Season, Sex and Size related Diet of Grass Goby, *Zosterisessor ophiocephalus* (Pallas, 1814) in the Eastern Adriatic Sea

Ishrana glavoča travaša, Zosterisessor ophiocephalus (Pallas, 1814), u istočnom dijelu Jadranskog mora, ovisno o godišnjem dobu, spolu i veličini

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Abstract

Feeding habits of Grass goby, *Zosterisessor ophiocephalus* (Pallas, 1814) related to season, sex and size were examined during one year. Stomach contents of 302 specimens were examined, of which 91 stomachs were empty. The sampled population consisted of 136 females (45.03%) ranging from 6.8 to 19.3 cm (13.30 ± 2.24), 156 males (51.65%) ranging from 6.1 to 20.2 cm (14.14 ± 2.91) and 10 unspecified (3.31%) individuals of a length of 5.6 to 16.0 cm (10.07 ± 2.91). Grass goby is mainly carnivorous. The dietary preferences among sexes and sizes did not differ statistically. Results indicate that Grass goby is an opportunistic species that feeds on wide spectrum on prey. Decapods were the most important prey (IRI =3208.49), followed by Osteichthyes (IRI=490.03).

KEY WORDS

diet composition
stomach contents
the Adriatic Sea
grass goby

Sažetak

Tijekom jedne godine ispitivane su prehrambene navike glavoča travaša, Zosterisessor ophiocephalus (Pallas, 1814), ovisno o godišnjem dobu, spolu i veličini. Pregledan je želučani sadržaj 302 uzorka, od čega je 91 želudac bio prazan. Uzorkovana populacija sastojala se od 136 ženki (45,03 %) u rasponu od 6,8 do 19,3 cm (13,30 ± 2,24), 156 mužjaka (51,65 %) u rasponu od 6,1 do 20,2 cm (14,14 ± 2,91) i 10 jedinki neodređenog spola (3,31 %) dužine od 5,6 do 16,0 cm (10,07 ± 2,91). Glavoč travaš je uglavnom mesožder. Prehrambene sklonosti među spolovima i veličinama nisu se statistički razlikovale. Rezultati pokazuju da je glavoč travaš oportunistička vrsta koja se hrani širokim spektrom plijena. Deseteronošci su bili najvažniji plijen (IRI =3208,49), a slijede Osteichthyes (IRI=490,03).

KLJUČNE RIJEČI

sastav ishrane
sadržaj želuca
Jadransko more
glavoč travaš

1. INTRODUCTION / *Uvod*

Grass goby, *Zosterisessor ophiocephalus* (Pallas, 1814) (Gobiidae) is an inshore species that usually occurs in brackish waters of estuaries and lagoons of the Mediterranean and Black Sea including Adriatic Sea and Sea of Azov [1]. It is widespread in the Adriatic Sea, especially in Novigrad and Karin. The Novigrad Sea, Croatia (44°12'N, 15°30'E) is an estuarine embayment of 29 km ^{2} on the northeastern Adriatic coast receiving freshwater from the Zrmanja river and a few nearby seasonal creeks. It is connected to the Velebit channel to the north by the narrow Maslenica channel, and to the Karin Sea to the south by the Karin channel. Salinity in the study site ranges from 10 to 30 PSU [2], and water surface temperature of the Zrmanja estuary ranges from 6.7 to 26.6°C [3]. Sea bottom types include rocky bottom covered with algae, muddy and sandy ground partially covered with seagrass and gravel ground [4]. Seagrass meadows and associated soft substrates provide an essential habitat for life and reproduction of this species [5], [6]. The primary prey items for *Z. ophiocephalus* are small fish and larger crustacean [7]. The grass goby is an iteroparous species living up to 5 years and reaching sexual maturity at 2 or 3 years of age [8], [9].

Feeding habits of grass goby have been studied in the Mediterranean Sea [10], [11] the Black Sea [12], the northeastern Atlantic [7] and the Adriatic Sea [13]. Dispite its wide distribution along the Adriatic coast, there is no information about the feeding ecology of this species. Estimates of food consumption of grass goby is needed to determine its trophic level in Adriatic Sea. Therefore, the present study aimed to report feeding habits of *Z. Ophiocephalus* in eastern Adriatic Sea related to season, sex and size .

2. MATERIALS AND METHODS / *Materijali i metode*

A total of 1229 individuals of *Zosterisessor ophiocephalus* were caught on a monthly basis between September 2010 and August 2011 in Novigrad Sea (44°12'03.03"N; 15°32'01.01"E, eastern Adriatic Sea) (Figure 1.). The fish were caught with a small coastal trawl made for such needs. Measured from the beginning to the end of the wing, the length of the net is 25 m and is of different heights. At the beginning of the wing the height of the net is 70 cm, and at the central part, together with the bag 500 cm. The size of the mesh on the wings of the net is 8 mm (from node to node), and in the middle with the central sac 4 mm [14]. The net was pulled by two men, always from the direction of the entrance to the bay towards its end, i.e. the coast. The average area covered by the net in one pull was about 500 m2. The net was always dragged in the morning from the opening (entrance) to the bottom (end) of the investigated bay. The working depth ranged from 4 to 0 m. Laboratory work included measure of total length down to the nearest 0.1 mm using Vernier caliper, body weight using digital scale down to the nearest 0.1g. In order to determine possible seasonal changes in feeding habits of grass goby subsample of 302 stomachs were examined (20-25 samples per mount). Stomachs of grass goby were removed opened and their content was identified to the possible lowest taxon and weighed to the nearest 0.01 g. In order to overcome the limitations of single indices the diet of *Z. Ophiocephalus* was described as follows:

The main food items were determined using the index of relative importance (IRI) [15] : IRI= %F (%N + %W), where %F is number of stomachs in which a food item was found x 100/total number of stomachs with food; %N is number of each prey item x 100/total number of preys in all stomachs; %W is wet weight of each prey item x 100/total weight of stomach contents

The various preys were classified according to their preponderance (frequency, number, weight) using the Main Food Index values "MFI" (Hand Food Index) [16].

MFI = [(% N + % F) / 2] * % W

The diet index (Q) [17]: Q = % N * % W,

The fullness index (%Jr): %Jr = (Wp/W) × 100, where Wp is the weight of prey items calculated as a difference between the weights of full and empty stomachs (Wsf-Wse); W refers to a total body weight.

The vacuity index (%V) was calculated as: %V = E/N × 100, where E is the number of empty stomachs and N is the total number of stomachs analyzed

The abundance (%N): %N = np/Np × 100, where Np is the number of prey specimens in a specific group and Np is the number of all determined prey groups

The frequency of occurrence (%F): %F = n/N × 100, where n is the number of stomachs containing a certain prey and N is the total number of analyzed stomachs containing any kind of prey.

3. STATISTICAL ANALYSES / *Statistička analiza*

A one-way analysis of similarity (ANOSIM, PRIMER, Primer-e ltd.) was used to test whether the diet among fish by sex and size differed statistically. The analysis is based on the presence/ absence using Bray- Curtis similarity.

4. RESULTS / *Rezultati*

Samples for stomach analyzes consisted of 136 females (45.03%) ranging from 6.8 to 19.3 cm (13.30 ± 2.24), 156 males (51.65%) ranging from 6.1 to 20.2 cm (14.14 ± 2.91) and 10 unspecified (3.31%) individuals of a length of 5.6 to 16.0 cm (10.07 ± 2.91). Of the total number of analyzed stomachs (N=302), 91 was completely empty, which yielded vacuity index (% V) of 30.13%. By analyzing the annual change of the index, the highest value was recorded in winter (4.96%) and the lowest in the autumn (1.98%) (Figure 1). In 29 stomachs, fully digested remains of food could not be determined, while the 181 stomach contained at least a small amount of food that could be determined. In addition, the fullness index (% Jr) for the total sample was relatively high at 4.42%.

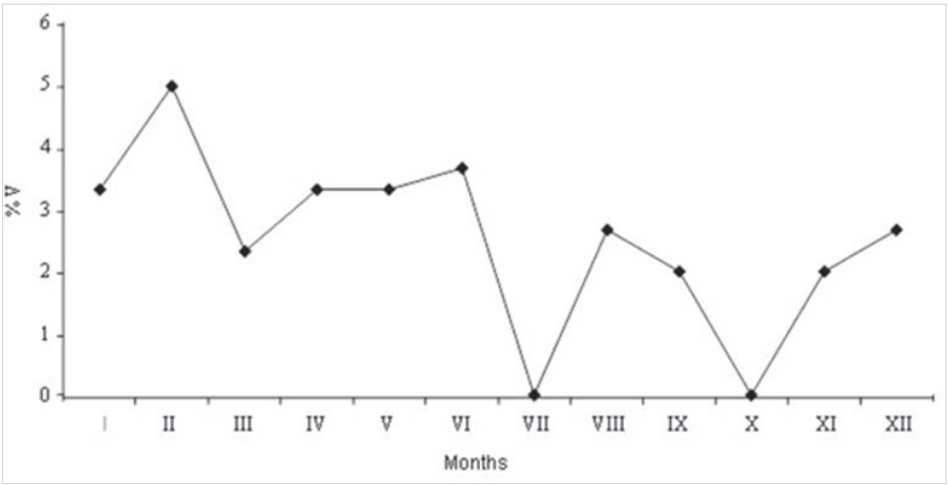

Figure 1 Monthly fluctuation of stomach vacuity coefficient (%V) of Grass goby *Zosterisessor ophiocephalus* from Novigrad Sea (September 2010 - August 2011)

Slika 1. Prikaz mjesečnog kolebanja koeficijenta praznoće probavila (%V) kod glavoča travaša, Zosterisessor ophiocephalus u Novigradskom moru (rujan 2010. – kolovoz 2011.)

Table 1 Values of mass percentage (%W), abundance (%N) and frequency of occurrence (%F) of prey for analyzed sample of Grass goby, *Zosterisessor ophiocephalus* from the Novigrad Sea (N=302)

Tablica 1. Vrijednosti postotka mase (%W), brojnosti (%N) i postotka učestalosti pojavljivanja (% F) plijena za analizirani uzorak glavoča travaša, Zosterisessor ophiocephalus u Novigradskom moru (N= 302)

Food item	N(%N)	W(%W)	F(%F)
PHAEOPHYTA			
Total PHAEOPHYTA	1 (0,02)	0,003 (0,01)	1 (0,55)
SPERMATOPHYTA			
Zostera noltei (dijelovi)	151 (3,31)	1,03 (2,09)	85 (46,70)
Zostera sp.	7 (0,15)	0,37 (0,76)	5 (2,74)
Total SPERMATOPHYTA	158 (3,46)	1,39 (2,85)	90 (49,45)
MOLLUSCA			
Cephalopoda	2 (0,04)	2,38 (4,87)	2 (1,09)
Ukupno Cephalopoda	2 (0,04)	2,38 (4,87)	2 (1,09)
Gastropoda			
Bittium reticulatum	1 (0,02)	0,07 (0,15)	1 (0,54)
Retusa truncatula	2 (0,04)	0,91 (1,85)	2 (1,09)
Gastropoda (unidentifield)	1 (0,02)	0,58 (1,19)	1 (0,54)
Total Gastropoda	4 (0,08)	1,63 (3,19)	4 (2,17)
Bivalvia	2 (0,04)	3,02 (6,16)	2 (1,09)
Total Bivalvia	2 (0,04)	3,02 (6,16)	2 (1,09)
Total MOLLUSCA	8 (0,17)	6,97 (14,24)	8 (4,39)
ANNELIDA			
Polychaeta sp.	31 (0,68)	0,03 (0,06)	2 (1,09)
Oligochaeta sp.	172 (3,77)	0,34 (0,70)	59 (32,4)
Annelida (unidentifield)	2 (0,04)	0,03 (0,05)	1 (0,55)
Total ANNELIDA	205 (4,50)	0,39 (0,81)	62 (34,06)
SIPUNCULIDA			
Sipunculida (unidentifield)	1 (0,02)	0,01 (0,03)	1 (0,54)
Total SIPUNCULIDA	1 (0,02)	0,01 (0,03)	1 (0,54)
CNIDARIA			
Hydrozoa	1 (0,02)	0	1 (0,54)
Total CNIDARIA	1 (0,02)	0	1 (0,54)
ARTHROPODA			
Liocarcinus sp.	1 (0,02)	0,04 (0,09)	1 (0,55)
Pachygrapsus marmoratus	3 (0,06)	1,27 (2,60)	3 (1,64)
Total BRACHYURA	4 (0,08)	1,32 (2,70)	4 (2,19)
Penaeus kerathurus	3 (0,06)	1,33 (2,72)	3 (1,64)
Penaeidea (unidentifield)	17 (0,37)	5,52 (11,29)	15 (8,24)
Caridea (unidentifield)	4 (0,08)	0,24 (0,49)	2 (1,09)
Hippolytae inermis	3 (0,06)	1,29 (2,65)	3 (1,64)
Total CARIDEA	7 (0,15)	1,54 (3,14)	5 (2,74)
Total CRUSTACEA (unidentifield)	54 (1,18)	9,72 (19,87)	40 (21,97)
Total ARTHROPODA	114 (2,50)	29,18 (59,61)	94 (51,64)
Zosterisessor ophiocephalus	4 (0,08)	7,40 (15,12)	4 (2,19)
Symphodus ocellatus	1 (0,02)	2,19 (4,48)	1 (0,55)
Gobius sp.	1 (0,02)	1,02 (2,09)	1 (0,55)
OSTEICHTHYES (unidentifield)	13 (0,28)	2,81 (5,75)	16 (8,79)
Total OSTEICHTHYES	19 (0,42)	13,44 (27,45)	8 (4,39)
Total VERTEBRATA	38 (0,82)	26,88 (54,91)	16 (8,79)
Fish scales	1568 (34,41)	0,21 (0,42)	18 (9,89)
TELEOSTEI (bony fish)	4 (0,08)	0,01 (0,02)	2 (1,09)
TELEOSTEI (eggs)	130 (2,85)	0,003 (0,007)	2 (1,09)
AMORFNA TVAR	69 (1,51)	24,25 (49,55)	69 (37,91)
Total (other)	1771 (38,87)	24,47 (50,0)	91 (50,0)

Table 1 presents different groups of prey: total body weight (% W), abundance (% N), and frequency of occurrence (% F). This is indicative of diverse nutrition in the Novigrad Sea. In the analyzed stomachs, remains of Vertebrates and Invertebrates were found, such as Mollusca, Cnidaria, Annelida, Sipunculida, Arthropoda and Osteichthyes.

ANOSIM analysis of stomach content of Grass goby with respect to their individual size is depicted on MDS graph based on Bray-Curtis similarity coefficients (Figure 2.). Statistical analysis did not produce any homogenous groups and there is no significant difference in feeding between individuals smaller than 12 cm and those larger than 12 cm respectively (ANOSIM: R=-0.396).

Analysis of stomach content of Grass goby with respect to sex did not yield homogenous groups (Figure 3.). Analysis of similarities between samples shows no significant difference in food composition between sexes (ANOSIM: R=-0.484).

The values in Table 2 show that Arthropoda are main component of diet (MFI=1614.21, Q=149.17), while Vertebrata (MFI=264.09, Q=45.42), according to coefficient Q are supplementary diet. Mollusca (MFI=32.54, Q=2.50) are necessary,

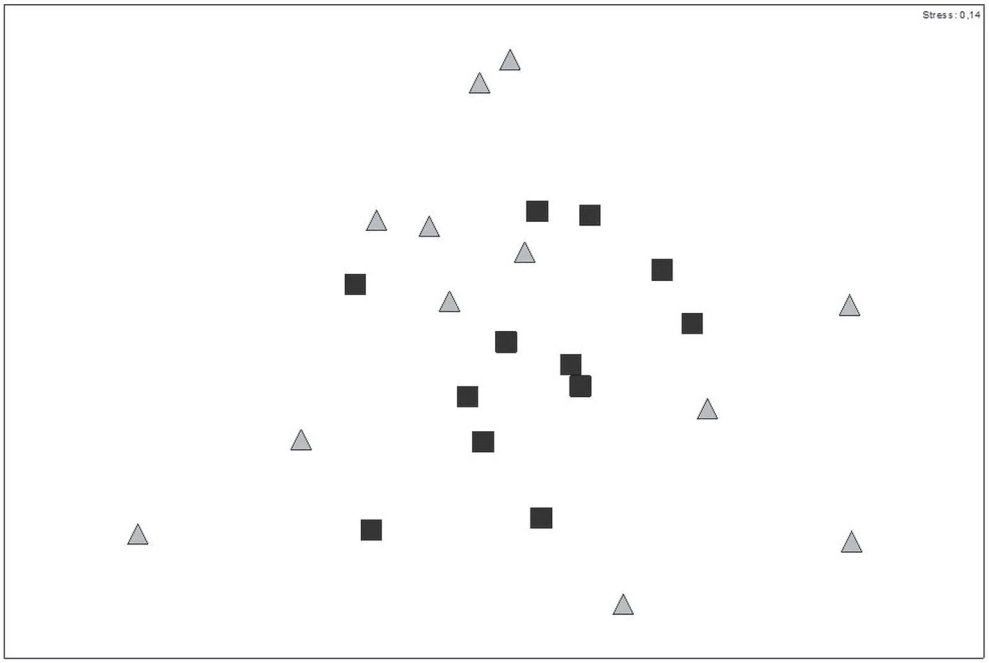

Figure 2 MDS ordinate graph of food composition of Grass goby, *Zosterisessor ophiocephalus* from the Novigrad Sea related to the size of individuals (▲<12,0 cm, ■> 12,0 cm; stress: 0,14)

Slika 2. MDS ordinacijski prikaz usporedbe sastava ishrane glavoča travaša, Zosterisessor ophiocephalus u Novigradskom moru obzirom na veličinu jedinki (▲ 12,0 cm; ■> 12,0 cm; stres: 0,14)

Table 2 Values of Index of relative importance (IRI), main food index (MFI) and diet index (Q) of prey of Grass goby, *Zosterisessor ophiocephalus* from the Novigrad Sea

Tablica 2. Vrijednost koeficijenta relativnog značaja (IRI), koeficijenta osnovnih tipova hrane (MFI) i koeficijenta hranjivosti (Q) plijena glavoča travaša, Zosterisessor ophiocephalus u Novigradskom moru

Category of prey	IRI	MFI	Q
Phaeophyta	0,01	*	*
Spermatophyta	312,76	75,59	9,90
Mollusca			
Cephalopoda	5,40	2,78	0,21
Gastropoda	3,41	1,72	0,13
Bivalvia	6,82	3,52	0,27
Total Mollusca	63,37	32,54	2,50
Annelida	181,01	15,69	3,66
Sipunculida	0,02	*	*
Cnidaria	0,01	0	0
Arthropoda			
Decapoda - Penaeidae	142,61	72,12	5,54
Decapoda - Caridea	9,06	4,56	0,48
Decapoda - Brachyura	6,13	3,08	0,23
Crustacea (neidentificirano)	462,82	230,16	23,55
Total Arthropoda	3208,49	1614,21	149,17
Vertebrata			
Osteichthyes			
Zosterisessor opiocephalus.	33,43	17,28	1,32
Symphodus ocellatus	2,47	1,28	0,09
Gobius sp.	1,16	0,59	0,04
Total Vertebrata	490,03	264,09	45,42

but with low Q occur only randomly in diet. Furthermore, high values of MFI (75.59) and Q (9.90) are given for Spermatophyta, which indicate that it is supplementary or randomly ingested with its animal prey by Grass goby in Novigrad Sea.

Quantitative analysis of changes in diet of Grass goby during different season's coefficients IRI, MFI and Q were compared. Evident is that groups Arthropoda and Spermatophyta dominate in diet throughout the year. Also, group Annelida is found in stomachs during all seasons. During summer, along with Arthropoda, Osteichthyes are found in prey (Figure 2.). The values of coefficients for basic food groups (Table III.) during winter season show that necessary food consists of Arthropoda, Mollusca and Spermatophyta. During spring season Arthropoda, Annelida and significantly more, Osteichthyes, are part of diet.

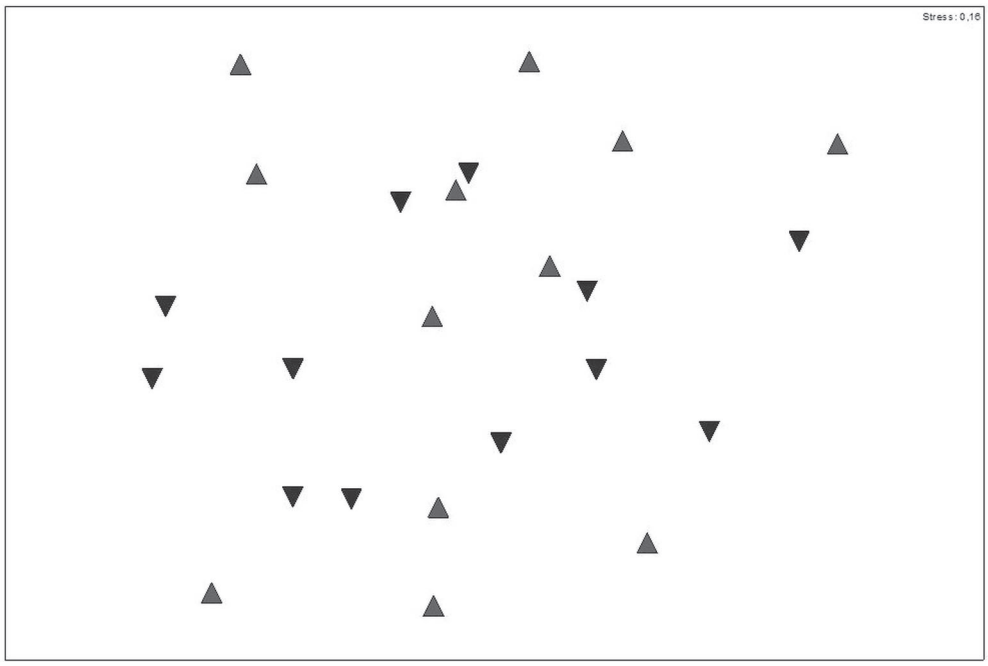

Figure 3 MDS ordinate graph of food composition of Grass goby, *Zosterisessor ophiocephalus* from the Novigrad Sea related to the sex (▲females, ▼males; stress: 0,16)

Slika 3. MDS ordinacijski prikaz usporedbe sastava ishrane glavoča travaša, Zosterisessor ophiocephalus u Novigradskom moru obzirom na spol jedinki (▲ženke, ▼mužjaci; stres: 0,16)

Table 3 Seasonal values of main food index (MFI) of prey of Grass goby, *Zosterisessor ophiocephalus* from the Novigrad Sea

Tablica 3. Sezonske vrijednosti koeficijenta osnovnih tipova hrane (MFI) plijena glavoča travaša, Zosterisessor ophiocephalus u Novigradskom moru

Category of prey	Autumn	Winter	Spring	Summer
Phaeophyta	-	-	-	0,004
Spermatophyta	2497,75	21,65	1705,04	98,71
Mollusca	-	-	-	-
Cephalopoda	1,94	-	-	-
Gastropoda	0,08	24,60	166,30	-
Bivalvia	-	27,88	-	2,45
Annelida	0,13	21,95	6798,24	23,31
Spinculida	-	0,19	-	-
Cnidaria	-	-	-	-
Arthropoda	-	-	-	-
Decapoda- Brachyura	0,007	-	166,30	5,60
Decapoda - Penaeidae	2,00	35,16	332,60	8,01
Decapoda - Caridea	255,46	-	-	3,62
Crustacea (unidentified)	1,29	1686,44	498,90	849,65
Vertebrata	-	-	-	-
Osteichthyes (unidentified)	-	-	156,30	2,35
Zosterisessor opiocephalus.	-	-	166,30	52,99
Symphodus ocellatus	-	-	-	5,63
Gobius sp.	-	-	-	2,28

In summer season abundance of Arthropoda and Osteichthyes in diet decreases rapidly.

5. DISCUSSION / *Rasprava*

In the available literature, complete nutrition of grass goby, regarding prey, diet intensity and seasonal variation in diet, was known only from the work of Hajji et al. [10] for the Mediterranean Sea area. Bell & Harmelin-Vivien [18] analyzed nutrition on 49 species from Gobiidae family in the Mediterranean Sea, but there is only a mention of grass goby as the mesophagous carnivores. Until this research, for the Adriatic Sea there is no data on the feeding habits of grass goby.

In this paper, food composition and feeding regime of Grass goby from the Novigrad Sea, collected on meadows of seagrass (*Zostera noltei*, Hornemann, 1832) is described. Results confirm wide range of prey (8 basic food categories), which indicates that Grass goby is opportunistic predator feeding on variety of prey. Similar number of prey categories along with detailed description of prey species is given by Hajji et al. [10] for Mediterranean populations of this species. Franco et al. [19] in

their research in Venetian lagoon, state prey groups for all gobiid species, where majority of prey falls into Arthropoda group (order Amphipoda). Analysis of Grass goby from research area shows crustaceans and other benthic organisms to be the most abundant prey items. Firstly, decapod crustaceans as the most important prey group (to a lesser extent during autumn), than bony fish (Osteichthyes), as well as ringed worms (Annelida). Small fish belonging to the genus *Symphodus* (Rafinesque, 1810), *Gobius* (Linnaeus, 1758) and *Zosterisessor* (Whitley, 1935), along with decapod crustaceans belonging to family Penaeidae (Rafinesque, 1815), represent the necessary prey (most abundant during winter season and before spawning), while in research of Hajji et al. [10] fish is the second most abundant group of prey. Interesting fact is that in the stomachs of larger individuals of Grass goby there are present juveniles of same species, indicating cannibalism. There are reports of cannibalism for this species in the available literature. The cannibalism in species is mentioned in literature *Knipowitschia panizzae* (Gobiidae) [20]. Considering the fact that these are preliminary reports in the captivity conditions, research confirmed the hypothesis that females of this species are more aggressive during pre-spawning period [20]. Hence, the authors made a connection with intra-population relations. Regarding the increased aggressiveness of females of Grass goby in the pre-spawning period [21], changes in ecological parameters (temperature, salinity, habitat degradation, lack of food or inability to distinguish prey item) are considered as causes of cannibalism in the Novigrad Sea area. Results also indicate great abundance of Spermatophyta (*Zostera noltei*) in the stomach content of the investigated species, although sea grass is of little dietary value. Incidentally, meadows of sea grass are a habitat for this species, so the abundance of plant material in stomachs should be taken with caution. Given that Grass goby is a predator that feeds mainly at dusk because its visual acuity is at best at that time [22], and that fish swallow its prey whole ([23], the probable reason for greater abundance of *Z. Noltei* in the stomach content is accidental ingestion with the targeted prey. Other prey groups in this research, like Mollusca, Phaeophyta, Cnidaria and Sipunculida are regarded as occasional prey. This research confirmed the hypothesis that neither sex nor size of Grass goby influence its diet in the Novigrad Sea area. However, Hajji et al. [10] in their investigation of diet of Grass goby from the Mediterranean Sea recorded a decrease in the crustacean abundance, as well as an increase in the abundance of molluscs and small fish as prey in larger individuals. Ontogenetic changes in diet of this species may be the result of increase in the ability to catch larger prey by older individuals [24]. Franco et al. [25] by comparing two largest gobiid species, *Gobius niger* (Linnaeus, 1758) and *Z. Ophiocephalus* noted difference in diet, where Grass goby feeds on larger prey. Also their conclusion is that both species are carnivorous with Amphipods as main prey, and that they are ambush predators with particular technique developed for mobile prey. The comparison with results of Hajji et al. [10] shows that in the Mediterranean Sea the majority of prey of Grass goby are crustaceans such as Isopod *Idotea balthica* (Pallas, 1772.), decapods *Trachysalambria curvirostris* (Stimpson, 1860) and *Processa edulis* (Risso, 1816). Furthermore, important components of diet of Grass goby for the mentioned area are molluscs *Bittium reticulatum* (Da Costa, 1778), also found in this research, *Cerithium vulgatum* (Bruguière, 1792) and fish species *Engraulis encrasicolus* (Linnaeus, 1758), *Lithognathus mormyrus* (Linnaeus, 1758) and *Sardinella aurita* (Valenciennes, 1847) as the secondary prey [10]. Crustaceans are the main food component of most Mediterranean Gobiid species, e. g. *Gobius auratus* (Risso, 1810) [26], *Gobius cobitis* (Pallas, 1814) [27], [11] and *Deltentosteus quadrimaculatus* (Valenciennes, 1837) [28], [29].The results of this research are in accord with other published research regarding feeding habits of Grass goby, which also state crustaceans, molluscs and small fish as main food groups, both in the Novigrad and in the Mediterranean Sea [11], [30], [31], for the north-eastern Atlantic Ocean [32] and the Black Sea [12]. Furthermore, similar food composition and prey species abundances was recorded for black goby, *Gobius niger* (Linnaeus, 1758) and rock goby, *Gobius paganellus* (Linnaeus, 1758) from Black Sea [7] the north-eastern Atlantic Ocean [33] and the Mediterranean Sea [34]. Feeding intensity oscillated during the year from maximum in the spawning season (February-May) to minimum (September-December). Also, seasonal fluctuations in feeding intensity were recorded, which was inversely proportional to the coefficient of stomach vacuity [35]. The percentage of empty stomachs decreased, i. e. the feeding intensity increased before and after the spawning season. Requirements for energy storage during spawning, as well as energy recovery after the spawning, may give the explanation for the observed seasonal fluctuation of the feeding intensity [10]. Crustaceans were the main prey throughout the whole year and the abundance of molluscs was highest during winter and spring, which is very similar to the results of various research on the Mediterranean populations of this species [10].

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