fishes

Article

An Insight into the Feeding Ecology of *Serranus scriba*, a Shallow Water Mesopredator in the Northern Adriatic Sea, with a Non-Destructive Method

Ana Lokovšek [1,2,*], Martina Orlando-Bonaca [1], Domen Trkov [1] and Lovrenc Lipej [1]

1 Marine Biology Station Piran, National Institute of Biology, Fornače 41, SI-6330 Piran, Slovenia
2 Jozef Stefan International Postgraduate School, Jamova Cesta 39, SI-1000 Ljubljana, Slovenia
* Correspondence: ana.lokovsek@nib.si; Tel.: +386-(0)59-232-920

Abstract: *Serranus scriba* is a common member of the coastal fish community in the Adriatic Sea, but knowledge about its feeding ecology is scarce. The aim of this paper is to present new evidence about its food preferences and feeding habits. An innovative non-destructive method of fecal pellet analysis was used for this study. This method does not require sacrificing specimens and the fish can be released back into the sea alive after the laboratory work. The results demonstrated that *S. scriba* mainly preys on decapods, followed by polychaetes, isopods, fish, mollusks and swarming shrimps. The calculated index of trophic diversity (ITD) value of 0.89 indicates that it is an opportunistic feeder that feeds on a wide range of different prey. According to the calculated trophic level of 3.43, which is higher than that of other members of the community, *S. scriba* is also an important piscivorous predator. With age, *S. scriba* undergoes an ontogenetic shift. The proportion of crustaceans, gastropods and polychaetes decreases with age and body size, while the proportion of fish increases.

Keywords: coastal fishes; feeding habits; diet; non-destructive method; non-lethal method; *Serranus scriba*; painted comber; northern Adriatic

Citation: Lokovšek, A.; Orlando-Bonaca, M.; Trkov, D.; Lipej, L. An Insight into the Feeding Ecology of *Serranus scriba*, a Shallow Water Mesopredator in the Northern Adriatic Sea, with a Non-Destructive Method. *Fishes* **2022**, *7*, 210. https://doi.org/10.3390/fishes7040210

Academic Editor: Abbott Peter Klimley

Received: 8 June 2022
Accepted: 19 August 2022
Published: 20 August 2022

1. Introduction

The northern Adriatic Sea is a shallow coastal sea, where infralittoral habitats are home to rich fish assemblages, with many members closely associated with the seabed [1]. Anthropogenic activities can lead to habitat disturbances, changes in fish community structure and changes in ecosystem functioning [2–5]. Therefore, some fish groups can be used as a tool to assess changes in habitat conditions. Currently, robust methodologies are being developed in an effort to assess the most suitable indicator species for the evaluation of the status of coastal fish assemblages [6].

The most important coastal fish families in the Mediterranean Sea in terms of abundance and frequency of occurrence include Gobiidae, Labridae, Bleniidae, Sparidae, Tripterygiidae, Atherinidae, Mugilidae, Mullidae, Pomacentridae, Sygnathidae and Serranidae [1]. The family Serranidae includes 538 species from temperate and tropical seas [7]. This study focuses on one of the smaller members of the group, the most common member of the family in the coastal area of the Mediterranean, the painted comber (*Serranus scriba* (Linnaeus, 1758)). *S. scriba* is a subtropical species that inhabits rocky habitats with algal covers and seagrass meadows, distributed in the Mediterranean Sea, the Black Sea and the eastern Atlantic Ocean from the Bay of Biscay to Mauritania [8,9]. *S. scriba* is very common throughout the Mediterranean Sea, and is usually found at a depth of 0 to 30 m [10]. Normally it does not grow larger than 200 mm, but the largest specimen captured to date measured 323 mm and weighed 456.7 g [11]. *S. scriba* is not commercially exploited in the Mediterranean area, but it is often caught as by-catch by recreational fishermen [12]. Despite its wide distribution, the ecology and feeding habits of the species are poorly known. According to some studies, *S. scriba* is often predating on crustaceans, especially decapods [10] and smaller necto-benthic and crypto-benthic fish species [13,14]. It has also been documented that the removal of a mesopredator such as *S. scriba*, may result in the proliferation of small fishes and could, thus, affect the populations of small invertebrates [15]. Studies on the feeding ecology of *S. scriba* in the Adriatic Sea have not been conducted yet, although species is widespread and common in the area. *S. scriba* is supposed to play an important role in the trophic web [16–18]; therefore, there is a need for in-depth knowledge of its food and feeding habits.

Feeding ecology describes the diverse feeding modes and morphological, physiological and senso-neural adaptations to the prey type and the prey abundance in the habitat [19–21] and contributes to the understanding of resource partitioning [22,23], habitat selection [21], predation [24–27], evolution [28], competition, trophic ecology [29,30] and energy transfer within and between ecosystems [31–33].

The feeding habits of fish species are usually based on the examination of stomach contents [34–36], which requires a large number of sacrificed fish. Therefore, it is ethically questionable and particularly unsuitable for the study of endangered or rare fish, and species with low population densities or inhabiting marine protected areas [37–39]. Alternatives to traditionally used method of stomach content analysis are non-lethal methods, among which the most effective method is stomach flushing [40]. Although stomach flushing is one of the most efficient non-lethal methods used to date [40–42], this procedure can cause mortality of up to 60% in some fish species [42] and can have a negative effect on fish condition [41]. Most non-lethal methods cannot be regarded as non-destructive, because the process of obtaining samples is still quite invasive and can cause injuries or even death [42]. In our study, we used a non-destructive method that is less invasive and does not harm the fish.

The aim of this study is to present the first data on the feeding ecology of *S. scriba* in the northern Adriatic Sea and to propose the application of a recently developed non-destructive and non-lethal method for isolating undigested prey from feces in order to study the fish diet. The main goals of the paper are (i) to identify and categorize the prey items of *S. scriba* in the northern Adriatic Sea, (ii) to estimate the trophic level (TROPH) of *S. scriba* and compare it with other species in the community and that respective to other studies, and (iii) to calculate the index of trophic diversity (ITD).

2. Materials and Methods

2.1. Study Area

The Gulf of Trieste is the northernmost part of the Adriatic Sea, stretching across the coasts of Italy, Slovenia, and Croatia. It extends from Cape Savudrija to Grado and is partially enclosed by the Istrian peninsula to the south. It covers an area of 551 km^2 and includes a water volume of 9.5 km^3 [43]. The Gulf is very shallow, with an average depth of only 18.7 m and a maximum depth of 37 m. The area is known for the highest amplitudes between high and low tides (average = 88 cm; [44]) and the lowest temperatures in winter [43]. Water temperature and salinity are strongly influenced by river outflows. In winter, the temperature can drop down to 6 °C and in summer it warms up to 26 °C, while the average salinity is around 37–38 [45,46]. Between April and September, temperature stratification occurs in the water column, with a seasonal thermocline in spring [43].

The Slovenian part of the Gulf of Trieste accounts for one third of the entire surface of the Gulf [47]. The coastal relief varies from steep rocky cliffs to gradual sloping beaches. The lower part of the coast, in particular, has already been heavily modified due to anthropogenic development and urbanization. Today, only one fifth of the coastline remains in its natural state [44,47]. The bottom along the coast is mainly rocky and consists of alternating layers of flysch, sandstone and soft marl.

2.2. Fieldwork

Between August 2020 and June 2021, 150 specimens of *S. scriba* were collected in shallow Slovenian waters, at a depth range of 0.5–5 m. The sampling sites were selected according to the benthic habitat type, since *S. scriba* predominantly inhabits a rocky bottom with different algal cover, sometimes also bordering with seagrass meadows (mostly *Cymodocea nodosa*). Fish were caught at eight locations in the Slovenian part of the Adriatic Sea (Figure 1, Table 1). The majority of specimens were caught in front of the National Institute of Biology-Marine Biology Station in Piran, for a total of 44 fish, 22 of which were caught in autumn between the end of September and October 2020 and 22 in spring, between the end of April and June 2021. The rest were caught at other localities (Table 1). Additionally, at the Cape Piran, Cape Ronek and Pacug sites, the parallel transect method [1,48] was used to monitor the density of painted comber populations. A measuring tape (50 m long) was laid on the rocky bottom at depths ranging from 1.5 to 4 m. After a few minutes, when the fish had become accustomed to the diver's presence [49], all the fish were counted 1 m to the left and then 1 m to the right of the transect line. The data were transformed into densities expressed per 100 m^2.

Before specimen collection, we took 15 min each time to observe feeding behavior. We were interested in how *S. scriba* approaches and grasps its prey, where it hunts and what interactions they have with each other and with other species. During the observation, the snorkeler calmly floated on the surface of the water, carefully observing the action while moving the bait along the seafloor.

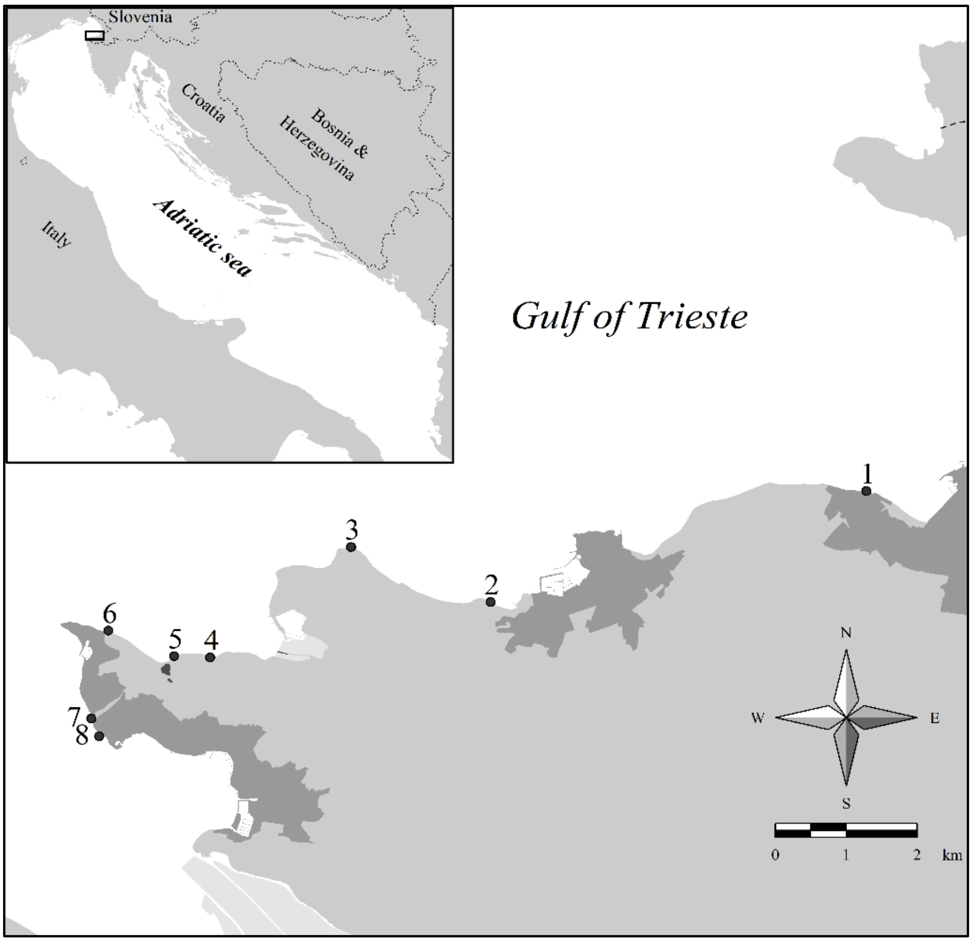

Figure 1. Map of the study area with 8 sampling sites for *Serranus scriba* (for the names of the sites see Table 1).

Table 1. Coordinates of the sampling sites for *S. scriba* in Slovenian coastal waters, sampling dates and number of fish collected.

Sampling Site	Site Name	Latitude (N)	Longitude (E)	Sampling Dates	Total *n* of Fish
1	Žusterna	45.547027	13.708841	27 May 2021, 11 June 2021	4
2	Belveder	45.532796	13.640692	24 June 2021	13
3	Cape Ronek	45.540174	13.615569	25 May 2021	11
4	Pacug	45.525771	13.589926	28 May 2021	18
5	Fiesa	45.525989	13.583407	5 June 2021	28
6	Cape Piran	45.529401	13.571537	20 May 2021	5
7	Marine Biology Station Piran	45.517830	13.568325	22 September 2020, 30 September 2020, 1 October 2020, 11 May 2021, 20 May 2021, 26 May 2021, 27 May 2021	44
8	Bernardin	45.515486	13.569735	31 May 2021, 1 June 2021, 2 June 2021, 3 June 2021	27

Fish were collected by snorkeling, using a barbless hook (size 6, Crivit Lasercut Selection) with a bait. During the first few days of fieldwork, different baits were tested, from pieces of squid, snails of the genus *Gibbula*, chunks of anchovy, to hermit crabs, which proved to be the best bait to attract *S. scriba*. The hook was attached to a 3 m long nylon line (0.20 mm, 3.1 kg, Crivit Specimen Line) hanging from a modified wooden pole. After the fish took the bait (hook and bait were completely in its mouth), we achieved that the hook was anchored in the oral cavity of the fish with a short pull on the nylon. Therefore, the fish did not swallow the hook, which could lead to internal injuries of the esophagus or stomach and thus death. As soon as the fish was hooked, we pulled it over the surface of the water as quickly as possible and took it off the hook. On one snorkeling session, 1–15 *S. scriba* individuals were caught and placed in buckets of filtered seawater. Each fish was placed in its own bucket with filtered seawater (through a 125-µm sieve) to remove any impurities that might affect the results. The specimens were transported to the laboratory of the National Institute of Biology-Marine Biology Station in Piran as soon as possible (in less than one hour after the sampling was completed).

2.3. Laboratory Work

At the laboratory of the National Institute of Biology-Marine Biology Station in Piran, each bucket was labeled with the serial number of the fish and equipped with an aerator to supply air. The aerator was placed above the bottom of the bucket to prevent the feces from fragmenting due to air bubbles.

The fish were then accurately weighed using the Sartorius CP 225D balance and measured with caliper to the nearest millimeter. Total body length (TL, length from the tip of the snout to the end of the caudal fin), standard body length (SL, length from the tip of the snout to the base of the tail) and fork length (FL, length from the tip of the snout to the center of the fork in the tail), were measured. The measured fish were classified into age groups of 1, 2, 3 and 4 years according to the study of Tuset et al. [50].

The buckets were then left covered for at least 24 h. During this period, all fish digested the prey and defecated. After 24 h, no feces containing prey items were excreted; only white, "empty" feces were excreted by a few fish after this period. Thin, white, stringy feces are an indicator of an empty gut. The fecal pellets were then carefully removed using a modified pipette and stored in 70% ethanol. Fecal pellets from each fish were stored separately in a labeled vial with the serial number of the fish. The contents of the bucket were filtered through a 125 µm sieve to capture any remaining pieces of prey and fish that were released back into coastal waters. Almost all the fish survived the procedure, but two out of 150 fish died in the buckets due to internal injuries after swallowing the hook. When released, the fish were in good condition and swam away immediately.

The contents of the fecal pellets were examined under an Olympus SZx16 stereo microscope with an Olympus DP74 camera. Fecal pellets consist of undigested prey items and peritrophic membranes. The undigested prey items were determined to the lowest possible taxonomic level and counted. The number of prey specimens was identified by the presence of its typical body parts, such as carapace or claws, in the sample. For example, when we found a pair of claws or a carapace of an anomurid crab of the genus *Pisidia*, we assumed that the fish had caught and digested just one *Pisidia* sp. specimen. In addition to taxa with hard body parts, taxa with soft bodies were also recognized by their undigested parts such as outer body layers (e.g., polychaetes or fish eggs). The prey items were identified using identification keys for the marine fauna of the Mediterranean and northwestern Europe [51–53]. Each prey was measured and photographed under an Olympus SZx16 stereo microscope with an Olympus DP74 camera. Fish species were determined through the identification of otoliths in pellets, using the Atlas of Otoliths for the Western Mediterranean [54] and the AFORO online database [55]. According to the formulas in the AFORO online data base [55], we calculated the total length of prey fish using the lengths of the otoliths.

2.4. Data Analyses

The prey frequency of occurrence (prey occurrence in the fecal pellets) (%PF; [56]) was calculated as follows: %PF = ns/NS, where ns represents the number of fecal pellets with prey s and NS the number of total fecal pellets. Additionally, the numerical index (%PN; [56]) of prey in the fecal pellets was calculated: %PN = ni/NI ∗ 100, where ni represents the total number of prey belonging to taxon i, and NI represents the total number of all prey in all taxonomic units.

The prey were divided into four categories: main prey, secondary prey, complementary prey and accidental prey (Table 2), as listed by Hureau [57].

Table 2. Prey categories according to Hureau [57] (%PF = frequency of occurrence, %PN = numerical index).

%PN	%PF	Prey Category
>50	>30 <30	Main preferential prey Main occasional prey
10 < %PN < 50	>10 <10	Secondary frequent prey Secondary frequent prey
1 < %PN < 10	>10 <10	Complementary prey of 1st order Complementary prey of 2nd order
<1		Accidental prey

Diet diversity was expressed by the index of trophic diversity (ITD), which is a modified Shannon-Wiener diversity index (H′; [58]):

$$\mathrm{ITD} = 1 - \mathrm{H}'.$$

The ITD value ranges from 0 to 1, where 0 means no diversity and 1 means maximum diversity. ITD was calculated at the consistent taxonomic level that is at the order level.

TrophLab, a stand-alone Microsoft access for estimating trophic levels, was downloaded from www.fishbase.org (accessed on 20 July 2021) [59] and used to calculate the TROPH index of the species studied. The trophic levels of *S. scriba* were calculated [59,60] as:

$$TROPHi = 1 + \Sigma DCij * TROPHj,$$

where DCij represents the proportion of prey j in the diet of species i and TROPHj represents the partial trophic level of prey j. To demonstrate the importance of each taxon in the diet, the SIMPER function in the R programming environment [61] was used:

$$[ijk] = abs(x[ij] - x[ik])/sum(x[ij] + x[ik]),$$

where x represents the abundance of prey taxon i in samples j and k. The index is the sum of the individual contributions of all prey taxa of species S:

$$d[jk] = sum\ (i = 1 \ldots S)\ d[ijk].$$

The SIMPER function performs a pairwise comparison of prey groups and returns the average contributions of each taxon to the overall Bray-Curtis diversity index (Available at: https://www.rdocumentation.org/packages/vegan/versions/2.4-2/topics/SIMPER; accessed on 26 July 2021). Spearman's correlation and multivariate analysis (Bray-Curtis similarity for differences between fishes, locations and between ages for *S. scriba*) were performed in R (R 4.0.2 software package; R Development Core Team 2008, Vienna, Austria) using the PRIMER v7+ (PERMANOVA software, Albany, New Zeland) [61,62] package. A p-value of <0.05 was chosen to determine the statistical significance of the trend.

3. Results

3.1. S. scriba Density and Biometry

On performed visual parallel transects, *S. scriba* density varied between 6 and 12 ind./100 m^2 at different localities and depths. The highest density was calculated at Cape Ronek, where 11–12 ind./100 m^2 were observed at 1.5 m depth (Table 3). Average density at Cape Ronek was 11 ind./100 m^2 and 6.5 ind./100 m^2 in Pacug and 7 ind./100 m^2 at Cape Piran (Table 3). Based on the results for the Slovenian part of the Gulf of Trieste, the calculated average density of *S. scriba* is 8.34 ind./100 m^2. The total length of the 150 specimens caught, ranged from 108 mm to 217 mm, while the weight ranged from 17 g to 163 g (Table 4). According to the length structure of the fish, we estimated that most fish were 1 or 2 years old (Table 5), indicating that the majority of caught fish were juveniles.

Table 3. Painted comber densities at sampling locations in Slovenian waters.

Sampling Location	Date	Length (m)	Depth (m)	Average Density (ind./100 m^2)
Cape Piran	20 May 2021	50	1.5 3.0–3.5	7 8
Cape Ronek	25 May 2021	50	1.5 3.8	11.5 10
Pacug	23 June 2021	50	1.5 3.5	7 6.5

Table 4. Average minimal and maximal sizes (TL = total length, FL = fork length, SL = standard length) and weight of 150 specimens of *S. scriba*.

	TL	FL	SL
Average size (mm)	140.38 (SD = ±19.12)	137.31 (SD = ±18.28)	117.23 (SD = ±16.50)
Max. size (mm)	216.98	213.62	180.02
Min. size (mm)	108.30	107.40	68.88
Average weight (g)		42.68 (SD = ±21.74)	
Min. weight (g)		17.00	
Max. weight (g)		163.80	

Table 5. Total length (TL) and groups of 150 specimens of *S. scriba*.

Age	TL (mm)	N	%
1+	108–130	50	33.3
2+	130–152	60	40.0
3+	152–170	29	19.3
4+	170–200	11	7.3

3.2. Feeding Habits of S.scriba

A total of 32 taxa were identified as prey items in the fecal pellets of *S. scriba* (Table 6). The most abundant prey of *S. scriba* were crustaceans (%PN = 69.21%, %PF = 98.67%), followed by polychaetes (%PN = 12.63%, %PF = 40.67%), mollusks (gastropoda: %PN = 4.66%, %PF = 17.33%; bivalvia: %PN = 0.67%, %PF = 2.67%) and fish (%PN = 6.82%, %PF = 20.67%; Table 6). Among crustaceans, the most abundant and also most frequent prey items were decapods (PN% = 46.75%, %PF = 96.67%), followed by isopods (PN% = 13.64%, %PF = 37.33%). The most common prey items found was *Pisidia* sp. which alone accounted for 18.80% of all prey. Polychaetes, mainly vagile species (suborder Errantia), accounted for 12.63% of prey items, which is the second most frequent prey (Tables 2 and 6). Teleost fishes (6.82% of total prey items) constituted the complementary prey of 1st order (Table 2). The following fish species were identified (see Table 6): *Atherina hepsetus* Linnaeus, 1758, *Gobius fallax* Sarato, 1889, *Symphodus ocellatus* (Linnaeus, 1758), *S. cinereus* (Bonnaterre, 1788), *Pomatoschistus bathi* Miller, 1982, *Mullus surmuletus* Linnaeus, 1758 and *Gobius cruentatus* Gmelin, 1789. In some cases, only fish vertebrae were found in the fecal pellets and species identification was not possible. In addition to prey with hard parts, prey with soft body structure were also identified in the feces such as fish eggs (N% = 5.99, %PF = 11.33%) and cuticles of polychaete worms. The diet of *S. scriba* specimens was compared between age groups. The majority of the captured S. scriba in our study were less than 3 years old and less than 173 mm long (TL). While the adult specimens of *S. scriba* prey mainly on decapods, isopods and fish, the diet of juveniles consists of polychaetes and small crustaceans such as mysids, amphipods and shrimps. Two-year old and older individuals tend to supplement their diet with epibenthic and nectobenthic fish, which coincides with the increase of the trophic level with age (up to 3+) (Table 7). The proportion of fish in the diet increased from an initial 3.97% at age 1 to around 10% by age 4. The proportion of crustaceans decreased from 71.52% to 40.66% between the 1st and the 4th year (Figure 2). The proportion of eggs in the diet increased from 1.99% in 1-year-old individuals to 35.16% in 4-year-old individuals. The average calculated trophic diversity index (ITD) was 0.89 (on a scale of 0 to 1, where 0 means no diversity and 1 means highest diversity). No statistically significant difference was found between the diet and age/length composition of *S. scriba* at the different sampling sites (Bray-Curtis, $p < 0.05$).

During the fieldwork, it was noted that *S. scriba* specimens are more active in the morning and evening, when they were observed to be feeding actively. Our personal observations also demonstrated that *S. scriba* usually monitors visually open areas and upon detection of a passing prey (or a bait) performs a short but very fast burst chase and then upon completion of prey pursuit occupies another waiting spot. The waiting spot was usually within heterogenous rocky reefs, but we also observed them waiting in *Posidonia oceanica* meadows or hiding in algae. In many cases, *S. scriba* was observed lurking behind a rock or under a boulder, waiting for a prey to come close enough to grab it and suck it into their mouths. The prey was consumed with a quick suction. If the prey was too large, it was usually spat out several times before consumption. This activity often attracted several other *S. scriba*, who then competed for the prey. Larger, dominant *S. scriba* often exhibited aggressive behavior and chased off smaller specimens. On the other hand, on a few occasions, younger *S. scriba* (juvenile and subadult individuals) were also observed cooperating and hunting between rocks in groups of up to 6 individuals. Regarding interspecific interactions, in a few cases *S. ocellatus* was observed to clean parasites from

S. scriba. The latter stood vertically with their heads down and allowed *S. ocellatus* to remove parasites.

Table 6. Numerical index (%PN) and frequency of occurrence (%PF) of particular prey taxa of *S. scriba* in the northern Adriatic Sea.

Taxa	%PF (*n*= 150)	%PN	Prey Category (Hureau 1970)
CRUSTACEA (total)	98.67	69.21	Main preference prey
AMPHIPODA (total)	2.67	0.83	Accidental prey
Caprellidae	2.67	0.83	
CIRRIPEDIA	2.00	0.50	Accidental prey
Crustacea indeterminata	1.33	0.33	
DECAPODA (total)	96.67	46.75	Secondary frequent prey
ANOMURA (total)	79.33	25.45	Secondary frequent prey
Anomura indeterminata	5.33	1.66	
Pisidia sp.	56.67	18.80	
Porcellana platycheles	6.00	1.66	
BRACHYURA (total)	30.67	7.65	Complementary prey of 1st order
Brachyura indeterminata	16.67	4.16	
Pilumnus hirtellus	12.67	3.16	
Majidae	1.33	0.33	
CARIDEA (total)	11.33	3.33	Complementary prey of 1st order
Athanas nitescens	2.67	0.67	
Caridea indeterminata	8.67	2.66	
Decapoda indeterminata	38.00	10.32	
ISOPODA (total)	37.33	13.64	Secondary frequent prey
Anthuridae	2.67	0.83	
Idoteidae	0.67	0.17	
Isopoda indeterminata	18.67	5.32	
Sphaeromatidae	20.67	7.32	
MYSIDA	15.33	3.99	Complementary prey of 1st order
OSTRACODA	2.67	0.67	Accidental prey
TANAIDACEA (*Tanais dulongii*)	8.67	2.50	Complementary prey of 2nd order
POLYCHAETA (total)	40.67	12.63	Secondary frequent prey
Polychaeta-Errantia	39.33	11.81	
Polynoidae	0.67	0.17	
Spirorbis sp.	2.67	0.65	
MOLLUSCA	5.33		Accidental prey
BIVALVIA	2.67	0.67	Accidental prey
GASTROPODA	17.33	4.66	Complementary prey of 1st order
TELEOSTEI	20.67	6.82	Complementary prey of 1st order
Atherina hepsetus	5.33	1.50	
Gobius cruentatus	2.67	1.00	
Gobius fallax	3.33	1.33	
Mullus surmuletus	0.67	0.17	
Osteichtyes indeterminata	8.00	2.00	
Pomatoschistus bathii	2.00	0.50	
Symphodus cinereus	0.67	0.17	
Symphodus ocellatus	0.67	0.17	
Eggs	11.33	5.99	Complementary prey of 1st order

Table 7. Trophic level (TROPH) of different age groups of *S. scriba*.

Age	TROPH	SD
1+	3.42	±0.53
2+	3.46	±0.56
3+	3.48	±0.57
4+	3.36	±0.56

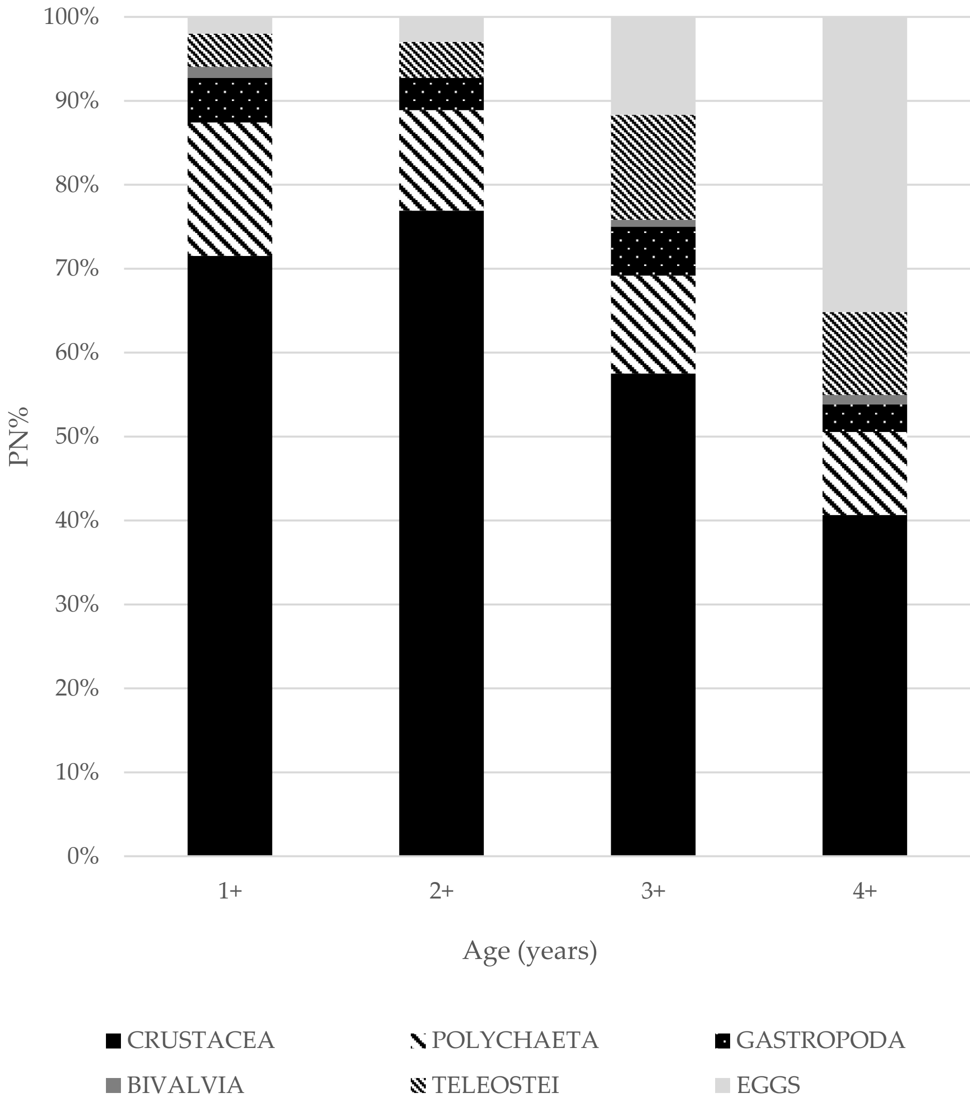

Figure 2. Comparison of prey proportions (%PN) in *S. scriba* diet.

4. Discussion

4.1. Advantages and Disadvantages of Non-Destructive Methods of Sampling and Analyses

A recently developed non-destructive method [63] was tested in this study on *S. scriba* to detect prey in fecal pellets. The advantage of the fecal examination method is that it can be used on smaller-sized fish, even smaller than 15 mm [63], and that the survival of all specimens makes these procedures suitable for use in protected marine areas and for the study of protected species or species with low population densities [37,40], and also renders the research more ethical without compromising quality. Another modern method that is also non-lethal and frequently used for diet estimation is stable isotope analysis; however, this method cannot provide taxonomic resolution and is more useful for describing a long-term assimilated diet [64]. This method can be complementary to stomach contents analysis or analysis of feces. Advantage of this non-destructive method is also shorter handling time, since a shorter handling time means a higher chance of survival and a less stressful experience for the animal [42,63]. The method is easy to use on less mobile, small and medium-sized coastal fishes, as the fish can be placed in buckets overnight and complex aquarium equipment is not needed. The method is far more complicated to use on open-water fish or larger fish, which cannot be housed in buckets or similar small containers. Nevertheless, most members of coastal fish assemblages are small to medium in size, and therefore the feeding habits of many species can be studied using this method.

While the fieldwork for the capture of fish proved to be particularly time consuming, since it required 4 to 6 h of snorkeling to catch 10 specimens of *S. scriba,* it should be noted that long capturing times may be connected with the use of a barbless hook, which can reduce catch efficiency, but it also significantly reduces unhooking time, stress and hooking injuries [65–67]. Another important factor is the size of the hook, as larger hooks decrease the incidence of deep hooking and consequently prevent serious injuries and bleeding [68–71]. In our study, we used large and thin hooks (size 6). Only 2 fish out of 150 died due to hooking. Thus, our fishing technique proved to be suitable for the non-destructive diet analysis method. The diameter of the nylon line is another important factor. We used nylon line, 0.20 mm in diameter, because *S. scriba* has sharp teeth that can bite through thinner thread.

In other studies, *S. scriba* were caught using traditional fishing gears, such as floating nets and longlines, but these methods are more likely to injure or kill the fish [10,14,17,72–74]. Working with live fish requires appropriate living conditions for the captured specimens, which means regular water changes, adequate oxygen levels and appropriate water temperature.

During our sampling surveys, mostly juvenile and sub-adult fish were caught, since young fish are less wary and more curious than older, experienced fish. Due to the predominance of juveniles in the sample, the data on trophic levels and prey proportions for all *S. scriba* individuals are biased to some extent, so it is better to consider trophic levels and prey proportions separately for each length (age) class.

Prey items in the fecal pellets of *S. scriba* were sometimes so decomposed that identification down to the lowest taxonomic units was impossible, but because their prey consisted mainly of crustaceans, polychaetes, fish and other taxa with hard, distinguishable body parts that are not so easily decomposed, identification of family, class or order was still possible (see Figures 3 and 4). Even some soft parts of prey, such as the cuticle of polychaetes and fish eggs, were found, demonstrating that species with softer bodies can also be recognized in feces. The quality of the fecal examination method was tested on five dead adult specimens of *S. scriba* obtained as bycatch from fishermen. These individuals were dissected and the stomach contents were isolated to compare the stage of decomposition in the stomach and feces of *S. scriba*. We were able to confirm that the stage of decomposition was similar. Fish exanimated by the non-destructive method probably defecated faster due to stress and consequently the prey did not decompose well, making it easier for us to determine the prey [75]. This was confirmed by the finding of whole juvenile *Gobius* sp. (Figure 4B), whole tanaids of species *Tanais dulongii* (Figure 3D), whole sphaeromatids (Figure 3C), anthurids and some whole *Pisidia* sp. in the feces. We did not find any soft or hard prey in the stomachs examined that was not also found in the feces. Soft prey was also not found in the diet of *S. scriba* by the authors of other studies, regardless of the method used [10,14,17]. Thus, the non-destructive method does not lead to worse results. Even with the traditional method of stomach content analysis, there is always a possibility of overestimating the proportions of prey that decompose more slowly [35,76,77].

Some differences in the results of previous studies are due to the fact that they did not always use standardized methodology to study feeding ecology in ichthyology. To determine the importance of the prey, it is best to use a numerical index as well as frequency of occurrence and categorize the prey as regards to these two criteria (see Table 2). These parameters applied for food spectra analysis are set accordingly and are used to quantitatively describe and graphically represent diet [35,78–80].

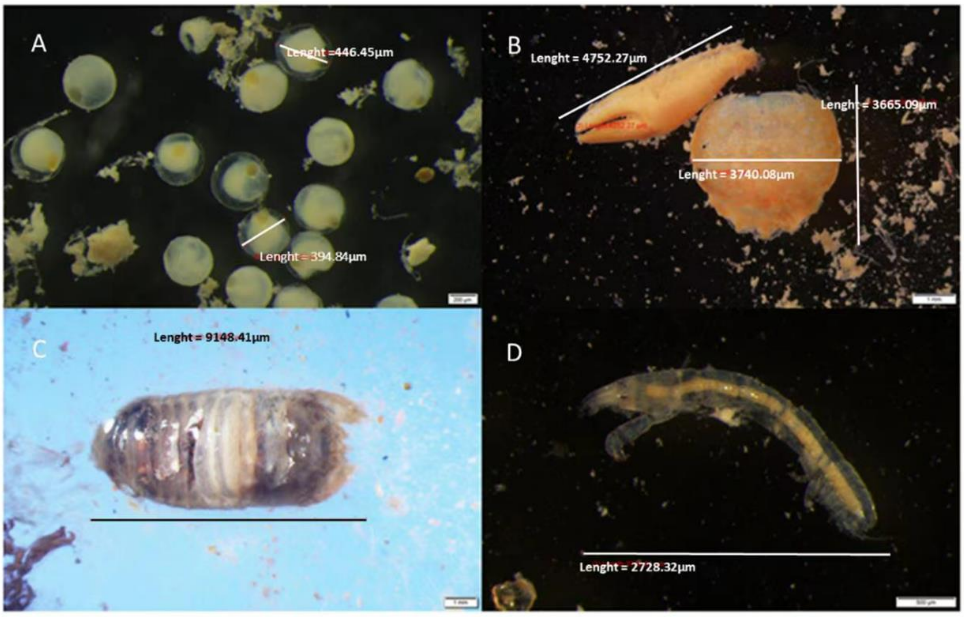

Figure 3. Prey items isolated from feces of *S. scriba*: (**A**) unidentified eggs, (**B**) carapace and claws of *Pisidia* sp., (**C**) isopod from family Sphaeromatidae and (**D**) *Tanais dulongii*.

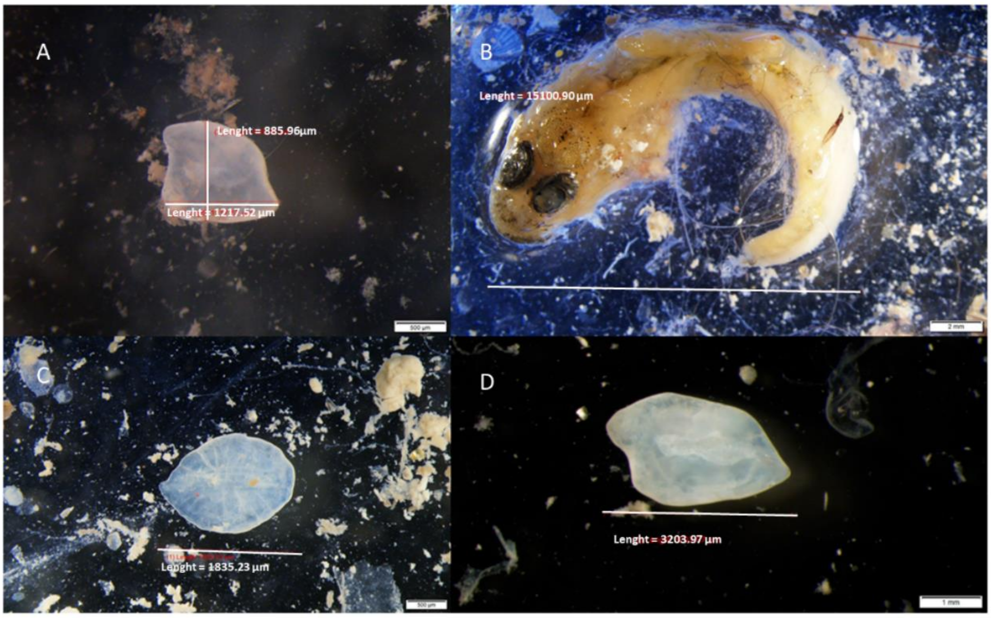

Figure 4. Otoliths and undigested *Gobius* sp. found in fecal pellets of *S. scriba:* (**A**) otolith of *Gobius cruentatus*, (**B**) *Gobius* sp., (**C**) otolith of *Atherina hepsetus* and (**D**) *Gobius fallax*.

4.2. Feeding Habits and Trophic Levels of S. scriba in the Northern Adriatic and in Other Mediterranean Areas

Our results indicate that *S. scriba* in the northern Adriatic Sea feeds on both slow-moving and fast-moving prey [13]. The calculated average trophic diversity index value (ITD) indicates a highly diverse diet. The calculated trophic levels demonstrated that in general, adults feed on higher trophic level than juveniles. This change in diet during development is referred to as an ontogenetic shift and has been confirmed for another *Serranus* species [81]. According to literature data [50], around 50% of individuals are sexually mature at 173 mm, which indicates that the majority of individuals used in the study were juveniles. Given the observed trend towards an increasing proportion of fish in the diet with body size, it is reasonable to assume that this proportion and therefore trophic level is even higher for older and larger *S. scriba*. The value of trophic level for 4-year-old *S. scriba* and older is, however, most likely biased due to the very small sample of these individuals and is not representative.

According to the analyses of otoliths found in the fecal pellets of *S. scriba* (see Figure 4), *G. fallax* was highlighted as the most common fish prey (Table 6), a finding that is attributed to its high abundance in the area [31] and its suitable shape and size for consumption by *S. scriba*. The suitable size of the prey is determined by limits of visual detection and size of the jaw apparatus [82–85]. Consuming one large prey means better energy efficiency than feeding on numerous small prey, but it is more time consuming, as prey handling time is longer [81]. Even though fish prey is not as important as regards to %PN and %PF, their mass and volume are larger than other prey and they are therefore a very important part of the diet. Piscivores generally reorient their prey head-first and lying on their side before swallowing it [84,86]. Therefore, the greatest width of the prey fish when swallowed is body depth (i.e., maximum distance between the dorsal and ventral portions of the fish). Prey with lower body depth is preferred, as it is easier to catch and consume [86]. Larger individuals of *S. scriba* are able to prey on fish species with greater body depth (*S. ocellatus*) (Figure 4), while smaller individuals prefer fish with shallow body-depth (*Gobius* sp.), as they are easier to capture and consume. Price et al. [87] have shown that handling time increases with prey body depth. Longer handling time increases the risk of losing the prey and being exposed to predators [88]; therefore, greater body depth is an anti-predatory adaptation [86].

Predators most often prey on fish that are well below the maximum ingestible size [82]. In wrasses, we noticed another anti-predatory behavior; in the presence of *S. scriba,* lateral positioning and display of the dorsal and ventral fin have been observed both in the wild and in captivity (*pers. observation*). Such a display makes the prey fish appear larger and is often sufficient to deter the predator from swallowing it [87,89]. Wrasses have a greater body depth than gobies at the same body length; therefore, wrasses are consumed by larger individuals of *S. scriba,* as observed in our study. Interestingly, smaller wrasses such as *Symphodus ocellatus* were observed to be removing parasites from *S. scriba,* although the latter is its potential predator (our study). Such behavior, i.e., approaching potentially dangerous clients, has been studied on cleaning gobies [90–92].

Our observations regarding specimens of *S. scriba* often predating from behind rocks, or lurking under boulders, are in accordance with previous studies [93,94]. The observed hunting behavior is known as the “sit and wait” predation mode and “burst chase” prey pursuit mode [19,94]. Vandewalle et al. [94] described that the majority of individuals occupy waiting spot above or within the algal cover or an overhang within heterogeneous rocky reefs, but they have also been observed hiding in algal thalli or *Posidonia oceanica* meadows, bordering sandy substrate.

S. scriba were observed searching for smaller crustaceans in cavities, holes and crevices under stones. Therefore, it was not surprising that crabs of the genus *Pisidia* were the most common prey items among the preyed crustaceans (see Figure 3), occurring in more than 50% of the examined samples (%PF = 56.67%, %PN = 18.80%). A crab, *Pilumnus hirtellus,* occurred in more than one tenth of the samples (% PF = 12.67%, %PN = 3.16%), while other crustaceans in the fecal pellets were mostly too decomposed to be identified to a species level. Isopods were a secondary common prey, mostly species of the family Sphaeromatidae, which are common inhabitants of rocky bottom communities [95]. Among complementary prey, well-preserved, almost whole specimens of *Tanais dulongii* (Tanaidacae), a widely distributed amphipod species along the entire Slovenian coast [96], were found.

Analyses of the diet of *S.scriba* have been conducted in different parts of the Mediterranean Sea and Canary Islands (Tables 8 and 9). In general, all the results confirm that crustaceans, especially decapods, are the main food source for *S. scriba*, while fish mainly represent secondary food. The highest proportion of fish in the diet was observed in a study from the Canary Islands (%PN = 22.64%), while this proportion was the lowest in our study (%PN = 6.82%). In terms of frequency of occurrence, fish were present in 38.32%

of the stomachs of *S. scriba* from the Canary Islands, 30.1% of those from the Tyrrhenian Sea and 20.67% of the fecal pellets of *S. scriba* from the northern Adriatic (see Table 8). The proportion of polychaetes in the diet of *S. scriba* was significantly higher in the northern Adriatic compared to other areas (%PN = 12.65%, %PF = 40.67%). In the Tyrrhenian Sea and in the Atlantic, authors observed a significantly higher proportion of caridean shrimps than observed in our study.

Table 8. Diet of *Serranus scriba* in various parts of the Mediterranean and Canary Islands (%PN = numerical index, %PF = frequency of occurrence).

	Our Research		**Moreno-Lopes et al., 2002**		**Arculeo et al., 1993**	
	Northern Adriatic, Gulf of Trieste		**Lanzarote, Atlantic Ocean**		**Thyrrenian sea, Gulf of Palermo**	
N=	**150**		**351**		**244**	
	%PN	**%PF**	**%PN**	**%PF**	**%PN**	**%PF**
CRUSTACEA (total)	69.21	98.67	75.08	95.52	60.9	
AMPHIPODA	0.83	2.67	0.98	1.87	0.7	0.02
CIRRIPEDIA	0.50	2.00				
Crustacea indeterminata	0.33	1.33				
DECAPODA (total)	46.75	96.67	60	82.24		
ANOMURA	25.45	79.33	13.44	23.36		
Galatheidae					18.6	24.1
Paguridae					0.02	<0.1
Porcellanidae					3.4	2.2
BRACHYURA (total)	7.65	30.67	21.64	46.73	0.4	12.9
CARIDEA (total)	3.33	11.33	24.92	37.38	49.8	27.7
ISOPODA	13.64		0.33	0.93	3.1	
MYSIDA	3.99	15.33			1.5	0.1
OSTRACODA	0.67	2.67				
STOMATOPODA			0.33	0.93		
TANAIDACEA	2.50	8.67	2.50	8.67		
MOLLUSCA	5.33		1.97	5.61	0.9	
BIVALVIA	0.67	2.67				
CEPHALOPODA			0.98	2.80	0.2	1.5
GASTROPODA	4.66	17.33	0.98	2.80	0.7	<0.01
POLYCHAETA (total)	12.63	40.67	0.33	0.93	0.8	0.7
TELEOSTEI	6.82	20.67	22.64	38.32	10.2	30.1
Teleostei indeterminata	2.00	8.00	11.15	20.56		
Atherinidae						
Atherina sp.	1.50	5.33	2.3	6.65		
Blennidae						
Blennidae indeterminata			0.33	0.93		
Parablennius pilicornis			1.31	2.80		
Scartella cristata			0.33	0.93		
Gobiesocidae						
Lepadogaster sp.			0.66	0.93		
Gobiidae						
Gobius cruentatus	1.00	2.67				
Gobius fallax	1.33	3.33				
Gobius niger			0.66	1.87		
Pomatoschistius bathi	0.50	2.00				

Table 8. *Cont.*

	Our Research		Moreno-Lopes et al., 2002		Arculeo et al., 1993	
	Northern Adriatic, Gulf of Trieste		Lanzarote, Atlantic Ocean		Thyrrenian sea, Gulf of Palermo	
N=	150		351		244	
	%PN	%PF	%PN	%PF	%PN	%PF
Labriidae						
Centrolabrus trutta			0.33	0.93		
Labridae indeterminata			2.62	5.61		
Symphodus cinereus	0.17	0.67				
Symphodus ocellatus	0.17	0.67				
Mullidae						
Mullus surmuletus	0.17	0.67				
Serranidae						
Serranus sp.			0.33	0.93		
Scorpaenidae						
Scorpaena maderensis			0.66	1.87		
Sygnathidae						
Sygnathus sp.			0.66	1.87		
Synodontidae						
Synodus synodus			0.33	0.93		
Trypterygiidae						
Tripterygion delaisi			0.66	1.87		
Eggs	5.99	11.33				

Table 9. Trophic levels of *Serranus scriba* in the Mediterranean Sea.

Authors and Year of Publication	Sampling Location	TROPH	TL (mm)
Our study, 2021	Northern Adriatic, Gulf of Trieste	3.43 ± 0.52	108–217
Karachle and Stergiou, 2017	Northwest Aegean Sea	3.94 ± 0.63	106–236
Stergiou and Karpouzi, 2002	(Review–average from various locations in Mediterranean)	3.79	50–230
Khoury, 1984	Tyrrhenian Sea, Gulf of Napoli	3.8	
Arculeo et al., 1993	Tyrrhenian Sea, Gulf of Palermo	3.87	100–230
Vasilki, 2016	South-West Lesvos, Aegan Sea,	3.8	173+

The trophic levels for *S. scriba* in different parts of the Mediterranean Sea were calculated (see Table 9) and ranged from 3.43 ± 0.52 in the northern Adriatic (our study) to 3.94 +/± −0.63 in the Aegean Sea [29]. The TROPH values for the same species may vary between sampling sites, seasons and years [97], and such changes in feeding habits may be influenced by changes in habitats [97] and prey availability [98]. The TROPH value also varies between different sizes and phases of the predator's biological cycle [99] as well as sampling time [48,99]. These parameters should be taken into account when interpreting and comparing results for the same geographic area. Indeed, if we compare two results from the Aegean Sea [14,100], we may assume that the differences in TROPH values are probably due to differences in fish body lengths (see Table 9), which may explain the difference in the values for the same region. Moreover, the trophic level for *S. scriba* calculated in our study (TROPH = 3.43 ± 0.53) is most similar to that of *Labrus merula* (TROPH = 3.47 ± 0.55), *Symphodus ocellatus* (TROPH = 3.4 ± 0.51), *Mullus surmuletus* (TROPH = 3.44 ± 0.53), *Diplodus annularis* (TROPH = 3.4 ± 0.46), *Diplodus sargus* (TROPH = 3.38 ± 0.51) and *Diplodus vulgaris* (3.5 ± 0.46), as calculated by Stergiou and Karpouzi [29]. All these species feed on decapod crustaceans, polychaetes, bivalves and echinoderms [29], which means that *S. scriba* may compete with them for available food resources. Furthermore, according to Stergiou and Karpouzi [29], the trophic level of *S. scriba* adults (3.7 ± 0.58) is higher than the trophic levels of other members of the coastal fish community (i.e., Sparidae, Labridae, Bleniidae and Gobiidae), that are abundant in the northern Adriatic Sea [1]. Therefore, *S. scriba* can be considered as a top predator of the community. As one of the most abundant piscivorous species on the rocky bottom in the coastal zone of Slovenian waters, it could play an important role as a predator of the goby family, especially of *Gobius cruentatus* and *Gobius fallax,* which are among the most numerous fish species in the area [101]. *S. scriba* feeds also on juvenile *Chromis chromis*,, a key fish species with an important role in transferring carbon, nitrogen and phosphorus from pelagic systems to the littoral zone [102,103].

4.3. Implications for Conservation

Fish are a crucial bioindicator of the ecological integrity of aquatic systems at different levels, from microhabitat to catchment; thus, they represent an important monitoring tool [104]. Species that are suitable bioindicators have high specificity and fidelity, i.e., they are found only in a particular type of environment and are widespread and abundant in that environment [105]. Because *S. scriba* is site-faithful [74], widespread throughout the Mediterranean [73], easy to collect and identify [104], and feeds opportunistically, it is suitable as a bioindicator species.

Data on feeding habits are essential for species and habitat conservation [103,106]. *S. scriba* is known as one of the nine most aggressive predators in the Adriatic fish communities, as defined by approaching, attacking and lure ingestion [93]. Aggressive predators play a very important role in the environment, as their behavior is the primary organizing force shaping the assembly of fish communities and driving preference and occupancy of heterogeneous and homogenous benthic habitats [104]. The most aggressive predators in Adriatic fish communities were found to be nine taxa of families Serranidae (3), Gobidae (3), Sparidae (2) and Labridae (1) [93].

The Adriatic Sea is a heavily exploited part of the Mediterranean basin, where the number of large apex predators, such as sharks and rays, have declined dramatically over the past two centuries [107] and mesopredators (mid-range predators in the middle of a trophic level [3] that typically prey on smaller animals), have taken over their role [108]. The loss of an aggressive mesopredator, such as *S. scriba*, may result in a drastic change in the fish community, including an increase in prey populations, and may have a major impact on the ecosystem as a whole [109]. *S. scriba* is an abundant opportunistic predator in the coastal fish community and is helping to maintain stability of the ecosystem [110,111] due to its generalist foraging strategy. Although nowadays the species does not require special protection, efforts should be made to maintain the overall variety and diversity of marine habitat types in the northern Adriatic Sea. Anthropogenic impacts on marine ecosystems should be monitored regularly and appropriate conservation actions taken before populations declines are recorded. For monitoring fish assemblages, it is recommended to use non-destructive methods such as a visual census whenever possible.

5. Conclusions

S. scriba is an important opportunistic mesopredator of the northern Adriatic rocky bottom fish communities. It preys on a wide range of different prey such as small epibenthic invertebrates and small coastal fishes (e.g., gobies, wrasses and *Atherina* spp.). While younger *S. scriba* tend to feed on small invertebrates such as polychaetes, mysids, and shrimp, they later undergo an ontogenetic shift and feed on a higher trophic level (with fish and decapod crustaceans). This paper supports previous research on the feeding habits of *S. scriba* and confirms the usefulness of the new non-lethal method [63] for studying the diet of small- to medium-sized coastal fish.

Author Contributions: Conceptualization, A.L., L.L. and D.T.; methodology, A.L., L.L. and D.T.; investigation, A.L., L.L., D.T. and M.O.-B.; writing—original draft preparation, A.L., D.T. and M.O.-B.; writing—review and editing, A.L., L.L., D.T. and M.O.-B.; project administration and funding

acquisition, A.L., L.L., D.T. and M.O.-B. All authors have read and agreed to the published version of the manuscript.

Funding: The authors acknowledge the financial support from the Slovenian Research Agency (ARRS), research core funding No. P1-0237.

Institutional Review Board Statement: Our research was conducted at the Marine Biology Station Piran of the National Institute of Biology of Slovenia, where researchers have the government authorization for fish culture and experimentation. Moreover, *Serranus scriba* is not on the list of protected species in Slovenia and therefore no special permits are required for catching and studying the species. We confirm that ethical cost of the research is balanced by the scientific value of the research. Knowledge about food and feeding habits of *S. scriba* is important for understanding local ecosystem dynamics, it helps us identify changes in the environment at an early change and contributes to the general advancement of knowledge. The authors assure that the present research complies with the commonly accepted '3Rs': replacement of animals by alternatives wherever possible, reduction in number of animals used, and refinement of experimental conditions and procedures to minimize the harm to animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available, since they originate from the research program funded by the Slovenian Research Agency (ARRS).

Acknowledgments: The authors would like to thank Milijan Šiško, Tihomir Makovec, Leon Lojze Zamuda, Luka Preložnik, Jure Zaman, Urška Bizjak, Gaja Jenko, Mojca Pungerčar, Doroteja Erhatič and Romina Bonaca for their assistance during the fieldwork and laboratory work. Special thanks to Valentina Pitacco and Milijan Šiško for their help in the statistical analyses.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Orlando-Bonaca, M.; Lipej, L. Factors affecting habitat occupancy of fish assemblage in the Gulf of Trieste (Northern Adriatic Sea). *Mar. Ecol.* **2005**, *26*, 42–53. [CrossRef]
2. IRP; Fletcher, S.; Lu, Y.; Alvarez, P.; McOwen, C.; Baninla, Y.; Fet, A.M.; He, G.; Hellevik, C.; Klimmek, H.; et al. Governing Coastal Resources. In *Implications for a Sustainable Blue Economy*; Report of the International Resource Panel; United Nations Environment Programme: Nairobi, Kenya, 2021.
3. Airoldi, L.; Balata, D.; Beck, M.W. The Gray Zone: Relationships between habitat loss and marine diversity and their applications in conservation. *J. Exp. Mar. Biol. Ecol.* **2008**, *366*, 8–15. [CrossRef]
4. Elliott, M.; Borja, A.; Cormier, R. Activity-footprints, pressures-footprints and effects-footprints—Walking the pathway to determining and managing human impacts in the sea. *Mar. Pol. Bul.* **2020**, *155*, 111201. [CrossRef] [PubMed]
5. Borja, A.; Menchaca, I.; Garmendia, J.M.; Franco, J.; Larreta, J.; Sagarminaga, Y.; Schembri, Y.; González, R.; Antón, R.; Micallef, T.; et al. Big Insights From a Small Country: The Added Value of Integrated Assessment in the Marine Environmental Status Evaluation of Malta. *Front. Mar. Sci.* **2021**, *8*, 638232. [CrossRef]
6. Sarrazin, V.; Kuhs, V.; Kullmann, B.; Kreutle, A.; Pusch, C.; Thiel, R. A sensitivity-based procedure to select representative fish species for the Marine Strategy Framework Directive indicator development, applied to the Greater North Sea. *Ecol. Indic.* **2021**, *131*, 108161. [CrossRef]
7. Nelson, J.S. *Fishes of the World*; John Wiley and Sons: Hoboken, NJ, USA, 2006.
8. Bauchot, M.L. Poissons osseux. Fiches FAO d'identification pour les besoins de la pêche. (rev. 1). Méditerranée et mer Noire. *Zone Pêche.* **1987**, *37*, 891–1421. (In French)
9. Maigret, J.; Ly, B.; Maigret, S. Marine fishes of Mauritania. *France Sci. Nat.* **1986**, *213*, 77.
10. Moreno-Lopes, A.; Tuset, J.V.; González Garcia-Diaz, M.M. Feeding habits of *Serranus scriba* (Osteichthyes, Serranidae). *Bol. Mus. Munic. Funchal.* **2002**, *53*, 5–17.
11. Zorica, B.; Pallaoro, A.; Sinovčić, G.; Keč, V.Č. Recent data of maximum age and length of painted comber *Serranus scriba* (Linnaeus, 1758) in Mediterranean Sea. *Acta Adriat.* **2010**, *51*, 223–226.
12. Heemstra, P.C.; Anderson, W.D., Jr.; Lobel, P.S. Groupers (seabasses, creolefish, coney, hinds, hamlets, anthiines, and soapfishes). In *FAO Species Identification Guide for Fishery Purpouses. The Living Marine Resources of the Western Central Atlantic*; Bony Fishes Part 1 (Acipenseridae to Grammatidae); Food and agriculture organization of the United Nations: Rome, Italy, 2013; Volume 2, pp. 1308–1369.
13. Arculeo, M.; Froglia, C.; Riggio, S. Food partitioning between *Serranus scriba* and *Scorpaena porcus* (Perciformes) on the infralittoral ground of the South Tyrrhenian Sea. *Cybium* **1993**, *17*, 251–258.

14. Vasiliki, M. An Estimation of the Diet of the Species *Serranus scriba* (Linnaeus, 1758) in the Area of Nisiopi, in South-West Lesvos. *J. Environ. Eng. Sci.* **2016**, *5*, 593–600. [CrossRef]
15. Deudero, S.; Pinnegar, J.K.; Polunin, N.V.C. Spatial variation and ontogenic shifts in the isotopic composition of Mediterranean littoral fishes. *Mar. Biol.* **2004**, *145*, 971–981. [CrossRef]
16. Heemstra, P.C.; Randall, J.E. Groupers of the world. *FAO Fish. Synop.* **1993**, *16*, 1.
17. Pinnegar, J.K.; Polunin, N.V.C. Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. *Oecologia* **2000**, *122*, 399–409. [CrossRef] [PubMed]
18. Labropoulou, M.; Eleftheriou, A. The foraging ecology of two pairs of congeneric demersal fish species: Importance of morphological characteristics in prey selection. *J. Fish. Biol.* **1997**, *50*, 324–340. [CrossRef]
19. Saikia, S.K. On the methodology of feeding ecology in fish. *Eur. J. Ecol.* **2016**, *2*, 35–46. [CrossRef]
20. Ferry, L.; Cailliet, G. Sample size and data analysis: Are we characterizing and comparing diet properly. In Proceedings of the Feeding, Ecology and Nutrition in Fish. International Congress on the Biology of Fishes, San Francisco, CA, USA, 14–18 July 1996; pp. 71–80.
21. Wootton, J.T. Effects of disturbance on species diversity: A multitrophic perspective. *Am. Nat.* **1998**, *152*, 803–825. [CrossRef]
22. Ross, S.T. Resource partitioning in fish assemblages: A review of field studies. *Copeia* **1986**, *2*, 352–388. [CrossRef]
23. Guedes, A.P.P.; Araújo, F.G. Trophic resource partitioning among five flatfish species (Actinopterygii, Pleuronectiformes) in a tropical bay in south-eastern Brazil. *J. Fish Biol.* **2008**, *72*, 1035–1054. [CrossRef]
24. Wetherbee, B.M.; Cortés, E.; Bizzarro, J.J. Food consumption and feeding habits. In *Biology of Sharks and Their Relatives*, 1st ed.; Jeffrey, C., Carrier, J.A., Musick, M.R., Heithaus, Eds.; CRC Press: Boca Raton, FL, USA, 2012; pp. 225–246.
25. Motta, P.J.; Wilga, C.D. Advances in the study of feeding behaviors, mechanisms, and mechanics of sharks. In *The Behavior and Sensory Biology of Elasmobranch Fishes: An Anthology in Memory of Donald Richard Nelson*, 1st ed.; Timothy, C.T., Samuel, H.G., Eds.; Springer Science-Business Media, B.V.: Dordrecht, The Netherlands, 2001; pp. 131–156.
26. Martin, R.A.; Hammerschlag, N.; Collier, R.S.; Fallows, C. Predatory behaviour of white sharks (*Carcharodon carcharias*) at Seal Island, South Africa. *J. Mar. Biolog. Assoc.* **2005**, *85*, 1121–1136. [CrossRef]
27. Frid, A.; Marliave, J. Predatory fishes affect trophic cascades and apparent competition in temperate reefs. *Biol. Lett.* **2010**, *6*, 533–536. [CrossRef] [PubMed]
28. Collar, D.C.; O'Meara, B.C.; Wainwright, P.C.; Near, T.J. Piscivory limits diversification of feeding morphology in centrarchid fishes. *Int. J. Org. Evol.* **2009**, *63*, 1557–1573. [CrossRef]
29. Stergiou, K.I.; Karpouzi, V.S. Feeding habits and trophic levels of Mediterranean fish. *Rev. Fish Biol. Fish.* **2002**, *11*, 217–254. [CrossRef]
30. Svanback, R.; Bolnick, D.I. Intraspecific competition drives increased resource use diversity within a natural population. *Proc. R. Soc.* **2007**, *274*, 839–844. [CrossRef] [PubMed]
31. Nakano, S.; Murakami, M. Reciprocal subsidies: Dynamic interdependence between terrestrial and aquatic food webs. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 166–170. [CrossRef]
32. Baxter, C.V.; Fausch, K.D.; Murakami, M.; Chapman, P.L. Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology* **2004**, *85*, 2656–2663. [CrossRef]
33. Baxter, C.V.; Fausch, K.D.; Saunders, C.W. Tangled webs: Reciprocal flows of invertebrate prey link streams and riparian zones. *Freshw. Biol.* **2005**, *50*, 201–220. [CrossRef]
34. Hynes, H.B.N. The food of fresh-water sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a Review of Methods Used in Studies of the Food of Fishes. *J. Anim. Ecol.* **1950**, *19*, 36–58. [CrossRef]
35. Hyslop, E.J. Stomach contents analysis—A review of methods and their application. *J. Fish Biol.* **1980**, *17*, 411–429. [CrossRef]
36. Cortes, E. A critical review of methods of studying fish feeding based on analysis of stomach contents: Application to elasmobranch fishes. *Can. J. Fish. Aquat. Sci.* **1997**, *54*, 726–738. [CrossRef]
37. Baker, A.M.; Fraser, D.F. A method for securing the gut contents of small, live fish. *Trans. Am. Fish. Soc.* **1976**, *105*, 520–522. [CrossRef]
38. Crossman, E.J.; Hamilton, J.G. An apparatus for sampling gut contents of large, living fishes. *Environ. Biol. Fishes* **1978**, *3*, 297–300. [CrossRef]
39. Light, R.W.; Adler, P.H.; Arnold, D.E. Evaluation of gastric lavage for stomach analyses. *N. Am. J. Fish. Manag.* **1983**, *3*, 81–85. [CrossRef]
40. Kamler, J.F.; Pope, K.L. Nonlethal methods of examining fish stomach contents. *Rev. Fish. Sci. Aquac.* **2001**, *9*, 1–11. [CrossRef]
41. Hartleb, C.F.; Moring, J.R. An improved gastric lavage device for removing stomach contents from live fish. *Fish. Res.* **1995**, *24*, 261–265. [CrossRef]
42. Meehan, W.R.; Miller, R.A. Stomach flushing: Effectiveness and influence on survival and condition of juvenile salmonids. *J. Fish. Res. Board Can.* **1978**, *35*, 1359–1363. [CrossRef]
43. Boicourt, W.C.; Kuzmić, M. The inland sea: Circulation of Chesapeake Bay and the Northern Adriatic. *Mar. Ecol. Prog. Ser.* **1999**, *303*, 81–129. [CrossRef]
44. Ogrin, D. Podnebje in izredni vremenski dogodki ob Tržaškem zalivu pred letom 1841. *Geogr. Obz.* **2012**, *3*, 23–30. (In Slovene)
45. Bailey, R.G. *Ecoregions—The Ecosystem Geography of the Oceans and Continents*, 2nd ed.; Springer: New York, NY, USA, 1998; pp. 196–200.

46. Bićanić, Z. Undersurface Salinity Minimum Participation in the Process of Making Deep Adriatic Sea-water. *Hrvat. Geogr. Glas.* **1998**, *60*, 123–134.
47. Turk, R. An assessment of the vulnerability of the Slovene coastal belt and its categorisation in view of (in)admissible human pressure, various activities and land-use. *Ann. Ser. Hist. Nat.* **1999**, *9*, 37–50.
48. Lipej, L.; Orlando-Bonaca, M. Assessing Blennioid fish populations in the shallow Gulf of Trieste: A comparison of four in situ methods. *Period. Biol.* **2006**, *108*, 23–29.
49. Labrosse, P.; Kulbicki, M.; Ferraris, J. Underwater visual fish census surveys: Proper use and implementation. In *Reef Resources Assessment Tools*; Secretariat of the Pacific Community: Noumea, New Caledonia, 2002.
50. Tuset, V.M.; Garcia-Diaz, M.M.; Gonzalez, J.A.; Lorente, M.J.; Lozano, I.J. Reproduction and growth of the painted comber *Serranus scriba* (Serranidae) of the Marine Reserve of Lanzarote Island (Central-Eastern Atlantic). *Estuar. Coast. Shelf Sci.* **2005**, *64*, 335–346. [CrossRef]
51. Riedl, R. *Fauna e flora del Mediterraneo. Dalle Alghe ai Mammiferi; una Guida Sistematica alle Specie che Vivono nel mar Mediterraneo*, 1st ed.; Franco Muzzio Editore: Padova, Italy, 1991. (In Italian)
52. Falciai, L.; Minervini, R. *Guida dei Crostacei Decapodi d'Europa*, 1st ed.; Franco Muzzio Editore: Padova, Italy, 1992.
53. Hayward, J.P.; Ryland, S.J. *Handbook of the Marine Fauna of North-West Europe*, 2nd ed.; Oxford University Press: Oxford, UK, 2017. (In Italian). [CrossRef]
54. Tuset, V.M.; Lombarte, A.; Assis, C.A. Otolith atlas for the western Mediterranean, north and central eastern Atlantic. *Sci. Mar.* **2008**, *72*, 7–198. [CrossRef]
55. Lombarte, A.; Chic, Ò.; Parisi-Baradad, V.; Olivella, R.; Piera, J.; Garcia-Ladona, E. A web-based environment for shape analysis of fish otoliths. The AFORO database. *Sci. Mar.* **2006**, *70*, 147–152. [CrossRef]
56. Macdonald, J.S.; Green, R.H. Redundancy of variables used to describe importance of prey species in fish diets. *Can. J. Fish. Aquat.* **1983**, *40*, 635–637. [CrossRef]
57. Hureau, J.C. Biologie Comparée de Quelques Poissons Antarctiques (Nototheniidae). Musée Océanographique: Monaco-Ville, Monaco, 1970; p. 68. (In French)
58. Shannon, C.E.; Wiener, W. *The Mathematical Theory of Communication*, 1st ed.; University of Illinois Press: Urbana, IL, USA, 1963.
59. Pauly, D.; Froese, R. Trophic levels of fishes. In *FishBase 2000: Concepts, Design and Data Sources*, 1st ed.; The International Center for Living Aquatic Resources Management (ICLARM): Manila, Philippines, 2000; p. 127.
60. Pauly, D.; Froese, R.; Palomares, M.L. Fishing down aquatic food webs: Industrial fishing over the past half-century has noticeably depleted the topmost links in aquatic food chains. *Am. Sci.* **2000**, *88*, 46–51. [CrossRef]
61. Clarke, K.R. Non-parametric multivariate analyses of changes in community structure. *Austral. J. Ecol.* **1993**, *18*, 117–143. [CrossRef]
62. Clarke, K.R.; Gorley, R.N. *Primer v6: User Manual/Tutorial. Plymouth*; PRIMER-E: Auckland, New Zealand, 2006.
63. Trkov, D.; Lipej, L. A non-destructive method for assessing the feeding habits of coastal fish. *Mediterr. Mar. Sci.* **2019**, *20*, 453–459. [CrossRef]
64. Zorica, B.; Ezgeta-Balić, D.; Vidjak, O.; Vuletin, V.; Šestanovič, M.; Isajović, I.; Čikeš Keč, V.; Vrgoč, N.; Harod, C. Diet Composition and Isotopic Analysis of Nine Important Fisheries Resources in the Eastern Adriatic Sea (Mediterranean). *Front. Mar. Sci.* **2021**, *8*, 609432. [CrossRef]
65. Muoneke, M.I.; Childress, W.M. Hooking mortality: A review for recreational fisheries. *Rev. Fish. Sci. Aquac.* **1994**, *2*, 123–156. [CrossRef]
66. Wilde, G.R. Tournament-associated mortality in black bass. *Fisheries* **1998**, *23*, 12–22. [CrossRef]
67. Alos, J.; Palmer, M.; Grau, A.M.; Deudero, S. Effects of hook size and barbless hooks on hooking injury, catch per unit effort, and fish size in a mixed-species recreational fishery in the western Mediterranean Sea. *ICES J. Mar. Sci.* **2008**, *65*, 899–905. [CrossRef]
68. Broadhurst, M.K.; Gray, C.A.; Reid, D.D.; Wooden, M.E.L.; Young, D.J.; Haddy, J.A.; Damiano, C. Mortality of key fish species released by recreational anglers in an Australian estuary. *J. Exp. Mar. Biol. Ecol.* **2005**, *321*, 171–179. [CrossRef]
69. Carbines, G.D. Large hooks reduce catch-and-release mortality of blue cod, *Parapercis colias* in the Marlborough Sounds of New Zealand. *N. Am. J. Fish. Manag.* **1999**, *19*, 81–85. [CrossRef]
70. Bacheler, N.M.; Buckel, J.A. Does hook type influence the catch rate, size, and injury of grouper in a North Carolina commercial fishery? *Fish. Res.* **2004**, *69*, 303–311. [CrossRef]
71. Cooke, S.J.; Barthel, B.L.; Suski, C.D.; Siepker, M.J.; Philipp, D.P. Influence of circle hook size on hooking efficiency, injury, and size selectivity of bluegill with comments on circle hook conservation benefits in recreational fisheries. *N. Am. J. Fish. Manag.* **2005**, *25*, 211–219. [CrossRef]
72. Chopin, F.S.; Arimoto, T.; Inoue, Y. A comparison of the stress response and mortality of sea bream *Pagrus major* captured by hook and line and trammel net. *Fish. Res.* **1996**, *28*, 277–289. [CrossRef]
73. Alos, J.; Palmer, M.; Balle, S.; Grau, A.M.; Morales-Nin, B. Individual growth pattern and variability in *Serranus scriba*: Bayesian analysis. *ICES J. Mar. Sci.* **2010**, *67*, 502–512. [CrossRef]
74. March, D.; Palmer, M.; Alós, J.; Grau, A.; Cardona, F. Short-term residence, home range size and diel patterns of the painted comber *Serranus scriba* in a temperate marine reserve. *Mar. Ecol. Prog. Ser.* **2010**, *400*, 195–206. [CrossRef]
75. Beulig, A.; Fowler, J. Fish on prozac: Effect of serotonin reuptake inhibitors on cognition in goldfish. *Behav. Neurosci.* **2008**, *122*, 426. [CrossRef]

76. Amundsen, P.A.; Hernandez, J. Feeding studies take guts–critical review and recommendations of methods for stomach contents analysis in fish. *J. Fish Biol.* **2019**, *95*, 1364–1373. [CrossRef]
77. Baker, R.; Buckland, A.; Sheaves, M. Fish gut content analysis: Robust measures of diet composition. *Fish Fish.* **2014**, *15*, 170–177. [CrossRef]
78. Buckland, A.; Baker, R.; Loneragan, N.; Sheaves, M. Standardising fish stomach content analysis: The importance of prey condition. *Fish. Res.* **2017**, *196*, 126–140. [CrossRef]
79. Amundsen, P.A.; Klemetsen, A. Diet, gastric evacuation rates and food consumption in a stunted population of Arctic charr, *Salvelinus alpinus* L., in Takvatn, northern Norway. *J. Fish Biol.* **1988**, *33*, 697–709. [CrossRef]
80. Greenwell, C.N.; Coulson, P.G.; Tweedley, J.R.; Loneragan, N.R. Regional differences in the feeding of the ambush predator *Neosebastes pandus* and comparisons of diets in the Scorpaenidae, Triglidae and Platycephalidae. *J. Fish Biol.* **2018**, *93*, 95–109. [CrossRef]
81. Morato, T.; Santos, S.R.; Andrade, P.J. Feeding habits, seasonal and ontogenetic diet shift of blacktail comber, *Serranus atricauda* (Pisces: Serranidae), from the Azores, Northeastern Atlantic. *Fish. Res.* **2000**, *49*, 51–60. [CrossRef]
82. Gill, A.B. The dynamics of prey choice in fish: The importance of prey size and satiation. *J. Fish Biol.* **2003**, *63*, 105–116. [CrossRef]
83. Mihalitsis, M.; Bellwood, D.R. A morphological and functional basis for maximum prey size in piscivorous fishes. *PLoS ONE* **2017**, *12*, e0184679. [CrossRef]
84. Christensen, B. Predator foraging capabilities and prey antipredator behaviours: Pre-versus postcapture constraints on size-dependent predator-prey interactions. *Oikos* **1996**, *76*, 368–380. [CrossRef]
85. Hoyle, J.A.; Keast, A. The effect of prey morphology and size on handling time in a piscivore, the largemouth bass (*Micropterus salmoides*). *Can. J. Zool.* **1987**, *65*, 1972–1977. [CrossRef]
86. Reimchen, T.E. Evolutionary attributes of headfirst prey manipulation and swallowing in piscivores. *Can. J. Zool.* **1991**, *69*, 2912–2916. [CrossRef]
87. Price, S.A.; Claverie, T.; Near, T.J.; Wainwright, P.C. Phylogenetic insights into the history and diversification of fishes on reefs. *Coral Reefs.* **2015**, *34*, 997–1009. [CrossRef]
88. Nilsson, P.A.; Brönmark, C. Prey vulnerability to a gape-size limited predator: Behavioural and morphological impacts on northern pike piscivory. *Oikos* **2000**, *88*, 539–546. [CrossRef]
89. Wootton, R.J. Biology of the sticklebacks. *Hydrobiology* **1978**, *63*, 434. [CrossRef]
90. Darcy, G.H.; Maisel, E.; Ogden, J.C. Cleaning preferences of the gobies *Gobiosoma evelynae* and *G. prochilos* and the juvenile wrasse *Thalassoma bifasciatum*. *Copeia* **1974**, *147*, 375–379. [CrossRef]
91. Wicksten, M.K. Behaviour of cleaners and their client fishes at Bonaire, Netherlands Antilles. *J. Nat. Hist.* **1998**, *32*, 13–30. [CrossRef]
92. Soares, M.C.; Bshary, R.; Cardoso, S.C.; Côté, I.M.; Oliveira, R.F. Face your fears: Cleaning gobies inspect predators despite being stressed by them. *PLoS ONE* **2012**, *7*, e39781. [CrossRef] [PubMed]
93. Kruschel, C.; Schultz, S.T. Aggressive predation drives assembly of Adriatic fish communities. *Diversity* **2020**, *12*, 130. [CrossRef]
94. Vandewalle, P.; Casinos, A.; Viladiu, C.; Osse, J. Suction Feeding Strategies of Two Species of Mediterranean Serranidae (*Serranus cabrilla* and *Serranus scriba*). *Neth. J. Zool.* **1999**, *49*, 81–95. [CrossRef]
95. Vieira, P.E.; Queiroga, H.; Costa, F.O.; Holdich, D.M. Distribution and species identification in the crustacean isopod genus *Dynamene* Leach, 1814 along the North East Atlantic-Black Sea axis. *ZooKeys* **2016**, *635*, 1–29. [CrossRef]
96. Fišer, C. Prispevek k poznavanju škarjevk (Tanaidacea: Peracarida: Crustacea) v slovenskem morju. *Nat. Slov.* **2004**, *6*, 11–17. (In Slovene)
97. Karachle, P.K.; Stergiou, K.I. The effect of season and sex on trophic levels of marine fishes. *J. Fish Biol.* **2008**, *72*, 1463–1487. [CrossRef]
98. Politou, C.Y.; Papaconstantinou, C. Feeding ecology of Mediterranean poor cod, *Trisopterus minutus capelanus* (Lacepede), from the eastern coast of Greece. *Fish. Res.* **1994**, *19*, 269–292. [CrossRef]
99. Tudela, S. Ecosystem effects of fishing in the Mediterranean. FAO Fisheries Department (EP/INT/759/GEF). *General fisheries Comission for the Mediterranean Studies and Reviews.* **2000**, *74*, 44.
100. Karachle, P.K.; Stergiou, K.I. An update on the feeding habits of fish in the Mediterranean Sea (2002–2015). *Mediterr. Mar. Sci.* **2017**, *18*, 43–52. [CrossRef]
101. Lipej, L.; Mavrič, B.; Orlando-Bonaca, M.; Uhan, J.; Makovec, T.; Trkov, D. *Raziskave Ribjih Združb v Akvatoriju Krajinskega Parka Strunjan: Zaključno Poročilo 2015*; Nacionalni Inštitut za Biologijo: Morska Biološka Postaja: Piran, Slovenija, 2015; p. 32. (In Slovene)
102. Jennings, S.; Renones, O.; Morales-Nin, B.; Polunin, N.V.C.; Moranta, J.; Coll, J. Spatial variation in the 15N and 13C stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: Implications for the study of trophic pathways. *Mar. Ecol. Progr. Ser.* **1997**, *146*, 109–116. [CrossRef]
103. Pinnegar, J.K. Why the damselfish *Chromis chromis* is a key species in the M editerranean rocky littoral—A quantitative perspective. *J. Fish Biol.* **2018**, *92*, 851–872. [CrossRef]
104. Chovanec, A.; Hofer, R.; Schiemer, F. Fish as bioindicators. *Trace Met. Contam. Environ.* **2003**, *6*, 639–676. [CrossRef]
105. Gerhart, A. Bioindicator species and their use in biomonitoring. *Env. Mon.* **2002**, *1*, 77–123.

106. Simpfendorfer, C.A.; Heupel, M.R.; White, W.T.; Dulvy, N.K. The importance of research and public opinion to conservation management of sharks and rays: A synthesis. *Mar. Freshw. Res.* **2011**, *62*, 518–527. [CrossRef]
107. Ferretti, F.; Osio, G.C.; Jenkins, C.J.; Rosenberg, A.A.; Lotze, H.K. Long-term change in a meso-predator community in response to prolonged and heterogeneous human impact. *Sci. Rep.* **2013**, *3*, 1057. [CrossRef] [PubMed]
108. Gül, G.; Demirel, N. Evaluation of the comprehensive feeding strategy and trophic role of overexploited mesopredator species in the Sea of Marmara (northeastern Mediterranean). *Estuar. Coast. Shelf Sci.* **2021**, *259*, 107448. [CrossRef]
109. Gianni, F.; Bartolini, F.; Airoldi, L.; Mangialajo, L. Reduction of herbivorous fish pressure can facilitate focal algal species forestation on artificial structures. *Mar. Environ. Res.* **2018**, *138*, 102–109. [CrossRef]
110. Sergio, F.; Newton, I.; Marchesi, L.; Pedrini, P. Ecologically justified charisma: Preservation of top predators delivers biodiversity conservation. *J. Appl. Ecol.* **2006**, *43*, 1049–1055. [CrossRef]
111. Estes, J.A.; Terborgh, J.; Brashares, S.J.; Power, E.M.; Berger, J.; Bond, J.W.; Carpenter, R.S.; Essington, T.E.; Holt, D.R.; Jackson, B.C.; et al. Trophic downgrading of planet Earth. *Science* **2011**, *333*, 301–306. [CrossRef] [PubMed]