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Infralittoral fish assemblages in the Zrmanja estuary, Adriatic Sea

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The infralittoral fish assemblage was investigated inside the temperate Zrmanja river estuary, Adriatic Sea. The specimens were caught using a beach seine at three stations and four times over the year. A total of 10 035 individuals, belonging to 47 species and to 17 families were represented in the catch, out of these Atherina boyeri *(60.3%),* Symphodus ocellatus *(14.4%) and* Pomatoschistus marmoratus *(7.5%) were most abundant, comprising 82.2% of the total catch. Marine and estuarine species represented 89.6 and 10.4%, respectively, while freshwater species were not observed in samples. Differences in fish assemblages were observed with respect to sampling dates and stations mostly due to different temporal and spatial distributions of juvenile fish settlers. Juveniles of open sea spawners* (L. mormyrus, P. erythrinus) *were found with increased distance from the river mouth. As they progressively grow, they disperse throughout the estuary. Juveniles of resident species* (Pomatoschistus sp.) *stayed closer to the river mouth. Permanent estuary residents comprised 11 species, 33 species were opportunists and the remaining 3 were transients. The data thus show that even in small temperate estuariesthe characteristics of river inflow have the potential to alter spatio-temporal patterns in infralittoral fish assemblages.*

Key words: infrallitoral fish assemblages, juveniles, distribution, temperate estuary

INTRODUCTION

Estuaries are important nursery habitats for marine juvenile fish and are characterized by high fish species richness and abundance of juveniles (CLARIDGE *et al*., 1986; LENANTON & POTTER, 1987; REINA-HARVAS & SERRANO, 1987; ROBERTSON & DUKE, 1990; TZENG & WANG, 1992; THIEL *et al*., 1995).

The value of tropical estuaries as habitats for marine animals is relatively well studied (REINA-HARVAS & SERRANO, 1987; TZENG & WANG, 1992). However, little is known about the biodiversity, community structure, temporal variations and life history of fish in temperate latitude estuaries, particularly along the Mediterranean coast. Previous studies conducted in the Adriatic analyzed fish infralittoral assemblages on rocky shores (DULČIĆ *et al*., 1997; GUIDETTI & BUSSOTTI, 2000) and on sandy bottom beaches (DULČIĆ *et al*., 2004, 2005). Unfortunately, there are no data about fish community structure in an Adriatic estuary although there are four large Croatian rivers that belong to the Adriatic Sea basin and all are located in interesting fishing areas.

Understanding juvenile fish distribution patterns is important for estimating the relative value of different estuarine habitats, as well as for assessing habitat restoration or rehabilitation needs (HANNAN & WILLIAMS, 1998). The main objective of this study was to test the hypothesis that there is no difference in fish assemblages within a small temperate estuary. Results obtained in this study could be useful for giving a better understanding of fish community structure in a temperate Mediterranean estuary.

MATERIAL AND METHODS

The karstic Zrmanja River (69 km) belongs to the Adriatic Sea basin. Its bottom is graveled and has steady flow with few cascades and rapids, with a karst stream running partly underground. The estuary of the Zrmanja River (φ 44° 11' N, λ 15° 34' E) empties into the Novigrad Sea (mean depth 28 m, length 11km, width 5 km) that is connected to the Adriatic and the adjacent Karin Sea (length 3.8 km, width 2.4 km) by narrow channels (Fig.1).

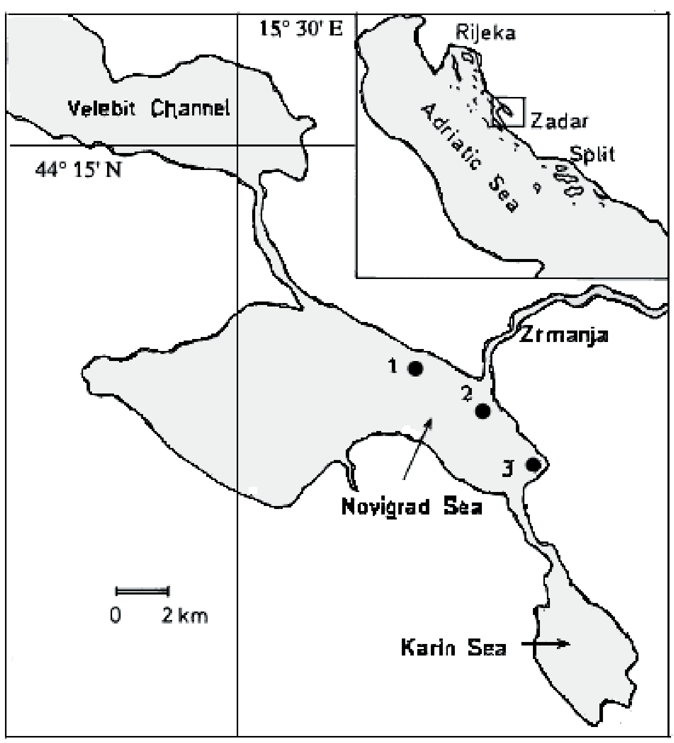

Fig. 1. Study area of the Zrmanja river estuary with three stations (1, 2 and 3) where spatial and temporal changes in fish assemblages were studied

The estuary (approx. 500 m wide at the river mouth) is highly stratified with a salt wedge and a sharp halocline. The fresh water Zrmanja river inflow determines the depth of the halocline. In summer salinity was high due to low river inflow. The highest inflow (mean 65 m^3s^{-1}) and lowest salinity occur in the December – April period (BURIĆ *et al*., 2004). Annual temperature varies from 6.7 – 26.6 ºC (mean 16.4 ºC), while the salinity ranged from 0 – 38 (mean 19.0). Nutrient concentrations are low and transparency high, characteristics of an oligotrophic system (BULJAN, 1969). Three sampling stations were selected in different parts of the estuary (1-2 km apart). The depths of the sampling stations ranged from 0.5 to 2.5 m and the substrate was heterogeneous rocky, sandy and muddy. *Zostera noltii* meadows were present at Station 1 and in clumps on Station 3, while this species was not observed on Station 2.

The fish fauna was sampled on 4 occasions: (A) August 25th 2003, (B) November 8th 2003, (C) February 20th 2004 and (D) May 20th 2004. Fish samples were collected using a 50 m long beach seine. Net depth at the beginning of the wings was 30 cm, while at the central part it was 250 cm including the sac. The wings had an 8 mm mesh size, while the mesh size of central sac was 4 mm. The net was always hauled from the deeper part of a sampling station perpendicularly toward the shore. At each sampling station four replicate net deployments (100 - 200 m apart) were made. The maximum working depth was up to 1.5 m.

The collected organisms were sorted and preserved in 4% formalin (pH 8.5-9.0). Fish species were identified according to JARDAS (1996). Total length (to the nearest 0.1 cm) and weight (to the nearest 0.01 g) were measured for each individual.

Physical-chemical parameters were measured three times before each net deployment; temperature was measured with a mercury thermometer and salinity with a laboratory inductive salinometer (accurate to ± 0.1) 20-30 cm beneath the surface.

Fish assemblage structure was analyzed using the PRIMER software package (CLARKE & WARWICK, 2001). Species richness (S) as simply a count of species and abundance (N) values were recorded. Data were log transformed and the Bray-Curtis similarity matrix was used to generate 2-dimensional ordination plots with the non-metric multidimensional scaling (nMDS) technique (CLARKE, 1993). The ANOSIM test for a replicated two-way crossed experimental layout was used for testing differences in species assemblage between seasons and sites (CLARKE & WARWICK, 2001). The α level for rejecting the null hypothesis was set at 0.05. All species that constituted less than 3% of total sample were omitted from the analysis.

Differences between sampling dates and stations with respect to temperature, salinity, biomass, species richness, total abundance, Pielou's evenness (J') and Shannon Wiener diversity (H) were tested with ANOVA using the Minitab software package. Prior to the analysis, homogeneity of variance was tested using Levene's test.

RESULTS

Differences were observed in abiotic parameters with respect to sampling date and station (Table 1).

Table 1. Mean values of salinity and temperature at three station on four occasions together with total number of individuals and total number of species sampled in the Zrmanja estuary

		Salinity		Temperature	
Source	df	MS	F	MS	F
Date (T)	3	1175.1	9401.1**	316.6	6020.8**
Station (ST)	2	106.0	847.6**	0.9	17.2**
TxST	6	10.1	80.9**	0.2	3.7**
Residual	30	0.1		0.1	
Levene			ns		ns

The lowest salinity values at all sampling stations were recorded in February, while August was characterized with the highest salinity values. With respect to sampling station, the lowest salinity values at all sampling dates were recorded at station 2. Although temperature differences were present between sampling times and stations, they were less pronounced with respect to stations. Since the relationship between sampling date and station was significant, for both salinity and temperature, changes in these abiotic parameters were not fully explained by each independent variable (Table 2).

A total of 10 035 fishes, belonging to 17 families and 47 species were caught at three stations in the Zrmanja estuary. The majority of caught specimens were juveniles (with already formed scales and fins, but which had still not reached first maturity), with the exception of about 10% of *Atherina boyeri* and *Pomatoschistus marmoratus* as adult individuals in the total sample. Sixteen species were found at all three stations (Table 3).

*Table 2. Results of ANOVAs comparing salinity and temperature at three station on four dates (*p < 0.05; **p < 0.01; ns: not significant, p >0.05)*

Occasions	Salinity			Temperature			Total no.	Total no.
	ST1	ST2	ST3	ST1	ST2	ST3	individuals	species
August	34.0	32.0	35.0	25.1	25.2	25.3	1 804	20
November	16.0	8.0	16.0	11.4	11.2	11.6	2 669	27
February	8.0	6.0	9.0	12.2	11.9	12.8	2 456	18
May	27.0	20.0	27.0	18.1	17.2	18.1	3 106	35

Table 3. List of all sampled fish species (+ present; - absent) with their total abundances at stations (1, 2 and 3) and dates: August (A), November (B), February (C), May (D) with habitat utilization: residents (R), opportunists (O) and transients (T)

Species	Habitat util.	Total	Station 1				Station 2				Station 3			
			A	B	C	D	A	B	C	D	A	B	C	D
Clupeidae														
Sardina pilchardus	T	17												+
Sprattus sprattus	T	27							+	+				
Engraulidae														
Engraulis encrasicolus	T	2								+		+		
Syngnathidae														
Sygnathus abaster	O	13			+		+		+	+				
Syngnathus acus	O	2										+		
Syngnathus tenuirostris	O	58		+	+	+		+	+	+		+	+	+
Syngnathus typhle	O	14	+			+	+			+	+	+	+	+
Nerophis maculatus	O	3						+		+				
Nerophis ophidion	O	14				+	+	+						+
Hippocampus ramulosus	O	1									+			
Serranidae														
Serranus hepatus	O	1										+		
Serranus scriba	O	10	+			+	+							
Mullidae														
Mullus barbatus	O	27		+		+		+		+		+		+
Mullus surmuletus	O	16				+		+		+				

Table 3. Cont'd

Species	Habitat util.	Total	Station 1				Station 2				Station 3			
			A	B	C	D	A	B	C	D	A	B	C	D
Sparidae														
Boops boops	O	1	+											
Diplodus annularis	O	101	+	+		+	+	+		+	+	+		+
Diplodus vulgaris	O	2				+				+				
Lithognathus mormyrus	O	40	+								+			
Pagellus erythrinus	O	10				+				+		+		
Centracanthidae														
Spicara flexuosa	O	38				+				+				+
Spicara smaris	O	59	+			+					+			
Labridae														
Symphodus cinereus	O	37	+	+	+	+	+						+	+
Symphodus ocellatus	O	1447	+	+	+	+	+	+	+	+	+	+	+	+
Symphodus roissali	O	98		+	+	+		+		+		+	+	+
Symphodus tinca	O	2				+								
Gobiidae														
Gobius niger	R	185	+	+		+	+	+	+	+	+	+	+	+
Gobius cobitus	R	2										+		+
Knipowitchia caucasica	R	7						+						
Pomatoschistus bathi	R	286		+	+	+		+	+	+		+	+	+
Pomatoschistus marmoratus	R	748				+	+	+	+	+	+	+	+	+
Pomatoschistus minutus	R	257			+			+	+	+				+
Zebrus zebrus	O	1							+					
Zosterissessor ophiocephalus	R	211	+	+	+	+	+		+	+	+	+	+	+
Callionymidae														
Callinonymus fasciatus	O	28								+			+	+
Callionymus risso	O	151					+	+	+		+	+		
Blenniidae														
Lipophrys dalmatinus	O	2							+					+
Lipophrys pavo	R	14					+			+				+
Parablennius sanguinolentus	R	7	+	+		+					+			
Parablennius tentacularis	R	11	+	+	+	+				+	+	+	+	+
Centrolophidae														
Centrolophus niger	O	1				+								
Mugilidae														
Chelon labrosus	O	1								+				
Liza aurata	O	1						+						
Atherinidae														
Atherina boyeri	O	6055	+	+	+	+	+	+	+	+	+	+	+	+
Bothidae														
Arnoglosus laterna	O	1							+					
Pleuronectidae														
Platichthys flesus	R	24								+				
Soleidae														
Solea kleini	O	1								+				
Buglossidium luteum	O	1									+			
Total species		47	13	12	10	23	13	16	14	26	14	18	12	21

Eight species were present throughout the year and comprised almost 90% of the total catch, while three of them (*Atherina boyeri,* 60.3%; 6 055 individuals ranging from 2.4 to 7.6 cm; *Symphodus ocellatus,* 14.4% and *Pomatoschistus marmoratus,* 7.5%) made up majority of that percentage. Juveniles of marine euryhaline species appear to be the most common in our samples. We observed only four (*Lipophrys pavo*, *Platichthys flesus*, *Pomatoschistus bathi*, *P. minutus*) solely estuarine species (their entire life-cycle occurs in the estuary), while true freshwater species were never recorded. Juveniles of some marine species (*B. boops*, *B. luteum*, *H. ramulosus*, *L. mormyrus*) were found only in August, while others like *S. cinereus* or *P. erythrinus* were found at Station 3 only in November. Estuarine species, including *A. laterna* (Lt = 9.1 cm; Wt = 5.58 g) and *Knipowitchia caucasica* (Lt = 2.6 cm; Wt = 0.14 g), were recorded in February.

The biomass of fish samples in the Zrmanja estuary varied temporally and spatially (Tables 4, 5).

Species richness varied between sampling dates, but did not show variations between sampling stations. ANOVA revealed a significant interaction between sampling date and station for a number of individuals. Pielou's evenness index varied only between stations, while the Shannon-Wiener diversity index showed both temporal and spatial variations.

The nMDS plots of fish assemblages in the Zrmanja estuary showed differences in fish abundance (N) with respect to sampling date and station (Fig. 2). Station 2 differed in fish community composition from Stations 1 and 3 (Fig 2a). With respect to dates, assemblages sampled in colder months were similar to each other, while there appears to be difference in community structure between those sampled in the warmer part of the year (Fig 2b). According to the results of the 2-way crossed ANOSIM test, fish assemblage abundances differed among stations (ρ_{av}=0.49, *P*=0.001) and sampling dates (ρ_{av}=0.51, *P*=0.001).

Table 4. Total values of biomass, species richness (S) and total abundance (N), and mean values of Pielou's evenness (J) and Shannon Wiener diversity (H) of fish collected by four replicate hauls at three station on four dates

Occasions	Biomass			S			N			J'			H		
	ST1	ST2	ST3	ST1	ST2	ST3	ST1	ST2	ST3	ST1	ST2	ST3	ST1	ST2	ST3
August	1448.0	640.6	661.7	13	13	14	1192	171	441	0.35	0.75	0.52	0.81	1.80	1.19
November	807.7	442.8	594.9	12	16	18	1047	929	693	0.31	0.49	0.31	0.54	0.83	0.63
February	1654.9	507.9	551.1	10	14	12	1735	350	371	0.30	0.54	0.67	0.58	1.09	1.22
May	2641.3	1428.5	1320.4	23	26	21	1315	1072	719	0.48	0.66	0.49	1.19	1.61	1.21

*Table 5. Results of ANOVAs comparing biomass, species richness (S), total abundance (N), Pielou's evenness (J) and Shannon Wiener diversity (H) of fish at three station on four dates (*p <0.05; **p < 0.01; ns: not significant: p >0.05)*

		Biomass		S		N		J'		H	
Source	df	MS	F	MS	F	MS	F	MS	F	MS	F
Date (T)	3	233998	5.3**	77.0	10.3**	26843	1.0ns	0.1	2.6ns	1.2	6.7**
Station (ST)	2	301643	6.86**	0.4	0.06ns	233660	8.5**	0.2	5.2**	0.9	5.2**
TxST	6	12211	0.28ns	1.6	0.2ns	25131	0.9ns	0.03	0.9ns	0.1	0.6ns
Residual	30	43949		7.4		27471		0.04		0.2	
Levene			ns		ns		ns		ns		ns

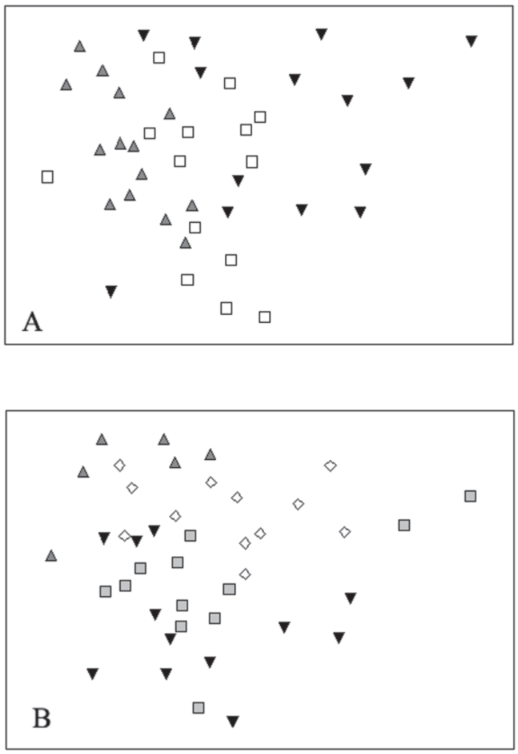

Fig. 2. A) nMDS ordination plot (stress = 0.21) of three stations in the Zrmanja river estuary: (▲ Station 1, ▼Station 2, ■ Station 3); B) nMDS ordination plot (stress = 0.21) of sampling dates (▲ A - August, ▼ B - November, ■ C - February, ◊ D - May)

Species were places in three categories on the bases of habitat use: permanent residents (R) which spend their entire life within the estuary and are highly adapted for estuarine conditions by possessing specialized physiological adaptations; opportunists or secondary residents (O) that spend only a part of their life history in the estuary, usually as juveniles, and generally have few physiological adaptations for estuary life and transients (T), often stenoterm and stenohalin species that only occasionally enter the estuary, usually when estuarine conditions are very similar to those of the open sea. In terms of relative contribution of species and individuals to each residential category, permanent estuary residents comprised 12 species (Gobiidae, Blenniidae, Pleuronectidae) that were represented by 1 752 individuals (17.46% of the catch). A total of 33 species represented by 8 237 fish (82.08% of the total) were considered to be opportunists. The remaining 3 species (Clupeidae, Engraulidae) represented by 46 individuals (0.46% of the catch) were considered to be transients.

Juveniles of open sea spawners (Sparidae, Centracanthidae, Serranidae) were found at Station 1 or 3. For example, a total of 40 juveniles of *L. mormyrus*, ranging from 1.8 to 4.4 cm L_t, were recorded in August at Station 1. Furthermore, 70% of 1 447 juveniles of *S. occelatus* were found also at Station 1 in August (ranging from 2.3 to 4.9 cm L_t), although larger individuals (ranging from 3.8 to 13.4 cm L_t) were recorded on all sampling dates and at all stations. Juveniles of *D. annularis* ranging from 1.5 to 2.4 cm were found at Station 1 and 3, while larger ones ranging from 2.2 to 10.3 cm were recorded throughout the estuary. At the other side, juveniles of estuary spawners, mainly resident species (Gobiidae, Blennidae) were recorded at Station 2.

DISCUSSION

The fluctuating nature of the estuarine environment determines its fish community structure. Results of the present study indicate that in the temperate Zrmanja estuary, fish exhibits both spatial and temporal variations.

Due to lack of temporal replicates we cannot make conclusions about seasonal differences in fish assemblages. However, the fish assemblage sampled in February (winter) in the Zrmanja estuary was low in diversity and composed of only the most physiologically tolerant species such as *A. boyeri* (BARDIN & PONT, 2002). JARDAS (1996) indicated that early spring and late summer (period of increasing temperature) were main spawning periods for the most common Adriatic fish species. The Zrmanja estuary, in May (spring) and November (fall) samples, received recruits of many species that spawn in the open sea, due to the timing of their settlement and recruitment. For example, more than 70% of juvenile *S. ocellatus* were observed in August and November, following early summer spawning, while *A. boyeri* entered the Zrmanja estuary in May. BARDIN & PONT (2002) previously reported spring immigration of *A. boyeri* and *Pomatoschistus spp*. into a

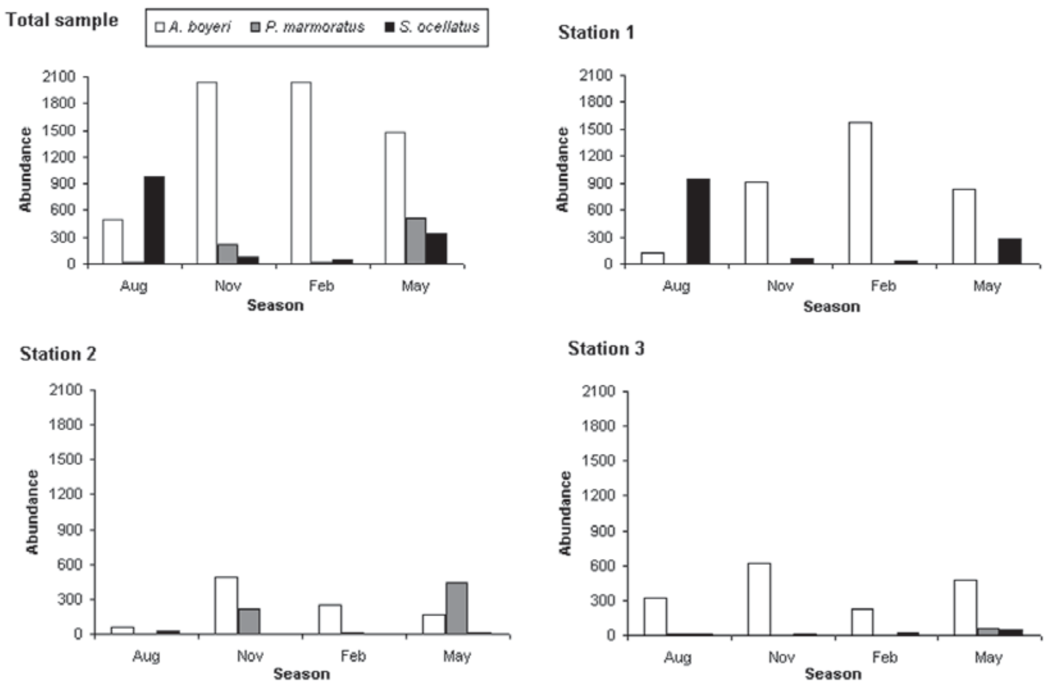

Fig. 3. Spatial and temporal distribution of three of the most abundant fish species in the Zrmanja river estuary fish assemblage

Mediterranean lagoon. Furthermore, *C. niger*, *C. labrosus* and *S. kleini*, all winter spawners, were found only in samples collected in February. Ecological separation of the dominant species by settlement timing resulted in the fact that species did not compete for the same niche (TZENG & WANG, 1992).

Only a few species dominate total abundances; for example, the atherinid comprised more than 60% of the total abundance in this study. In mangrove forests, gobiid (CLYNICK & CHAPMAN, 2002) and mugilid species (KUO *et al*., 1999) dominated the samples, showing once again a domination of one or two fish families in estuaries. These dominant taxa, like *A. boyeri* and *S. ocellatus* in this area, are important in the trophic support of fishery populations (VEGA-CENDEJAS *et al*., 1994) since they belong to the productive and low trophic level species with high ecological efficiency (DULČIĆ *et al*., 1997).

On the other side, we found that 82% of the caught species were opportunists. Five species, namely *A. laterna*, *C. labrosus*, *L. aurata*, *S. kleini* and *Z. zebrus*, were found only once and only at ST2 which is strongly influenced by the Zrmanja River. Therefore, we characterized them as rare species with a narrower spatial distribution. However, many other species (38.3%) were also present only at one of three stations or only once. Although the remaining species (48.9%) had wider temporal and spatial distributions, most of them were more abundant only at one station or only on one date. *L. mormyrus* juveniles were most abundant in August after their settlement from ichthyoplankton. Similarly, *P. bathi* and *P. marmoratus* were most abundant at ST2 in May, while *P. minutus* was the most numerous at ST2 in November. It seems that in a temperate estuary the majority of species only occasionally occupy some parts of the estuary following certain life-cycle phases such as settlement, recruitment, or spawning.

Recruitment of such small juvenile fish is limited by the supply of larvae that settle in the first seagrass they encounter (BELL et *al*., 1988). HANNAN & WILLIAMS (1998) concluded that settling fish do not discriminate between seagrass beds of different structure; in fact, they settle in any available shelter. In our study, some marine species, such as *B. boops* or *S.*

tinca, were found only at ST1 that is nearest to the real marine environment, and has meadows of *Zostera noltii*. The preference by small labrids for substrates colonized by macroalgae, as observed at that station, was known not only for the Mediterranean (GARCIA-RUBIES & MACPHERSON, 1995), but is also a common feature in other temperate waters (CHOAT & AYLING, 1987). Large-sized juveniles of some open sea spawners were distributed throughout the entire Zrmanja estuary because most of these were presumably old enough to move widely. Also, those large juveniles may easily leave seagrass shallows for alternative habitats in case of some accidental situation (HANNAN & WILLIAMS, 1998). Juveniles of resident species (found in such a habitat during their whole life cycle) always stayed around low salinity stations and were quantitatively dominant there. However, in spring when the strongest northern winds occur in the Zrmanja canyon (BULJAN, 1969) juveniles of resident species are carried directly to marine areas by the currents generated.

HANNAN & WILLIAMS (1998) stated that ocean-spawned juveniles, after settlement, tend to stay closer to the entrance of the estuary, while juveniles of lagoon spawners are widely distributed within the lagoon. Although the majority of species found in this study could be classified by spawning area (inshore or offshore waters), very little is known about their specific spawning habitats or larval transportation routes. In relation to our investigated habitat, we found that fewer species of open sea spawners (*L. mormyrus*, *P. erythrinus*, *S. flexuosa*) are found with increased distance from the river mouth. As they progressively grow, they disperse throughout the estuary, and such juveniles recruit at later times and at larger sizes far from the river mouth. On the contrary, juveniles of estuary spawners, mainly resident species (*Pomatoshistus* sp.), stayed closer to the river mouth or near the shoreline with the main influence from river outflow.

The results of this study contribute to the knowledge of the Zrmanja estuary biodiversity and indicate spatial and temporal variations in infralittoral fish assemblages inside a small temperate estuary. Observed variations can be attributed to temporal shifts in faunal composition, immigration and emigration in terms of settlement and recruitment of both marine and estuarine fishes and their trophic interactions. However, due to insufficient replicates, it is necessary to proceed with investigations on wider temporal and spatial scales including manipulation experiments, in order to determine the effects of biological interaction and environmental factors in structuring fish assemblages in these environments.

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Infralitoralne zajednice riba na ušću Zrmanje, Jadransko more

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SAŽETAK

Infralitoralne zajednice riba su proučavane na području ušća rijeke Zrmanje, u Jadranskom moru. Jedinke su ulovljene malom obalnom mrežom potegačom na tri postaje, u četiri uzorkovanja tijekom jedne godine istraživanja. Ukupno 10 035 jedinki svrstanih u 47 vrsta i 17 porodica, je bilo zastupljeno u uzorku, od čega su *Atherina boyeri* (60,3%), *Symphodus ocellatus* (14,4%) i *Pomatoschistus marmoratus* (7,5%) bile najzastupljenije vrste, sačinjavajući 82,2% od ukupnog uzorka. Prave morske i estuarijske vrste riba su bile zastupljene s 89,6, odnosno s 10,4%, dok slatkovodne ribe nisu uopće ulovljene. Utvrđene su prostorne i vremenske razlike u zajednicama riba zbog različite vremenske i prostorne raspodjele nedoraslih riba. Nedorasle jedinke onih vrsta koje se mrijeste u otvorenom moru (*L. mormyrus*, *P. erythrinus*) su nađene s povećanom udaljenošću od samog ušća rijeke. Kako su navedene jedinke rasle, tako smo ih nalazili različito rasprostranjene na širem području ušća. Nedorasle jedinke rezidentnih vrsta (*Pomatoschistus* sp.) su ulovljene blizu samog ušća. Stalni su estuarijski rezidenti sačinjavali 11 vrsta, 33 vrste su bile oportunisti, a preostale 3 tranzitne vrste. Podaci pokazuju da čak i mali estuariji umjerenih geografskih širina imaju potencijal pojačati prostorno-vremenske razlike u zajednicama infralitoralnih riba.

Ključne riječi: infralitoralne zajednice riba, nedorasle jedinke, raspodjela, estuarij umjerenih geografskih širina