

# Summer Fish Migrations in the River Neretva (South-Eastern Adriatic Coast, Croatia) as a Consequence of Salinization

## *Ljetne migracije riba u rijeci Neretvi (jugoistočna obala Jadrana, Hrvatska) nastale kao posljedica salinizacije*

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### Summary

River Neretva Estuary, located in the middle South-Eastern Adriatic coast, is a very important traditional fishery ground and biodiversity-rich ichthyologic area. Historically, the estuary situated in Croatia and Bosnia and Herzegovina was composed of wetlands, lakes, and lagoons, but in the 20th century, it was changed into a large agriculture area, settlements and port facilities. The major flow was channelled with large banks to prevent floods. All these activities enabled significant intrusion of seawater 20 km into the inland area. This led to the marinization of major flow freshwater ecosystems. This article presents data on summer fish migrations along with the major flow of River Neretva, during the May-September period. The temperature and salinity showed that seawater started to enter inland major flow from middle May up to the end of September. In total, the 1,429 fish individuals were caught. The numerically dominant species were *Atherina boyeri* (23.58%), *Sardina pilchardus* (21.62%), *Sparus aurata* (11.20%), *Chelon ramada* (8.19%) and *Solea solea* (7.63%). Dominant species in the total mass were: *Chelon ramada* (43.45%), *Solea solea* (14.27%), *Sparus aurata* (10.14%), *Chelon aurata* (7.66%) and *Anguilla anguilla* (3.75%). The major result of this study points to the fact that major Neretva flow of 20 km in length from the river mouth is under seasonal summer impact of seawater, which leads to salinization and complete marinization of this ecosystem during the warm period of the year. The marinization of the large Neretva flow area creates new nursery and feeding grounds for marine estuarine opportunist fish species, such as small pelagics European pilchard and anchovy, and also enlarge these grounds for the marine estuarine dependent species, such as grey mullets, sea breams, and flatfishes.

### KEY WORDS

fish migrations  
summer salinization  
fish species composition  
River Neretva estuary

### Sažetak

Ušće rijeke Neretve, smješteno usred jugoistočne obale Jadranskog mora, vrlo je važno tradicionalno ribolovno tlo i ihtiološko područje bogato biološkom raznolikošću. Povijesno, ušće smješteno u Hrvatskoj i Bosni i Hercegovini bilo je sastavljeno od močvarnih područja, jezera i laguna, ali u 20. stoljeću pretvoreno je u veliko poljoprivredno područje, naselja i lučke objekte. Glavni tok bio je usmjeren uz pomoć velikih nasipa kako bi se spriječila poplave. Sve ove aktivnosti omogućile su znatan prodor morske vode 20 km u unutrašnjost. To je dovelo do marinizacije slatkovodnih ekosustava glavnih tokova. Ovaj članak daje podatke o ljetnim migracijama ribe u glavnom toku rijeke Neretve, u razdoblju od svibnja do rujna. Temperatura i salinitet pokazali su da je morska voda počela ulaziti u glavni tok u unutrašnjosti od sredine svibnja do kraja rujna. Ukupno je ulovljeno 1429 jedinki ribe. Brojčano dominantne vrste bile su *Atherina boyeri* (23.58 %), *Sardina pilchardus* (21.62 %), *Sparus aurata* (11.20 %), *Chelon ramada* (8.19 %) i *Solea solea* (7.63 %). Dominantne vrste u ukupnoj masi bile su: *Chelon ramada* (43.45 %), *Solea solea* (14.27 %), *Sparus aurata* (10.14 %), *Chelon aurata* (7.66 %) i *Anguilla anguilla* (3.75 %). Glavni rezultat ovog istraživanja upućuje na to da je glavni tok Neretve u dužini od 20 km od ušća rijeke pod ljetnim sezonskim utjecajem morske vode, što dovodi do salinizacije i potpune marinizacije ovog ekosustava tijekom toplog razdoblja godine. Marinizacija velikog područja toka Neretve stvara novo mrijestilište i hranilišta za morske estuarijski oportunističke vrste riba, poput malih pelagičnih riba, kao što su srdele i inčuni, a također proširuje ovo područje za morske estuarijski ovisne vrste, poput cipla, komarče i iverka.

### KLJUČNE RIJEČI

migracije riba  
ljetna salinizacija  
struktura ribljih vrsta  
ušće rijeke Neretve

## 1. INTRODUCTION / Uvod

Estuaries are one of the areas with the highest biological productivity and important role in the functioning of both marine and inland aquatic systems. They serve as nursery

grounds for many marine species and provide a migratory route for fish species [11]. Due to their position within the drainage basin, these areas are characterized by a high level of

human activity that can compromise their ecological integrity, because of increasing urban and industrial development [10, 27]. Although a very important ichthyologic and biodiversity area, the Neretva Delta (Croatia, Bosna and Herzegovina) has not been comprehensively researched with its marine and freshwater ecosystems. Most of the research partially addressed either the recruitment of the fish juveniles [5, 13, 19], individual marine species ecology [3, 21], the ecological significance of some freshwater fish endemic species [19, 31], or the evaluation of significant habitats like Parila lagoon [35]. The recent biodiversity changes and invasions of fish and crabs reported ongoing invasions of blue crab, *Callinectes sapidus* in the brackish ecosystems [14, 30] and records of several invasive marine fish [23] and freshwater fish [15, 22, 34]. A holistic approach is challenging because of the constant changes in the habitat of the Neretva Delta, which also resulted in changes of the composition of water fauna due to changes in significant ecological factors, particularly salinity. The occurrence of salinization of major Neretva flow, with an increase in salinity during summer, due to seawater intrusion into the upper regions, has not been studied at the fauna change level. The developed models of sea intrusion to major Neretva flow showed that seawater should protrude 25 km from the river mouth, occupying complete riverbed from the depth of 1 m to the bottom at 8-10 meters [29]. Thus, major Neretva flow during the summer season represents the largest transitional ecosystem area in the whole Neretva estuary. However, this transitional ecosystem is of temporary nature and depends on the quantity of water flow, which is unpredictable as it depends on dam management in the middle Neretva reach. The flow quantity of 500 m<sup>3</sup>/sec is the freshwater amount needed to throw out seawater from the major flow to the river mouth [29]. This flow quantity started with higher electricity production which usually begins in October and finishes in April-May, but without strict dates. This also means that major Neretva flow is the largest transitional ecosystems during the summer season when the flow is under the process of salinization, but this also means that all marine flora and fauna vanished during the winter season, either dying or migrating to the sea. The similar is happening with freshwater organisms during the summer marinization period. A similar impact of lower river flow was described for the Upper Tagus Estuary in Portugal [37].

There is very little information on reproductive and food fish migrations in the Neretva River. In the last 10 years, there has

been an increase in the number of thinlip grey mullet, *Chelon ramada* in the Neretva delta and its migration to upper parts of the delta was recorded [20, 38]. However, most of the research to date has been carried out in the area of the Neretva mouth [3, 4, 5, 6] and concerned the dynamics of sand smelt populations and three grey mullet species. Recently, the migrations of *Petromyzon marinus* from the sea to the freshwater parts of the Neretva Estuary was described [24].

The main objective of this study was to investigate the marine fish migrations through the Neretva River from May to the end of September when the sea deeply penetrates the Neretva River upstream causing salinization and marinization of the freshwater areas and ecosystems.

## 2. MATERIAL AND METHODS / Materijal i metode

### 2.1. Study area / Područje istraživanja

The study was conducted in the major flow of the Neretva River (43.027773, 17.553131), 1 km downstream of the town of Opuzen and 10 km from the river mouth (Figure 1). The river area of 500 meters length was sampled with gill nets and ell traps. The study area is part of the typical flow of the Neretva from the town of Metković to the river mouth. The right side of the river is of soil and mud mixture, and depths of 5-6 meters begin right along the coast, with a steep slope. As a result, this area is poorly overgrown with aquatic plants, and immediately after the reed zone, it plunges towards the bottom. The left side of the river has been protected by a 300 m long rock embankment to prevent erosion, which significantly changed the ecosystem's features. This part of the coast has a slight slope towards the bottom of the river, which in some parts has areas overgrown with aquatic plants and algae, and rich in juvenile fish and small freshwater fish. Once a week, measurements of the temperature and salinity of the River Neretva were carried out, over the entire depth profile from the surface to the bottom (about 10 meters), by YSI 850 multimeter probe.

### 2.2. Sampling and fish analysis / Uzorkovanje i analiza ribe

Monitoring of the fish migrations was carried out with two smaller gillnets (mesh sizes 40 and 28 mm and 50 m long) and one gillnet (mesh size 72 mm; length 100 m). Smaller gillnets were connected into one and were laid across the Neretva River throughout the study. Large gillnet was laid down the river from smaller depths in U-shape with curved ends. The 30-meter big-scale sand smelt gillnets (mesh size 20 mm) were laid along the

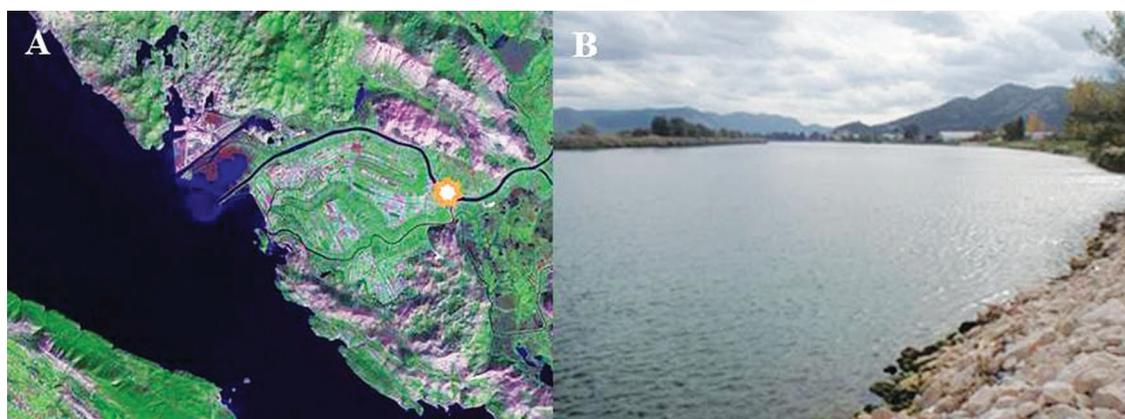


Figure 1 Map (A) of the Neretva Estuary with the position of the sampling site (star); (B) photo of the sampling site  
Slika 1. Karta (A) ušća rijeke Neretve s položajem mjesta uzorkovanja (zvijezda); (B) fotografija mjesta uzorkovanja

right bank of the Neretva River. Also, five heart-shaped steel traps were used, which were laid along both banks of Neretva. The diameter of the mesh was 50 mm. They were laid to the depths of 2-8 meters and inspected once a week with the addition of bait (old bread). Five traditional eel traps were used, which were inspected once a week. They were laid along the left and right banks of the river to a depth of 2-5 meters.

After the catch, all the fish were kept in iced water. Within three hours the following measurements were taken: total length with an ichthyometer (mm) and total mass with a precision scale (0.01 g). Weekly and monthly qualitative and quantitative analysis of the structure of the fish community and abundance and quantity of individuals and species were made. Total and monthly distribution of all dominant fish species (those that participated with >1% in the total sample) was calculated. The length-weight relationship was calculated using equation  $W=abL^b$ ; where  $W$  represents wet weight (g),  $a$  regression intercept,  $L$  total length (cm) and  $b$  regression slope [18]. Fultons condition factor was calculated following Froese (2006):  $K=100*W/L^3$ .

Age was determined from the number of scale annuli from 30 individuals. Scales from individuals of different length classes were removed, cleaned, dried, mounted between microscope slides and photographed under a stereomicroscope with a camera. Each scale was read by three different readers, taking it into the account that two reads need to be verified as same to determine the age. The annuli were interpreted according to Bagenal and Tesch (1978), the edge of the scale was treated as the last annuli and the area between the previous annuli and edge of the scale was treated as the annual growth increment.

Statistical data analysis and charting were done using the statistical programs Statsoft Statistica 10.0 and Microsoft Excel. The data required to establish the age key was taken from [www.fishbase.org](http://www.fishbase.org) for the Adriatic Sea or from own age data.

### 3. RESULTS / Rezultati

#### 3.1. Water temperature / Temperatura vode

Figure 2 shows the temperature profile of the Neretva River during the monitoring period from May to the end of September 2012. It can be seen that the temperature is uniform throughout the profile only at the beginning of May and they indicate the "fresh" character of the Neretva waters. As early as mid-May, temperatures higher than the surface layers appear at five-meter depth, which indicates the beginning of the penetration of the sea in the bottom layers. Already in the middle of May, this temperature threshold is established at about three meters' depth, pointing to the clear character of the water profile that remains throughout the summer. During the summer months, the temperature of the fresh-brackish layer to a depth of about three meters ranges from 18 to a maximum of 21 °C during the afternoon due to warming from the sun. During the night the temperature drops for 1-2 °C. The temperature of the sea layer, which stabilizes during the summer at 2.8-3 meters' depth, ranges from 23 to 25 °C and has the temperature characteristics of the coastal sea.

#### 3.2. Water salinity / Salinitet vode

The depth profile of Neretva salinity during the monitoring period from May to the end of September is shown in Figure 3. The picture shows that the freshwater profile of Neretva during monitoring was found only in early May when salinity through the whole column was 0.3 psu. Already at the end of May, a two-layer character of the water column was formed in the Neretva riverbed. Thus, at the end of May, the boundary of the fresh-brackish layer fluctuates at a depth of about 5 meters. Early at June, this limit moves to the depth of 3 m, at which salinity greater than 35 psu was recorded, which is a typical salinity of the Adriatic coastal water. More precise salinity measurements were performed only for depths of up to half a meter and at

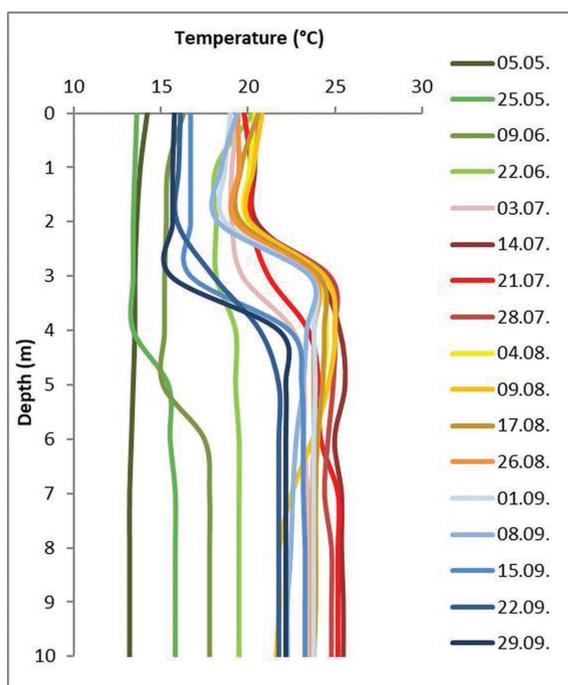


Figure 2 The temperature profile of the River Neretva column in the sampling site in the May-September period  
Slika 2. Temperaturni profil stupca rijeke Neretve na mjestu uzorkovanja u razdoblju od svibnja do rujna

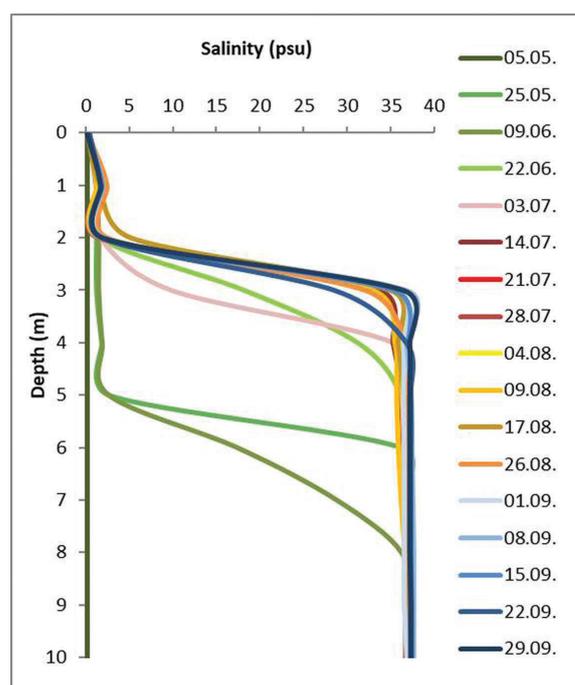


Figure 3 Salinity profile of the River Neretva column in the sampling site in the May-September period  
Slika 3. Profil saliniteta stupca rijeke Neretve na mjestu uzorkovanja u razdoblju od svibnja do rujna

the boundary of two layers of water. Measurements have shown that during the summer months, the layer of typical freshwater is only a few centimetres deep. During midday, this layer is found to be only 2-3 cm. Already at 5 cm salinity increases to 1.5-2 psu, so it enters the category of water which is not recommended in intensive agriculture. The boundary layer between the brackish (salinity water up to 30 psu) and seawater (all salinity above 35 psu) is formed in Neretva major flow from early June at a depth of only about 2.8 meters. And below conditions of the marine ecosystem prevail (hence not the estuarine transitional brackish ecosystem). This part can be further stratified into sections where salinity is from 5 to 10 psu, therefore areas that can be inhabited by some freshwater fish tolerating elevated salinity (carp, perch, etc.), and salinity areas of 10-25 psu inhabited by typical estuarine species (mullet, sea breams, flatfish, eel, sand smelt, etc.).

### 3.3. Composition of fish species / *Sastav ribljih vrsta*

In total, 1,429 individuals were caught during the sampling period. The catch was composed of 31 marine fish species and 8 freshwater fish species (Table 1). Most individuals (536) and species (22) were caught in August, and least individuals (40) and species (6) were caught in May (Figure 4). The numerically dominant species were: big-scale sand smelt *Atherina boyeri* (23.58%), European pilchard *Sardina pilchardus* (21.62%), gilthead sea bream *Sparus aurata* (11.20%), thinlip grey mullet *Chelon ramada* (8.19%) and common sole *Solea solea* (7.63%) (Figure 5). The dominant species were all marine fish species. Among the freshwater fish species, the most abundant was *Gambusia holbrooki* (4.55%). Total monthly biomass of the all caught individuals was highest in September (20.878 kg; 31.77%) (Figure 6). Dominant species in the total mass were: thinlip grey mullet (43.45%), common sole (14.27%), gilthead

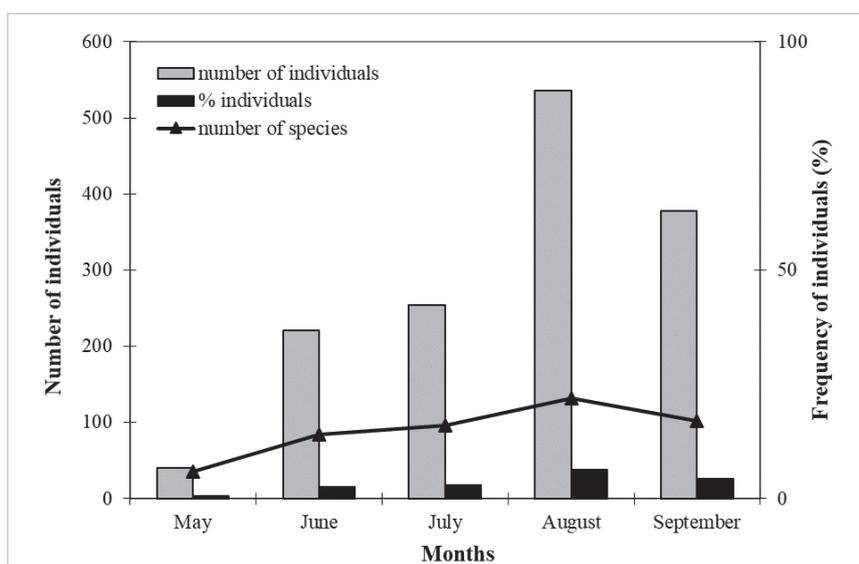


Figure 4 Monthly number of the fish species and individuals caught in the sampling area  
Slika 4. Mjesečni broj vrsta riba i jedinki ulovljenih u području uzorkovanja

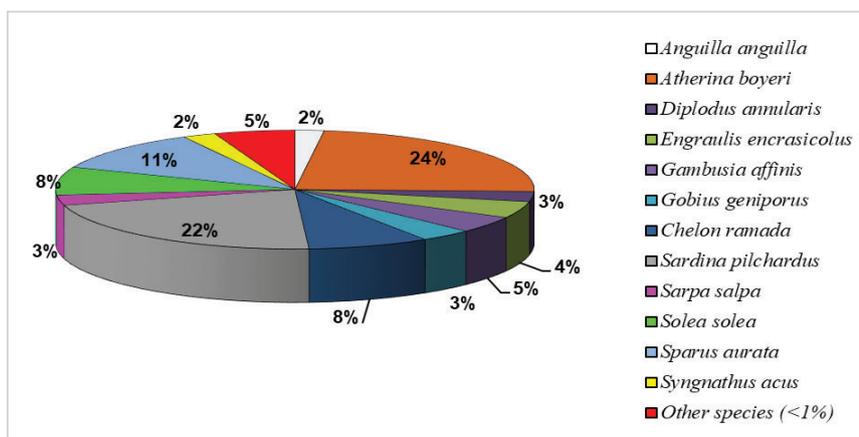


Figure 5 Percentage of the number of individuals of the specific fish species in the total catch  
Slika 5. Postotak broja jedinki određene vrste riba u ukupnom ulovu

Table 1 List of fish species and structure of their catch in River Neretva from May to September (N - number of individuals; W - total weight in grams)  
 Tablica 1. Popis vrsta riba i struktura ulova u rijeci Neretvi od svibnja do rujna (N - broj jedinki; W - ukupna težina u gramima)

SPECIES	May N (Wg)	June N (Wg)	July N (Wg)	August N (Wg)	September N (Wg)	TOTAL N specimens (%)	TOTAL Wg (%)
<i>Anguilla anguilla</i>	0	7 (659.67)	8 (1074.72)	4 (32.76)	9 (699.73)	28 (1.96)	2466.88 (3.75)
<i>Atherina boyeri</i>	0	95 (464.31)	52 (233.53)	117 (580.83)	73 (370.05)	337 (23.58)	1648.72 (2.51)
<i>Carassius gibelio</i>	0	0	0	0	3 (424.93)	3 (0.21)	424.93 (0.65)
<i>Dicentrarchus labrax</i>	0	0	0	1 (746)	0	1 (0.07)	746 (1.14)
<i>Diplodus annularis</i>	1 (17.19)	1 (17.83)	4 (200.6)	16 (556.4)	18 (852.74)	40 (2.80)	1644.76 (2.50)
<i>Diplodus puntazzo</i>	0	0	1 (8.45)	0	1 (72.61)	2 (0.14)	81.06 (0.12)
<i>Engraulis encrasicolus</i>	0	7 (19.14)	39 (195.29)	10 (54.26)	8 (35.02)	64 (4.48)	303.71 (0.46)
<i>Gambusia affinis</i>	0	0	0	65 (7.99)	0	65 (4.55)	7.99 (0.01)
<i>Gasterosteus aculeatus</i>	0	0	0	2 (1.44)	0	2 (0.14)	1.44 (0.00)
<i>Gobius geniporus</i>	0	21 (100.89)	8 (48.1)	10 (88.17)	11 (80.2)	50 (3.50)	317.36 (0.48)
<i>Gobius niger</i>	0	0	3 (34.2)	2 (26.26)	0	5 (0.35)	60.46 (0.09)
<i>Ictalurus punctatus</i>	0	1 (123.07)	0	0	3 (304.38)	4 (0.28)	427.45 (0.65)
<i>Knipowitschia croatica</i>	0	0	0	3 (0.56)	0	3 (0.21)	0.56 (0.00)
<i>Labrus merula</i>	0	0	1 (62.38)	2 (93.19)	0	3 (0.21)	155.57 (0.24)
<i>Lichia amia</i>	0	0	0	0	1 (108.46)	1 (0.07)	108.46 (0.17)
<i>Lithognathus mormyrus</i>	1 (276.23)	1 (410.61)	1 (196.98)	4 (838.19)	0	7 (0.49)	1722.01 (2.62)
<i>Chelon aurata</i>	0	0	0	0	14 (5032.25)	14 (0.98)	5032.25 (7.66)
<i>Chelon ramada</i>	18 (5396.59)	27 (6851.98)	36 (8585.91)	10 (1952.07)	26 (5765.65)	117 (8.19)	28552.2 (43.44)
<i>Mugil cephalus</i>	0	0	0	0	1 (1112.54)	1 (0.07)	1112.54 (1.69)
<i>Mullus barbatus barbatus</i>	0	0	1 (48.37)	5 (128.38)	3 (63.1)	9 (0.63)	239.85 (0.36)
<i>Parablennius gattorugine</i>	0	0	1 (10.53)	0	0	1 (0.07)	10.53 (0.02)
<i>Platichthys flesus</i>	0	1 (270.86)	3 (642.69)	3 (589.59)	0	7 (0.49)	1503.14 (2.29)
<i>Rutilus basak</i>	0	1 (9.06)	0	2 (1.14)	0	3 (0.21)	10.2 (0.02)
<i>Sardina pilchardus</i>	5 (19.16)	29 (107.73)	64 (231.74)	110 (453.55)	101 (395.31)	309 (21.62)	1207.49 (1.84)
<i>Sarpa salpa</i>	0	0	0	40 (1690.40)	0	40 (2.80)	1690.49 (2.57)
<i>Scardinius plotizza</i>	0	0	0	0	2 (4.29)	2 (0.14)	4.29 (0.01)
<i>Solea solea</i>	14 (806.85)	21 (1149.09)	14 (1234.99)	46 (4733.46)	14 (1451.55)	109 (7.63)	9375.94 (14.27)
<i>Sparus aurata</i>	0	1 (1.91)	18 (414.14)	51 (2140.77)	90 (4104.73)	160 (11.20)	6661.55 (10.14)
<i>Sprattus sprattus</i>	1 (3.42)	8 (30.64)	0	0	0	9 (0.63)	34.06 (0.05)
<i>Syngnathus abaster</i>	0	0	0	32 (7.21)	0	32 (2.24)	7.21 (0.01)
<i>Tinca tinca</i>	0	0	0	1 (160.89)	0	1 (0.07)	160.89 (0.24)
TOTAL N (W g)	40 (6519.44)	221 (10216.79)	254 (13222.62)	536 (14883.6)	378 (20877.54)	1429	65719.99
%N (%W)	2.80 (9.92)	15.47 (15.55)	17.77 (20.12)	37.51 (22.65)	26.45 (31.77)		
TOTAL SPECIES	6	14	16	22	17	31	
% species	19.35	45.16	51.61	70.97	54.84		

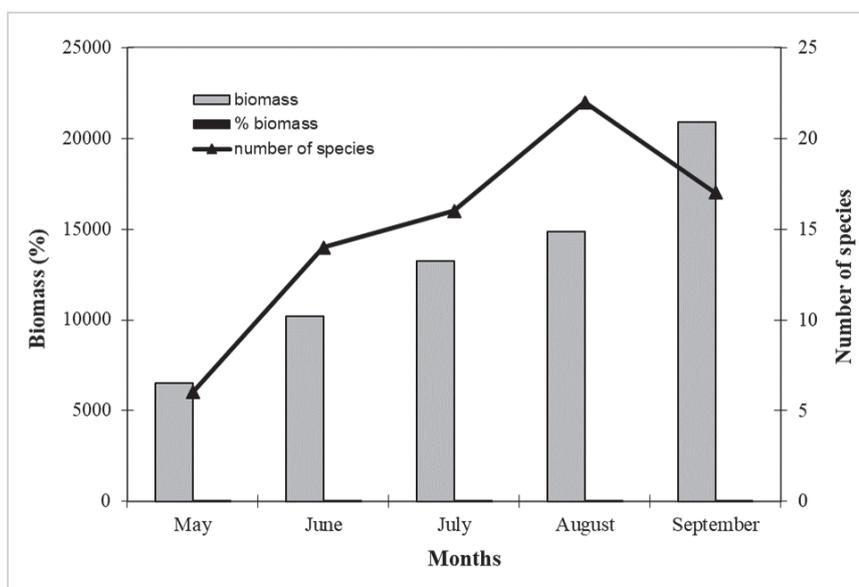


Figure 6 Monthly mass of the fish species and individuals caught in the sampling area  
Slika 6. Mjesečna masa vrsta riba i jedinki ulovljenih na području uzorkovanja

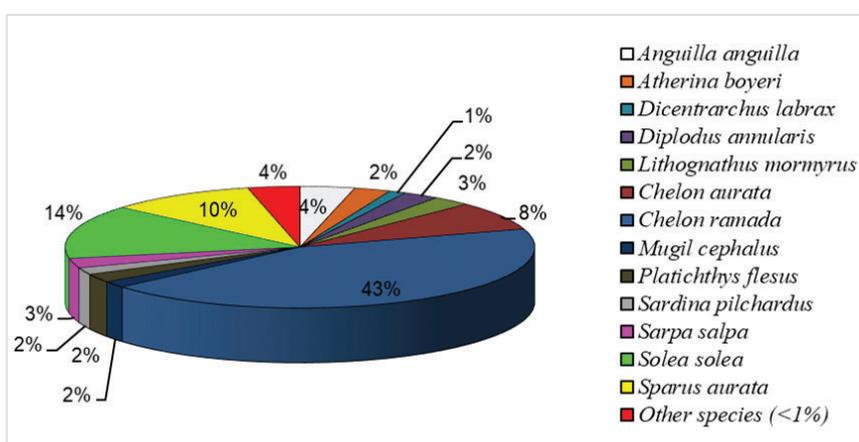


Figure 7 Mass percentage of the individuals of specific fish species in the total catch  
Slika 7. Postotak mase jedinki određene vrste riba u ukupnom ulovu

seabream (10.14%), golden grey mullet *Chelon aurata* (7.66%) and European eel, *Anguilla anguilla* (3.75%) (Figure 7). Thinlip grey mullet and common sole were caught uniformly throughout the 5 months of sampling, while gilthead sea bream was caught only from July to September, with young fish dominating in catches.

#### Big-scale sand smelt, *Atherina boyeri* / *Gavun oliga*

The total of 332 individuals (1.6 kg in total) were caught. The species was dominant in number and well represented in mass. It was caught in all months of the survey except May, and most in August (35.2%) (Figure 8A). Individuals were collected with a total length of 6.4 cm to 12.0 cm ( $9.2 \pm 0.63$  cm) and weighing 1.65 g to 7.34 g ( $4.97 \pm 0.75$  g). Most individuals belonged to the 9 cm (64.8%) length class (Figure 8B). The exponent  $b$  from the equation of length-mass ratio has a value of 1.580, indicating a negative allometric growth (Figure 8C). The age structure indicates that the majority of the population found in the study area is composed of 3-year-old big-scale sand smelt (Figure 8D).

#### European pilchard, *Sardina pilchardus* / *Srdela*

In the study area, 306 individuals (1.2 kg in total) were caught. The European pilchard was dominant in number and well represented

in mass. It was caught in all months of the survey, most notably in August (35.9%) (Figure 9A). Individuals were caught with a total length of 5.5 cm to 12.2 cm ( $8.4 \pm 0.88$  cm) and weighing 0.91 g to 10.23 g ( $3.95 \pm 1.31$  g). Most individuals belonged to the 9 cm (41.50%) length class (Figure 9B). The exponent  $b$  from the equation of length-mass ratio has a value of 3.147 ( $R^2=0.815$ ) and indicates positive allometric growth (Figure 9C). The age structure indicates that the entire population found in the study area were composed of juvenile specimens, aged 0+ years.

#### Gilthead seabream, *Sparus aurata* / *Komarča*

Total of 158 individuals (6.7 kg in total) were caught during all months of the survey except May. Most were caught in September (57.0%) (Figure 10A). Individuals were caught with a total length of 6.1 cm to 25.8 cm ( $14.33 \pm 2.79$  cm) and weighing from 1.91 to 263.82 g ( $42.16 \pm 31.69$  g). Most individuals belonged to the 13-17 cm (80.3%) length classes (Figure 10B). The exponent  $b$  from the equation of the long-mass ratio has a value of 3.221 ( $R^2=0.981$ ) and indicates positive allometric growth (Figure 10C). The age structure indicates that the majority of the affected population in the study area is composed of juvenile gilthead seabream aged 1 year (86.7%) (Figure 10D).

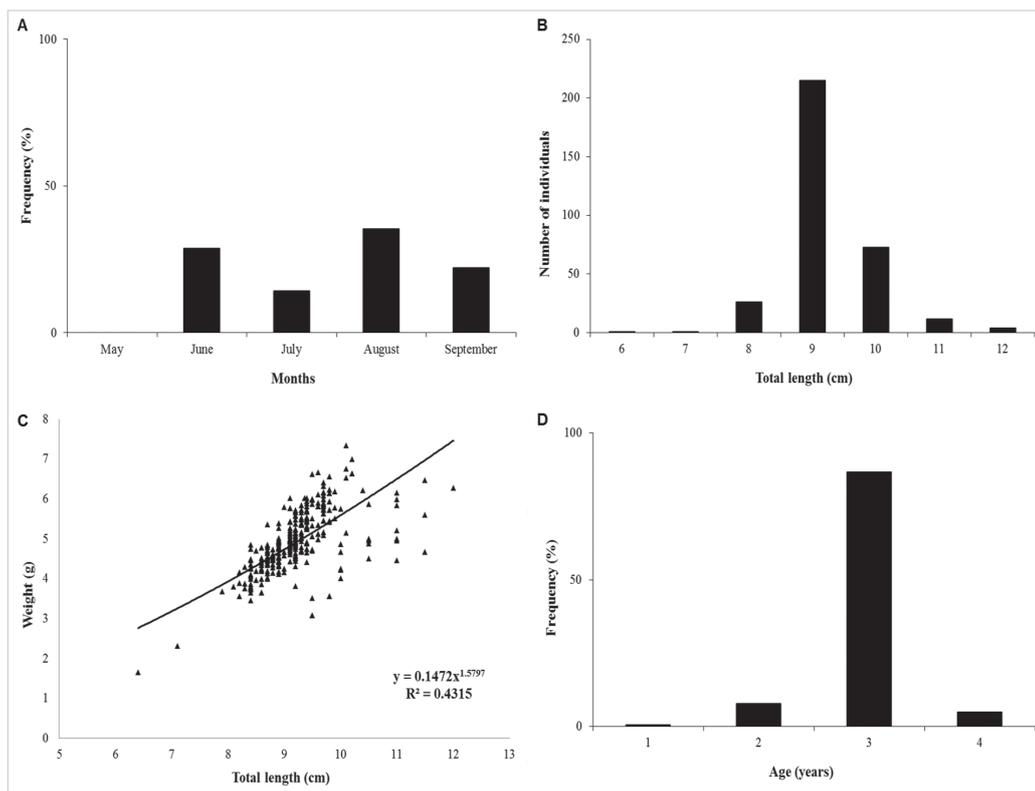


Figure 8 The structure of sand smelt, *Atherina boyeri* catch: A) frequency of monthly catch; B) body length structure; C) length-weight relationship and D) age structure  
 Slika 8. Struktura gavuna olige, ulov *Atherina boyeri*: A) učestalost mjesečnog ulova; B) struktura duljine tijela; C) odnos duljine i težine i D) doba struktura

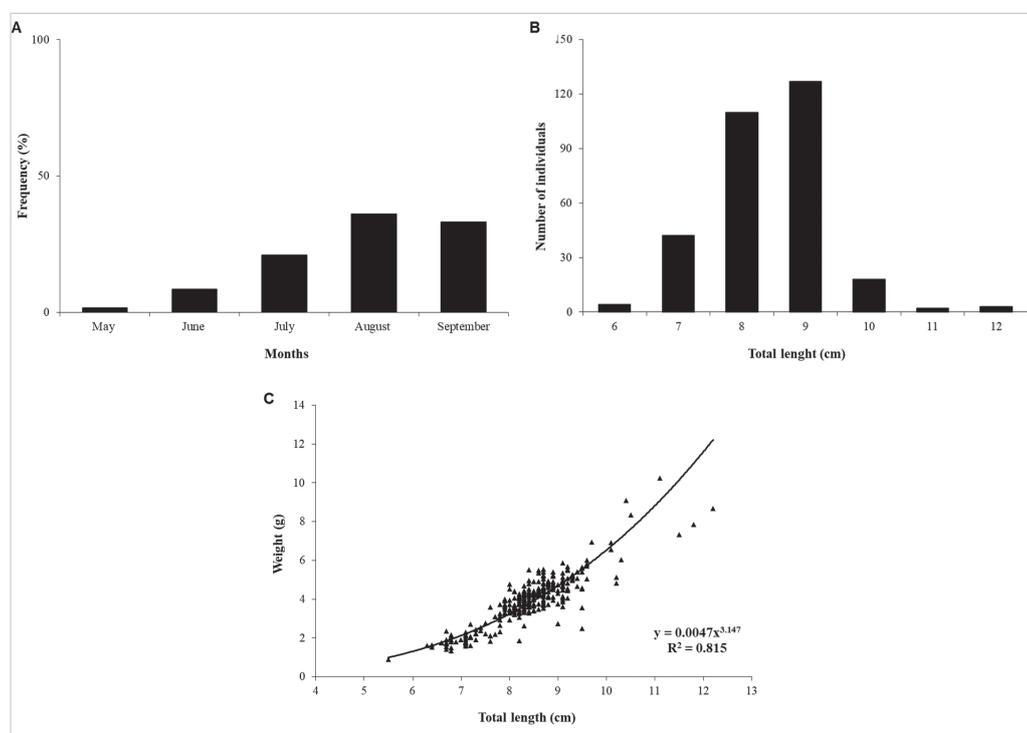


Figure 9 The structure of European pilchard, *Sardina pilchardus* catch: A) frequency of monthly catch; B) body length structure and C) length-weight relationship  
 Slika 9. Struktura ulova srdele, ulov *Sardina pilchardus*: A) učestalost mjesečnog ulova; B) struktura duljine tijela i C) odnos duljine i težine

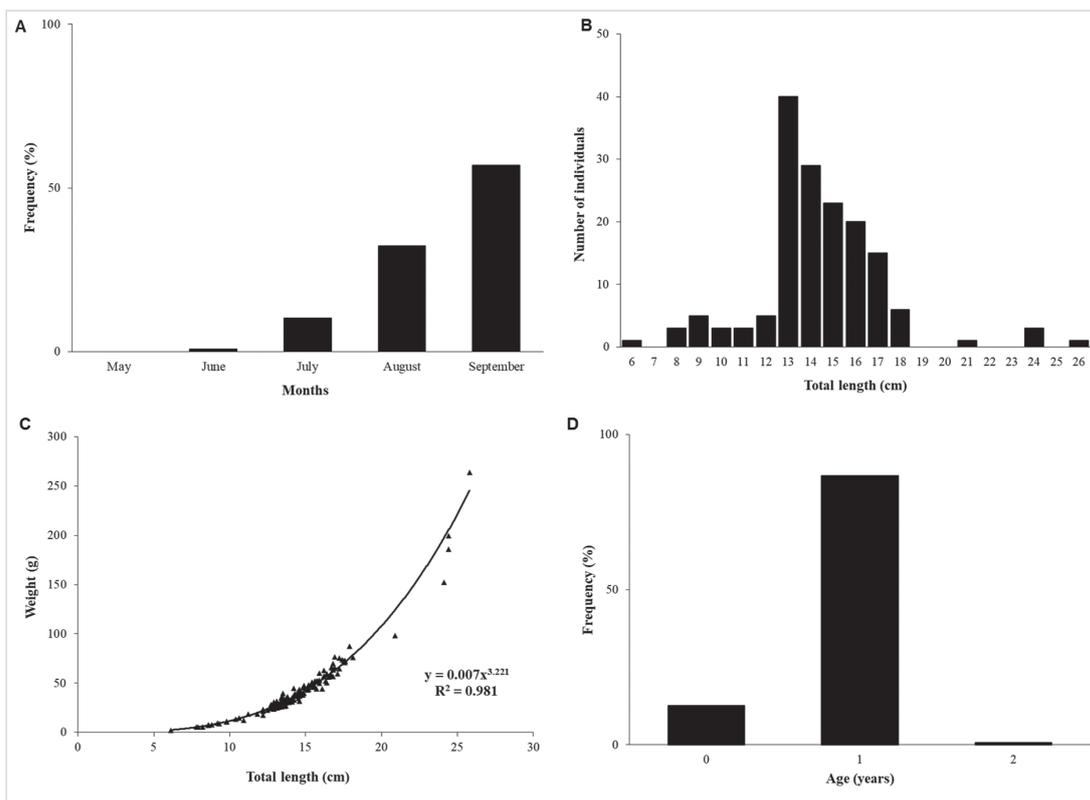


Figure 10. The structure of gilthead seabream, *Sparus aurata* catch: A) frequency of monthly catch; B) body length structure; C) length-weight relationship and D) age structure.

Slika 10. Struktura komarče, ulov *Sparus aurata*: A) učestalost mjesečnog ulova; B) struktura duljine tijela; C) odnos duljine i težine i D) doba struktura.

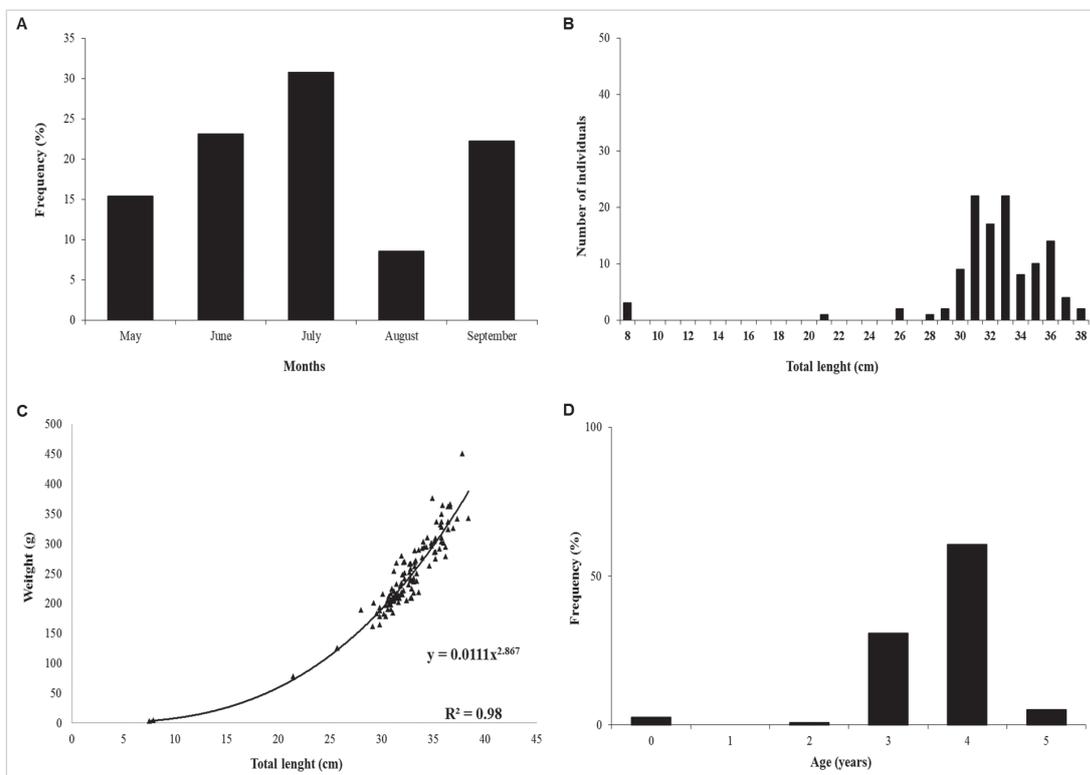


Figure 11 The structure of thinlip mullet, *Chelon ramada* catch: A) frequency of monthly catch; B) body length structure; C) length-weight relationship and D) age structure

Slika 11. Struktura cipla balavca, ulov *Chelon ramada*: A) učestalost mjesečnog ulova; B) struktura duljine tijela; C) odnos duljine i težine i D) doba struktura

### Thinlip grey mullet, *Chelon ramada* / *Cipal balavac*

Thinlip grey mullet, with a catch of 117 individuals and 28.6 kg of mass, was one of the dominant species, both numerically and in biomass. It was caught in all months of the survey, most notably in July (30.8%) (Figure 11A). Individuals were captured with a total length of 7.5 cm to 38.4 cm ( $32.09 \pm 4.72$  cm) and a mass of 3.59 g to 450, 32 g ( $244.04 \pm 70.71$  g). Most individuals belonged to the length class 31-33 cm (52.51%) (Figure 11B). The exponent  $b$  from the equation of length-mass ratio has a value of 2.867 ( $R^2=0.984$ ) and indicates a negative allometric growth and increased growth in length (Figure 11C). The age structure indicates that the majority of the thinlip grey mullet aged 3 and 4 years (91.3%) (Figure 11D).

### Common sole, *Solea solea* / *List*

With a catch of 109 individuals (9.4 kg in total), the common sole was one of the dominant species both in number and in biomass. It was caught in all months of the survey, most notably in August (42.2%) (Figure 12A). Individuals were captured with a total length of 15.1 cm to 25.9 cm ( $21.01 \pm 2.63$  cm) and a mass of 3.59 g to 172.95 g ( $86.02 \pm 34.89$  g). Most individuals belonged to the 19-20 cm (31.20%) length class (Figure 12B). The exponent  $b$  from the equation of length-mass ratio has a value of 3,209 ( $R^2=0.958$ ) and indicates positive allometric growth (Figure 12C). The age structure indicates that the majority of the population in the study area was composed of the common sole of 1 and 2 years of age (99%) (Figure 12D).

### European anchovy, *Engraulis encrasicolus* / *Inćun*

Total of 57 individuals (total 0.3 kg) were caught and this species was relatively well numerically represented during the study. It was caught in all months of the survey except May, and most in July (56.1%) (Figure 13A). Individuals were collected with a total length of 7.1 cm to 12.4 cm ( $9.9 \pm 1.27$  cm) and weighing from 1.48 g to 10.72 g ( $5.33 \pm 2.00$  g). Most individuals belonged to the 10 cm (43.9%) length class (Figure 13B). The exponent  $b$  from the equation of length-mass ratio has a value of 3.004 ( $R^2=0.806$ ) and indicates positive allometric growth, that is, increased growth in mass (Figure 13C). The age structure indicates that the entire population found in the study area is composed of juvenile European anchovies (0+ and 1+ years) (Figure 13D).

### Slender goby, *Gobius gobiporus* / *Glavoč bjelaš*

Total of 50 Individuals (total 0.317 kg) of slender goby were caught from June to September (Figure 14A). The total length of individuals ranged from 6 to 11.8 cm ( $8.13 \pm 1.58$  cm) and weight 2.91 to 15.39 g ( $6.34 \pm 3.95$  g). Most of the individuals belong to the 7-8 cm length classes (Figure 14B). The value of exponent  $b$  (2.931) ( $R^2=0.887$ ) indicates negative allometric growth (Figure 14C).

### Annular seabream, *Diplodus annularis* / *Špar*

Total of 40 individuals (1.6 kg in total) were caught during all months of the survey, most notably in August (40.0%) (Figure 15A). Individuals were caught with a total length of 4.9 cm to

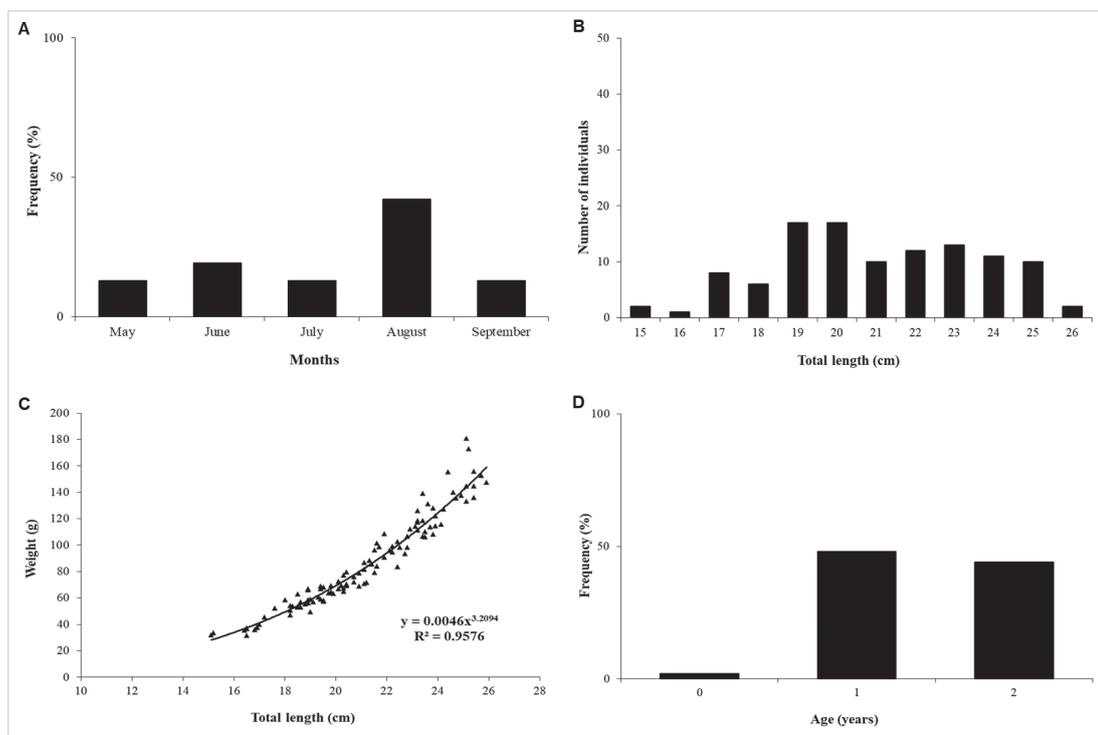


Figure 12. The structure of common sole, *Solea solea* catch: A) frequency of monthly catch; B) body length structure; C) length-weight relationship and D) age structure

Slika 12. Struktura lista, ulov *Solea solea*: A) učestalost mjesečnog ulova; B) struktura duljine tijela; C) odnos duljine i težine i D) dobna struktura

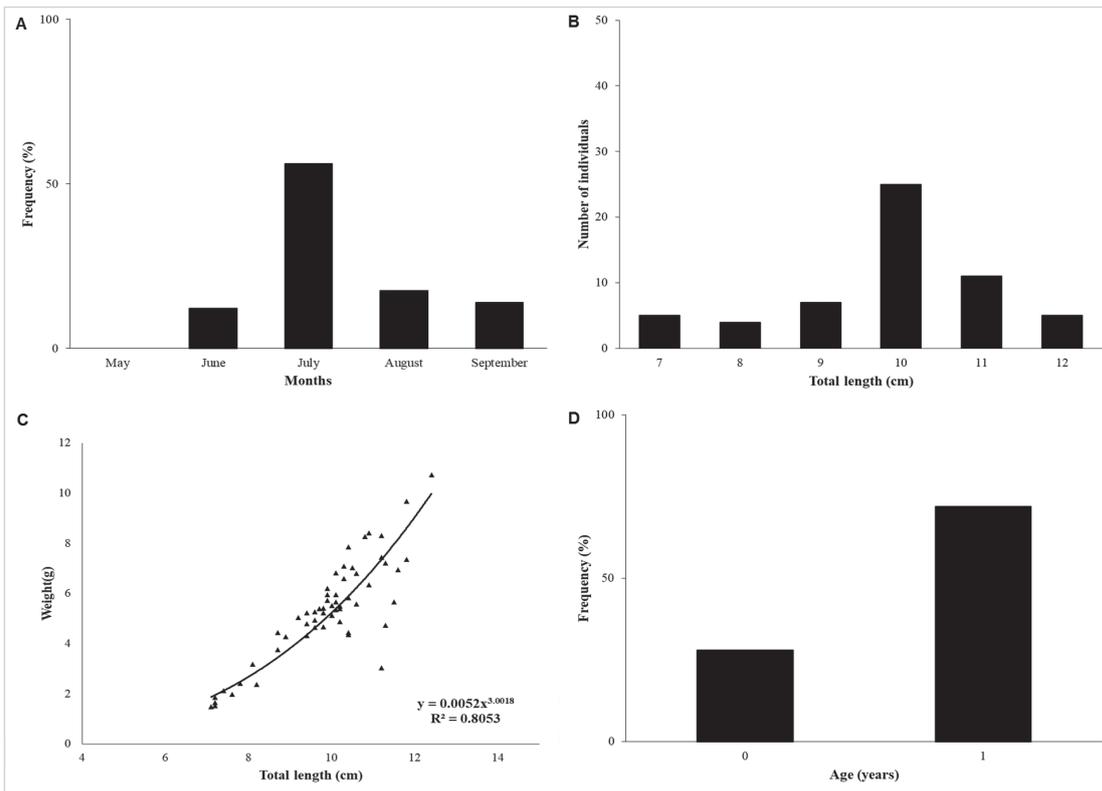


Figure 13 The structure of European anchovy, *Engraulis encrasicolus* catch: A) frequency of monthly catch; B) body length structure; C) length-weight relationship and D) age structure

Slika 13. Struktura inćuna, ulov *Engraulis encrasicolus*: A) učestalost mjesečnog ulova; B) struktura duljine tijela; C) odnos duljine i težine i D) doba struktura

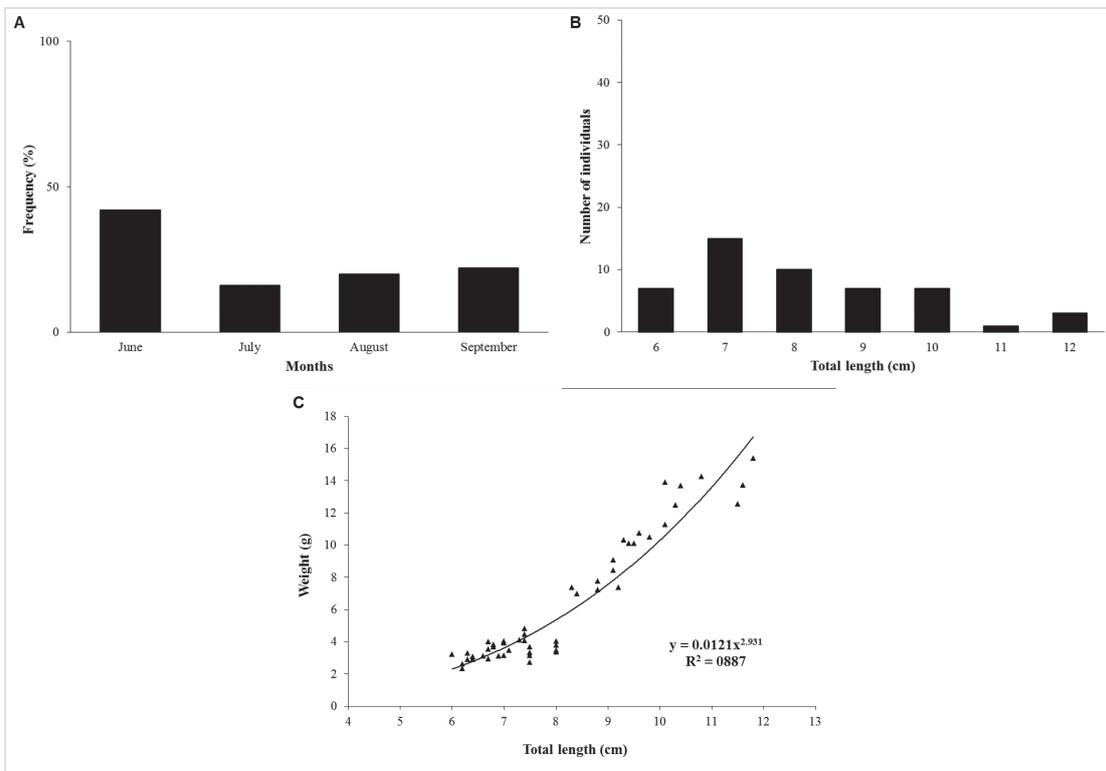


Figure 14 The structure of slender goby, *Gobius geniporus* catch: A) frequency of monthly catch; B) body length structure and C) length-weight relationship

Slika 14. Struktura glavoča bjelaša, ulov *Gobius geniporus*: A) učestalost mjesečnog ulova; B) struktura duljine tijela i C) odnos duljine i težine

19.1 cm ( $13.13 \pm 2.48$  cm) and weighing 1.8 g to 85.76 g ( $41.12 \pm 20.86$  g). Most individuals belonged to the 11-14 cm (62.25%) length classes (Figure 15B). The exponent  $b$  from the equation of length-mass ratio has a value of 3.012 ( $R^2=0.971$ ) and indicates positive allometric growth, that is, increased growth in mass (Figure 15C). The age structure indicates that the majority of the population found in the study area was at 3 years of age (37.5%) (Figure 15D).

#### European eel, *Anguilla Anguilla* / *Jegulja*

In the study area, 28 individuals (2.5 kg in total) were caught and the eel was relatively well represented both numerically and in mass. It was caught in all months of the survey except May, and most in September (32.1%) (Figure 16A). Individuals were captured with a total length of 9.5 cm to 64.2 cm ( $34.1 \pm 10.22$  cm) and weighing 1.78 g to 489.18 g ( $88.10 \pm 86.12$  g). Most individuals belonged to the 35 cm (46.4%) length class (Figure 13B). The exponent  $b$  from the equation of length-to-mass ratio has a value of 3.038 ( $R^2=0.992$ ) and indicates positive allometric growth, that is, increased mass growth (Figure 16C). The age structure indicates that the majority of the population found in the study area was composed of juvenile eels 2 years of age (Figure 16D).

#### Eastern mosquitofish, *Gambusia holbrooki* / *Gambuzija*

In the study area, eastern mosquitofish inhabited the area along both shores and is probably the most abundant fish species in the Neretva River. A total of 35 individuals (8.0 g total) were selected to determine the species accurately. Individuals were

caught with a total length of 2.0 cm to 4.1 cm ( $2.8 \pm 0.33$  cm) and weight from 0.11 g to 0.86 g ( $0.23 \pm 0.12$  g). Most individuals belonged to the 3 cm (88.6%) length class. The exponent  $b$  from the equation of length-mass ratio has a value of 2.971 ( $R^2=0.869$ ) and indicates negative allometric growth. The majority of the population was 2 years old.

#### Other marine and freshwater fish species / *Ostale morske i slatkovodne ribe*

In addition to the presented most abundant, another 13 species were caught (Table 1). They were poorly represented both numerically (5.45% in total) and by mass (3.58% in total). These are Sparidae (sand steenbras, *Lithognathus mormyrus* and sharpsnout seabream, *Diplodus puntazzo*), labridae (brown wrasse, *Labrus merula*), Gobiidae (black goby, *Gobius niger*), Blenniidae (tompot blenny, *Parablennius gattorugine*), Clupeidae (sprat, *Sprattus sprattus*), Carangidae (leerfish, *Lichia amia*), Moronidae (European bass, *Dicentrarchus labrax*), Mugilidae (flathead grey mullet, *Mugil cephalus*, and golden grey mullet, *Chelon aurata*), Mullidae (red mullet, *Mullus barbatus*), Pleuronectidae (European flounder, *Platichthys flesus*) and black-striped pipefish, *Syngnathus abaster*. Most of these species were represented by 1-2 individuals and can be considered as incidental catches. European bass and leerfish are typical transitional predator species. In addition to the more detailed described mosquitofish, several freshwaters species were recorded in the study area. These are Prussian carp, *Carassius gibelio*, three-spined stickleback, *Gasterosteus aculeatus*, American catfish, *Ictalurus punctatus*, Neretva dwarf

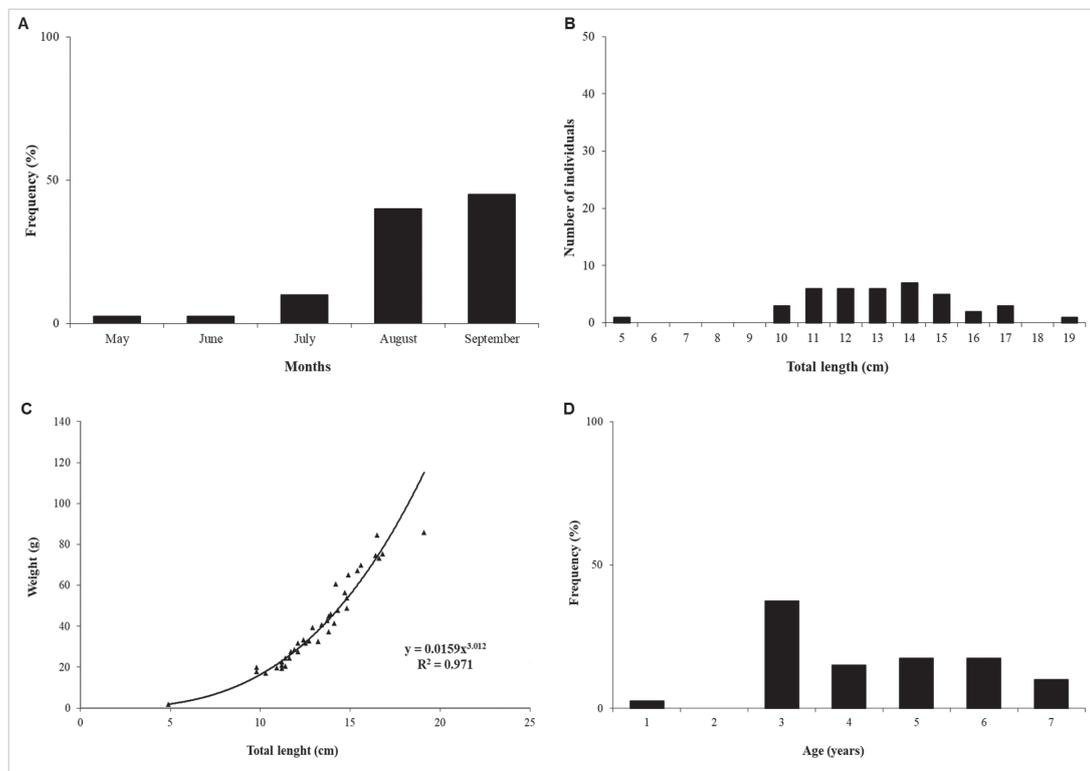


Figure 15 The structure of annular seabream, *Diplodus annularis* catch: A) frequency of monthly catch; B) body length structure; C) length-weight relationship and D) age structure

Slika 15. Struktura špara, ulov *Diplodus annularis*: A) učestalost mjesečnog ulova; B) struktura duljine tijela; C) odnos duljine i težine i D) dobnna struktura

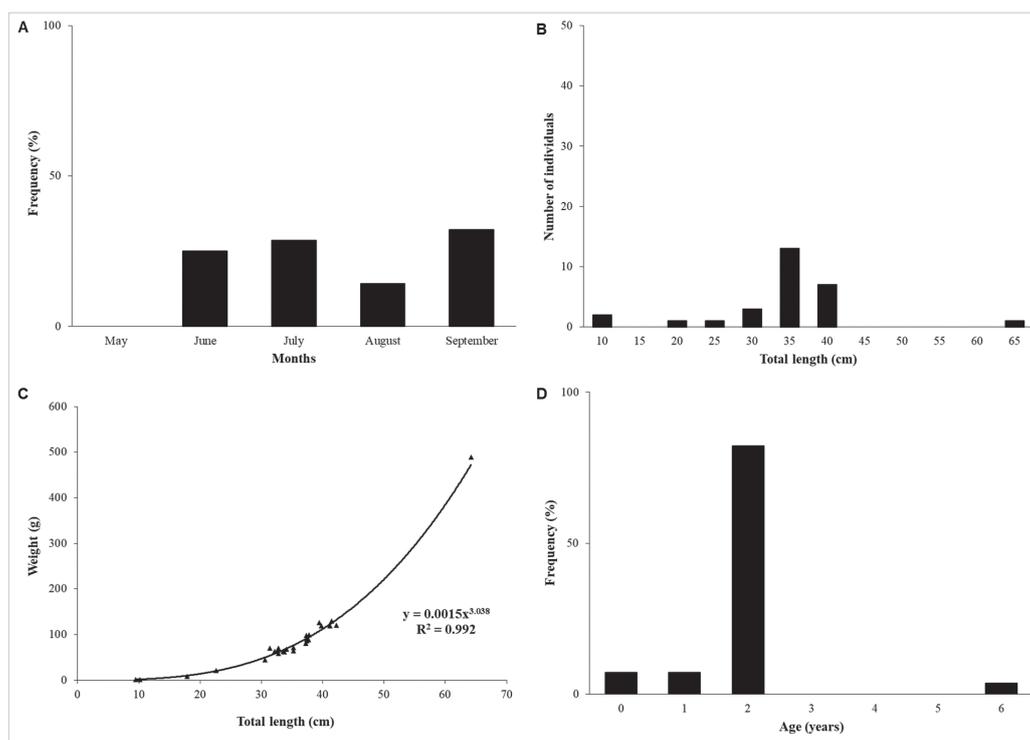


Figure 16 The structure of European eel, *Anguilla anguilla* catch: A) frequency of monthly catch; B) body length structure; C) length-weight relationship and D) age structure

Slika 16. Struktura jegulje, ulov *Anguilla anguilla*: A) učestalost mjesečnog ulova; B) struktura duljine tijela; C) odnos duljine i težine i D) dozna struktura

goby, *Knipowitschia croatica*, Basak, *Rutilus basak*, Neretva rudd, *Scardinius plotizza* and tench, *Tinca tinca*. All of these species were mostly caught only once during the entire 5-month survey, with only 1-3 individuals.

#### 4. DISCUSSION / Rasprava

The major flow of the River Neretva was freshwater from the surface to the bottom at the beginning of May. The seawater started to intrude in the middle of May and stay until the end of September when this research was accomplished. According to hydrological models, the 500m<sup>3</sup> of flow is needed to kick out the seawater from the major riverbed and flows bellow 180 m<sup>3</sup> enable intrusion of the seawater 20 km inland, including an area in Bosnia and Herzegovina [39] while during the summer season the flows are bellow 80m<sup>3</sup> [29]. The measurements of salinity in this study showed that only a few centimetre of the river water column was below 0.3 psu, what is drinkable and irrigation water characteristics. The water column from 10 cm to 200 cm represents brackish water of different increasing salinities, while water below 2,8 m to the bottom is typical seawater of 35 psu and higher. This means that major Neretva flow is not the only estuarine brackish ecosystem, but also a typical marine ecosystem with typical marine biota during the summer period. This is supported with catching of typical marine fish, like a brown ray, *Raja miraletus* or octopus (*Octopus vulgaris*) 20-25 km from the river mouth (<https://caportal.net/2019/11/07/foto-raza-kroz-neretvu-stigla-do-visica/>). As a result of human activities salinization is occurring all over the world and is expected to be worse due to climate changes and increased water demand [9]. Such salinized rivers have characteristics of degraded freshwater systems but also represents habitats for

salt-tolerant faunas. Loss of biodiversity, changes in community composition and a decline in the species richness such as insects represents consequences that can occur in more salinized rivers [26]. On the opposite side, salinization creates new habitat for the marine species, serving as a large new nursery ground for the younger stages, as was documented by this study.

The highest number of fish species and individuals were caught in August. These numbers were smallest during May, increasing during June and July, and started to decrease in September. The numerically dominant species in the investigated area were two small pelagic species: the big-scale sand smelt (23.58%) and the European pilchard (21.62%). While a big-scale sand smelt is a common inhabitant in the Neretva Estuary [3] and typical estuarine resident, the presence of the younger cohort of European pilchard point to the importance of the Neretva Estuary as a nursery ground for the small marine pelagic species. This is enhanced with a documented presence of two other small pelagic, European anchovy and sprat, in the study area. Similar findings were published for other European estuaries, i.e. Gironde Estuary (France), where densities of all the studied species (i.e. European anchovy, sprat or European seabass) significantly increased in recent time, accompanied by changes in water temperature and seawater intrusion [33]. The effects of global changes in this estuarine system favour its nursery function for marine juveniles and some evidence of effects at a wider scale (community and ecosystem levels) may also alter the structure and functioning of this system [16]. The intrusion of the seawater inside the River Gironde, modify the potential habitat area for marine fish and positively affect the abundance of juvenile species using the system as a nursery ground [33], as described for the Neretva Estuary. This

classified all three small pelagic species as marine estuarine-opportunists, and migration into and out estuaries is often seasonal [25]. The next group of highly abundant species belong to the marine estuarine-dependent guild; the species which rely on the protected waters of estuaries for providing a suitable nursery habitat [7]. These species in Neretva Estuary are gilthead seabream (11.20%), the thinlip grey mullet (8.19%) and the common sole (7.63%). Among the freshwater fish species, most abundant was *Gambusia holbrooki* (4.55%). It is documented that this species tolerates estuarine salinities [1, 36] and this is a reason while is considered as highly invasive in Neretva Estuary [22].

The most interesting fish finding is a high number of small pelagic species like European pilchard and anchovy in the major flow. Subsequently, these small fish attracted large pelagic species, like bluefish and pompano, which were caught by sport fishermen [22] in the area of Neretva mouth. These changes should have impacts on predator-prey interactions and competition. Recent studies have shown that *Argyrosomus regius* could colonize the entire estuarine area, which can cause a high niche overlap. His colonization can increase of predation pressure and cause food competition with other species (e.g. European sea bass) which at the end can affect the functioning of the whole estuary, including the nursery function [12]. However, during our survey, these large marine predators were not sampled in a higher number, but interviews with sport fishermen indicated their presence in major Neretva flow.

The sampled big-scale sand smelt in the major Neretva flow was 3 years old. This is confirmation of previous study hypothesis on sand smelt in the coastal waters (mouth of River Mala Neretva), where most sand smelts were in their first and second year, while the number of older individuals was very low due to their potential migration upstream [3]. Recent findings confirm this hypothesis for the River Neretva waters. The big-scale sand smelt is typical estuarine resident species, as reported for Porto-Lagos Lagoon (Greece) [28] or Venice lagoon [17].

The reasons for salinity increase in the last 30 years in the Neretva Estuary are: exploitation of sand at the mouth of the Neretva River and reducing the summer flow due to the operation of hydropower plants. Although it is clear that these two activities coincide with an increase in salinity, due to the lack of more serious scientific and professional research, it is difficult to assess their impact on the salinity process of the Neretva waters [39]. Rough estimations of the major Neretva flow property for the 20 km river length (app. 100m width, app. average 10 m depth) during extended summer season show that 80% of water column (from 3 to 10 m depth) represent typical marine ecosystem, while 19% represent estuarine ecosystem (from 0.3 m to 2.5-3m depth) and only 1% remains as a freshwater ecosystem (surface to 0.3 m). This is documented with a dominance of typical small marine pelagic fish and several other typical marine fish species followed with typical estuarine resident species like sand smelt and estuarine dependent species, like younger gilthead sea bream and several grey mullet species. The freshwater ichthyofauna is tightened in the few several centimetres of the water column and dominated with mosquitofish.

The main result of this study points out that major Neretva flow of 25 km in length from the mouth is under strong seasonal summer impact of seawater, thus leading to its salinization and complete marinization of this ecosystem during the warm

period (May-October) of the year. This is immediately followed by an abrupt change during higher river water flow in the October-May period, which forced marine fish to migrate back to sea, while sessile marine and brackish organisms vanished from the major flow. However, this event creates new transitional ecosystem and additional nursery ground, which favours migration of smaller marine estuarine opportunist pelagic species and estuarine dependent marine species juveniles and adults, contributing to potential better recruitment of marine fish species in the Neretva Estuary.

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