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Recruitment and food composition of juvenile thin-lipped grey mullet, *Liza ramada* (Risso, 1826), in the Neretva River estuary (Eastern Adriatic, Croatia)

Vlasta BARTULOVIĆ[1] *, Branko GLAMUZINA[1], Davor LUČIĆ[4],
Alexis CONIDES[2], Nenad JASPRICA[4] and Jakov DULČIĆ[3]

[1] *University of Dubrovnik, Department of aquaculture, Ćira Carića 4, 20 000 Dubrovnik, Croatia*

[2] *Hellenic Centre for Marine Research, Agios Kosmas, Hellinikon, 16604 Athens, Greece*

[3] *Institute of Oceanography and Fisheries, P. O. Box 500, 21 000 Split, Croatia*

[4] *University of Dubrovnik, Institute for Marine and Coastal Research, Kneza Damjana Jude 12, 20 000 Dubrovnik, Croatia*

**Corresponding author, e-mail: vlasta@unidu.hr*

The present paper describes the recruitment and food composition of thin-lipped grey mullet, Liza ramada *(Risso, 1826) juveniles in the estuary of the Neretva River in the southeastern Adriatic. Thin-lipped mullet appeared in the Port of Ploče in February. The relationships between total length, standard length, and weight showed a remarkably good fit to the expected allometric model, with b=3 for total length. Twelve different animal food categories were identified. The greatest diversity was recorded in April at the Port of Ploče when 10 categories were present. In addition, six diatom taxa were noted. Harpacticoid copepods were the most common prey (39%) in these samples, followed by insects (32%) and cladocerans (12%). All other prey accounted for less than 4%. Insects represented 99% of prey in the stomachs of specimens sampled from the freshwater Crna Rijeka River. Thin-lipped mullet juveniles were not found on sandy beaches or natural bays around the port. It thus appears that juveniles find the port area to be a habitat with the favorable characteristics of a brackish water nursery: of predators, lower salinity, and high food availability. These all promote better survival and recruitment in this area of the Croatian Adriatic.*

Key words: thin-lipped grey mullet, *Liza ramada*, juveniles, food, recruitment

INTRODUCTION

The thin-lipped grey mullet, *Liza ramada* (Risso, 1826) is a catadromous pelagic species found in various habitats, from shallow brackish and marine waters to lagoons, estuaries and river deltas. It tolerates salinity extremes, as well as abrupt water-quality changes (THOMSON, 1990). *L. ramada* is distributed along all coastlines, with especially high abundance found in river deltas (JARDAS, 1996).

Like most Mugilidae, this species reproduces at sea, after which fry undertake a trophic migration shoreward to continue their development (KOUTRAKIS *et al.,* 1994) in food-rich lagoons, rivers, and even lakes (THOMSON, 1966). Spawning in the southeastern Adriatic reaches a peak in October (BARTULOVIĆ, 2006), and juveniles appear along the middle-Adriatic coast in February (JUG-DUJAKOVIĆ, 1988) and March (KATAVIĆ, 1980).

Food and feeding of mullet fry of several species have been described by a number of authors, including: CARDONA (1999) in Minorca; DE SILVA & WIJEYARATNE (1977), and PEREIRA & DE SILVA (1978) in Negombo Lagoon (Sri Lanka); TORRICELLI *et al.* (1988), and TOSI & TORICELLI (1988) in the Arno River; ZISMANN *et al.* (1975) in Haifa Bay; ALBERTINE-BERHAUT (1973) in the Gulf of Marseilles; FERRARI & CHIEREGATO (1981) in the Po River delta; GISBERT *et al.* (1995) in the Canal Vell lagoon (Spain). Unlike adults, juveniles generally are planktonphagous (ALBERTINI-BERHAUT, 1973, 1975; TOSI & TORRICELLI, 1988; PYSAREUSKAYA & AKSEMOVA, 1991).

Recent research along the Croatian coast indicates an increase in the population of *L. ramada* in the Neretva River estuary (unpublished data). This paper evaluates recruitment and the composition of available food as possible explanations.

MATERIAL AND METHODS

Juvenile fish were sampled between January and May 2004 at three stations in the Neretva River estuary, Croatia (Fig. 1). Station 1 was in the Port of Ploče, along a 30-year-old seaside promenade exposed to the open flow of municipal sewage and rainwater drainage. Depth at this site

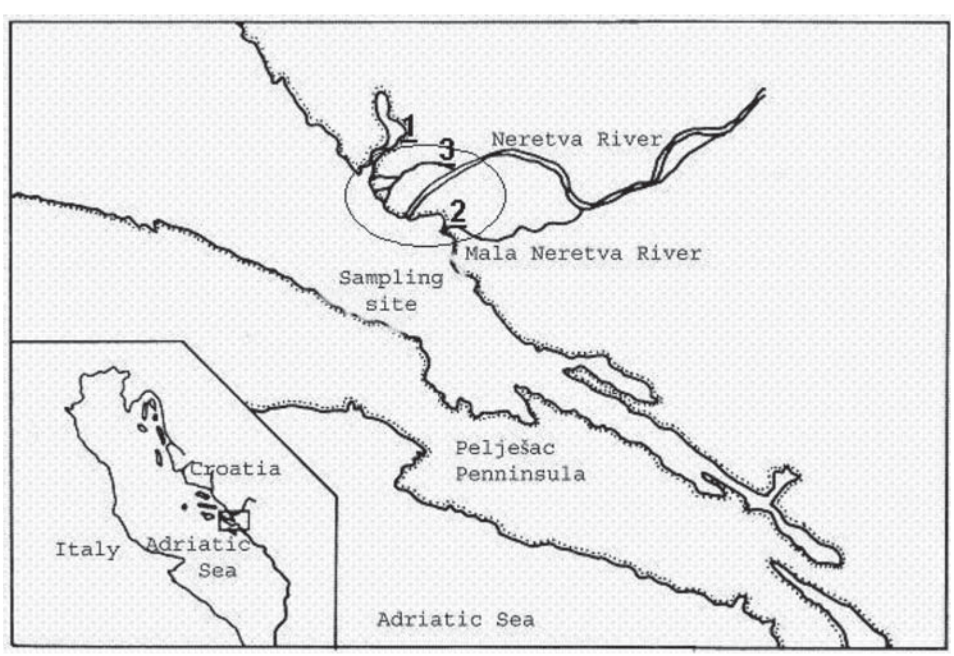

Fig. 1. Map of the estuary of the Neretva River with sampling stations (1- Port of Ploče; 2- mouth of the Mala Neretva River; 3- Crna Rijeka River)

is 1 - 2 m and the bottom is composed mainly of small rocks.

Station 2 was in the Mala Neretva River estuary on a shallow beach composed of fine sand. Depth varied from a few to twenty centimeters.

Station 3 was in the Crna Rijeka River, near the village of Rogotin, where the coastline and bottom is composed mainly of artificially added rocks covered with mud. Depth varied from 0.5 to 1 m.

Temperature and salinity were measured with portable digital instruments (WTW).

The characteristics of the stations demanded different types of gear for effective sampling. Owing to the depth and nature of the bottom, a small net mounted on a 2-m-long pole was used at stations 1 and 2. A beach seine was used to sample at Station 3.

Fish were preserved in 4 % formalin immediately after sampling, then transported to the laboratory where total (TL) and standard (SL) lengths were measured to the nearest 0.01 mm with a digital caliper and body wet weight (W) was measured to the nearest 0.0001 g with an analytical balance.

The relationship between TL and W was calculated for all specimens according to the commonly used power function (RICKER, 1975):

$$W = a\,(TL)^b$$

where *W* is wet weight (g); *TL* is total length (cm); *a* and *b* are parameters.

Growth over sampling intervals was calculated using Chou's estimator (CHOU, 1968):

$$\textit{Rate of increase (or decrease)} = \pm\sqrt[n]{\frac{\textit{final value}}{\textit{initial value}}}$$

where *n* is the number of intervals (n = 4); and initial value and final value refer to either weight or length, as appropriate.

Prey composition in the stomachs of 50 randomly selected fish from each sample from Station 1, 18 fish from Station 2 and 13 fish from Station 3 was determined to the lowest possible taxon with a binocular microscope at 400x. Sub-samples of stomach contents were examined using an inverted microscope (Olympus IX 71) at 400x to determine micro-algal taxa. Diatoms were identified following standard techniques (BATTARBEE, 1986).

The relative numerical contribution of each micro-algal taxon was determined from 50 randomly selected fields of vision. Frequency of occurrence was determined for each prey category (HYNES, 1950; PAIS, 2002).

Prey composition was analyzed by Principal Components Analysis to define similarities in the presence (or absence) of certain items, as well as similarities between stomach contents among months. Similarities were examined using a simple clustering technique based on the Bray-Curtis dissimilarity index (ALDENDERFER & BLASHFIELD, 1984; LUDWIG & REYNOLDS, 1988). The Olds Rank Correlation Criterion was used to detect any similarity in food composition between sampling dates (OLDS, 1938; ROGERS, 1968). This criterion, applied only to the most abundant zooplankton groups (Cladocera and Harpacticoida) and Insecta, is given as:

$$r' = 1 - \frac{6\sum_{i=1}^{n} d^2}{n(n^2 - 1)}$$

where *n* is the number of ranks; and *d* is the difference between rank values.

RESULTS

Temperature and salinity

Sea-surface temperature at the Port of Ploče (January-May) varied from 7.2 °C in February to 22.8 °C in May; and from 9.3 °C to 21.8 °C at a depth of 1 m. Over the same period salinity varied from 10.3 to 35.9 at the surface and from 21.5 to 37 at 1 m (Fig.2).

When juveniles appeared at sandy beaches (Station 3), the temperature was 22 °C and salinity was 8. Juveniles started to migrate to freshwater during April, when the temperature at Station 2 was 14.4 °C and salinity was 1.2.

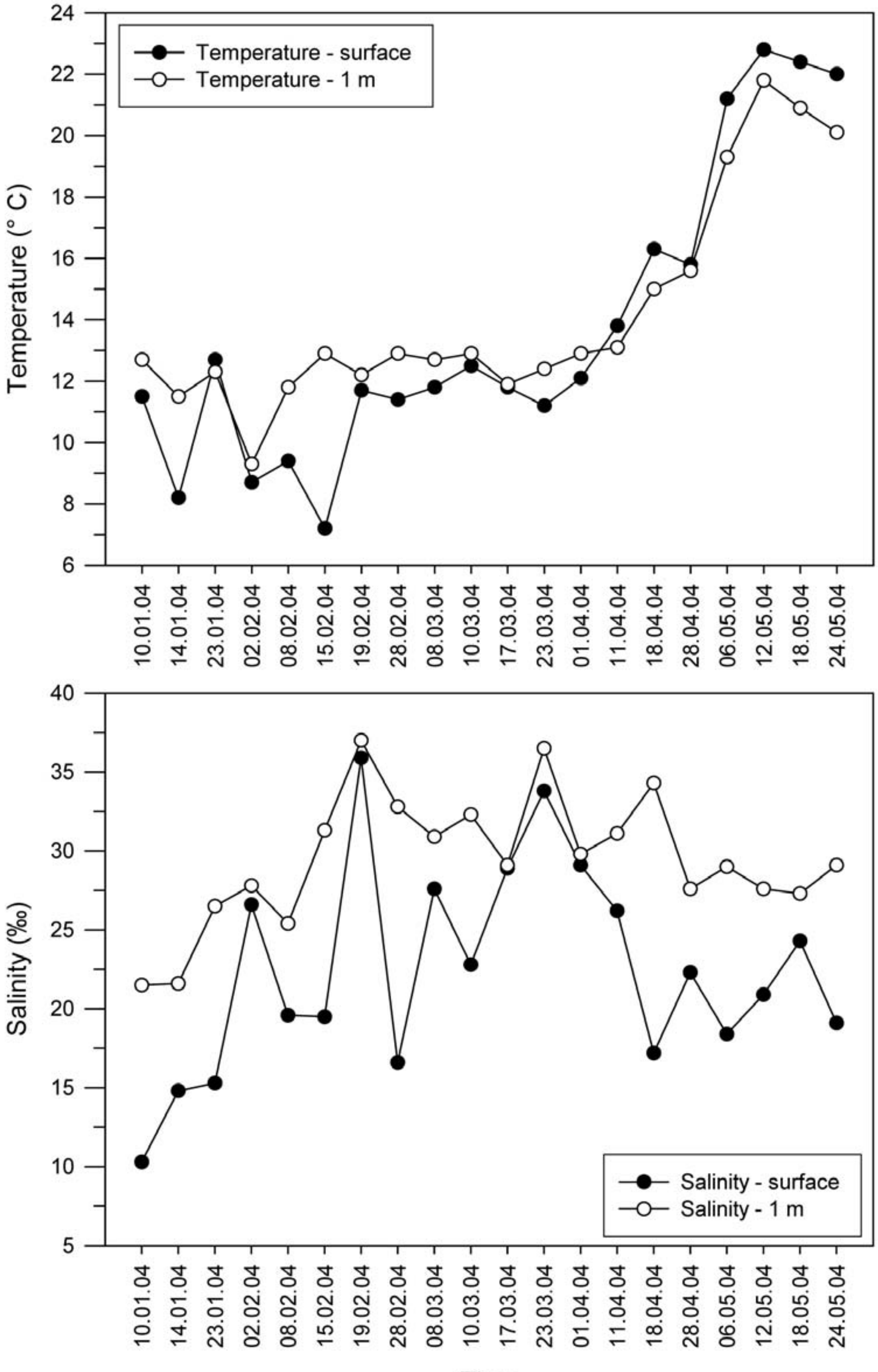

Fig. 2. Temperature and salinity at the station in the Port of Ploče during January-May 2004

Characteristics of recruitment

Thin-lipped grey mullet juveniles were first caught in the Port of Ploče in February. The fish remained in the area for the next three months. The first appearance of juveniles on the sandy beaches of the Mala Neretva River estuary was in April, and they started to migrate to freshwater in May, when they were caught in Crna Rijeka River, approximately 5 km upstream.

Juveniles in Ploče in the February - April period were dominated by fish of 20 - 25 mm TL. Smaller fish were found in February and larger ones from March onwards. Total lengths of juveniles from the other stations were between 20 - 25 mm in April and May.

The formulae relating weight to, respectively, total length and standard length were:

$$Weight(g) = 0.0094[TL, cm]^{3.001},\ r^2 = 0.932, std.error = \pm 0.008g$$

$$Weight(g) = 0.0167[SL, cm]^{3.103},\ r^2 = 0.941, std.error = \pm 0.008g$$

Each of these relationships (Fig. 3) shows a good fit to the expected allometric model in which $b = 3$.

Growth

Growth was estimated at +2.5% in terms of length and +7.5% in terms of weight (Fig. 4). Growth was low, even negative, during the first 50 days of recruitment. This corresponds with a period of generally low temperatures. A higher rate of growth was observed in April and afterwards, when sea water temperature exceeded 15 °C.

Food Composition

Algae

Only diatoms were recorded in gut samples on 27 March in Ploče, and again on 22 April in Ploče and also at the Mala Neretva River estuary station. The frequency of microalgae

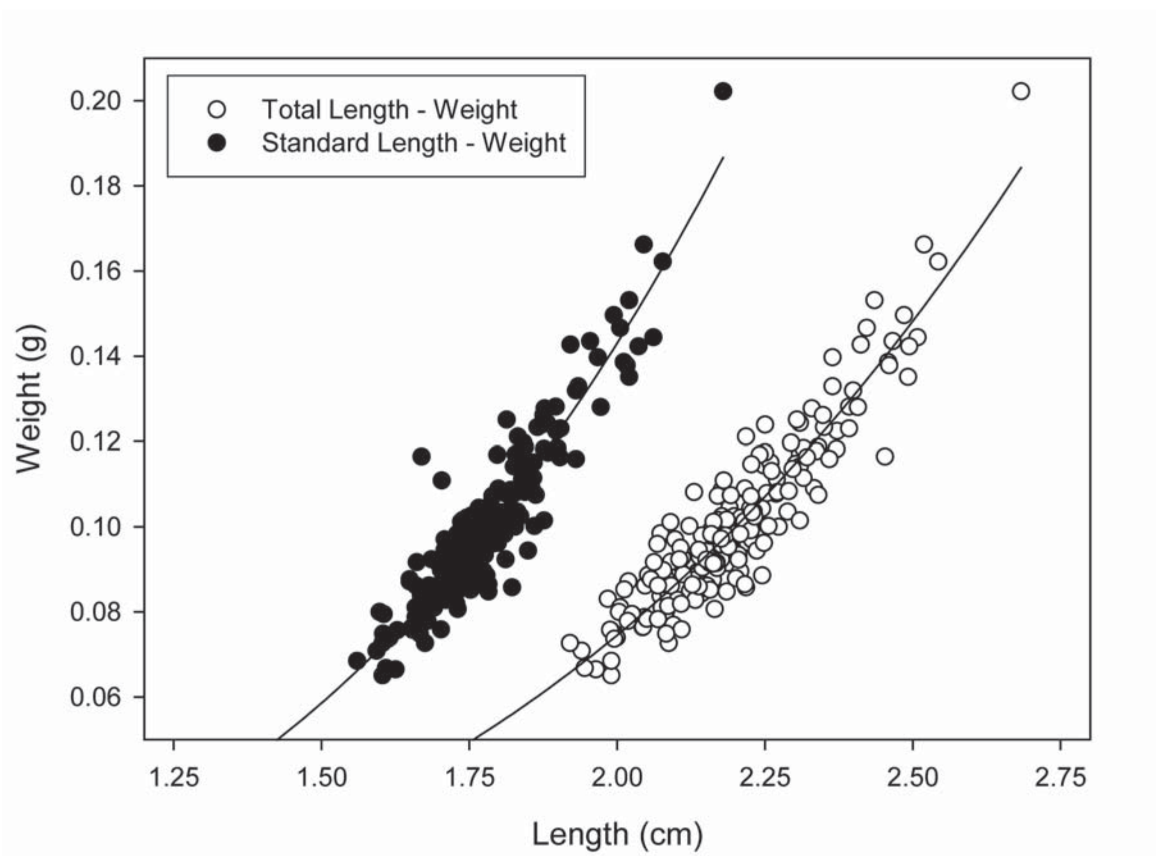

Fig. 3. Length - weight relationships of thin-lipped grey mullet, Liza ramada*, juveniles from the Neretva River estuary*

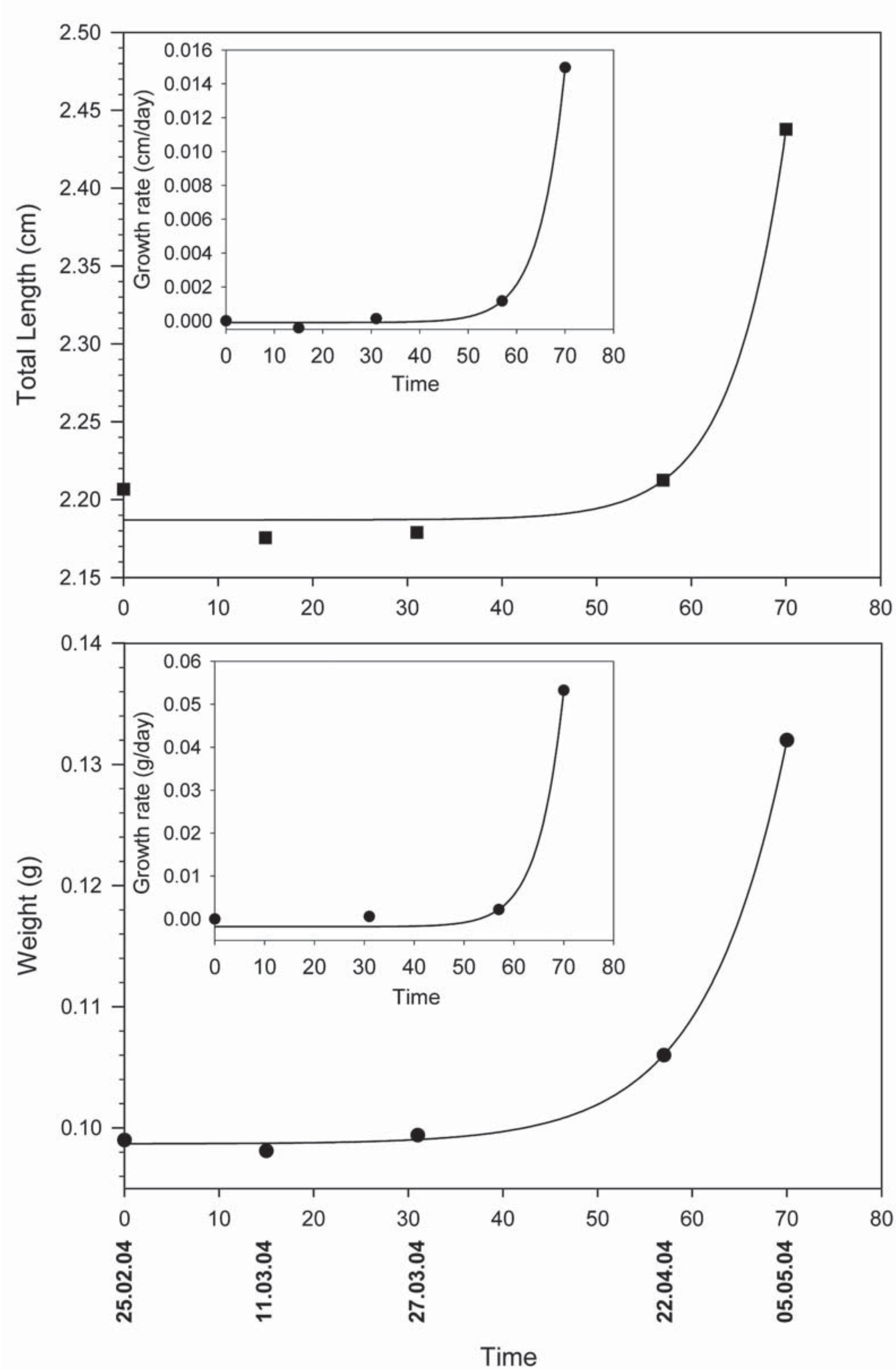

Fig. 4. Weight and length intervals and growth rates of thin-lipped grey mullet, Liza ramada*, juveniles from the Neretva River estuary*

occurrence was 32%. Whenever microalgae were found in stomach contents they were massively present. In total, six diatom taxa were noted. These included five pennates: *Achnanthes longipes*, *Licmophora flabellata*, *L. gracilis*, *Nitzschia* cf. *distans*, and *Synedra fulgens*. One centric, *Melosira nummuloides*. *N.* cf. *distans*, was most common, accounting for 49% of all diatoms.

Fish length in March and April ranged from 19.5 - 26.8 mm (mean 22.25 ± 1.49 mm). Diatoms were abundant in the stomach of fish larger than 22.5 mm (one-way ANOVA, N=89; P<0.002). Unidentified microalgae (2 - 20 μm), and parts thereof, were rarely present.

Fauna

Twelve different animal food items were identified, with the greatest diversity recorded in April at Ploče when 10 categories were present (Table 1). The minimum (one) was recorded in May.

Different development stages of harpacticoids (Copepoda) and insects represented the bulk of animal prey. Small or large aggregations of their eggs were found in gut contents, probably owing to fish having ingested gravid females.

Harpacticoids were the most common prey (39%) at Ploče, followed by insects (32%) and cladocerans (12%). All other categories accounted for less than 4% (Fig. 5).

Table 1. Average number and percent contribution of prey in the stomachs of thin-lipped grey mullet, Liza ramada*, juveniles from Ploče harbor*

	25 February		11 March		27 March		22 April		05 May	
No of caught fish	489		611		456		348		142	
No of analyzed fish	50		50		50		50		50	
	avg ± SD	%	avg ± SD	%	avg ± SD	%	avg ± SD	%	avg ± SD	%
Diatoms					mass		mass			
Bivalve larvae	0.1±0.2	<1								
Polychaete larvae							0.1±0.2	<1		
Cladocera	0.3±0.9	4			0.7±1.7	5	9.2±16.5	39		
Cirripedia larvae							2.5±4.9	11		
Ostracoda							0.3±1.0	1		
Oithona copepodites					0.1±0.3	1	0.1±0.3	<1		
Oncea copepodites					0.1±0.3	1	0.1±0.2	<1		
Harpacticoidea total	0.4±0.9	12	10.2±23.7	97	3.2±6.4	70	0.5±2.0	4		
Nauplii			21.7±27.6	68	6.7±9.7	49				
Copepodities	0.2±0.9	4	8.8±27.4	28	2.3±3.4	17	0.9±2.7	4		
Adults	0.3±0,8	8	0.1±0.3	1	0.6±0.7	4	0.1±0.3	<1		
Gammaridea juv.	0.3±0,6	<1	0.1±0.3	<1			0.1±0.2	<1		
Insecta	5.3±3.8	83	0.8±1.1	2	3.2±3.7	23	10.3±8.5	44	8.0±1.0	100
Harpacticoidea eggs	rare		abundant				abundant			
Insect eggs	abundant				abundant		rare			

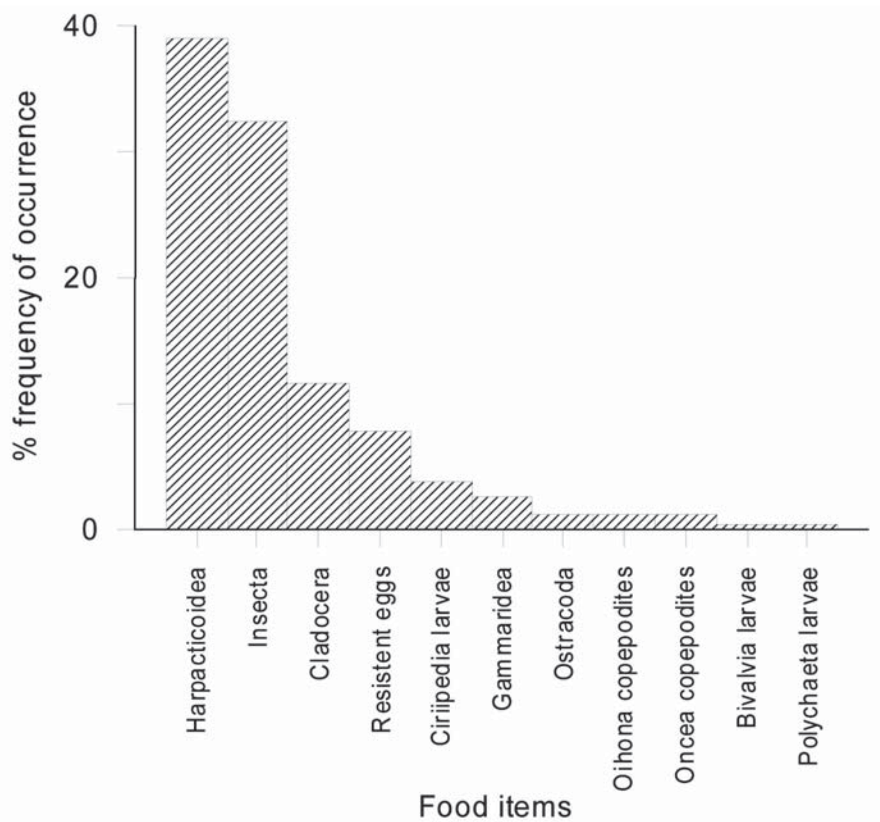

Fig. 5. Pareto diagram of the relative frequency of occurrence of the various food item categories in the stomach contents of thin-lipped grey mullet, Liza ramada, *in the Port of Ploče*

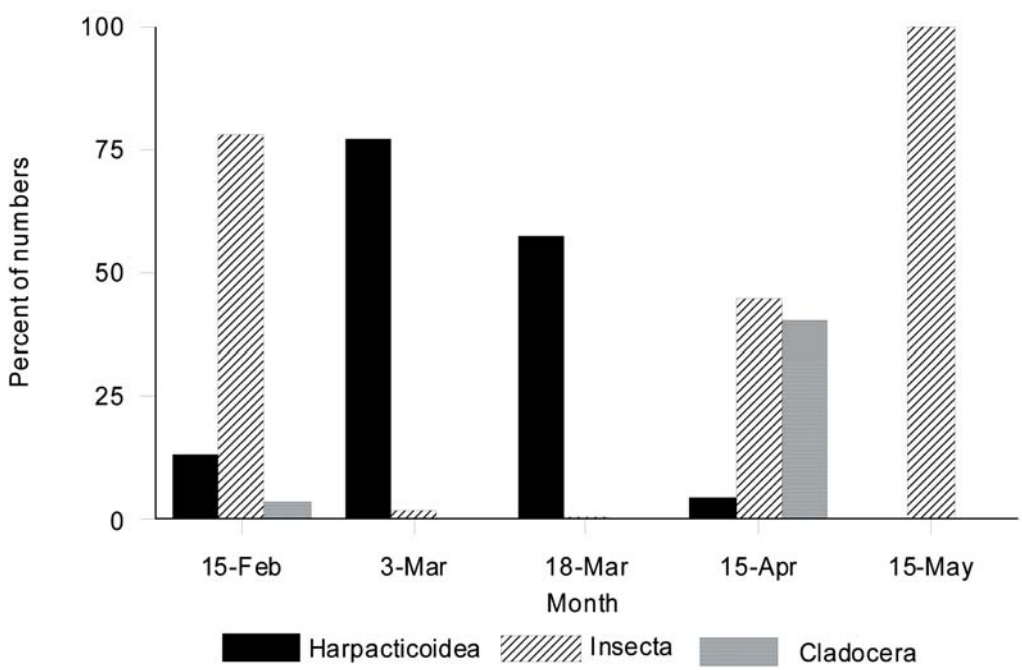

Fig. 6. The occurrence of the 3 major zooplankton groups in the diet of thin-lipped grey mullet, Liza ramada, *juveniles (in percentage)*

Percentage abundance of the most common prey exhibited clear monthly variations (Fig. 6). Harpacticoid naupliar stages dominated in March, copepodites in April and adults, represented by the free-living meiobenthic genera *Cletodes* and *Haloschizopera*, in February (Table 1).

Insects were pronouncedly dominant in February and May. In April, insects and the cladoceran *Podon polyphemoides* dominated gut contents (Fig. 6), and the average abundance of all prey was very high (Table 1).

Harpacticoids were the most common animal prey (84%) in the stomachs of 18 specimens caught in the Mala Neretva River estuary, followed by insects (10%) and nematodes (6%). The average number of prey, however, was less than in Ploče: 1.33 ± 1.54 harpacticoids, 0.56 ± 1.20 insects, and 0.28 ± 0.67 nematodes. Harpacticoid nauplii and copepodites represented 33 and 29% of the total number, respectively. Sand was found in the guts of 13 specimens.

Insects represented 99% of prey in the stomachs of thirteen specimens sampled from the Crna Rijeka River. *Daphnia* spp made up the small remaining percentage. The number of insects in the stomachs was high (12.77 ± 5.83).

Food composition data showed strong dissimilarity between sampling dates, with all clustering accounting for less than 50% similarity. Moreover, the Olds Criterion was negative in all cases, indicating negligible similarity between food compositions at successive sampling dates (Fig.7).

Cluster analysis between sampling dates showed higher similarity between February and May. Otherwise, similarity was less than 50%.

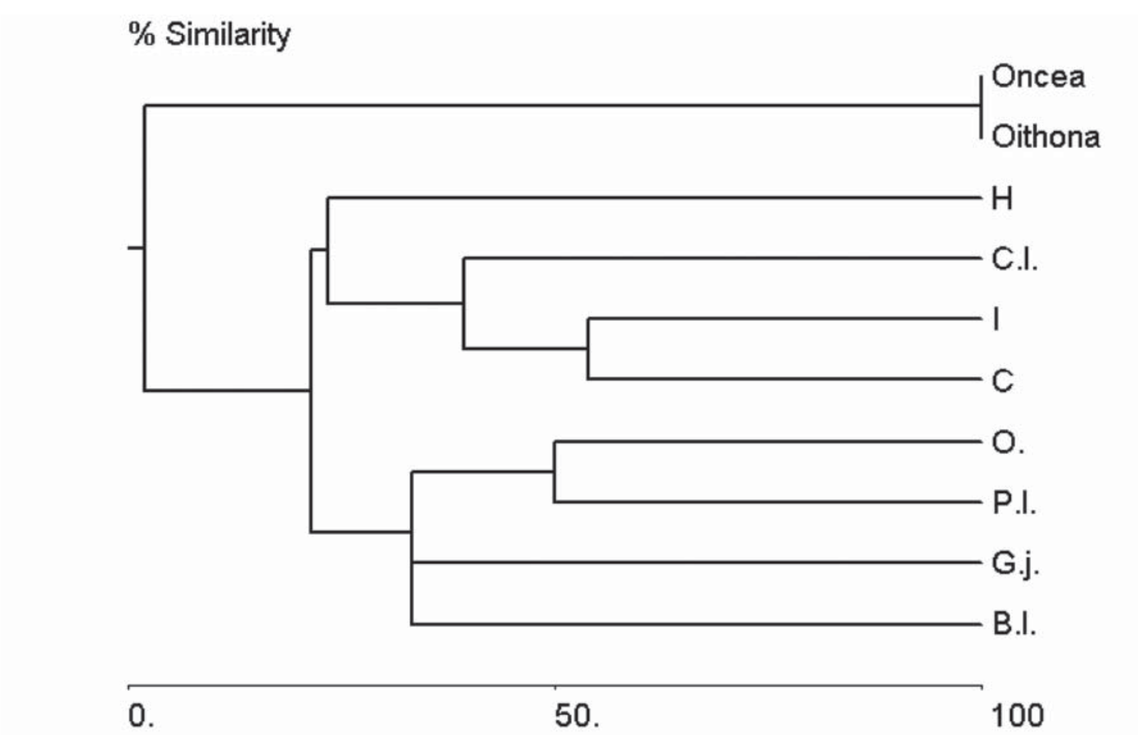

Fig. 7. Clustering scheme of the food item appearance pattern, based on prey numbers, in the diet of thin-lipped grey mullet, Liza ramada*, according to the Bray-Curtis Similarity Index. Legend:* B.l.. Bivalvia larvae; P.l.. Polychaeta larvae; C.. Cladocera; C.l..Cirripedia larvae; O.. Ostracoda; Oithona. Oithona copepodites; Oncea. Oncea copepodites; H.. Harpacticoidea. G.j.. Gammaridea juveniles; I.. Insecta

DISCUSSION

Thin-lipped grey mullet juveniles in the Neretva River estuary were first observed at the end of February in the Port of Ploče, a habitat with lower salinity. Length ranged from 19 to 23 mm. This is consistent with observations from the middle Adriatic (near Šibenik, 150 km north of the Neretva River) on sandy or gravel beaches (JUG-DUJAKOVIĆ, 1988). In the same general area KATAVIĆ (1980) caught the highest number of juveniles (in the Pantan River estuary) in March.

Published reports of first appearance differ according to geographical location. In Porto Lagos lagoon (Northern Greece), juveniles appeared in early January at a TL of 14.6 - 18.3 mm (KOUTRAKIS *et al.,* 1994). Juveniles appeared along the coast of Israel in January, with the main period between the end of February and the beginning of March (ZISMANN & BEN-TUVIA, 1975). TORRICELLI *et al.* (1982), working in the Arno River (Italy), noted that juveniles appeared in January while, at the mouth of the Magra River (Italy), they had already appeared in December (GANDOLFI *et al.,* 1981). ROSSI (1986) found juveniles in October in the Po River Delta (Italy), and CAMBRONY (1984) concluded that January is the time of first appearance in Languedoc-Roussillion (France). VIDY & FRANC (1992) stated that, in Tunis, the season started in December.

Earlier appearances are more common in the southern parts of the Mediterranean, but this pattern does not seem to apply to the middle and northern Adriatic. The three main studies for the middle Adriatic (KATAVIĆ, 1980; JUG-DUJAKOVIĆ, 1988; the present paper) document recruitment during February – March. Recruitment is much earlier in the Northern Adriatic (Italian coast, Po River; ROSSI, 1986), generally in October. This might be explained by the higher temperatures of northwestern Adriatic lagoons that permit earlier maturation and spawning of *L. ramada*.

The present results on fry feeding habits differ from those of previous studies in which micro- and mesozooplankton were the main food (FERRARI & CHIEREGATO, 1981; TOSI & TORRICELLI, 1988; GISBERT *et* al., 1995). Although the present study confirms a carnivorous diet, microalgae were important in the nutrition of some specimens. FERRARI & CHIEREGATO (1981) noted that the diet of thin-lipped mullet less than 28 mm TL exclusively consisted of zooplankton. Our study showed a greater preference for diatoms. Microalgae occurred in the stomachs of fish up to 26.9 mm TL, and diatoms were abundant for those specimens larger than 22.5 mm TL. Most of the diatoms in stomach contents were large (>50 µm) littoral species typically found in a variety of substrata (SNOEIJS & KASPEROVIČIENE, 1996) and common members of spring plankton in the coastal middle and south Adriatic (PUCHER-PETKOVIĆ, 1979; JASPRICA & CARIĆ, 2001). SNOEIJS (1999) reported the same species to be frequent in many estuaries and inland seas.

Meiobenthic harpacticoids and insects were the most abundant prey identified in this study. However, in other studies they were considered also but other authors have noted them to be rare (FERRARI & CHIEREGATO, 1981; TOSI & TORRICELLI, 1988; GISBERT *et al.,* 1995). This could be explained by differences in habitat where fish were sampled.

Low similarity between prey items (Fig. 7) clearly indicates opportunistic feeding in this species. Thus, as insects were among the most abundant prey during certain periods of this study, they naturally would be expected to be the most targeted prey. This might explain the differences in prey preference reported by other authors.

Earlier reports (FERRARI & CHIEREGATO, 1981; TOSI & TORRICELLI, 1988) indicated that sandy beaches are generally the most typical habitat sought by juveniles before their migration to freshwater. However, in the present work they were not found on the sandy beaches and natural bays around the Neretva River estuary, but rather in the area of the Port of Ploče. This habitat was formed rather recently (mid 1970s) as a result of the development of the port facilities and the town that has grown around them. A major feature of this development is freshwater runoff that locally lowers salinity and sometimes carries a high number of insects, thereby making it very suitable for juveniles. The same features apply to the freshwater site (Rogotin) where insects dominated prey. An important caveat is that there are no comparative data on the distribution of *L. ramada* in this area before the port development.

The nature of the substrate and coastal morphology thus appear to be insufficient to attract juvenile mullet. Instead, the spring rains and subsequent runoff that reduce salinity and carry suitable food items into coastal waters are key factors. The fact that there is no organized collection and treatment of municipal runoff in this area thus has the somewhat unexpected benefit of enhancing the recruitment of mullet.

According to most published information, the general rule is that *L. ramada* appears in coastal areas and later migrates to freshwater nursery grounds. The present data suggest that, in the newly created ecosystem of the Port of Ploče, juveniles find a nursery ground that includes the sought-after characteristics of freshwater habitats, including an absence of predators, lower salinity, and high food availability. All foster better survival and recruitment of thin-lipped grey mullet juveniles and appear to be responsible for enhancing mullet stocks in this region of the Croatian Adriatic.

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Novačenje i sastav hrane mlađi cipla balavca *Liza ramada* (Risso, 1826) na ušću rijeke Neretve (istočni Jadran, Hrvatska)

Vlasta BARTULOVIĆ[1 *], Branko GLAMUZINA[1], Davor LUČIĆ[4], Alexis CONIDES[2], Nenad JASPRICA[4] i Jakov DULČIĆ[3]

[1] *Sveučilište u Dubrovniku, Odjel za akvakulturu, Ćira Carića 4, 20 000 Dubrovnik, Hrvatska*

[2] *Helenski Centar za istraživanje mora, Agios Kosmas, Hellinikon, 16 604 Atena, Grčka*

[3] *Institut za oceanografiju i ribarstvo, P.P. 500, 21 000 Split, Hrvatska*

[4] *Sveučilište u Dubrovniku, Institut za more i priobalje, Kneza Damjana Jude 12, 20 000 Dubrovnik, Hrvatska*

SAŽETAK

U ovom radu opisuju se novačenje i sastav hrane mlađi cipla balavca *Liza ramada* na ušću rijeke Neretve u jugoistočnom Jadranu. Cipal balavac se pojavljuje u luci Ploče tijekom veljače. Odnosi između ukupne dužine, standardne dužine i mase pokazuju izuzetno dobro poklapanje s očekivanim alometrijskim modelom, za vrijednost alometrijskog koeficijenta b=3 za ukupnu dužinu. Određeno je dvanaest različitih kategorija hrane. Najveća raznolikost, 10 skupina plijena, je utvrđena u travnju u luci Ploče. Dodatno je utvrđeno 6 taksonomskih kategorija alga kremenjašica. Harpaktikoidni veslonošci su najčešći plijen (39%) mlađi cipla balavca, zatim slijede kukci (32%) i rašljoticalci (12%). Na sav ostali plijen otpada manje od 4%. Kukci predstavljaju 99% plijena utvrđenog u probavilima jedinki prikupljenih iz Crne rijeke. Mlađ cipla balavca nije utvrđena na pješčanim plažama i zaljevima šireg područja ušća Neretve. Smatra se da je brojnost mlađi cipla balavca u luci Ploče u svezi s povoljnim uvjetima staništa kao što su: manji broj predatora, niža slanost i veća količina dostupne hrane. Sve navedeno pogoduje uspješnijem novačenju cipla balavca u ovom dijelu hrvatskog Jadrana.

Ključne riječi: cipal balavac, *Liza ramada*, mlađ, ishrana, novačenje