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Ichthyoplankton community structure in Boka Kotorska Bay (Southern Adriatic Sea)

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ABSTRACT

The state of biodiversity reflects the degree of stability and persistence of ecosystems, especially in cases of significant direct or indirect anthropogenic impacts on marine environment. The early life stages of fishes at three locations in the area of marina Porto Montenegro were analyzed during spring and summer of 2016, 2017 and 2018 by monthly dynamics (March-August). The aim of this work is to compare the diversity of species with existing data, especially after the formation of the exclusive marina and to estimate possible impact on the distribution and species composition. Investigation shows dominance of anchovy (*E. encrasicolus*) and sand steenbras (*Lithognatus mormyrus*). Total ichthyoplankton abundance was lowest during 2017 when total abundance was in range from 4-24 eggs/larvae per m^2 of sea surface, while during 2016 and 2018 total abundance was in range from 4-67 and 4-86 eggs/larvae per m^2 of sea surface, respectively. It can be concluded that the anthropogenic impact most likely caused the changes that led to the change in composition of dominant species between years and the negligible spawning of anchovy in 2017. Results pointed out the necessity of introducing of more intense management and protection measures of pelagic fish species in the area of Boka Kotorska Bay.

Keywords: fish larvae, ichthyoplankton, diversity, South Adriatic Sea

INTRODUCTION

Ichthyoplankton (early life development stages of fishes) represents the basis of fisheries biology studies, i.e. the abundance of reproductively mature fish population depends on the success in growth, development and survival of early fish development stages and conditions under which they live until they reach the first reproductive maturity. In order to determine the diversity of species, spawning and/or feeding zones, the ichthyoplankton analysis represents the essential foundation of the research, while understanding the effect of environmental factors and their changes to growth, development, survival and abundance of the species provide explanation to abundance and/or diversity changes and certain short-term and long-term forecasts as regards biomass or spatial distribution.

The importance of ichthyoplankton surveys is of particular importance for the assessment and forecasting of existing fisheries resources. It provides information on unexploited resources as well as on exploited resources (Ahlstrom & Moser, 1976). About 78 % of Mediterranean and Black Sea stocks assessed are currently fished at biologically unsustainable levels, although the percentage has slightly decreased since 2014 (88 %) (FAO, 2018). Although intensive efforts have being made to adopt a Joint fisheries management plan for the Adriatic Sea and the whole Mediterranean, further analysis of the anthropogenic impact on the early life stages of fishes will contribute to a better definition of management measures and mitigating the condition of overfished or endangered stocks.

The aim of this paper is to monitor the qualitative and quantitative composition of ichthyoplankton in the marina of Porto Montenegro. The study area is one of the significant spawning and nursery areas of numerous pelagic fish species (Mandić *et al.*, 2014). Following the intensive construction, urbanization and commercial development of the part of the area covered by this investigation, it is of utmost importance to understand the anthropogenic impact on possible structural changes in the composition of the early life stages of fish populations.

MATERIALS AND METHODS

The survey was conducted in the Tivat Bay in a total of 3 positions in the vicinity of the marina Porto Montenegro. Construction of the marina began after 2008, and today it is one of the most luxurious marinas for yachts with a nautical settlement in the Adriatic Sea. Earlier, the area was a military complex and an overhaul of a shipyard that represented a significant source of pollution and degradation of the area's natural potentials, especially marine area.

Two positions were monitored in the area of marina, very close to the coastal area (with max depth of 13 m) while the third (control) position is located in the central part of the Tivat bay as a sampling reference point (Fig. 1).

Figure 1. Study area with investigated positions (red dots)

The sampling was carried out using the WP2-type plankton net with cylinder diameter of 57 cm, mesh size of 200 μ and total length of 260 cm. The net was towed vertically 2 meters above the bottom up to the sea water surface. After sampling, the material was conserved in 2.5% buffered formaldehyde-seawater solution. Data on temperature and salinity were taken at each of the investigated positions. During the sampling, the current state of the sea and winds was recorded in the log book in order to determine as precisely as possible the effect of all factors on ichthyoplankton distribution and abundance. The material was examined by NIKON SMZ 800 binocular equipped with a camera, an external unit and a programme enabling the measuring and analysis of all relevant identification characteristics necessary for species determination.

Diversity of species for the research period was determined using the Shannon diversity index (H'). The Shannon diversity index was calculated at the species level by positions. Diversity indices are the measure of certain community attributes as they are often used as indicators of ecological conditions of an environment (Clarke & Warwick, 1994). The Shannon diversity index is one of the most frequently used diversity indices as it includes both the diversity of species and the components of evenness with which individuals are distributed among the different species. It is also the index with the highest sensitivity with regard to changes in presence of rare species in a sample.

RESULTS AND DISUCUSSION

During a three-year study of the qualitative composition of ichthyoplankton in the marina Porto Montenegro, early life development stages of 34 fish species were found, 6 specimens were determined only by genus, and 8 specimens remained undetermined due to poor quality or lack of adequate literature. From a total of 20 families, Sparidae, Labridae, Serranidae and Callionymidae are the dominant in terms of diversity. The most abundant in terms of abundance are species from the families Engraulidae, Sparidae and Serranidae.

The presence of species by month is shown in Table 1.

March-August 2016

In March 2016, it was found spawning of: *Serranus cabrilla*, *Boops boops*, *Engraulis encrasicolus*, *Coris julis*, *Serranus hepatus*, *Sparus aurata* and *Arnoglossus thori*. The abundance was very low and ranged from 4-8 eggs/larvae per m^2 of sea surface. All positions were positive for ichthyoplankton.

In April 2016, a more intensive spawning began. All positions were positive for ichthyoplankton with abundance ranging from 4-27 eggs/larvae per m^2 of sea surface. Analyzes revealed spawning of the following species: *Engraulis encrasicolus*, *Gaidropsarus mediterraneus*, *Boops boops*, *Coris julis*, Symphurus sp., Cepola sp., Gobius sp. and Mugil sp. while one larva remained undetermined.

In May 2016, fish spawning showed a similar intensity as in April and can be defined as relatively weak. The most numerous were the early life stages of a sand steenbras (*Lythognathus mormyrus*) with 23 eggs/m^2 of sea surface, followed by annular seabream (*Diplodus annularis*) whose abundance ranged from 4-11 eggs/m^2 of sea surface. In addition to the species mentioned, spawning of *Scomber japonicus*, *Serranus scriba* and *Callionymus lyra* was confirmed and characterized by very low intensity.

In June 2016, spawning of *Engraulis encrasicolus*, *Trachurus trachurus* and *Lepadogaster lepadogaster* was found. The abundance was very low and in range from 4-8 eggs/larvae per m^2 of sea surface. One position was negative for ichthyoplankton findings.

Table 1. Spawning of species by months and years

Species/month	**2016**						**2017**						**2018**					
	3	**4**	**5**	**6**	**7**	**8**	**3**	**4**	**5**	**6**	**7**	**8**	**3**	**4**	**5**	**6**	**7**	**8**
Engraulidae																		
Engraulis encrasicolus (Linnaeus, 1758)	x	x		x	x	x		x			x	x		x		x	x	x
Clupeidae																		
Sardinella auritta (Valenciennes, 1847)															x			x
Sparidae																		
Diplodus annularis (Linnaeus, 1758)			x					x	x								x	x
Diplodus puntazzo (Walbaum, 1792)										x							x	x
Diplodus sargus (Linnaeus, 1758)					x	x		x								x		
Boops boops (Linnaeus, 1758)	x	x												x				
Sparus aurata (Linnaeus, 1758)	x																	
Lithognathus mormyrus (Linnaeus, 1758)			x				x	x		x	x	x			x	x	x	x
Pagellus bogaraveo (Brünnich, 1768)					x													
Labridae																		
Coris julis (Linnaeus, 1758)	x	x												x			x	x
Thalassoma pavo (Linnaeus, 1758)										x							x	x
Ctenolabrus rupestris (Linnaeus, 1758)					x						x	x		x				
Xyrichtys novacula (Linnaeus, 1758)						x												
Symphodus melops (Linnaeus, 1758)														x				
Blennidae																		
Salaria pavo (Risso, 1810)							x											
Parablennius tentacularis (Brünnich, 1768)											x							
Serranidae																		
Serranus hepatus (Linnaeus, 1758)	x													x	x			x
Serranus scriba (Linnaeus, 1758)			x															
Serranus cabrilla (Linnaeus, 1758)	x																	
Anthias anthias (Linnaeus, 1758)																	x	
Epinephelus sp. (Bloch, 1793)												x						
Carangidae																		
Trachurus trachurus (Linnaeus, 1758)				x	x	x									x			

Soleidae																		
Buglossidium luteum (Risso, 1810)										x								
Mullidae																		
Mullus barbatus (Linnaeus, 1758)					x	x												
Mullus surmuletus (Linnaeus, 1758)						x												
Merlucciidae																		
Merluccius merluccius (Linnaeus, 1758)															x			
Scombridae																		
Sarda sarda (Bloch, 1793)														x				
Scomber japonicas (Houttuyn, 1782)			x					x							x			
Callionymidae																		
Callionymus lyra (Linnaeus, 1758)			x									x						
Callionymus pussilus (Delaroche, 1809)								x				x		x	x		x	
Callionymus sp. (Linnaeus, 1758)																	x	
Syngnathydae																		
Syngnathus acus (Linnaeus, 1758)						x												
Gobiescocidae																		
Lepadogaster lepadogaster (Bonnaterre, 1788)				x														
Lotidae																		
Gaidropsarus mediterraneus (Linnaeus, 1758)			x															
Bothidae																		
Arnoglossus thori (Kyle, 1913)	x						x	x										
Gobiidae																		
Gobius niger (Lacepède, 1800)																	x	
Gobius sp. (Linnaeus, 1758)		x			x					x		x		x				
Cepolidae																		
Cepola sp. (Linnaeus, 1758)		x																
Cynoglossidae																		
Symphurus sp. (Rafinesque, 1810)		x																
Mugilidae																		
Mugil sp. (Linnaeus, 1758)		x																x
undetermined		x			x			x		x		x					x	x

In July 2016, fish spawning becomes more intense. All positions were positive for ichthyoplankton findings with abundance ranging from 4-31 eggs/larva per m^2 of sea surface. Analyzes revealed spawning of: *Engraulis encrasicolus, Mullus barbatus, Ctenolabrus rupestris, Pagellus bogaraveo, Trachurus trachurus, Mullus barbatus, Diplodus sargus* and Gobius sp., while one egg remained undetermined.

In August 2016, fish spawning showed a similar intensity as in July with the exception of more intensive spawning of anchovies. The most abundant were early life stages of anchovy with 106 eggs/m^2 of sea surface, followed by red mullet (M. *barbatus*), whose abundance ranged from 4-11 eggs/larvae per m^2 of sea surface. In addition to the mentioned species, the spawning of *Xyrichthys novacula, Diplodus sargus, Mullus surmuletus, Sygnathus acus* and *Trachurus trachurus* has been confirmed and was characterized by a low spawning intensity.

An analysis of the qualitative and quantitative composition of ichthyoplankton in the period March-August 2016 revealed that a significant number of pelagic fish species spawned in the study area, although the spawning intensity was relatively low. The study showed no significant dominance of species. The reason for this is most likely the fact that planktonic eggs are carried by sea currents, and its abundance depends significantly on seawater movements. It is likely that many species spawned in other parts of the Tivat Bay and that their early stages were influenced by marine currents and reached the investigated area.

An interesting finding was the larva of *Lepadogaster lepadogaster* (shore clingfish), whose spawning has not been previously confirmed during many years of ichthyoplankton investigation in the Boka Kotorska Bay area.

March-August 2017

Analyses of the abundance of ichthyoplankton showed that spawning in March 2017 was sparse with low spawning intensity. From total of investigated 3 positions, two were positive for ichthyoplankton, with confirmed spawning of *Arnoglossus thori*, *Salaria pavo* and *Lithognathus mormyrus*. The abundance was low and ranged from 4-8 eggs larvae per m^2 of sea surface.

In April 2017, all three investigated positions were positive for ichthyoplankton findings. It was found spawning of: *Engraulis encrasicolus, Lithognathus mormyrus, Diplodus annularis, Calliponymus pussilus, Arnoglossus thori, Diplodus sargus, Thalassoma pavo, Scomber japonicus* while one specimen remained undetermined. The qualitative and quantitative composition of ichthyoplankton in April shows the dominance of anchovy and sand steenbras. The abundance of dominant species ranged from 12-24 eggs/larvae per m^2 of sea surface. After dominant species, D. *annular*is and S. *hepatus* were most abundant with abundance of 12 eggs/larva m^2 of sea surface, while the abundance of other species was in the range from 4-8 eggs/larva m^2 of sea surface.

In May 2017, all positions were positive for ichthyoplankton findings, with spawning of only one species – *D. annularis*. The abundance was in range from 4-16 eggs/m^2 of seasurface.

In June 2017, all positions were positive on ichthyoplankton findings. Spawning of *D.puntazzo, L. mormyrus, Buglossidium luteum, Thalassoma pavo* and *Gobius* sp. was confirmed with abundance ranging from 4-16 eggs/larvae per m^2 of sea surface. One larvae was undetermined due to the very bad condition of individual.

In July 2017 spawning was very scarce while abundance was in range from 4-12 eggs/larvae per m^2 of sea surface. It was found spawning of *Parablennius tentacularis, C. rupestris, E. encrasicolus, L. mormyrus* and *C. pussilus*.

August 2017 was characterized with higher species diversity compared to previous months. It was found spawning of *E. encrasicolus*, *L. mormyrus*, *C. pussilus*, *C. lyra*, *C. rupestris*, *Epinephelus* sp and *Gobius* sp., while abundance was in range from 4-28 eggs/larvae per m^2 of sea surface. Highest abundance was confirmed for *L. mormyrus*.

The most dominant species for the period March-August 2017 was L. *mormyrus*, followed by *D. puntazzo* and *C. pussilus*. Among larvae, *Ctenolabrus rupestris* was most dominant, while it is very interesting to note that spawning of E. *encrasicolus* was extremely scarce, especially when compared to all previous research (Mandić *et al.,* 2011; Mandić *et al.* 2014; Mandić *et al*., 2016).

A comparative analysis of the qualitative and quantitative composition of ichthyoplankton over the same period in 2016 revealed that the qualitative and quantitative composition of the species declined significantly. The reason for the significantly reduced species diversity and spawning intensity may be multiple. Most likely it is consequence of anthropogenic impact, ie intensive cruise tourism, devastation of the coastal area by intensive construction, overexploitation of fish stocks by commercial fishermen and/or illegal fishing, unresolved issue of municipal wastewater or waste disposal.

Given that previous ichthyoplankton studies in the Boka Kotorska Bay have shown that Tivat Bay is one of the main spawning areas of anchovy (Mandić *et al.* 2013), the fact that no spawning of this species was found in May 2017 is quite worrying, so special attention should be paid to reducing anthropogenic impact and other factors that affect degradation of species diversity.

March-August 2018

During March 2018, there were no early life stages of fishes at any of the investigated positions. In April 2018, spawning was scarce and of medium intensity. All three positions were positive for ichthyoplankton, with confirmed spawning of *S. hepatus. E. encrasicolus, S. sarda, C. rupestris, S. melops, B. boops, C. julis, C. pussilus* and *Gobius* sp.. The total abundance was in the range of 4-43 eggs/larvae per m^2 of sea surface. The dominance of *S. hepatus* was expressed in all positions.

In May 2018, all positions were positive for ichthyoplankton. *L. mormyrus* was dominant species with 30 eggs/m^2 sea surface. Spawning *of S. japonicus, S. hepatus, S. auritta, T. trachurus, C. pussilus* and *M. merluccius* was very scarce and with low spawning intensity.

In June 2018, spawning character was medium with total abundance ranged from 4-40 eggs/larvae per m^2 of sea surface. All three positions were positive for ichthyoplankton, dominated by *L. mormyrus* and *D. annularis*.

In July 2018, *E. encrasicolus* dominance was expressed with abundance of 55 eggs/m^2. In July, the highest species diversity was found, ie spawning of 11 different fish species was confirmed at all positions, which is an exceptional diversity given the limited area of the study area and the relatively small sampling depth. In addition to the dominant species, significant spawning was confirmed for *L. mormyrus, T. pavo*, and *D. puntazzo*, with an abundance of 8-35 eggs/larvae per m^2, while other species were found with very low abundance (4-8 eggs/larvae per m^2).

The highest quantitative composition of ichthyoplankton was found in August 2018.

Anchovy abundance ranged, depending on position, from 50-110 eggs/larvae per m^2. In addition to anchovy, significant abundance was found for *D. annularis*, *D. Puntazzo*, and *L. mormyrus* with an abundance of 20 eggs /larvae per m^2, while other species were found with abundance of 4-8 eggs/larvae per m^2.

Comparative analysis with the same survey conducted in March-May 2017 indicates similar species diversity and abundance, but confirms negligible spawning of anchovy, one of the most economically significant pelagic fish species in the Boka Kotorska Bay. This indicates that the anchovy spawning in the Tivat Bay was negligible and is most likely due to the anthropogenic factor, since the basic physic-chemical parameters monitored in this study were favourable and could not represent a limiting factor for spawning.

Historical data indicates that part of the investigated area is one of the main spawning areas of pelagic fish species, especially for *Engraulis encrasicolus*, *Diplodus annularis*, *Diplodus puntazzo*, *Diplodus sargus* and *Coris julis* (Merker & Vujošević, 1972; Mandić *et al*., 2014).

Analysis of the diversity indices showed that the Shannon-Wiener indice ranged from 0 to 1.99 (Fig. 2). Although it was to be expected that diversity indices would increase proportionally from March to August, this situation is visible only for 2018. In 2017, the highest diversity indices were determined for April and August, while in 2016, highest indices were deterimned for March. This data is consistent with the fact that early spring is the period when most species start spawning.

Dominant species with abundance are shown in Fig. 3 and Fig. 4, processed in PRIMER software using Bray Curtis similarity analysis (Clarke & Gorley 2006).

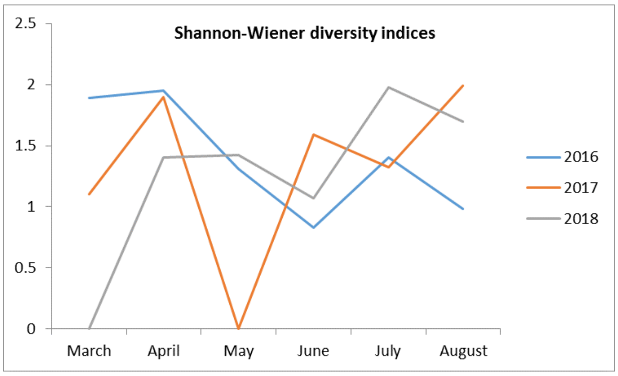

Figure 2. Shannon -Wiener diversity indices for investigated period

It is important to note that significant presence of microplastics in the samples was observed throughout the study period, and special attention should be paid to this problem in the following investigations. There are numerous negative impacts that microplastics have on the marine organisms, especially when it comes to larvae or juvenile fishes that can replace certain microplastics particles with prey.

Human activities in shallow sheltered bays and estuaries include dredging, development of ports and marinas, wind farm constructions and boating, and they can all affect the availability of pristine nursery and recruitment areas (Back *et al*., 2001; Sundblad & Bergströ, 2014; Larsson *et al.*, 2015, Macura *et al.*, 2016) . Although several shallow nearshore areas such as bays, estuaries and wetlands have been degraded or lost (Airoldi & Back, 2005), exact quantitative estimates of the effects on species distributions and abundances are often lacking (Macura *et al*., 2016). In particular, shallow near-shore habitats are utilized as reproductive areas (e.g., as spawning and nursery habitats) supporting larvae and juveniles of many fish species, including several commercially important species (Lotze *et al*., 2006; HELCOM, 2010; Ljunggren *et al*., 2005).

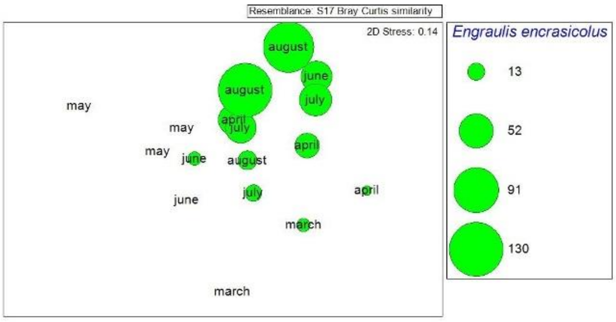

Figure 3. Abundance and presence of *E. encrasicolus* by surveyed months

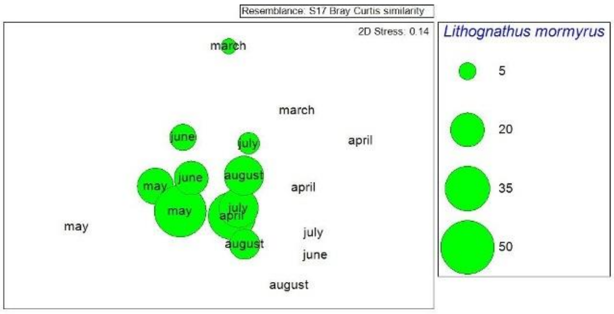

Figure 4. Abundance and presence of *L. mormyrus* by surveyed months

Therefore, a better understanding of how anthropogenic activities affect shallow habitats and the fish dependent on these habitats is essential for guiding management actions that aim to preserve, enhance or restore ecosystem services (Rönnbäck *et al*., 2007; Stål *et al*., 2008; Abelson *et al*., 2016).

It can be generally concluded that the overall diversity of the species in the study area is high, especially considering the relatively small area of the study. Significant fluctuations in the abundance and intensity of dominant species are undoubtedly the result of anthropogenic influence, and it is essential to introduce certain measures of protection and improvement. Therefore, innovative solutions are recommended to preserve diversity in the marina area, such as the placement of underwater artificial reefs, as formations where many species of fish find shelter from predators, food sources and potential spawning grounds.

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Struktura zajednica htioplanktona u Bokokotorskom zalivu (južno Jadransko more)

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SAŽETAK

Stanje biodiverziteta odražava stepen stabilnosti i otpornosti ekosistema,posebno u slučajevima značajnih direktnih i indirektnih antropogentih uticaja na morsku životnu sredinu. Rani stadijuma riba na tri lokacije u oblasti marine Porto Montenegro su analizirani mjesečnom dinamikom tokom proljeća i ljeta (mart-avgust) 2016, 2017 i 2018. godine. Cilj ovog rada je da uporedi diverzitet vrsta sa postojećim podacima, posebno nakon formiranja ekskluzivne marine i da se procijeni mogući uticaj na rasprostranjenost i sastav vrsta. Istraživanja su pokazala dominantnost inćuna (*E. encrasicolus*) i marmore (*Lithognatus mormyrus*). Ukupna brojnost ihtioplanktona je bila najniža tokom 2017.g. kada je ukupna brojnost bila u obsegu 4-24 jaja/larve po m^2 morske površine, dok je tokom 2016 i 2018.g. ukupna brojnost bila u opsegu 4-67 i 4-86 jaja/larve po m^2 morske površine. Može se zaključiti da je antropogeni uticaj vrlo vjerovatno izazvao promjene koje su uzrokovale promjene u sastavu dominantnih vrsta među ispitivanim godinama i zanemarivo mriješćenje inćuna u 2017.g. Rezultati ukazuju na neophodnost uvođenja intenzivnijeg menadžmenta i mjera zaštite pleagičnih vrsta u Bokokotorskom zalivu.

Ključne riječi: larve riba, ihtioplankton, diverzitet, južno Jadransko more