacific Subtropical Gyre | 200m, 800m | Davison & Asch, 2013 |
| *Chlorophthalmus agassizi* | 100 | 15% | Fiber | Northern Ionian Sea | 50-500m | this study |
| *Gadiculus argenteus* | 100 | 6% | Fiber | Northern Ionian Sea | 50-500m | this study |
| *Nettastoma melanura* | 20 | 5% | Fiber | Northern Ionian Sea | 50-500m | this study |
| *Peristedion cataphractum* | 24 | 4,2% | Fiber | Northern Ionian Sea | 50-500m | this study |

### Feeding habits

Knowledge on feeding habits is very important in fishery biology. The vacuity index for *C. agassizi* was higher than that reported by Anastasopoulou & Kapiris (2008). However, Kabasakal (1999) had a higher vacuity index (66%) than this study's result. The high number of empty stomachs may be related to the sampling period or the sampling area and might indicate reduced feeding activity, seasonal changes in prey availability or even a reproduction strategy. Our analysis showed the main and the most important for the species was fish which is in accordance with Anastasopoulou & Kapiris (2008), who research the diet of *Chlorophthalmus agassizi* 16 years ago and has also the same results as Kabasakal (1999). *C. agassizi* feeding habits showed a tendency towards a more generalized feeding on Fish and Crustacea. The vacuity index for *G. argenteus* was much lower than that found by Kabasakal (1999) (59%). This difference may be related to the sampling area. The species likely fed more actively or had greater success finding food in the Eastern Ionian Sea compared to the area of the North-eastern Aegean Sea. The diet composition of *Gadiculus argenteus* consisted mainly of crustacean, especially Euphausiacea identified, which was fully compliant with Albert (1993) and Mattson (1981) but partly compliant with Kabasakal (1999), as he found also fish (unidentified Myctophidae and Macrouridae) in *G. argenteus* diet. This difference in diet may be related to the sampling area. *G. argenteus* feeding strategy showed a tendency towards a generalized feeding behavior with a high within-phenotype contribution to the niche width for the Crustacea and Calanoida. This means that most of the individuals utilize many resource types of the above-mentioned prey simultaneously (Amundsen *et al*., 1996).

## Conclusion

The results of this study in the Eastern Ionian Sea were on average comparable with those reported by other authors. Microplastics were found in all the examined species. The occurrence of microplastics in these species, which are of minor commercial value, requires further attention, especially for potential threats to human health. The diet analysis showed no major changes with those previously reported by other authors. Given the limited information available on deep-sea fish species, this study contributes to enhance understanding of the impact and fate of microplastics within marine food webs and have better knowledge for their feeding habits. Further research is needed to assess long-term trends, identify potential sources of microplastics in the Mediterranean, and evaluate the ecological implications of microplastic ingestion by deep-water fish. While long-term monitoring and research are essential to fully understand the factors influencing deep-water fish diets.

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