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## A non-destructive method for assessing the feeding habits of coastal fish

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### Abstract

Studies on the feeding ecology of sympatric coastal species is important, especially in revealing different strategies to reduce competition. The aim of this study is to test the diet of littoral fish species with a non-destructive method, which does not necessitate the sacrifice of fish specimens. The clingfish *Lepadogaster lepadogaster* (Bonnaterre, 1788) was used to test this diet assessment method. Freshly caught specimens were delivered immediately to a specially designed box for collecting faecal pellets, supplied by an aerator. Clingfishes were left in the chambers for 24 hours to defecate. The pellets were carefully removed and fixed in 70% alcohol. The content of the pellets was analysed under stereomicroscope and prey items were determined and counted. The comparison with the existing studies showed similarity and consistency between their results, which proves the usefulness and applicability of the method for studying clingfish diet. The suitability of the proposed method was tested on related species *L. candollei* Risso, 1810, where all ingested prey items were also found and identified in faecal pellets, which confirms its effectiveness for diet analysis. A method of collecting faecal pellets immediately after defecation has proved to be more useful and effective than other nonlethal methods. After defecation, the specimens were released at the site where they were collected. Due to 100% survival as shown in this research, the proposed method is also suitable for studying the diet of rare and endangered fish species, and also coastal fish fauna in protected areas where traditional destructive sampling methods are not appropriate or allowed.

**Keywords:** Non-destructive method; feeding habits; fish assemblage; *Lepadogaster lepadogaster*.

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### Introduction

Research on the feeding habits of fish is crucial for understanding ecological processes at individual, population and community level (Sánchez-Hernández *et al.*, 2013). Factors related to ingestion and assimilation can affect the condition, growth, survival and colonization of species (Nunn *et al.*, 2012). This is why a successful protection and management plan are dependent on basic knowledge of ecological factors, known to affect feeding habits. The great majority of diet-related studies are based on the examination of stomach content; however, the major deficiency of this method is that the studied animals have to be killed and dissected. Thus, for many studied species, a high number of animals have to be sacrificed (Kamler & Pope, 2001; Sánchez-Hernández *et al.*, 2013), which in some populations can cause major changes in the population structure of fish species (Light *et al.*, 1983; Hartleb & Moring, 1995). In the case of endangered species, species of high commercial value or species with low population density, the sacrifice of specimens might be disapproved by the public (Baker & Fras-

er, 1976; Crossman & Hamilton, 1978; Light *et al.*, 1983; Haley, 1998). At the same time, such methods are banned in marine protected areas and for species protected by law it is hard and not likely to get the license to perform studies using lethal methods. In order to deal with these issues, researchers are trying to discover new nonlethal techniques. The most commonly used methods are based on the collection of stomach content samples, such as the use of gastroscopy (Dubets, 1954), tubes (White, 1930), suction of stomach content (Robertson, 1945), stomach flushing (Hyslop, 1980), emetics (Markus, 1932), removal of stomach content using forceps (Wales, 1962) and X-ray analysis (Stobbs, 1980). The efficiency of nonlethal methods strongly depends on the species, age and size, and also the size of the prey (Kamler & Pope, 2001).

Studies on closely related sympatric fishes are often difficult, especially when dealing with the feeding ecology of coastal species. Understanding such feeding relationships is very important since it could reveal different strategies to reduce competition such as resource partitioning. Clingfishes (family Gobiesocidae) are typical representatives of coastal fish assemblages. Three sym-

patric species have been recorded to date in the Slovenian coastal sea, namely, *Lepadogaster lepadogaster* (Bonna- terre, 1788), *L. candollei* Risso, 1810 and *Apletodon in- cognitus* Hofrichter & Patzner, 1997. None of them had been appropriately investigated in studied area in terms of their feeding ecology.

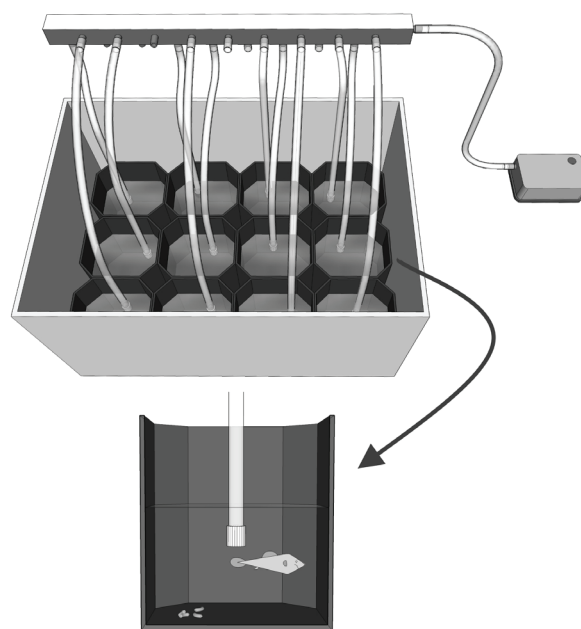
The aim of this study is to test the diet of the clingfish *L. lepadogaster* introducing a new non-destructive meth- od, which does not necessitate the sacrifice of fish spec- imens. The littoral coastal clingfish *L. lepadogaster* was used to test this diet assessment method because: a) there are some available data of *L. lepadogaster* feeding ecol- ogy published by Gibson (1972), Wilson (1981), King (1989), Mazé (2007) and Velasco *et al.* (2010), b) this clingfish species inhabits shallow coastal waters, usually above 1 m of depth (Hofrichter & Patzner, 2000), conse- quently it is possible to collected them by snorkelling and not only by SCUBA diving, c) species has short digestive tract and consequently rapid digestion (personal observa- tion), which is important, since method is based on faecal pellets.

## Material and Methods

Sampling of clingfish specimens was performed from January to November 2017 at different localities in the Slovenian part of the Gulf of Trieste. Specimens were collected at low tide during the day (9.00-18.00) by snor- kelling or SCUBA diving. They were caught by hand net and stored in 100 ml plastic chambers, with small holes on the cover that provided fresh water with oxygen, but small enough to prevent defecated faecal pellets to pass through. Basic ecological data on the sampling locality were described. Specimens were immediately (in less than 1 hour after the sampling was completed) delivered to the Marine Biology Station (National Institute of Biol- ogy), where they were cautiously measured to the nearest mm with a calliper and weighed to the nearest 10 mg using a Sartorius TE 1502S balance. Special attention was paid to the condition of clingfishes that were always kept moist during the measurements. After the measurements, each clingfish was placed in a 11x13x14 cm chamber, with filtered seawater (200 µm), supplied by an aerator (Fig. 1). The aerator was placed above the bottom to prevent defragmentation of faecal pellets. After every use, the chambers were disinfected in order to avoid the trans- mission of diseases and parasites.

Previously, we observed some specimens in aquaria to verify the defecation process. Specimens needed from 6 to 24 hours to produce faecal pellets (personal obser- vations). During defecation, water temperature ranged from 22 to 27°C. After the release of pellets, specimens may produce “false” pellets, which are made only of peritrophic membrane but with no content.

Clingfishes were left in the chambers for 24 hours. The pellets were carefully removed from the bottom of the chambers with a modified pipette and were fixed in 70% alcohol solution. Pellets consist of a peritrophic membrane and the internal part of undigested prey items.



**Fig. 1:** Box designed for collecting faecal pellets. It contains 12 smaller chambers in which fishes were placed, each equipped with filtered water and an aerator. Faecal pellets were subse- quently collected from the bottom of the chamber.

In certain cases, the pellets were broken and, thus, the content of the whole chamber was filtered through 125 µm mesh size plankton net. After defecation, the speci- mens were released at the site where they were collected.

The content of the pellets was analysed under an Olympus SZx16 stereomicroscope and photographed with an Olympus DP74 camera. Prey items were deter- mined to the lowest taxa and counted. The wet weight of the prey was calculated from size-weight correlation curves obtained from animals collected in the sampling area (unpublished data). They were weighed with Sartorius CP 225D balance to the nearest 0.01 mg and mea- sured under Olympus SZx16 stereomicroscope with an Olympus DP74 camera. A large proportion of digested prey items were whole or almost whole, while digested prey broken up into smaller parts, was recognized by typ- ical body parts such as carapace. The most digested prey items, such as polychaetes and amphipods were identi- fied by the jaws. For the feeding habits analyses, three different quantitative methods were used: frequency of occurrence (F%; Hynes, 1950), numerical abundance (N%; Macdonald & Green, 1983) and gravimetric com- position (B%; Macdonald & Green, 1983). In addition, the modified index of relative importance (IRI) (Pinkas *et al.*, 1971; Simenstad, 1979) was calculated from the formula:  $IRI = F\% (N\% + B\%)$ , where F% is the frequen- cy of occurrence, N% is numerical abundance and B% is gravimetric composition.

We also carried out some tests to verify if all ingested prey can be identified in faecal pellets. While specimens of *L. lepadogaster* did not feed normally under experi- mental conditions, we tried to ascertain it on 6 specimens of the closely related clingfish species *L. candollei*. Specimens were kept in chambers used for defecation and fed

with 2 specimens of 4 different taxa (*Pisidia* sp., *Athanas nitescens*, *Eualus* sp. and Gammaridea). Faecal pellets were collected within 24 hours after feeding and analysed.

## Results

Altogether, 116 specimens of *L. lepadogaster* were caught alive. Total length of the specimens ranged from 28.06 mm to 78.02 mm ( $57.19 \pm 7.92$  mm), while their weight ranged from 0.23 g to 5.85 g ( $2.08 \pm 0.87$  g). Sex ratio was 1:1 ( $\chi^2 = 0.1923$ ,  $p < 0.66$ ). All specimens survived the faecal pellet collection method. Altogether, 177 faecal pellets were produced and 836 prey items were found in the pellets. Almost all clingfishes (95.7%) produced from 1 to a maximum of 3 faecal pellets; on average, 1.59 pellets per specimen. In the faecal pellets, 4.72 prey specimens per pellet or 7.53 prey specimens per fish were found on average. In total, 47 taxa were recognized as prey items, which were grouped in 17 wider taxonomic groups (Table 1). Harpacticoid copepods (N% = 33.0) were by far the most numerous prey followed by amphipods (N% = 18.9) and decapods (N% = 15.7), which were also the most frequent prey in the samples in term of frequency of occurrence (both with F% = 69.4). The most important prey in terms of biomass were decapods (B% = 77.1) and amphipods (B% = 12.3), which together represent the great majority (89.4%) of biomass. Decapods were the most important prey in terms of relative impor-

tance (IRI% = 58.7), and were mainly represented by the crab *Pisidia* sp. Alternative prey groups were amphipods (IRI% = 19.8) such as species of the family Gammaridae and harpacticoid copepods (IRI% = 11.7). Gastropods constituted 4.9% of IRI.

The results of the effectiveness of the method, testing on relative clingfish species *L. candollei* showed that all 29 ingested prey items of 4 taxa (*Pisidia* sp., *Athanas nitescens*, *Eualus* sp. and Gammaridea), were found and identified in faecal pellets (Table 2).

## Discussion

### Clingfish diet analysis

Based on the wide range of prey found in the diet of *L. lepadogaster*, this clingfish species could be considered as carnivorous opportunist, which is consistent with the research of Gibson (1972), King (1989) and Velasco *et al.* (2010). In fact, the highly opportunistic foraging on a vast array of small preys is a general characteristic of many cryptobenthic fish species (Depczynski & Bellwood, 2003; Brandl *et al.*, 2018). *L. lepadogaster* share also other characteristics with cryptobenthic fishes such as small body size, short gut, rapid digestion and others (*sensu* Brandl *et al.*, 2018). A limited home range of cryptobenthic fish species, which naturally confines access to prey (Depczynski & Bellwood, 2003), may well explain their opportunistic feeding. The most important

**Table 1.** Major groups of food items found in the faecal pellets of *Lepadogaster lepadogaster* from North Adriatic Sea. F (%): relative frequency of occurrence; N (%): relative numerical abundance; B (%): relative gravimetric composition; IRI (%): index of relative importance.

Higher taxon	Lower taxon	F (%)	N (%)	B (%)	IRI (%)
Rhizaria	Foraminifera	1.8	0.2	< 0.1	< 0.1
	Placophora	0.9	0.1	< 0.1	< 0.1
Mollusca	Gastropoda	34.2	7.9	7.8	4.9
	Bivalvia	13.5	2.8	0.1	0.4
	Copepoda	38.7	33.0	0.1	11.7
	Amphipoda	69.4	18.9	12.3	19.8
	Decapoda	69.4	15.7	77.1	58.7
	Mysida	1.8	0.2	0.1	< 0.1
Crustacea	Anisopoda	2.7	0.5	< 0.1	< 0.1
	Isopoda	36.9	6.5	0.6	2.4
	Ostracoda	14.4	2.9	< 0.1	0.4
	Cirripedia	4.5	0.7	< 0.1	< 0.1
	Crustacea indeterminata	3.6	0.5	0.9	< 0.1
	Acari	19.8	5.6	< 0.1	1.0
Arachnida	Pantopoda	0.9	0.1	< 0.1	< 0.1
Annelida	Polychaeta	8.1	1.6	0.2	0.1
Echinodermata	Ophiuroidea	17.1	2.6	0.7	0.5
Indeterminata	Indeterminata	1.8	0.2	< 0.1	< 0.1

**Table 2.** Offered and ingested prey items and prey items, recognized in faecal pellets of 6 specimens of *Lepadogaster candollei* used to test the proposed nonlethal method.

	Offered prey	Ingested prey	Prey recognized in faecal pellets
Specimen 1	2x <i>Pisidia</i> sp.	2x <i>Pisidia</i> sp.	2x <i>Pisidia</i> sp.
	2x <i>Athanas nitescens</i>	2x <i>Athanas nitescens</i>	2x <i>Athanas nitescens</i>
	2x <i>Eualus</i> sp.	1x <i>Eualus</i> sp.	1x <i>Eualus</i> sp.
	2x Gammaridea	1x Gammaridea	1x Gammaridea
Specimen 2	2x <i>Pisidia</i> sp.	1x <i>Pisidia</i> sp.	1x <i>Pisidia</i> sp.
	2x <i>Athanas nitescens</i>	2x <i>Athanas nitescens</i>	2x <i>Athanas nitescens</i>
	2x <i>Eualus</i> sp.	1x <i>Eualus</i> sp.	1x <i>Eualus</i> sp.
	2x Gammaridea	1x Gammaridea	1x Gammaridea
Specimen 3	2x <i>Pisidia</i> sp.	2x <i>Pisidia</i> sp.	2x <i>Pisidia</i> sp.
	2x <i>Athanas nitescens</i>	2x <i>Athanas nitescens</i>	2x <i>Athanas nitescens</i>
	2x <i>Eualus</i> sp.	1x <i>Eualus</i> sp.	1x <i>Eualus</i> sp.
	2x Gammaridea	1x Gammaridea	1x Gammaridea
Specimen 4	2x <i>Pisidia</i> sp.	-	-
	2x <i>Athanas nitescens</i>	2x <i>Athanas nitescens</i>	2x <i>Athanas nitescens</i>
	2x <i>Eualus</i> sp.	1x <i>Eualus</i> sp.	1x <i>Eualus</i> sp.
	2x Gammaridea	1x Gammaridea	1x Gammaridea
Specimen 5	2x <i>Pisidia</i> sp.	-	-
	2x <i>Athanas nitescens</i>	2x <i>Athanas nitescens</i>	2x <i>Athanas nitescens</i>
	2x <i>Eualus</i> sp.	1x <i>Eualus</i> sp.	1x <i>Eualus</i> sp.
	2x Gammaridea	1x Gammaridea	1x Gammaridea
Specimen 6	2x <i>Pisidia</i> sp.	-	-
	2x <i>Athanas nitescens</i>	2x <i>Athanas nitescens</i>	2x <i>Athanas nitescens</i>
	2x <i>Eualus</i> sp.	1x <i>Eualus</i> sp.	1x <i>Eualus</i> sp.
	2x Gammaridea	1x Gammaridea	1x Gammaridea

prey group was Crustacea, represented by decapods and amphipods in particular, which was previously observed by Wilson (1981). Decapods were mainly represented by the crab *Pisidia* sp., while amphipods were dominated by gammarids. Both groups were frequently observed under stones in the habitat, where *L. lepadogaster* specimens were sampled (personal observation). The most preyed invertebrates in the diet of *L. lepadogaster* in terms of abundance were copepods, which is in accordance with King (1989) followed by amphipods, which were the most abundant prey items in studies of Gibson (1972), Mazé (2007) and Velasco *et al.* (2010). Despite the high abundance of copepods, their importance (IRI) is rather small compared to decapods and amphipods, due to their negligible biomass. The high abundance of decapods found in our study is due to the substantial availability of *Pisidia* sp. in the habitat (up to 26 specimens per 20 x 20 cm quadrant, unpublished data). The presence of a particular prey in the diet is highly dependent on the local occurrence of prey, as previously observed by Gibson (1972). Our data are in agreement with the observations of Wilson (1981), King (1989) and Velasco *et al.* (2010) (Table 3). Besides crustaceans, gastropods were the most abundant prey group, which is consistent with the stud-

ies of Wilson (1981), King (1989) and Mazé (2007). By comparing data from different studies a number of differences emerged, which are probably a consequence of the average size of the studied fish (decapods are more numerous in the diet of larger specimens, personal observations), local availability of prey, and also the size of the clingfish sample in terms of numbers, which can significantly affect the results (Gibson, 1972).

#### Accuracy of the method

Rapid digestion of *L. lepadogaster* (it excreted faecal pellets within 24 hours) is a result of its very short digestive tract, which has also been observed in other clingfish species such as *Sicyases sanguineus* Müller and Troschel, 1843 (Cancino & Castilla, 1988). In general, most of the specimens produced from 1 to 2 faecal pellets during 24 hours in captivity. Due to rapid digestion and a high proportion of full digestive tract, we assume that they feed very often. Prey was normally eaten whole (Day, 1880-1884), but in cases where a *Pisidia* sp. crab was too big and tried to defend itself, the clingfish tore off its claw and ate it (personal observation). Rapid di-

**Table 3.** Comparison of relative prey abundance (N%) data obtained by different authors.

Author		Wilson (1981)	King (1989)	Mazé (2007)	Velasco <i>et al.</i> (2010)	Present study
Number of specimens		42	47	178	6	116
Rhizaria	Foraminifera		1.6			0.2
	Placophora				25.0	0.1
Mollusca	Gastropoda	28.8	13.0	33.9		7.9
	Bivalvia		0.9	1.5		2.8
	Copepoda		43.4	1.4	12.5	33.0
	Amphipoda	21.2	10.9	48.7	37.5	18.9
	Decapoda	23.1	2.5	1.5		15.7
	Mysida					0.2
	Anisopoda					0.5
Crustacea	Isopoda	3.8	3.2	4.5	12.5	6.5
	Ostracoda	11.5	14.1	1.5	12.5	2.9
	Cirripedia					0.7
	Crustacea indeterminate					0.5
	Acari		7.5			5.6
Arachnida	Pantopoda					0.1
Annelida	Polychaeta		1.4	2.9		1.6
Echinodermata	Ophiuroidea		1.1	1.7		2.6
Insecta	Chironomidae larvae	11.5	0.4			
Indeterminata	Indeterminata			1.4		0.2
Other	Other			1.1		

gestion was reflected in poorly digested food, as well. A large proportion of digested prey items were consequently whole or almost whole, which facilitated the identification, quantification and measuring the prey. Cancino & Castilla (1988) found out that in the related clingfish species *S. sanguineus* some type of prey can even survive the passage through the digestive tract, due to its short gut and brief food passage time. The most digested prey items were identified based on the hard body parts (e.g. jaws) that did not degrade during digestion. During the reproductive season, eggs assumed to be *L. lepadogaster* eggs (based on size and shape), were found in prey samples, as well. Consequently, we could assume that if other soft body parts of organisms were present in the diet their remains would be also observed.

The comparison of our data of the *L. lepadogaster* diet with the data obtained in existing studies outside the Adriatic Sea of Wilson (1981), King (1989), Mazé (2007) and Velasco *et al.* (2010), who analysed stomach contents, showed similarity and consistency between studies, which proves the usefulness and applicability of the method for studying clingfish feeding ecology. Furthermore, even higher numbers of preyed taxa were identified with our method compared to methods used by other authors (Table 3), thus confirming the accuracy of

the tested method.

A method of collecting faecal pellets immediately after defecation has proved to be very useful and effective in other aspects as well. We did not obtain prey items from only a small proportion of specimens (4.3%), which is probably due to the fact that they had already defecated before being caught. As the specimens started to excrete empty peritrophic membranes after defecation, it is certain that all prey items in the digestive tract had already been defecated. The results of method testing with 6 specimens of *L. candollei*, showed that all ingested prey specimens were also found and identified in faecal pellets, which confirms the effectiveness of the proposed method for diet analysis. In addition, checking if all ingested prey items were found in stomachs when using other nonlethal methods of diet analysis is a proper test for evaluating effectiveness.

Other nonlethal methods (such as the use of gastroscopes, tubes, stomach suction, stomach flushing, emetics, forceps and others) vary in the ability to remove all stomach contents. The effectiveness of other nonlethal methods for studying fish diets depends on size, age and species of fish, and also on the size of the food particles in the stomach (Kamler & Pope, 2001). Due to the small size of *L. lepadogaster* and their relatively large-sized

prey, other nonlethal methods would probably be less efficient in studying this species or would even damage the specimens. Based on empty defecated peritrophic membranes, it appears that 24 hours suffice for the specimens of *L. lepadogaster* to defecate at a temperature of approximately 25°C. Consequently, on the next day the clingfishes could be released back into the sea at the place of capture. To prevent their mortality, it is important that clingfishes spend as little time as possible in stressful conditions. Most of the nonlethal methods require a lot of handling, while a strong positive relationship between handling-induced mortality and water temperature was previously demonstrated by Muoneke & Childress (1994) and Wilde (1998). Different techniques for obtaining stomach contents can also cause internal injuries (e.g. swim bladder rupture). Stomach flushing, as one of the most efficient nonlethal methods (Kamler & Pope, 2001), can cause 60% mortality in some fish species (Hartleb & Moring, 1995). Meehan & Miller (1978) found that stomach flushing can also have a negative effect on fish condition. Since the faecal pellets based method is a non-invasive technique and requires almost no handling of fish, there is less chance of injuries and, consequently, potential fish mortality is drastically reduced. This is supported by 100% fish survival in our experiments until the release to natural environment. The method is also suitable for fish of all sizes and was proved as effective on specimens smaller than 15 mm (*L. candollei*), while other methods (e.g. gastroscopes, tubes, stomach suction, stomach flushing and forceps) are unsuitable for such small specimens. Consequently, it is the only non-lethal method that can be used for very small specimens such as fish fry. Regular disinfection of all equipment (e.g. chambers, aerators) after every use also proved to be very important; disinfection prevents the transmission of disease and parasites to other fish. Compared to other nonlethal methods that can be rather expensive or require more than one handler (Kamler & Pope, 2001), the faecal pellets based method is easy to use (it requires 1 handler) and rather cheap. In fact, all the required equipment cost less than 100 €. Furthermore, the equipment used for collecting faecal pellets is also suitable for transport.

### Potential use of the method

The faecal pellets based method has a great potential for studying the fish diet of small and less mobile coastal fish species, which can be held in small chambers. With some modifications, bigger systems for collecting faecal pellets can be built, as well. This method is the most suitable for species feeding on hard-bodied prey, which is swallowed whole, have rapid digestion and poorly digest their prey. It turned out to be a very useful method for studying the feeding habits of *L. lepadogaster*. We also checked if we could obtain faecal pellets from other fish species of the coastal fish assemblage such as other clingfish species *L. candollei* and *A. incognitus* and certain gobies (e.g. *Gobius fallax* Sarato, 1889, *Gobius cruentatus* Gmelin, 1789, *Gobius cobitis* Pallas, 1814,

*Gobius paganellus* Linnaeus, 1758). The method proved to be useful, since in all cases we were able to find faecal pellets with recognizable prey remains for identification. The order of gobies (Gobiiformes) is one of the most diverse orders of percomorphs and also of fishes in general; they represent 5 to 10% of all teleost species (Patzner *et al.*, 2011). To this end, the faecal pellets based method has great potential for studying their diet, especially considering that gobies represent over 50% of the energy flow in coral reef habitats (Patzner *et al.*, 2011). Based on the weight of consumed prey and the weight of digested food residues in faecal pellets, the weight of assimilated food can also be obtained, which is a great advantage compared to other stomach content methods. This gives us a unique insight into the energy flow of certain habitats. In order to ascertain whether the method can potentially be used to study the diet of other representative species of the Mediterranean coastal fish assemblage, we preliminarily tested if we could obtain their faecal pellets in chambers and whether the prey remains are appropriate for identification. The method was proved to be useful for common fish species such as *Scorpaena porcus* Linnaeus, 1758, *Serranus scriba* (Linnaeus, 1758), *Tripterygion tripteronotus* (Risso, 1810), *Salapia pavo* (Risso, 1810) and even on the nectobenthic herbivore *Sarpa salpa* (Linnaeus, 1758). Due to its high survival rate, the proposed method is also suitable for studying the diet of rare and endangered fish species, and also coastal fish fauna in protected areas where traditional destructive sampling methods are not appropriate or allowed. As the method is nonlethal and relatively undemanding as regards fish handling, it is suitable for educational scopes as well.

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