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MASTER'S THESIS

DANIJEL KABLAR

UNIVERZA NA PRIMORSKEM
FAKULTETA ZA MATEMATIKO, NARAVOSLOVJE IN INFORMACIJSKE TEHNOLOGIJE

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(MAGISTRSKO DELO)

THE PREDATION IMPACT OF THE MEDITERRANEAN SHAG (*PHALACROCORAX ARISTOTELIS DESMARESTII*) ON THE POPULATION OF BLACK GOBY (*GOBIUS NIGER*)

(VPLIV PLENJENJA SREDOZEMSKEGA VRANJEKA (*PHALACROCORAX ARISTOTELIS DESMARESTII*) NA POPULACIJO ČRNEGA GLAVAČA (*GOBIUS NIGER*))

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The predation impact of the Mediterranean Shag (*Phalacrocorax aristotelis desmarestii*) on the population of Black Goby (*Gobius niger*)

(Vpliv plenjenja sredozemskega vranjeka (*Phalacrocorax aristotelis desmarestii*) na populacijo črnega glavača (*Gobius niger*))

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Izvleček:

Sredozemski vranjek (*Phalacrocorax aristotelis desmarestii*) je ribojeda morska ptica, ki živi na območju Mediterana in Črnega morja. Čeprav v Sloveniji ne gnezdi, zaradi neprimernih habitatov in človeških motenj, se čez poletje 11 % celotne populacije vranjeka nahaja vzdolž slovenske obale. Vranjek je zelo občutljiv na motnje s stani človeka, izlive nafte in uničenje habitatov. S tega razloga je uvrščen na seznamu priloge 1 (Annex 1) ptičje direktive (2009/147/EC).

Črni glavač (*Gobius niger*) predstavlja najbolj pomemben plen vranjeka s koeficijentom relativne pomembnosti od 65 % (IRI %-index of relative importance). Z metodo preiskave izbljuvkov smo analizirali 1000 otolitov črnega glavača iz dveh različnih lokalitet med poletjem 2018. Ugotovili smo da vranjek pleni glavače dolge od 3,48 do14,07 cm, s povprečno vrednostjo 8,77 cm. S stališča starosti je vranjek plenil starejše osebke od osebkov, ki smo jih mi dobili v vzorcu "narava", pred Morsko Biološko Postajo v Piranu . Vranjeki, ki so se nahajali na počivališču v Sečovljah so plenili manjše in mlajše glavače od vranjekov, ki so počivali v Strunjanu. Iz PCA analize je razvidno, da je razlika med velikostjo plena med Sečovljami in vzorcem iz narave manjša, kot je med Sečovljami in Strunjanom.

Key document information

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Keywords: Mediterranean shag, black goby, pellets, Slovenian part of the Adriatic Sea

Abstract: Mediterranean shag is a piscivorous marine bird that inhabits the Mediterranean and the Black Sea. During the summer 11% of its whole population can be seen along the Slovenian coast, roosting on artificial buoys from shellfish farms. Mediterranean shag is highly susceptible to the human disturbance, oil spills and habitat destruction, making him listed on Annex 1 of the Birds Directive (2009/147/EC). The black goby is represented as the favourite shag`s prey with IRI% (index of relative importance) being 65%. Using the pellet method, 1000 black goby otholits were analysed from two different locations during the summer of 2018. We calculated that Mediterranean shag preyed gobies in the size range from 3.48-14.07 cm in length with an average value of 8.77 cm. In terms of age it preyed older specimens than the ones we collected from the nature. Shags roosting in Sečovlje preyed upon smaller and younger gobies than the ones roosting in Strunjan. From the PCA was noticeable that there was less difference in prey size between Sečovlje and the samples from the nature, than it was between Strunjan and Sečovlje.

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To be allowed to use SCUBA equipment, at least one diving course has to be successfully finished. SCUBA diving is considered to be an extreme sport in which the consequences can result deadly if the dive is not conducted properly. The gear required to perform a dive is heavy (app. 25 kg) and it considerably restricts your motion. Once you are submerged your senses begin to “trick” you. Your vision is affected by the mask you are wearing and things underwater appear to be bigger and closer to you. The source of sound in the water is practically impossible to detect, as the sound through water travels faster than trough air. Despite the diving suit you sense cold and the deeper you go the colder it gets. Dealing with the previously described problems requires an immense amount of skill, coordination and patience. Those are the basic things you are dealing with in every dive, but to be able to perform any kind of work or research underwater one has to have an extreme skill and coordination because even writing underwater is unimaginably harder than someone might think. For those reason I would like to express a special gratitude to my mentor prof. Dr.

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Without you, my master thesis would not be possible!

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1 INTRODUCTION

1.1 MEDITERRANEAN SHAG IN THE NORTHERN ADRIATIC SEA

European shag (Linnaeus, 1761) (*Phalacrocorax aristotelis*) is a marine bird, which lives in the Western Palaearctic's coastal waters (del Hoyo et al. 1992; Cramp 1998). More than 2/3 of its entire population is situated in Europe. The overall population number is in constant decline, despite some populations being stable (BirdLife International 2019). Three European shag`s subspecies are known to exist and are distributed not to overlap with each other's breeding ranges (Xirouchakis et al. 2017). *Phalacrocorax aristotelis aristotelis* situated in western and northern Europe (in the Atlantic Ocean), *Phalacrocorax aristotelis riggenbachi* present in northern Africa (Atlantic coast of Morocco) and *Phalacrocorax aristotelis desmarestii* present in the Mediterranean as well as in the Black Sea (del Hoyo et al. 1992). The Mediterranean subspecies is relatively small and its population is estimated to 10000 breeding pairs, of which 7000 are situated in the western Mediterranean (Spanish, French and Italian islands) and 1000 pairs in the Black Sea (Aguilar and Fernandez 1999, Xirouchakis et al. 2017). They are highly susceptible to human disturbance which made the subspecies to be listed on Annex 1 of the Birds Directive (2009/147/EC).

Mediterranean shag is a medium large size black bird, from 65 to 80 cm in length with the wing length from 90 to 110 cm. Its feathers have greenish reflection and during the breeding season, it has a curved tuft on its head. Its clutch usually consists of 3 eggs, the incubation lasts 30 days and chicks fledge for 53 days (Aguilar and Fernandez 1999). It distinguishes from the great cormorant *(Phalacrocorax carbo*) by its smaller size and narrower beak (Henkens et al. 2010).

In Slovenia, Mediterranean shag was listed as a rare species, until the 1990s (Komisija za redkosti 1993). The fact that it does not breed in Slovenia, does not mean that the species is not present there. In the contrary, Mediterranean shag is present in Slovenia throughout the whole year (Geister 1995). An increase in sighting number is evidenced from the early 1990s, when during the winter bird count, only 0-10 individuals were counted (Sovinc 1994), but in the January waterbird censuses summarized by Božič (2007, 2008a, 2008b, 2010, 2011 and 2012), 39 to 312 birds were counted). During the summer, their number would drastically change. In 2004, Benussi (2005) estimated the shag`s number for the entire Gulf of Trieste to 1500-2000 birds. In 2012, 1500 individuals were counted only in the Slovenian part of the Gulf of Trieste, which makes 11% of the whole subspecies population (Bordjan et al. 2013).

Mediterranean Shags build their nests in the bushes of small, deserted islets (Sponza et al. 2010). Their nests are untidy and are made of seaweed and rotten twigs (Henkens et al. 2010). The lack of such breeding habitats in the Gulf of Trieste, as well as the fact that shags are highly susceptible to the human disturbance (Guyot 1993), make the Gulf of

Trieste unsuitable breeding habitat for the Mediterranean shag (Cosolo et al. 2011). Suitable breeding habitats are available in Croatian islets (Figure 1). The nearest are located in Vrsar, followed by Rovinj, Brijuni archipelago, Oruda, Silbanski grebeni in front of the town of Zadar and the Pelješac peninsula. According to IOO (2013), from 1500 up to 2000 pairs breed in those areas. In the late summer months 4000 birds of all age classes were counted in the communal roosts of the Gulf of Trieste, meaning that the majority of breeding population from the central and northern Adriatic migrate to the Gulf of Trieste (Škornik et al. 2012). As described by Sponza et al. (2013), migrations tend to be food related. Furthermore, marine birds are at high trophic level in the marine food webs, meaning that some alterations had been done in the marine ecosystem to force a predator to move/migrate in a more suitable area (Morat et al. 2011).

Figure 1. Important breeding locations in the central and upper Adriatic Sea (dots), as well as the three main roosting areas in the Gulf of Trieste (marked in white)(Sponza et al. 2103)

Mediterranean shag regurgitates pellets. These are composed of undigested food remains, which are often regurgitated in their roosting habitats. The composition of pellets varies from one species to another, depending on the food the individual predator is preying on. In pellets of terrestrial predators, mammal skulls, lower jaws, and skeletal and chitinous insect body parts are often found (SIMARINE-NATURA (LIFE10NAT/SI/141) 2013). In pellets of marine predators the most common undigested parts are cephalopod jaws, the remains of crab carapaces and legs and pharyngeal teeth and otoliths as fish`s skeletal parts (SIMARINE-NATURA (LIFE10NAT/SI/141) 2013). Otoliths are found in the fish`s inner ear cavity, and its functions are to aid in the auditory system and are the organ which provide balance to fish (Rodríguez Mendoza 2006). They are made from calcium carbonate in the aragonite form and their shape considerably differs among species. From one otolith it is possible to determine a fish to the species level, if the otolith is not severely damaged. The growth of fish could be also obtained by measuring otoliths. The period of fast growth is marked as the opaque zones and the period on slow growth are marked as the hyaline zones. These zones are called rings, and from them it is possible to access the fish`s age and length, annual growth rate, daily growth rate and mortality rate (Rodríguez Mendoza 2006).

1.2 FEEDING ECOLOGY OF THE MEDITERRANEAN SHAG

The European shag`s Atlantic subspecies (*Phalacrocorax aristotelis aristotelis*) preys on demersal, benthic and even pelagic fish, making it an opportunistic predator (Cosolo et al. 2012). Its feeding ecology has been already studied and well documented in various European countries. In Spain it preys on 16 species belonging to 10 families, dominated by Ammodytidae (<86%), in Norway it preys on 12 species belonging to 11 families, dominated by Gadidae (<70%), in Scotland had 3 families dominated by Ammodytidae (<81%) (Pearson 1968; Barrett and Furness 1990; Barrett 1991; Velando et al. 2005). However, the Mediterranean subspecies has quite different feeding habits. In Corsica, the Mediterranean shag preys mostly upon families Labridae and Ammodytidae (Guyot 1988), in the Riou Archipelago it preys upon 25 fish species from 12 families, with the most abundant families Atherinidae and Pomacentridae (Morat et al. 2011) and in Greece in mostly preys on families Gobiidae, Atherinidae and Labridae (Thanou 2013). In the Adriatic Sea, during the breeding season it preys upon five families equally: Sparidae, Gobiidae, Serranidae, Labridae and Maenidae (Cosolo et al. 2011), which are considered bentho-pelagic mobile species. During the post-breeding period in the Gulf of Trieste, it preys mainly upon the family Gobiidae (75%), Serranidae (13%), Cepolidae (3%) and Atherinidae (3%) (Lipej et al. 2016), which are demersal sedentary species. The diet of the European shag can be considered variable and depending on ecological properties of the location it inhabits (Figure 2). In the wild European Shag is estimated that it produces one pellet in every 3.7 days on average (Russell et al. 1995)

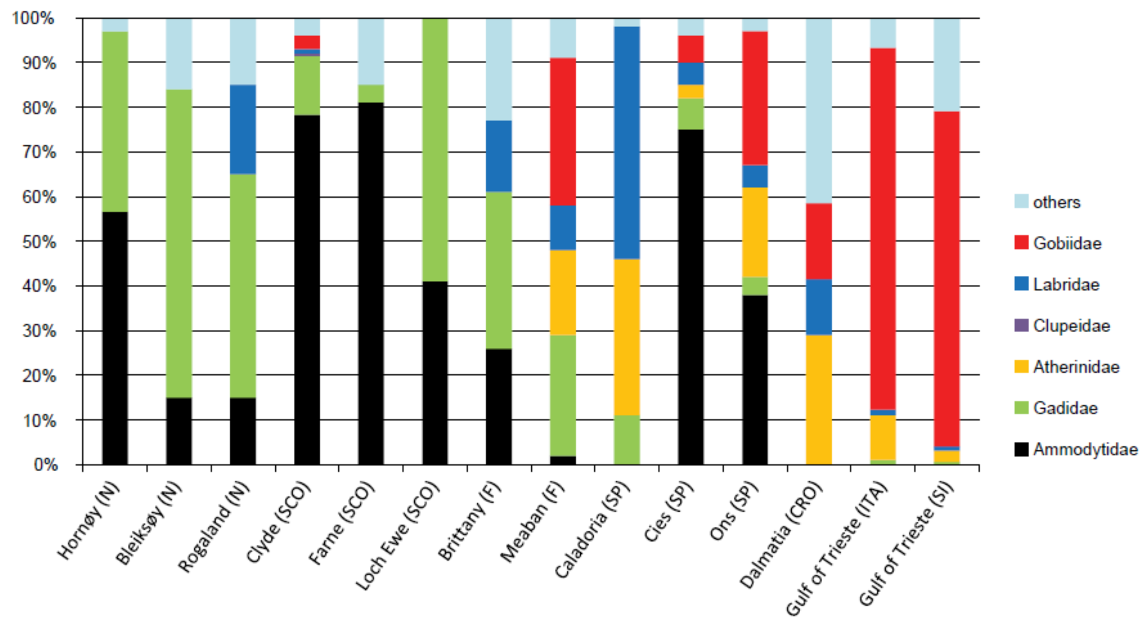

Figure 2. The diet of the European shag, *Phalacrocorax aristotelis*, in different locations in Europe (Lipej et al. 2016)

After the year 1981, the amount of demersal fish in the Adriatic Sea started to decline with values up to 67% in Albania, Montenegro, Bosnia–Herzegovina, Slovenia and Croatia due to overfishing (Sponza et al. 2010). Marine bird`s feeding strategies are usually driven by the abundance of accessible prey (Hunt 1991; Monaghan et al. 1994; Uttley et al. 1994). The overfishing effect in Croatia, led to the failure in feeding specialization in demersal fish, forcing the Mediterranean shag to migrate, after breeding, to a more suitable location, the Gulf of Trieste (Sponza et al. 2010).

1.3 BLACK GOBY

The black goby (*Gobius niger*) is a coastal, bottom-dwelling marine fish species. It inhabits the North Sea, the Baltic Sea, the Mediterranean Sea, the Black Sea, the Suez Canal and the Red Sea. Its area's southernmost point is the Cape Blanc in Mauritania (Carpenter et al. 2015). The black goby can be found in waters up to 70 meters of depth (Miller 1990). *G. niger* is easily found in estuaries and lagoons, as well as in seagrass meadows, over sand and muddy habitats. Its slow mobility and the male's black colour make him an easy prey to detect for marine birds such as the Mediterranean shag. The black goby is usually found on sedimentary bottom under rocks or dead bivalve parts. Its life span is confirmed to be at least 6 years (Ilkyaz et al. 2011), while the sexual maturity is reached the 2nd year of age (Miller 1986). Spawning season of *G. niger*, varies from one place to another (Boban et al. 2013). In the Tyrrhenian Sea (Italy) the spawning season, occurs from March until May, in Netherlands from April to June, in the Baltic Sea from May to August and in the Black Sea from April to September. The female can lay up to 2000 eggs, depending on the female`s age and size (Vesey and Langford 1985). Black goby preys on bivalves, crustaceans, small fish, polychaetes and gastropods (Miller 1986). Its population is rated as “least concern” on the IUCN red list and the population trend is stable, although, there is no quantified data about its population size (Carpenter 2015). Vesey and Langford (1985) noted that in a local trawl survey in England the black goby was the most common fish.

1.4 WORKING HYPOTHESIS

- Mediterranean shag (*Phalacrocorax aristotelis desmarestii*) preys on specimens of the black goby of specific age/length class,
- Mediterranean shag preys on larger/older individuals,
- With the predation on a certain age/length class, they make a selective pressure on the population of black goby in the Slovenian waters,
- Otoliths length and weight are proportionally correlated with the fish size,
- Otolith data from the pellets can be used to assess the consumed fishes biomass,
- From the otolith data we can calculate shag`s daily biomass consumption.

1.5 AIM OF THE STUDY

The aim of the study was to determine what is the age and length of the shag`s favourite prey, by using the otolith length and weight measurements from the shag's pellets. As its prey is predominantly the black goby, we were interested in the age class of prey specimens preyed by the shag. As Lipej et al. (2016) pointed out that 65% (IRI%–index of relative importance) of the Mediterranean shag`s prey are black gobies, we wanted to assess the predation capacity of the Mediterranean shag as well as its selective pressure on the population of black goby in Slovenian Sea. Based on the obtained results, nature conservation implications will be given.

2 STUDY AREA

2.1 GENERAL DESCRIPTION

The Gulf of Trieste is situated in the northern part of the Adriatic Sea, making it the northernmost part of the Mediterranean Sea. It covers an area of 550 km^2 and is a small part of the Gulf of Venice, spread across three countries: Italy, Slovenia and Croatia. The whole Slovenian coast is entirely part of the Gulf of Trieste and is 46 kilometres long. It is a shallow part of the Adriatic Sea with the maximum depth of 33 m measured in Piran (Orlando-Bonaca et al. 2008), although rarely exceeding 25 m (Lipej et al. 2016). The Gulf of Trieste is a part of the Mediterranean Sea that expresses semidiurnal, moderate tides, frequently up to 30 cm (Janeković and Kuzmić. 2005). During the winter, it is the coldest part of the entire Mediterranean basin, with water temperatures of 6 ºC while, during summer, it reaches up to 26 ºC (Cozzi et al. 2012). Due to a freshwater intake from the rivers Soča and Timavo from the Italian coast and rivers Dragonja and Rižana from the Slovenian coast, the salinity of The Gulf of Trieste varies from 25 to 35 (Cozzi et al. 2012). Koper and Piran bays are part of the Gulf of Trieste. Both of them are characterized by elevated sedimentation rates and silty clay as substrate. In the direction toward the open sea, the substrate changes from clay to 80% biogenic detritus (Ogorelec et al. 1991; Orlando-Bonaca et al. 2008). Water circulation in the Gulf of Trieste is counter-clockwise in deeper layers, as the current is coming into the Gulf from the Istrian coast and leaving by the Italian coast (Stravisi 1983). The circulation in the surface layer varies regardless to the presence or absence of the strong wind Bura (Stravisi 1983).

Along the Slovenian coast are present two natural monuments, Rt Madona and Debeli rtič and Nature reserve Strunjan, as well as two landscape parks Sečovlje Salina and Strunjan. Because of its unique characteristics, both salinas offer perfect habitats for various opportunistic species, as well as bird nesting and roosting habitats. However, both of them are inadequate for the Mediterranean shag, due to the lack of small islands.

2.2 ROOSTING AREAS OF SHAGS

A roosting area is defined as a place where bird gather, during the period they are inactive, to rest (Ward and Zahavi 2008). Mediterranean shag is a bird, which uses communal roosting habitats (Bordjan et al. 2013), defined as places where a group of intra or interspecific individuals, who feed in flocks or solitarily, gather to rest and is not of constant composition (Ward and Zahavi 2008). In the Gulf of Trieste, common roosting areas are shellfish farms, breakwaters, lighthouses, stranded trees and rocky isles (Benussi 2005). In Slovenian waters, preferred and mostly used roosting areas are mariculture facilities which farm *Mytillus galloprovincialis*. Three biggest shellfish farms in Slovenia are Sečovlje, Strunjan and Debeli rtič (Škornik 2012).

In this study, samplings were conducted in the two main areas, Sečovlje and Strunjan saltpans. As seen from Figure 3, the roost areas in Strunjan are closer to the open sea than the Sečovlje roosting area, face the land from the E and S and the buoys in Strunjan are 200 m distant from the land. However, Sečovlje saltpans are in a more closed bay, facing the open waters in NW direction only, the buoys are placed 400 m from the coast.

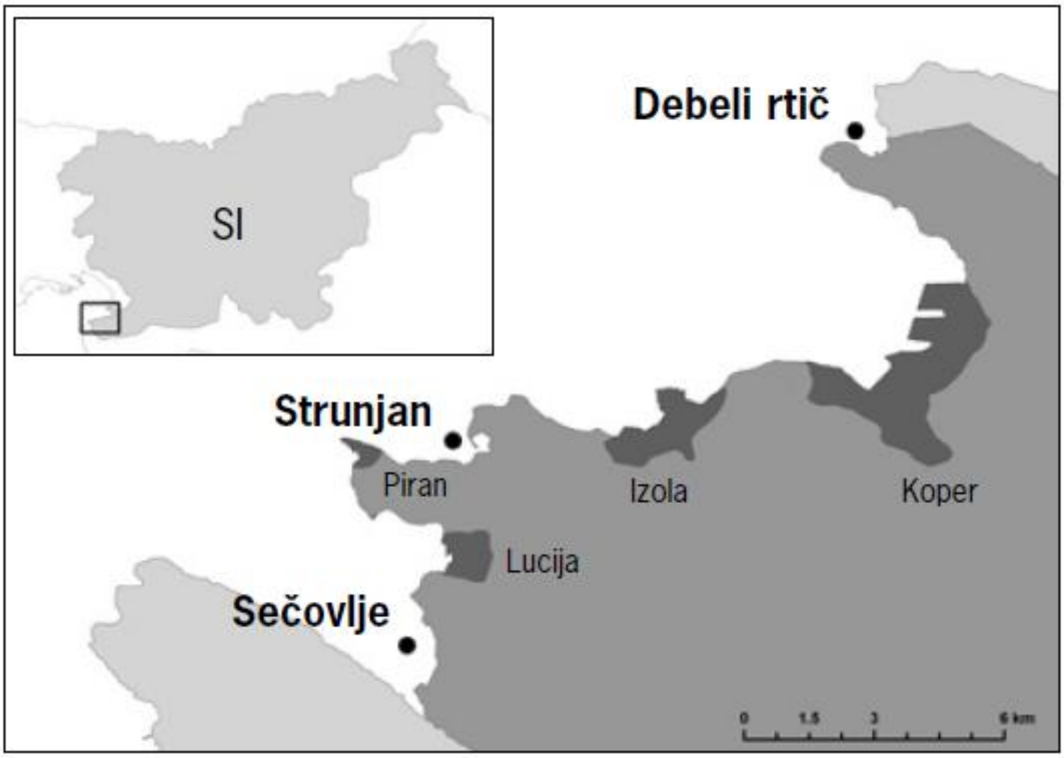

Figure 3. Study areas with Mediterranean shag`s (*Phalacrocorax aristotelis desmarestii*) roosting areas (Lipej et al. 2016)

3 MATERIAL AND METHODS

3.1 COLLECTION OF PELLETS

Mediterranean Shag`s pellets were collected from the floating buoys in two communal roosting areas in Slovenian water, the Strunjan Bay and Sečovlje saltpans, on two different occasions. On 19 July 2018, 21 pellets were collected from the floating buoys in Strunjan and on 23 July 2018, 14 pellets were collected from the floating buoys in Sečovlje. We approached the buoys by boat. Pellets were carefully collected by hand, wearing plastic gloves for antibacterial protection. Samples were stored in a plastic bag until the arrival at the lab of Marine Biology Station in Piran. Once arrived, the samples were moved from the plastic bag into a carton container and were left drying on the air.

3.2 COLLECTION OF GOBIES

Samplings of both surveys required the use of SCUBA equipment, a fish anaesthetic solution of quinaldine, an anaesthetic used in specific sampling of cryptobenthic fauna (Trkov et al. 2019), diluted with alcohol in a proportion 1:15, falcon tube, aquarium hand net and a diving net for the tube storage. To use the SCUBA equipment, at least one diving course is required, as it can easily turn to be a quite risky and even deadly sport. Once you are submerged your senses start to change. The vision is affected by the mask you are wearing and things underwater appear to be 1/3 bigger and ¼ closer to you. Sound in the water travels faster than in it does through the air, resulting in a constant noise pressure, with very little possibility of detecting its source. When fully equipped with the SCUBA gear you have approximately 25 additional kilograms on your back. An immense amount of skill, coordination and patience are required to be successful at diving properly with SCUBA gear. To perform any kind of work or research underwater is extremely difficult and has to be acknowledged.

As the black goby is a demersal fish, living under rocks or parts of dead shells, it`s collection is relatively easy. When a specimen was spotted, it was sprinkled with quinaldine solution, making them slower and easier to catch. When sprinkled with quinaldine, anesthetized targets were immediately forced out of their hiding place, where were caught with an aquarium hand net, and sprinkled some more with quinaldine. Afterwards, the samples were put in a falcon tube, with its own unique number, and were stored in the net. Samples were taken to Marine Biology Station (National Institute of Biology) in Piran and were stored at -20ºC.

3.3 ISOLATION OF OTOLITHS

Dried pellets were washed with water and the help of two sieves, with different mesh sizes. Isolated otoliths were collected carefully from the sieves by hand with tweezers with special care not to damage the otolith. The black goby otoliths were determined with the help of the determination key for otoliths (AFORO, 2018) and were stored in a plastic tube in 70% alcohol.
Sagittal otoliths from specimens caught in the wild were isolated with a scalpel and a pair of tweezers. After the removal of the fish`s intestine, with a clean scalpel cut, the lower part of its skull was perforated, making the otolith take possible. Every pair of otoliths was stored in a different glass vial and was ready for the morphometric measurements.

3.4 MORPHOMETRIC MEASUREMENTS

3.4.1 Otholits

A total of 1067 otoliths were analysed, 500 from Strunjan, 500 from Sečovlje and 67 from the live specimens. The length and width of the isolated black goby otoliths, both from the pellets and live specimens, were measured with the Olympus SZX16 stereomicroscope and photographed with Olympus DP-Soft SZX16 software (Figure 4). Measured otoliths were afterwards weighed with an electronic scale with accuracy 0.001 mg. Otoliths that were visibly eroded, broken or damaged in some other ways, were not included in the study, to reduce the potential error. Every otolith was measured from its rostrum to the postrostrum (figure 4).

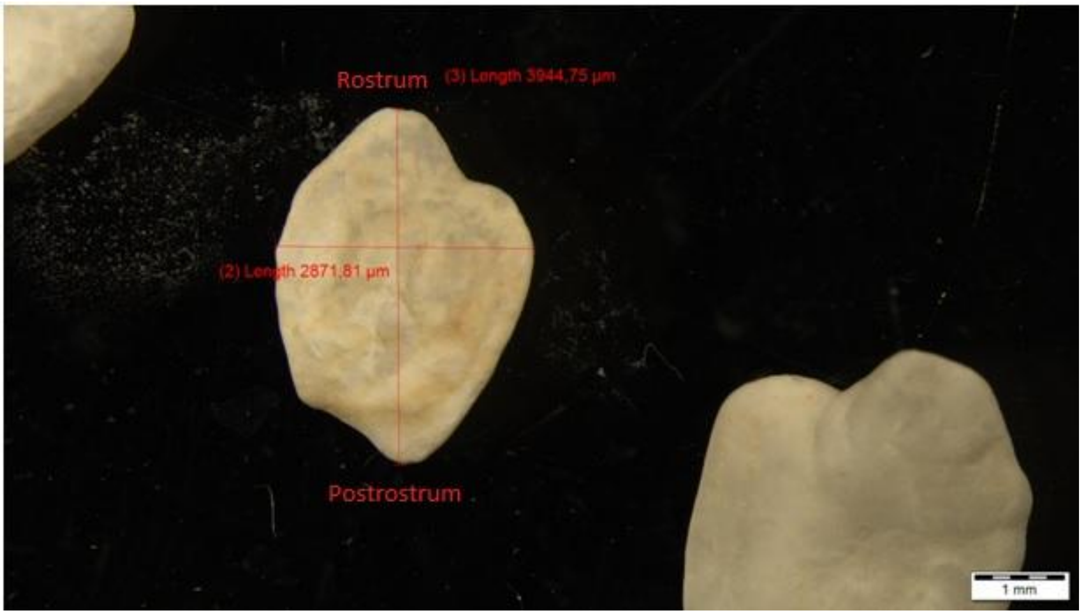

Figure 4. Measurements of the black goby otoliths by using the computer program Olympus cellSens

3.4.2 Gobies

Altogether, 67 specimens of black goby were obtained for the analysis. Collected gobies were first determined as *Gobius niger,* under a stereo microscope, with the help of the key for identification of in the Adriatic Sea (Kovačić, 2008). Every goby specimen was given an identification number for data management. Before weighing, the fish was dried with a paper towel, by gently pressing it on the fish`s surface, not to remove the mucus, only to absorb the water. Weighing was conducted with a 0.01g accuracy electronic scale. Three length measurements were measured on every specimen: head length (from the mouth to the end of the gill opercle), standard body length (from mouth to the end of the caudal peduncle; without the tail fin) and the total body length (from mouth until the end of the caudal fin). Specimen’s sex was determined by the shape and length of the genital papilla with a stereo microscope (Gandolfi et al. 1991). Genital papillas in males are slender like and considerably longer then the female`s, while female`s beside being shorter are almost round (Figure 5). To reduce the potential mistakes, the gonads were extracted from every specimen and were compared to the genital papillas to see if the result matched.

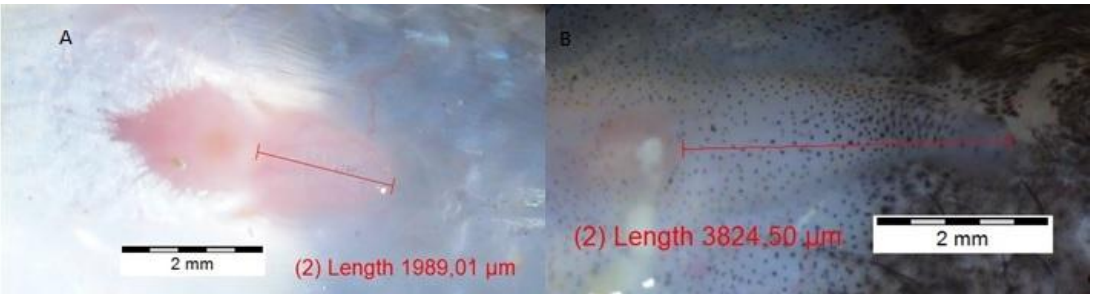

Figure 5. Black goby genital papillas; female`s papilla (A), male`s papilla (B)

3.5 DATA ANALYSIS

Based on the obtained data we prepared correlation diagrams between the otolith length and otolith weight both from the pellet samples and the caught specimens (fish). Otolith data were afterwards log-transformed and tested with linear regression in the programme Microsoft Excel. Linear regression data were displayed in the following order (p = x, 95% CI [Min, Max]). Otolith weight/length relationship was obtained by dividing otolith length by otolith weight. Those obtained data were grouped as follows: >1 (0.00-0.99), 1 (1.00-1.99), 2 (2.00-2.99), 3 (3.00-3.99), 4 (4.00-4.99), 5 (5.00-5.99) and 6 (6.00-6.99).

To assess the consumed biomass, an exponential curve was fitted to the total body length/otolith length diagram for the caught samples. The given formula was afterward used to calculate the fish weight from the pellet samples ($y=0.1088e^{1.0896x}$). Otolith length/ total body length diagram for the fish sample was created to obtain the formula

(W=0.3777TL + 0.5326), which was afterward used to convert otolith length data from the pellet samples into total body length data. To be certain that the used formulas were correct, the data on which they were obtained was proven statistically significant using linear regression.

The age of black goby was derived from the table from Fabi and Giannetti (1985). The table was reduced to constants which were multiplied by the total body length, to obtain the age of certain specimen. Data in the table was grouped according to age and total body length of the specimens. For the data to be comparable between the pellet samples and the caught specimens, it had to be converted in percents, due to a much smaller sample size of caught fish. Those data are grouped as described before >1 (0.00-0.99), 1 (1.00-1.99)...14(14.00-14.99).

Differences in total body length samples and total body weight samples from Strunjan and Sečovlje were tested for statistical significance using one-sample T-test with the programme IBM SPSS Statistics (IBM Corp. 2011).

The PCA (principal component analysis) biplot was created in R statistical environment (R Core Team, 2019) and ArcGIS software (ESRI, 2019) to obtain all Black goby morphometric variables simultaneously and thus investigate differences between pellet sample locations and caught specimens. To bias sample size in all comparing groups (nature, Sečovlje and Strunjan), a random subsampling technique was applied in R (R Core Team, 2019). In Strunjan and Sečovlje, ten (10) random replications of subsamples with the similar sample size as in the nature group were performed. Additionally, all subsamples were then compared for possible significant differences in the first PCA component, which loaded 96% of size variability, with the ANOVA analysis and Dunn post-hoc tests in R. Finally, additional 100 otoliths per site were tested from Sečovlje, Strunjan and Debeli rtič. Those otoliths belonged to the study of Lipej et al. (2016). Their size groups, determined with h-cluster analysis based on several otolith morphometric variables, were compared in similar locations along the Slovenian coast.

4 RESULTS

4.1 LENGTH/WEIGHT RELATIONSHIP OF OTOLITHS

4.1.1 Otoliths from pellets

Altogether, 1000 black goby otoliths from the pellets, were analysed, 500 from each location. A significant length/weight correlation was measured from the otoliths found in the pellets ($R^2 = 0.9187$; $p < 0.01$, 95% CI [2.61, 2.70 (Figure 6). Dividing the same length and weight values, from the pellets, by groups, it is noticeable that more than 90% of all measured otoliths are from a range from 1-3, with number two being the most abundant with 45% (Figure 7). The otolith weight/length mean value was 2.59 ± 0.86 mg/mm and the range spread from 0.75-6.10 mg/mm.

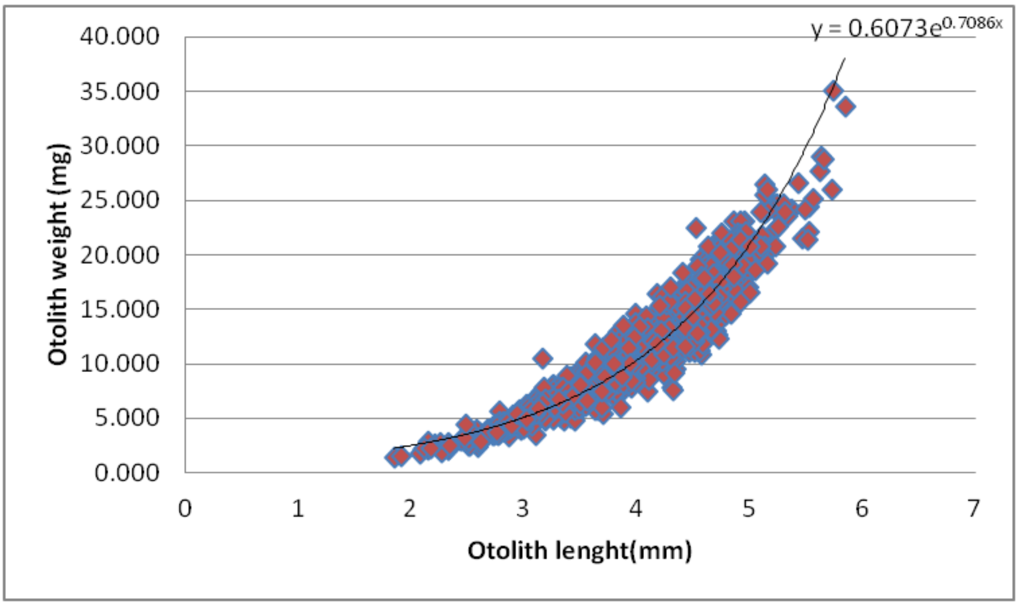

Figure 6. The relationship between otolith`s length and weight in otoliths, obtained from the pellets.

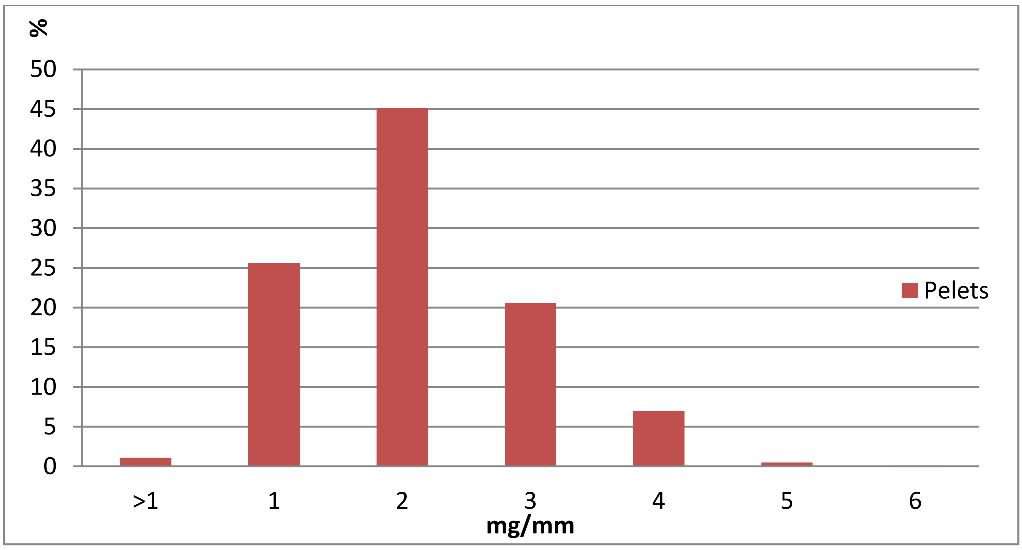

Figure 7. Otolith`s weight values divided by otolith`s length values, obtained from the pellets.

4.1.2 Otoliths from gobies

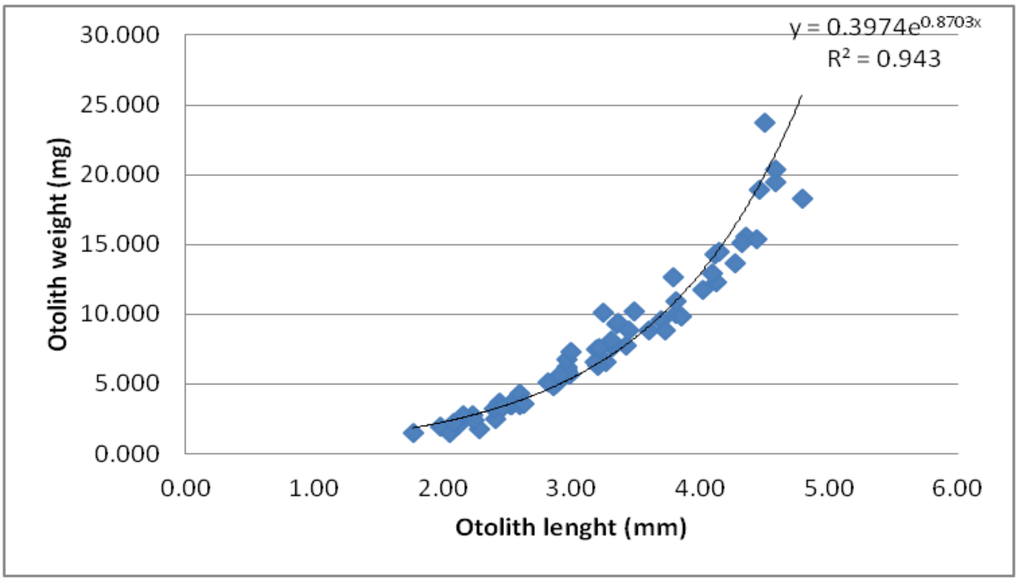

Figure 8. Graph showing the relationship between otolith`s length and weight, from the live specimens

From every black goby both otoliths were isolated, however, measurements were conducted only on one otolith. Positive correlation in length/weight ratio is noticeable even from the otoliths of live specimens ($p < 0.01$, 95% CI [2.63, 2.90]) (Figure 8). From the divided values of otolith length by otolith weight, it is evident that the ratio values 1, 2 and 3 are dominant, with the maximum values slightly above 25%, which altogether sums up to 75% of the total ratio values (Figure 9). From fish samples the mean otolith weight/length ratio value was 3.09 ± 1.35 mg/mm, ranging from the minimal value of 1.02 to the maximum value of 6.59 mg/mm.

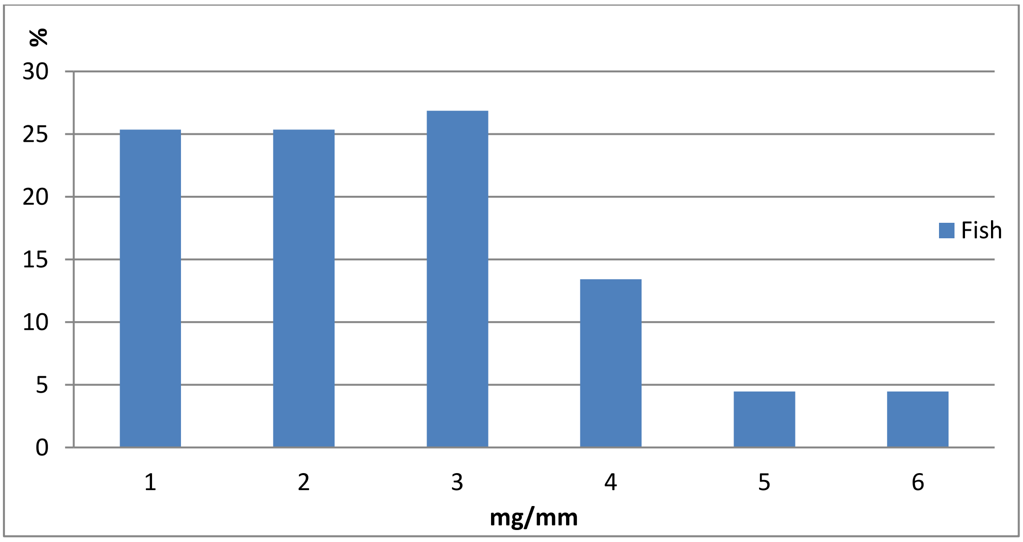

Figure 9. Otolith`s length and weight values grouped by their range values: 1 (0-0.99), 2 (2-2.99)...6 (6-6.59)

4.2 BIOMASS ASSESSMENT

A strong correlation ($p < 0.01$, 95% CI [2.919, 3.06]) between the total body length and weight of the caught samples was obtained, as shown in Figure 10. On the log-transformed data, a linear regression was applied which showed that R^2 value was 0.99.

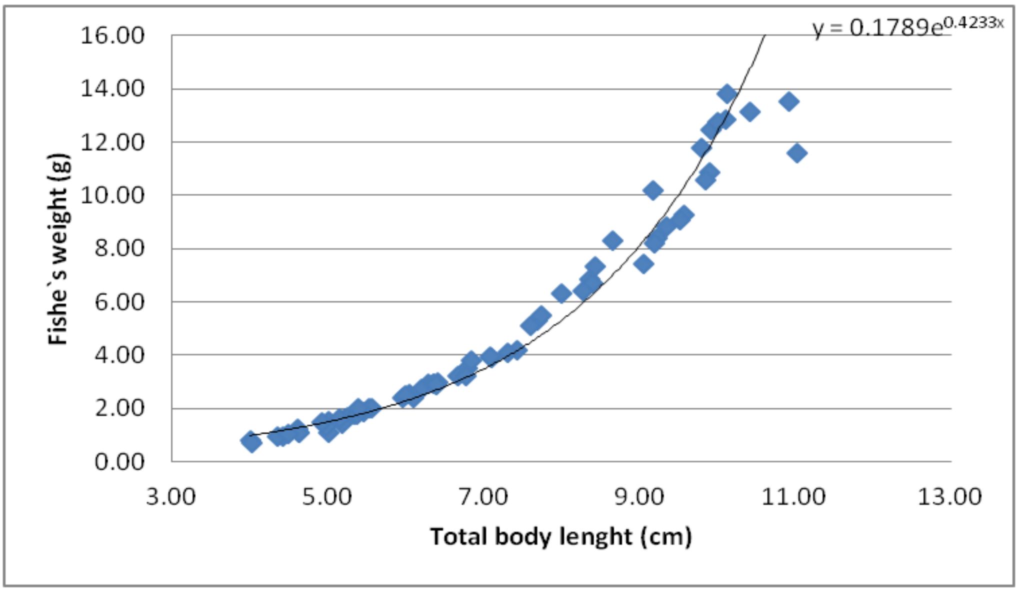

Figure 10 The relationship between fish`s total body length and weight.

A significant correlation in the total body length and otolith length was obtained with regression analysis ($p < 0.01$, 95% CI [0.80, 0.88]). To access the total body length for the samples from the pellets, the following formula was implied, W=0.3777TL + 0.5326, calculated and derived from the fish samples (Figure 11).

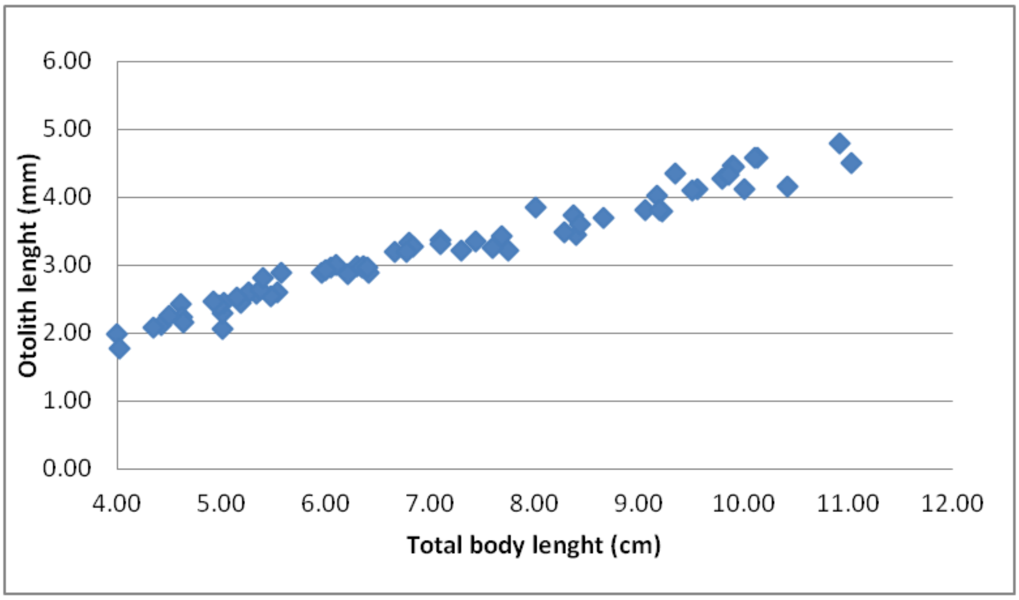

Figure 11. The relationship between fish`s body length and its otolith length.

The derived values ranged from 3.48 to 14.07 cm for both locations together, with the mean value 8.77 ± 1.85 cm, as for the fish samples, the total body length ranged from 4.01 to 11.03 cm with the mean value 7.05 ± 2.00 cm (Tables 1 and 2).

Table 1. The average values for both pellet locations, separated and together, and the fish samples.

AVERAGE	Strunjan	Sečovlje	Σ Pellets	Fish
Total body length (cm)	9.59±1.82	7.96±1.48	8.77±1.85	7.05±2.00
Otolith length (mm)	4.15±0.69	3.54±0.56	3.84±0.70	3.19±0.77
Otolith weight (mg)	12.78±5.64	8.21±3.58	10.49±5.25	7.97±5.19

Table 2. The minimum and the maximum values for both pellet locations, separated and together, and the fish samples

Min-Max	Strunjan	Sečovlje	Σ Pellets	Fish
Total body length (cm)	4.15-14.07	3.48-12.98	3.48-14.07	4.01-11.03
Otolith length (mm)	2.10-5.84	1.85-5.43	1.85-5.84	1.77-4.79
Otolith weight (mg)	2.09-35.07	1.38-26.53	1.38-35.07	1.48-23.79

To access the potential body weight for the samples from the pellets, we used an otolith length/total body weight formula ($p < 0.01$, 95%CI [3.28, 3.60]) from the fish samples ($y = 0.1088e^{1.0896x}$) (Figure 12). The mean body weight from the fish samples was 4.96 ± 3.98 g, ranging from 0.71-13.82 g. The mean body weight calculated for the pellet samples was 9.58 ± 8.08 g, with the minimum value of 0.82 g and the maximum value 63.11 g.

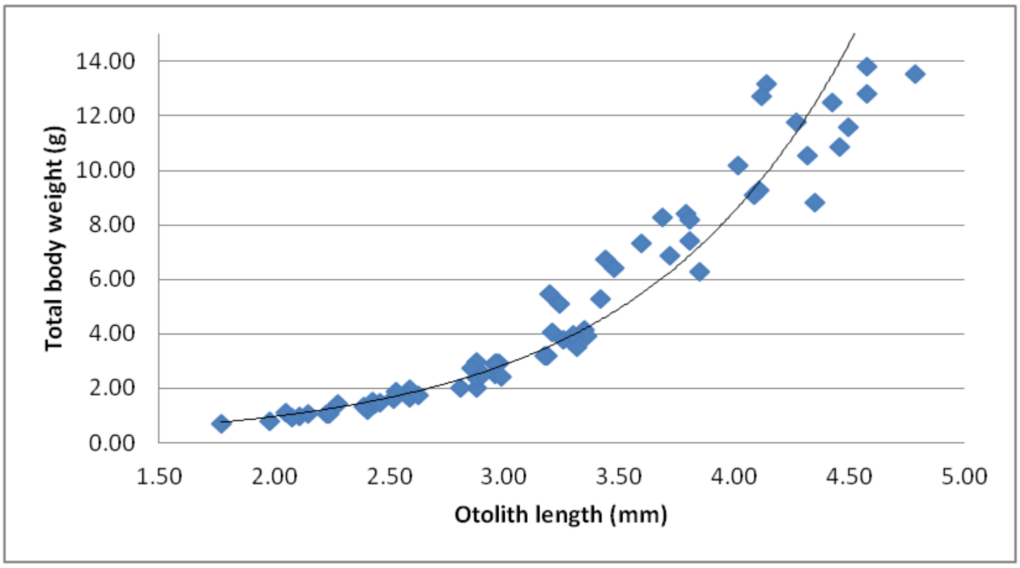

Figure 12. The relationship between fish`s otolith length and its total body weight.

4.3 SELECTIVE PRESSURE

The fish samples varied from 0 to 3 years of age (Figure 13). The predominant age group was the group 0, consisting of more than 50% of all individuals, followed by the group 1, 2 and then 3.

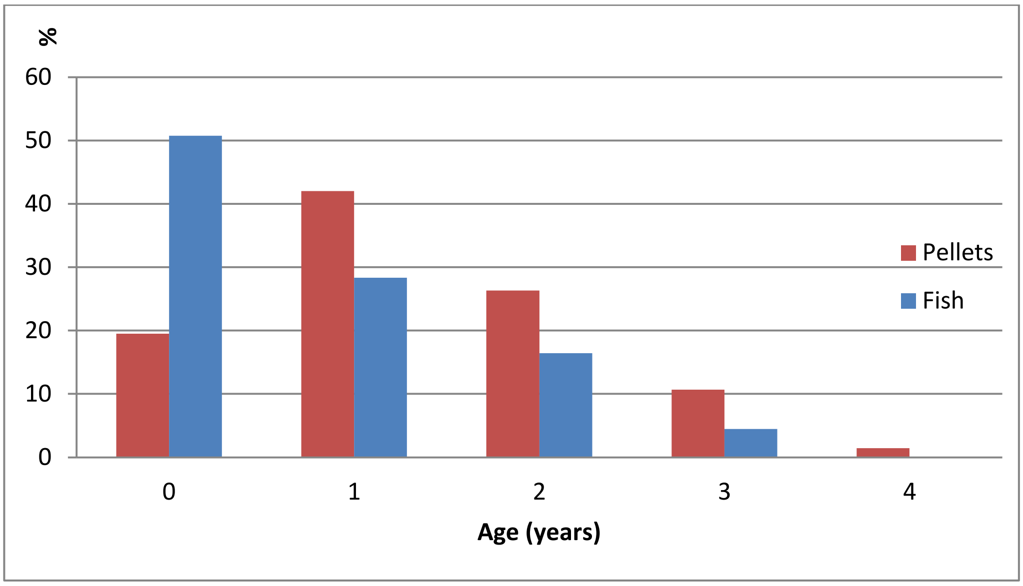

Figure 13. The comparison between age of the captured samples from the pellets and fish

The length groups 5, 6, 9 and 4, were the only groups with the frequency of occurrence with at least 15%. The group sizes of the above listed groups vary from the highest to the lowest, in that particular order (figure 14). It is also evident that shag preyed on larger specimens than are normally distributed in the marine environment (Table 4)

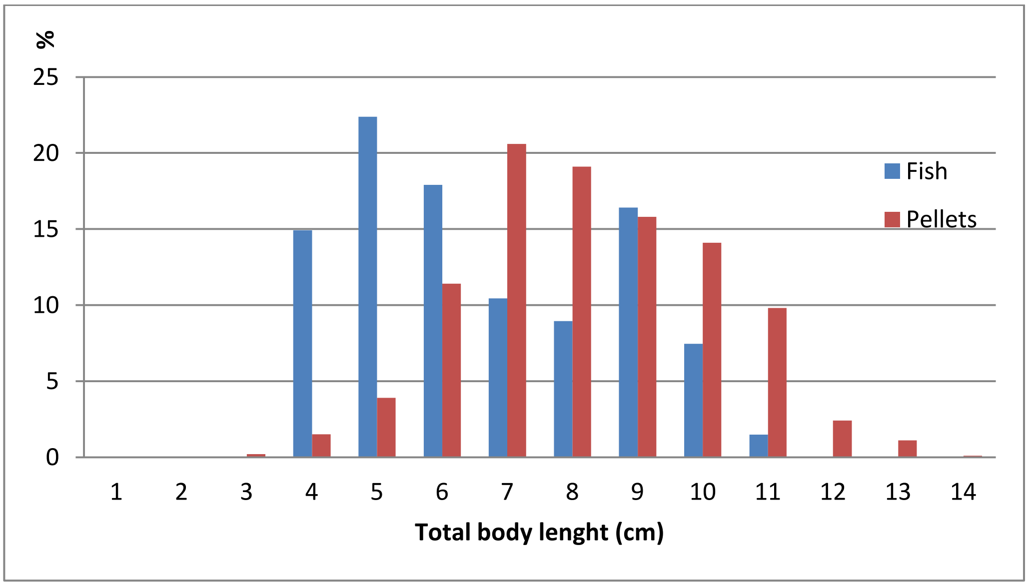

Figure 14. The comparison between fish and pellet samples in the terms of total body length

The age group 0 covers the length groups from up to 7 cm, the age group 1 from 6 cm to 10 cm, age group 2 from 8 cm to 11 cm and the age group 3 ranged from 9 cm to 10 cm of the fish`s total body length (Table 3). From table 4, is noticeable that Mediterranean shag tends to prey on older specimens then the ones we collected from the nature. The average goby age preyed by the Mediterranean shag was 1.33±0.95 years, while the average age for the ones we caught was 0.75±0.89 years (Table 4).

Table 3. The distribution of the black goby in terms of age and total body length from the live specimens (fish)

Total body length(cm)	Age 0	Age 1	Age 2	Age 3	Age 4	total
3						
4	10					10
5	15					15
6	7	5				12
7	2	5				7
8		4	2			6
9		4	5	1		11
10		1	2	2		5
11			1			1
12						
13						
14						
Total	34	19	10	3		

The preyed gobies from the pellet samples varied from 0 to 4 years of age (Figure 13). The most common age group was the group 1, consisting of slightly more than 40% of all specimens, followed by the group 2, the group 0, the group 3 and the scarce group was the group 4. More than 90% of all preyed gobies by the Mediterranean shag had the total body length between 6 and 12 cm, which cover all age groups. The most frequent length group was the group 7, followed by the groups 8 and 9, which had the frequency of occurrence also above 15% (Table 5).

Table 4. A comparison of size, age and weight of black goby (fish) between sampled locations.

Black goby	Size interval (cm)	Mean size (±STDEV) (cm)	Age (year)	Mean age (±STDEV) (year)	Weight (g)	Mean weight (±STDEV) (g)
Σ Preyed by shag	3.48-14.07	8.77±1.85	0-4	1.33±0.95	0.81-63.11	9.57±8.08
Strunjan	4.15-14.07	9.59±1.82	0-4	1.65±0.98	1.08-63.11	12.91±9.33
Sečovlje	3.48-12.98	7.96±1.48	0-4	1.00±0.80	0.81-40.37	6.24±4.65
Collected fish	4.01-11.03	7.05±2.00	0-3	0.75±0.89	0.71-13.82	4.96±3.98

Table 5. The distribution of the black goby (*Gobius niger*) in terms of age and total body length from pellets

Total body length(cm)	Age 0	Age 1	Age 2	Age 3	Age 4	total
3	2					2
4	15					15
5	39					39
6	64	50				114
7	55	151				206
8	13	120	58			191
9	6	54	78	19		158
10		31	57	51	2	141
11		14	52	24	9	98
12			15	4	4	24
13			3	8		11
14				1		1
Total	195	420	263	107	15	

4.4 DATA DIFFERENCE BETWEEN THE TWO SAMPLING LOCATIONS

Calculated data shows a slight deviation between the two sampling locations. From the otolith weight/length data is noticeable that in the area of Sečovlje roost, the Mediterranean shag preys smaller gobies than in Strunjan. In both locations the size class which correspond to the W:L ratio 2 is the most common; however, in Sečovlje the second most common is ratio 1, while in Strunjan is the ratio 3 (Figure 15).

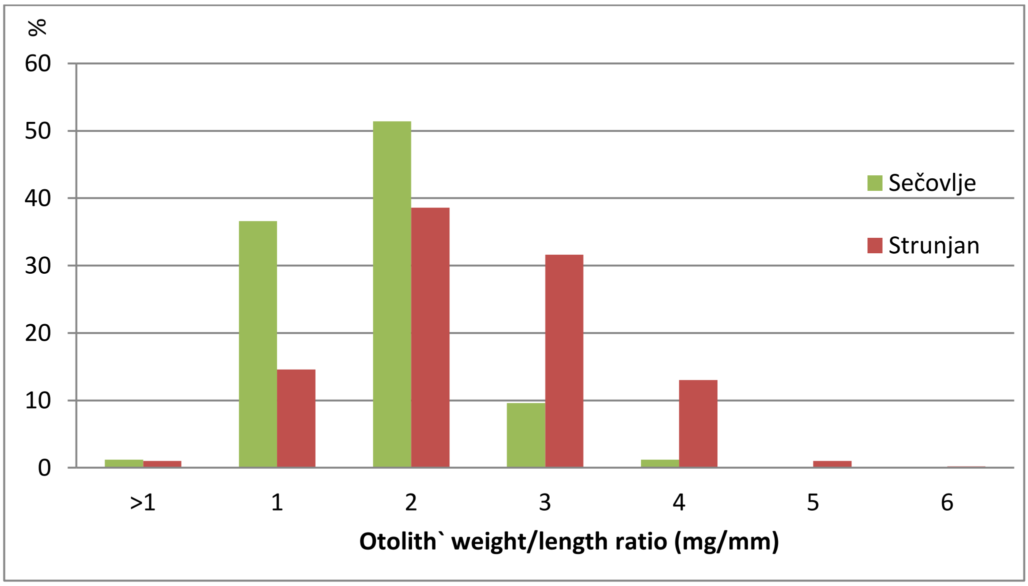

Figure 15. Comparison of data among the two sampling locations

In Strunjan the total body length extends from 4.15 to 14.07 cm, with the average value 9.59 ± 1.82, while in Sečovlje it starts at 3.48 and goes up to 12.98 cm, with the average value situated at 7.96 ± 1.48 (Table 4). The difference in the average length values is proven to be statistically significant with one-sample T-test (df=499, p<0.01) and it is 1.63 cm. In Sečovlje the length group 7 was the most common, followed by the group 8 and 6, altogether making 73.2%. The peak in Strunjan appears in larger size groups than in Sečovlje. The highest is the group 10 with 22.2%, followed by the group 9 with 21.2% (Figure 16).

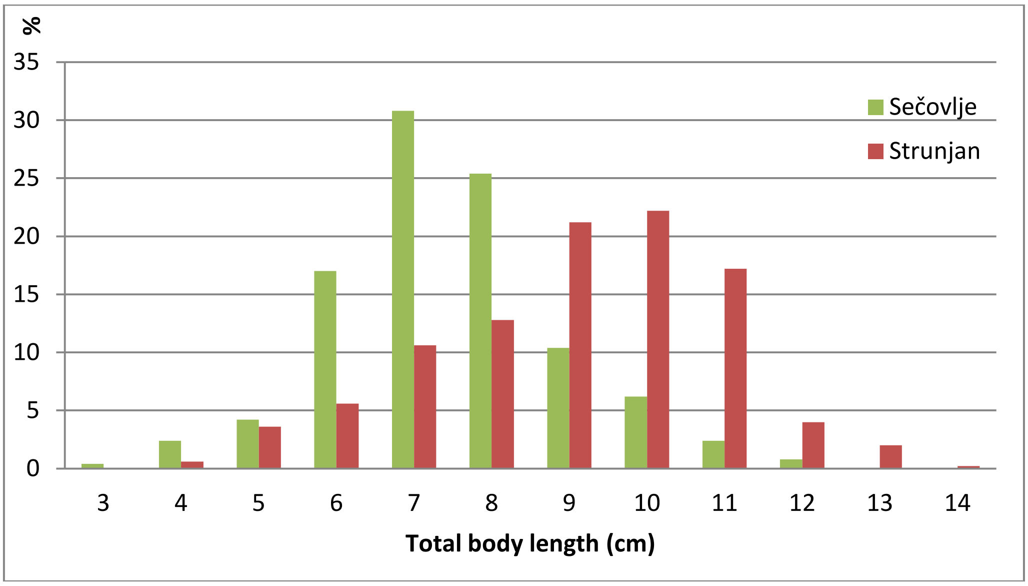

Figure 16. The comparison of data on total body lengths of fish found in both sampling locations.

The data dealing with fish weight shows a similar pattern. In Strunjan the smallest preyed fish weighed 1.08 g and the largest weighed 63.11 g. The average value was 12.91 ± 9.33 g. In the Sečovlje roost, the smallest caught fish weighed 0.81 g and the largest had 40.37 grams, in average 6.24 ± 4.65g (Table 4). From the results of one-sample T-test (df=499; p<0.01), the difference in average fish weights was statistically significant. It equalled 6.67 g, which is more than the average weight of the preyed fish in the Sečovlje saltpans (Table 4). In Sečovlje, the group 3-6 g represents the body weight peak with the frequency of 47% and the other groups with more than 5% are the groups 6-9 and 0-3 g, altogether making 86.2 % of all preyed gobies in Sečovlje. In Stunjan, there is not such peak present, as it was in Sečovlje. The data from Strunjan shows more even distribution among the groups. The most frequent group was the group 6-9 with 16.8%, followed by the group 3-6 with 15.8% and the group 9-12 with 15.2%. All other weight group had the frequency below 15% (Figure 17).

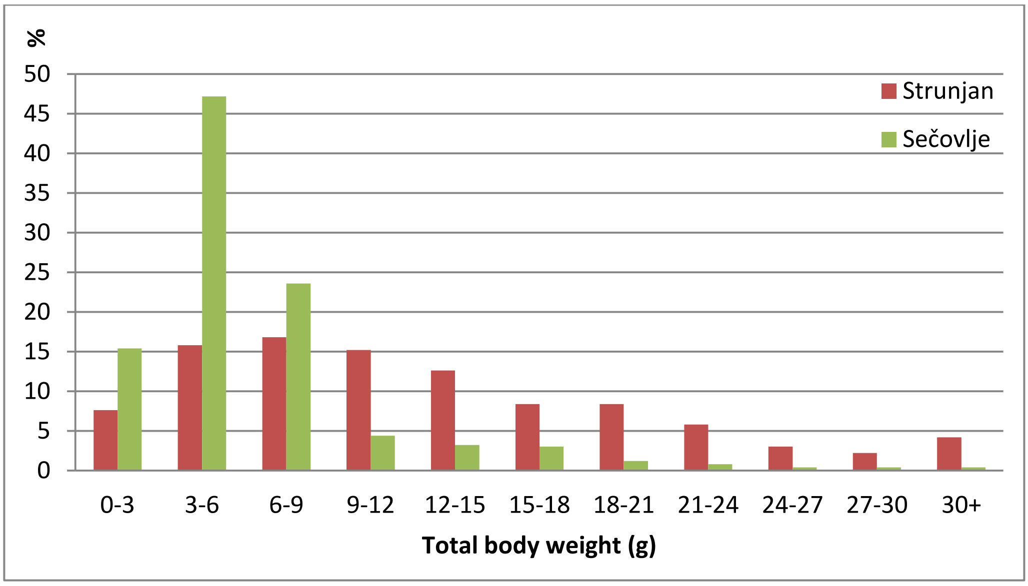

Figure 17. Data set of the total body weight divided by groups, from the two sampling locations

In terms of the age of the preyed gobies, the data between locations points out different results, although, the same five age groups (age group 0, 1, 2, 3 and 4) were present in both locations (Table 5 and 6).

Table 6. The distribution of the black goby in terms of age and total body length from Sečovlje.

Total body length(cm)	Age 0	Age 1	Age 2	Age 3	Age 4	total
3	2					2
4	12					12
5	21					21
6	48	38				86
7	41	112				153
8	9	80	38			127
9	2	18	26	6		52
10		7	13	11		31
11		2	6	3	1	12
12			3	1	1	4
13						
14						
Total	135	256	86	21	2	

Table 7. The distribution of the black goby in terms of age and total body length from Strunjan roost.

Total body length(cm)	Age 0	Age 1	Age 2	Age 3	Age 4	total
3						
4	3					3
5	18					18
6	16	12				28
7	14	39				53
8	4	40	19			64
9	4	36	52	13		106
10		24	45	40	1	110
11		12	45	21	8	86
12			13	4	3	20
13			3	8		11
14				1		1
Total	60	164	178	85	12	

In Sečovlje roost the most frequent preyed age group was the group of the 1 year old gobies with more than 50%, followed by the group 0 with 27%. In Strunjan, the Mediterranean shag preyed older individuals than in Sečovlje. Namely, the age group 2 was the most frequent with 35.6% and was followed by the age group 1 with 32.8 % (Figure 18).

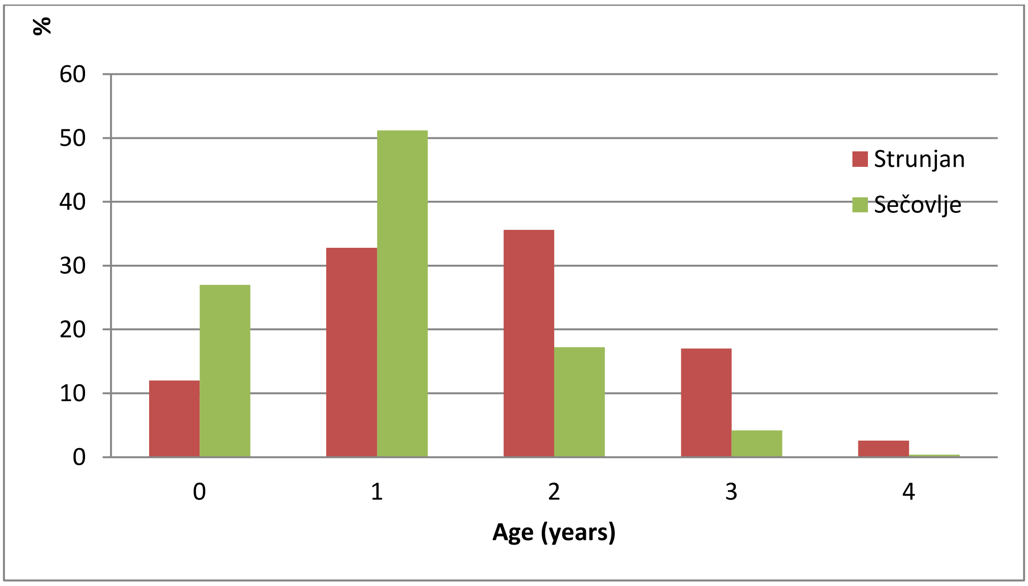

Figure 18. A comparison of preyed fish by age groups between two sampling locations.

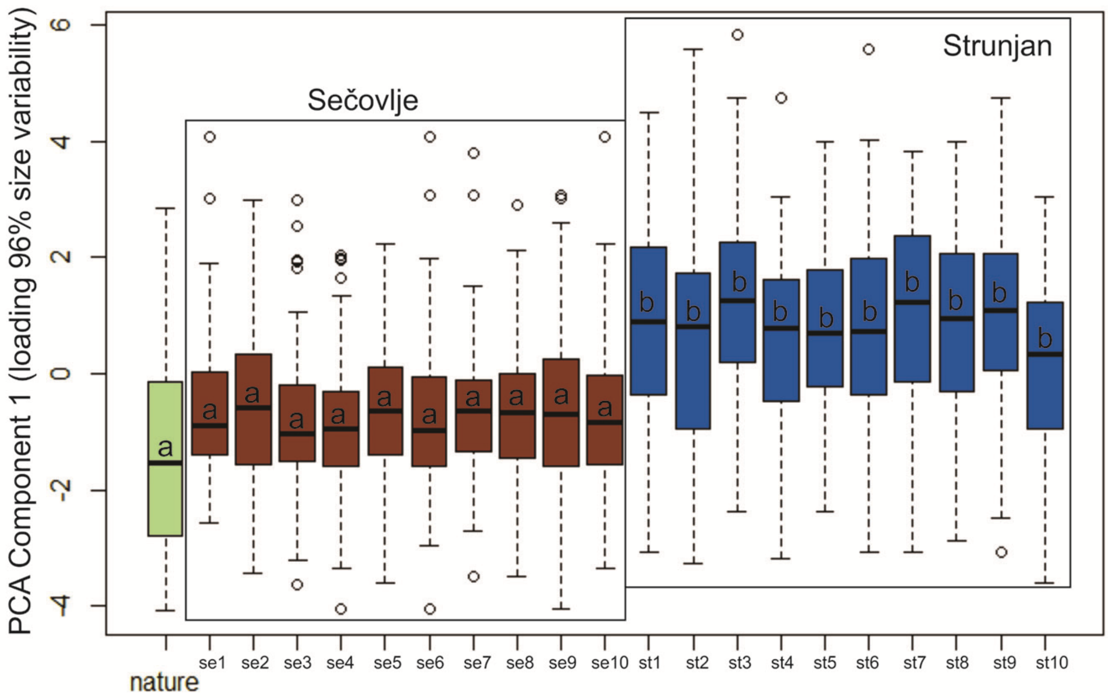

Figure 19. The difference between length classes from the Sečovlje bay (red bars) (blue bars), Strunjan bay and the samples caught in Piran ("nature", green bars).

By comparing the morphometric variables (total body length, fish weight, otolith length, otolith weight) represented with the first PCA component, clear differences between groups (factors) nature and Strunjan emerged. As mentioned above, black gobies caught in nature were of similar size as those preyed by the Mediterranean shag in Sečovlje (Figure 19). However, if we compare all sampling sites and morphometric variables, derived from the otoliths, in the multi-dimensional PCA environment, the same pattern is recognizable (Figure 20). In Strunjan the Mediterranean shag is preying on larger prey than in Sečovlje saltpans.

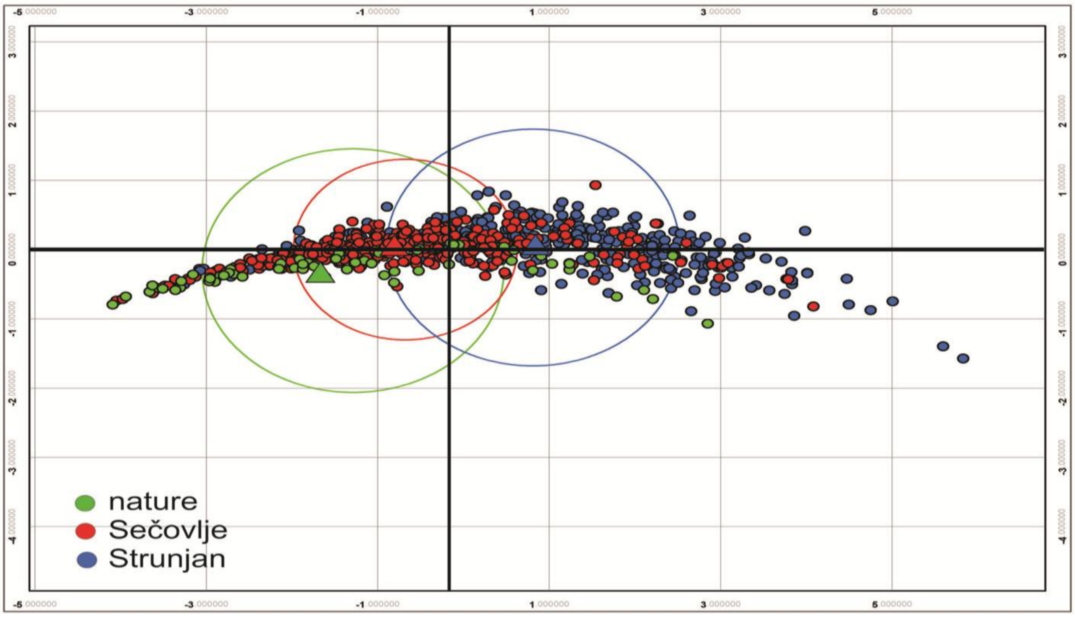

Figure 20. Difference in prey size parameters from Sečovlje, Strunjan and nature; x axis = PCA component 1 (loading 96% of size variability), y axis = PCA component 2 (loading 3% of size variability).

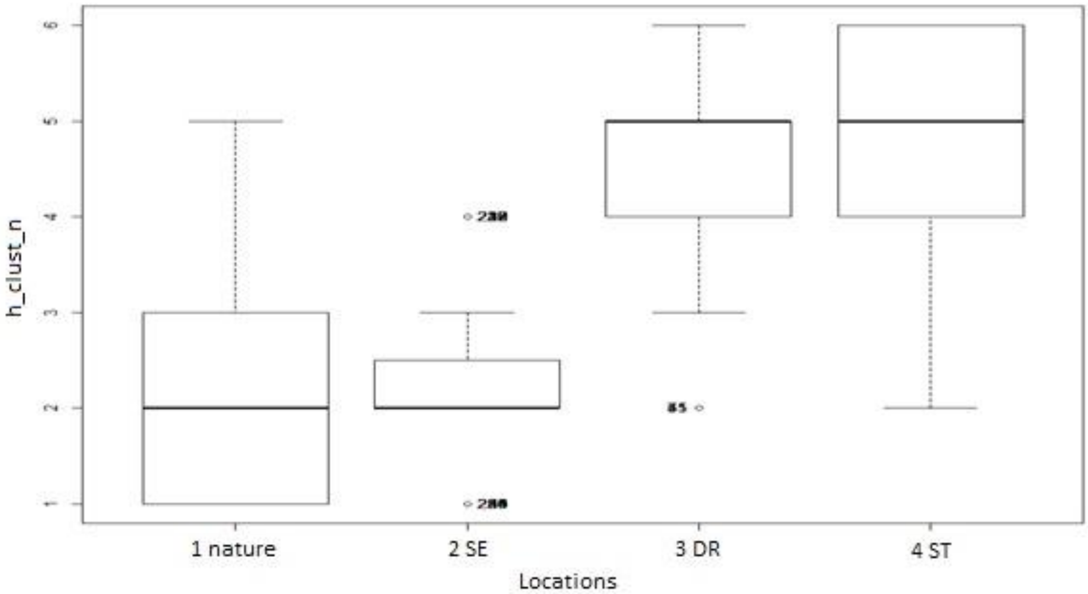

Figure 21. Difference in size classes (determined with h-cluster analysis of several morphometric variables) between Sečovlje (SE), Debeli rtič (DR), Strunjan (ST) according to the data obtained from Lipej et al. (2016) and the specimens caught from nature (narava)(this work)

To confirm our result a similar test was done on the otolith samples obtained from a previous study, performed by Lipej et al. (2016) and the result were more or less the same (Figure 21). Once again the result from Sečovlje saltpans was the one most similar to the size of fish caught in nature. Mediterranean shag preys larger fish in Strunjan bay and in Debeli rtič, than it does in Sečovlje.

5 DISCUSSION

5.1 CRITICISM OF THE USED METHODOLOGY

5.1.1 Pellets

The use of pellets in accessing the quantity and variety of bird food intake has become one of the most popular methods in analysing the food consumption of the piscivorous birds (Suter and Morel 1996). To collect them requires little effort and in most cases is the only available method for accessing shag`s diet. Pellet analysis is also a cheap and non-invasive method, which does not cause any disturbance to the birds. The method is practical for collecting large amount of samples in a short period of time (1 pellet contains 42 prey items on average, as shown by Lipej et al. (2016)). Laboratory work for the analysis is rather easy and requires little space. Pellets can be used to acquire a variety of information. They are used for calculate the prey species composition as well as the average length and mass of prey fishes and the estimated fish mass per pellet (Carss 1997). In some studies the daily intake was calculated for birds.

While choosing the pellet analysis for diet analysis some caution has to be taken into consideration as there are some potential drawbacks to the method. In the process of digestion otoliths tend to erode to some extent (smaller the fish greater the erosion) or could be even completely digested. This can lead to miscalculation in size estimation and is expressed in the wrong estimation of consumed fish`s biomass. Several authors pointed that in their study the results from analysed otoliths from pellets greatly varied from the otolith`s measurements before feeding shags (Johnstone et al. 1990). However, it seems that the results given from researches in captivity differ from the results in the wild. Shags from captivity regurgitated pellets once every day, while the results from Russel et al. (1995) shown that shag in the wild regurgitated once every 4 days. Suter and Morel (1996) reconstructed the fish sizes from pellets, considering only uneroded otoliths, and the results were the same as the results they gathered from fish school they caught in the same location.

5.1.2 Morphometry

To minimize the potential error in morphometry, every otolith was measured in the same position, vertically from the rostrum to the postrostrum, while the otolith was on its ventral side. Although, due to its roughness it was not always possible to measure length and width on the otolith`s ventral side. Morphometric measurements on caught fish specimens may have some bias. Several fish were larger in length than was the falcon tube in which they were frozen. Even after defrosting the specimen was still curved. While extracting otoliths from the live specimen, is possible that otoliths were damaged with scalpel or tweezers and then overlooked. To reduce the human bias, every measurement was conducted by the same person in the controlled environment.

5.2 PREDATION PRESSURE

Shag produces 1 pellet every 3.7 days. In the northern part of the Adriatic Sea, one Mediterranean shag`s pellet contains 41.2 prey items on average. During the period of one month the value of prey items, rises to 334.05. After a year, 4009 prey items are regurgitated from one shag only. In the Gulf of Trieste, during the summer months the number of Mediterranean shag reaches its peak at 2000 individuals. Multiplying the number of birds present in that area by the number of preyed items found in pellets on the yearly basis, we access the total number of preyed individuals for the Mediterranean shag in the Gulf of Trieste. The whole population of the Gulf of Trieste, on the yearly basis, consume 8.017.300 individuals. Almost half (49.40%) of preyed items, belongs to the black goby, resulting in 3.96 million black gobies being eaten by Mediterranean shags in the Gulf of Trieste. The average body weight for the black goby was estimated at 9.58 g from the pellet sample, and adding it to the number of preyed black gobies, the total of 37.942 kg or approximately 38 t of black gobies are consumed by the Mediterranean shag in the Gulf of Trieste every year

The black goby is the Mediterranean shag`s main prey with 50% in terms of abundance number in the Northern Adriatic (Lipej et al., 2016). Combined results from both studied locations confirm our hypothesis that the Mediterranean shag along the Slovenian coast prey on larger specimens than what we collected from the nature, in terms of length, age and weight. However, separated results show a difference in predation pressure between locations. Shags that roost in Sečovlje prey on smaller preys than shags roosting in Strunjan and Debeli rtič. It is possible that those differences between Sečovlje and Strunjan occur due to water transparency. Water transparency is highly affected by the resuspended material from the sea bottom. The maximum water depth in Strunjan is 14 m, while in Sečovlje is 12 m, meaning that the resuspended material in Strunjan has more space to distribute in the water column. The results from Secchi disc transparency method, for the past 8 years of monitoring, show that in Sečovlje the Secchi disc depth is 6.9 ± 2.2 m, while in Strunjan is 8.5 ± 2.8 m (NIB-MBP database). The visibility in Stunjan is better which probably allows to the Mediterranean shag to locate the prey from the surface and directly dive to collect it, while in Sečovlje Mediterranean shag is not able to detect the prey from the surface. It is possible that shags have to dive and search for the prey from underwater.

The other possible explanation could be that under the mariculture facilities the environmental conditions in term of available food, derived from mariculture, may affect the abundance and density of black gobies. In Sečovlje, despite the mussel farm, is also a fish farm present. The food excess from the fish farm makes that area suitable for the younger/smaller specimens to gain weight with little effort and is probably one of the reasons why are smaller specimens found in Sečovlje.
Our results from Sečovlje and nature suggest that shags roosting in Sečovlje prey on the approximately the same size of black gobies as were caught from the nature. However, the size of black gobies that we sampled, greatly differ from the size of gobies Fabi and Giannetti sampled in their study in 1985 (Figure 22 and 23).

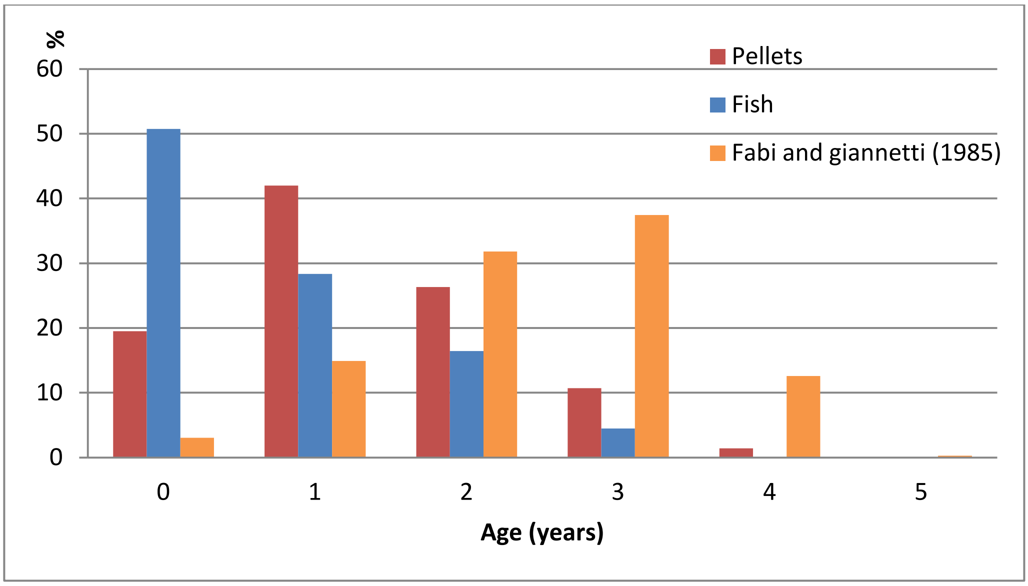

Figure 22. The comparison of age data between our results (Pellets and Fish) and the results of Fabi and Giannetti (1985).

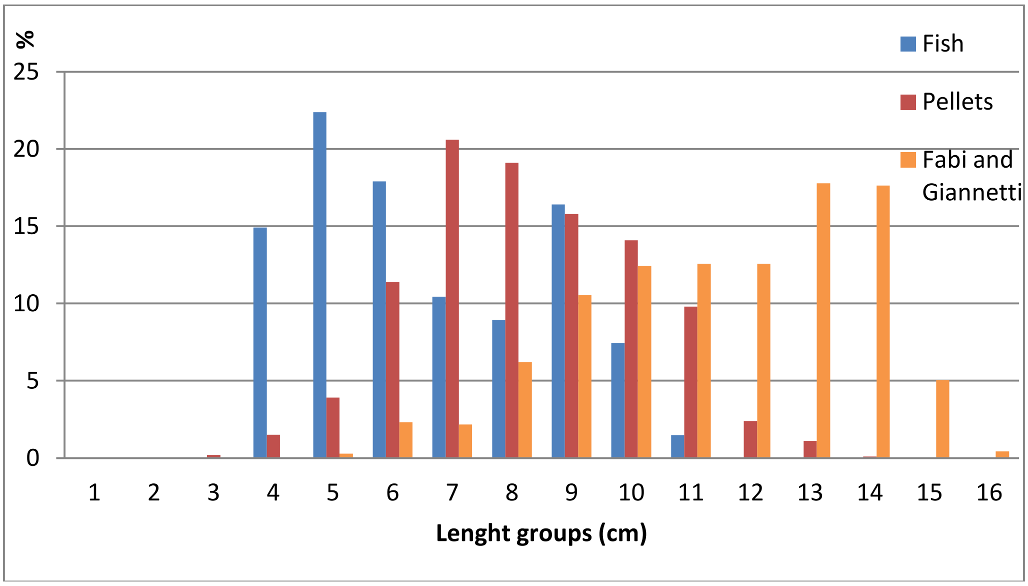

Figure 23. The relationship between our data and the data from Fabi and Giannetti (1985).

Those differences probably occurred due to different sampling methods. They collected their samples by trawl, while in this study sampling was done manually using SCUBA equipment. The differences may be attributed to the depth of samplings. Different substrates between the two sampling locations also play a role in the goby length. In central Adriatic are found larger specimens of black gobies (table 8). It is possible that in the northern Adriatic the black gobies do not reach the same length as they do in the central Adriatic, due to different environmental factors, or the predation pressure from the Mediterranean shag.

Table 8. Comparison of the maximum lengths of Black gobies, gathered by different authors (Boban et al. 2013).

Area	Study	Locality	TLmax (mm)
Adriatic Sea	Boban et al. (2013)	Adriatic Sea	145
	Tortonese (1975)	Adriatic Sea	150
	Fabi & Froglia (1983)	Adriatic Sea	165
	Fabi & Froglia (1984)	Adriatic Sea	160
	Fabi & Giannetti (1985)	Adriatic Sea	165
	Jardas (1996)	Adriatic Sea	160
	Locatello et al. (2002)	Venetian Lagoon	149
	Rasotto & Mazzoldi (2002)	Venetian Lagoon	139
	Mazzoldi & Rasotto (2002)	Venetian Lagoon	135
	This study	Slovenian coast	141

In their study, Bordjan et al. (2013), mention that the age distribution among shags in Strunjan during the summer was 50% juveniles and 50% adults. During the summer when the newborns arrive at the roosting habitats, in Sečovlje, the abundance of immature individuals was 80% (Škornik 2012). Immature individuals are forced to switch their location due to intraspecific competition which was observed in Strunjan, where a mature individual forced out of the larger buoy an immature individual (Bordjan et al. 2013). In Debeli rtič is presumably the same, as only 10% of roosting shags represents immature individuals (Škornik 2012). Thus Sečovlje saltpan is the only available location to move. This statement is supported by our results from Figures 19 and 21, from which is noticeable that shags roosting in Strunjan and Debeli rtič, feed on larger prey than shags roosting in Sečovlje. Those results are confirmed and fit well also with the results of Lipej et al. (2016), where shags roosting in Strunjan and Debeli rtič preyed on larger prey than shags roosting in Sečovlje (Figure 20). During the last bird counts, the number of shags present along the Slovenian coast has been increasing.

5.3 ASSESSMENT OF PREDATION CAPACITY OF SHAG

Several studies have been done about the shag`s pellet regurgitation. The results differ among in order of whether it was run in the wild or in captivity. In captivity, shags produce one pellet every day, and it was assumed to be its daily food intake (Russell et al. 1995). However, in the wild, shags produce one pellet every 1 to 7 days, with one pellet every 3.97 (approximately 4) days on average (Russell et al. 1995). While studying the Mediterranean shag`s diet, Lipej et al. (2016), discovered that a pellet contained 41.2 prey items on average, of which 49.40% belong to black gobies (20.35 otoliths). Dividing the number of otoliths by the average time it takes the shag to regurgitate one pellet, the number of potentially regurgitated otoliths for one day is accessed, counting 5.13 otoliths per day. From our study the calculated average biomass for consumed fish was 9.58 g for both locations together. From this data, the calculated average daily intake of Mediterranean shag for both locations is 49.14 g of black gobies. However, if we separate the results for both locations individually, in Sečovlje are daily consumed 32.01 g and in Strunjan 66.23 g. Not to be confused, those results represent the daily food intake for the black goby only. Fortunately, Lipej et al. (2016), in their study calculated that black goby abundance was 61.49%. Combining our result on the daily food intake for the black goby with their results on the abundance, the Mediterranean shag`s daily food intake totals 79.91 g/day. These results do not fit well with those of Sponza et al. (2010). They calculated shag`s daily food intake to range from 350 g/day to 400 g/day, from May to October, which is 5 times our result. Different results are due to different methods for obtaining the same result. They divided the calculated biomass with the number of pellets collected in the period of one month, while in this study we relied on the regurgitation of pellets and the morphometric measurements of the gathered otoliths. While transforming their data from otoliths to biomass, they used a formula calculated from the pellets belonging to the great cormorant (*Phalacrocorax carbo*). The great cormorant is a larger bird than the Mediterranean shag, meaning that it has to consume more food daily and is a pelagic predator. However, in this study the regurgitation of pellets was tested on the European shag (*Phalacrocorax aristotelis aristotelis*), so we believe that our results should be more precise hence the European and the Mediterranean shag belong to the same species.

5.4 NATURE CONSERVATION IMPLICATIONS AND POSSIBILITIES FOR RESEARCH UPGRADE IN THE FUTURE

To quantify the predation pressure that the Mediterranean shag is making on the overall population of black gobies, it would be of great value to continue gathering pellets monthly in the time of the shag's presence (the warmer part of the year), to check if there are any changes in term of monthly or seasonal differences. The observation of any kind of changes in the size or lengths of otoliths, from a constant monitoring, could indicate that an alteration in the population of black gobies had been done.

To calculate the daily intake of the Mediterranean shag, we believe that several studies on the pellet regurgitation, both in the captivity as well as in the wild, should be done in the Northern Adriatic Sea. It should consider 24h surveillance of the testing birds, to minimize the pellet loss, and to test if Mediterranean shags regurgitate only in their roosting habitats, or even in other places, or during flight. If the study on regurgitation is not possible to accomplish, a new approach to gain the data on the daily food intake of the Mediterranean shag is needed.

To test if our hypothesis regarding older shags roosting in Strunjan and younger in Sečovlje, is still valid, a similar study as the study from Bordjan et al. (2013), should be of great help. As their study was carried out in 2013, some new data would definitely help us in figuring out, if this trend is consistent and why is a difference present in preyed goby`s length from Sečovlje saltpans and Strunjan.

Since the Mediterranean shag is an endangered species, studies, which deal with its biology and ecology, are very important. It does not breed in the Gulf of Trieste due to inadequate habitats, although, the food availability in the studied area offer the potential to support a high number of specimens. This is confirmed also by the rising number of birds, which came to the studied area at the end of eighties of the last century. The use of artificial islets, as bird breeding habitats, shows good results for the conservation of waterbird species (Morris et al. 2001). The creation of a few islets in the gulf of Trieste could potentially help Shag`s population to start breeding in the Gulf of Trieste and potentially raise its population number.

6 CONCLUSIONS

From this study it is noticeable that the Mediterranean shags prey on different prey size specimens of the black goby among the two sampled areas. However, they are not preying on a certain length/age group, as specimens from different length/age structure were present in both sampled locations. Shags roosting in Strunjan tend to prey on larger/older prey in comparison with those roosting in Sečovlje, due to different biotic and abiotic factors. The fact that larger/older specimens of the shag`s population roost in Strunjan than in Sečovlje, this could be a possible explanation on why this difference is present. Although, comparing our results obtained from the pellets and the results obtained from fish samples from the nature, is reasonable to explain that shags roosting in Strunjan tend to feed on older population of black gobies. Due to a lack of information about the population size of black gobies inhabiting the Gulf of Trieste and the missing information on how the predation pressure from the Mediterranean shag affects black goby population, additional studies need to be made. Otoliths length, width and weight among them significantly correlate, as well as with the fish`s total length and weight. This correlation can be used to calculate the consumed fish`s biomass from the pellets. The pellet method used in this study helped us to understand the impact that the Mediterranean shag is doing on the different length and age classes of black goby in the Slovenian part of the Adriatic Sea.

7 SUMMARY

Mediterranean shag (*Phalacrocorax aristotelis desmarestii*) is a piscivorous marine bird that inhabits the Mediterranean and the Black Sea. Although it does not breed in Slovenia, due to inadequate habitats and human disturbance, during the summer 11% of its whole population can be seen along the Slovenian coast. Mediterranean shag is highly susceptible to the human disturbance, oil spills and habitat destruction, making him listed on Annex 1 of the Birds Directive (2009/147/EC). Mediterranean shag nests in bushes found on small isles. Nearest colonies are found in Croatia, with the biggest being on Brijuni Archipelago. After the breeding season, they migrate to the Gulf of Trieste, which is a suitable foraging habitat. The Gulf of Trieste is a shallow part of the Adriatic Sea with the depth of 33 m measured in Piran, although rarely exceeding 25 m. In Slovenia, shag roost on artificial buoys from shellfish farms. Mediterranean shag is considered to be an opportunistic predator. In the northern Adriatic Sea black goby (*Gobius niger*) is represented as the favourite shag`s prey with IRI% (index of relative importance) being 65%. The focus of this study was to determine the size range of the black goby upon which the Mediterranean shag is feeding, and test whether this size represents smaller or larger specimens from the population. Mediterranean shag regurgitates pellets, which consist of hard undigested parts of preyed specimens, mostly otoliths. The collection of regurgitated pellets and its analysis was proven a good method for analysing the diet of piscivorous birds. The method is cheap and offers minimal disturbance to the bird. From two communal roosting habitats in Slovenia, Sečovlje and Strunjan, 1000 black goby otoliths were analysed, 500 from each location. To test the normal distribution of gobies from their population, additional 67 live specimens were collected manually with the help of SCUBA equipment. Collected otoliths were measured in length, width and weight in the lab of Marine Biology Station in Piran. The collected fish samples were measured in total body length, total body weight and is gender were determined. After the extraction of otoliths, the same measurements were conducted as the one done to the otoliths from the pellets.

Strong correlation was found among the otolith’s length, weight and width, from every location. Fish biomass from the pellets was calculated from an equation of total body weight and otolith length derived from the samples caught in the wild. Total body length for the consumed fish was calculated from equation of total body length and otolith length, also derived from the samples caught in the wild. It was calculated that Mediterranean shag preyed gobies from 3.48-14.07 cm in length with the average value of 8.77 cm. The mean body weight calculated for the pellet samples was 9.58 g, with the minimum value of 0.82 g and the maximum value 63.11 g. The daily food intake for the Mediterranean shag was calculated to be 79.91 g/day. In terms of age it preyed older specimens than the ones we collected from the nature. The mean value of age class for the specimens caught from the nature was 0.75, and the average value for the ones preyed by shag was 1.33. Shags roosting in Sečovlje preyed upon smaller and younger prey than the ones roosting in

Strunjan. There was less difference in prey size between Sečovlje and the samples from the nature, than it was for Strunjan. Most probable explanations are that in Sečovlje roost younger/smaller individuals of the Mediterranean shag`s population, and the fact that in Strunjan the water is clear and the predator can locate the prey from air, thus collecting larger specimens.

8 POVZETEK V SLOVENSKEM JEZIKU

Sredozemski vranjek (*Phalacrocorax aristotelis desmarestii*) je ribojeda morska ptica, ki živi na območju Mediterana in Črnega morja. Čeprav v Sloveniji ne gnezdi, zaradi neprimernih habitatov in človeških motenj, se čez poletje 11 % celotne populacije vranjeka nahaja vzdolž slovenske obale. Vranjek je zelo občutljiv na motnje s stani človeka, izlive nafte in uničenje habitatov. S tega razloga je uvrščen na seznamu priloge 1 (Annex 1) ptičje direktive (2009/147/EC). Gnezdi v nizkem grmišču na nenaseljenih otokih. Najbližja primerna gnezdišča se nahajajo na Hrvaških otokih kot so Brijoni in otoki v okolici Rovinja in Vrsarja. Po koncu gnezditvene sezone vranjek migrira v Tržaški zaliv. Ta mu predstavlja idealno prehranjevalno območje, saj globina zelo redko presega 25 metrov. Vranjeki plenijo čez dan, medtem ko se ponoči nahajajo v svojih počivališčih. V Sloveniji so ta počivališča boje vzgajališča školjk. Vranjek velja za oportunistično vrsto, v Tržaškem zalivu najbolj pogosto pleni črnega glavača (*Gobius niger*)(indeks relativne pomembnosti = 65 %).

Cilj naloge je bil ugotoviti katere velikostne razrede črnega glavača pleni vranjek, in v kolikšnem številu. Opravili smo analizo izbljuvkov, skupkov trdih, neprebavljenih ostankov plena, med katerimi so tudi otoliti rib. Izbljuvke vranjeki izbljuvajo na počivališčih. Dostop do njih je zelo lahek in pri tem se ptic ne moti. Metoda preiskave izbljuvkov se je izkazala kot zelo dobra pri analizi prehrane ribojedih ptic.

Analizirali smo 1000 otolitov, izoliranih iz izbljuvkov vranjeka z dveh počivališč v Strunjanu in Sečovljah. Za testiranje normalne distribucije velikostnih razredov, prisotnih v populaciji črnega glavača, smo polovili 67 osebkov črnega glavača. Na izoliranih otolitih smo v laboratoriju na Morski Biološki Postaji v Piranu izmerili dolžino in širino, ter njihovo težo. Ulovljenim ribam smo izmerili dolžino in težo telesa, ter dolžino, širino in težo otolitov. Osebkom smo tudi določili spol po obliki genitalne papile. Ugotovili smo značilno korelacijo med dolžino, širino in težo otolitov z obeh lokacij, ter tudi pri ulovljenih osebkih iz narave. Biomaso uplenjenih rib smo preračunali iz enačbe korelacijskega diagrama med težo ulovljene ribe in dolžine otolitov. Dolžino ribe smo preračunali na podoben način, le da je namesto teže ribe v grafu bila dolžina ribe. Sredozemski vranjek v Sloveniji pleni osebke črnega glavača, ki merijo od 3,48 cm do 14,07 cm, s srednjo vrednostjo 8,77 cm. Srednja vrednost teže uplenjenih glavačev bila je 9,58 g iz razpona od 0,82 g do 63,11 g. Dnevni vnos za sredozemskega vranjeka znaša 79,91 g/dan. Glede na starost, je vranjek plenil starejše osebke od tistih, ki so bili ujeti iz narave. Poprečna starost ujetih glavačev znaša 0,75, medtem ko je poprečna starost uplenjenih osebkov 1,33. Vranjeki, ki počivajo v Sečovljah, plenijo manjše in mlajše osebke v primerjavi z vranjeki, ki počivajo v Strunjanu in na Debelem rtiču. Razvidno je tudi, da so uplenjeni glavači v Sečovljah bolj podobni vzorcu ujetih glavačev, kot so vzorci otolitov iz Strunjana. Verjetni razlogi, ki pojasnjujejo te razlike v velikosti uplenjenih osebkov so, da se v Sečovljah zadržujejo manjši/mlajši osebki populacije vranjeka kot so vranjeki, ki se nahajajo v Strunjanu. Druga možna razlaga je, da do razlike v velikosti uplenjenih glavačev pride zaradi prozornosti/motnosti vode, v olju, katerem lovijo plen. Globina v Strunjanu je manjša kot v Sečovljah, vidljivost pa je znatno bolja, kar pomeni da vranjek lahko zazna plen iz zraka in se potopi tarčno, medtem ko mora v Sečovljah iskati plen pod vodo.

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