Composition and Distribution of Ichthyoplankton in the Boka Kotorska Bay

Milica Mandić, Ana Pešić, Mirko Đurović, Aleksandar Joksimović, and Slobodan Regner

Abstract This paper provides an overview of all the data available on qualitative composition and distribution of ichthyoplankton in the area of the Boka Kotorska Bay. Although the research activities were not conducted continuously, the results showed a high level of diversity and abundance of certain species and proved that the Boka Kotorska Bay is one of the most important spawning areas and feeding grounds for juveniles of a number of pelagic fish species. Analysis of the plankton material resulted in identification of spawning of 40 different species from 7 genera and 20 families. Ichthyoplankton communities' diversity was analysed by two diversity indices: Shannon's diversity index (H') and Simpson's index (D). Early developmental stages of fish are one of the most sensitive phases in the fish lifecycle, so ichthyoplankton research is one of the main tasks of fisheries biology.

Keywords Biodiversity, Ichthyoplankton, South Adriatic, The Boka Kotorska Bay

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1 Introduction

Although the research of fish eggs and larvae had begun more than a century ago, the main research topics have not changed much since and they mainly concern the estimate of the adult population biomass and spatial distribution of eggs and larvae. Also, one of the main motives of these research activities is also to understand the influence of environmental factors, as well as their effect on changes in growth, survival and richness in the species under research. Factors that have an effect on fish juveniles, particularly those that influence survival of fish eggs and larvae are of particular importance for such research activities.

The most renowned work of a group of ichthyoplanktonologists in the early twentieth century (1905) resulted in issuing one of the most important publications – a monograph under the title *Uova, Larve e Stadi Giovanili di Teleostei* published under the serial *Fauna e Flora di Golfo di Napoli* [1]. There were 4 volumes, issued in several editions over the period of 25 years. Most of the material for the monograph was collected by Lo Bianco; however, his name did not appear as an author in any part of the monograph. The authors whose work contributed to making of this monograph are Sanzo, De Gaetani, Spartà, Cipria, Sella and Ciacchi.

In the early periods of ichthyoplankton research, in addition to these authors, the composition, quantity and distribution of the total ichthyoplankton or plankton stages of certain fish species or families were studied also by [2–5].

The ichthyoplankton research activities in the Adriatic date back to the early twentieth century. The earliest ichthyoplankton research were focused mainly on the plankton stages of certain fish species, with very few works dedicated to qualitative composition and total ichthyoplankton abundance. Gamulin [6] presented the data on pilchard eggs in the vicinity of Split, as well as in the entire Dalmatian Archipelago area. Apart from the data on pelagic and benthic fish populations, the expedition under the name Hyar, organised by the Institute for Oceanography and Fisheries from Split and the Institute for Marine Biology from Rovinj, provided the data on phyto- and zoo-plankton but also on ichthyoplankton in central and northern Adriatic. Gamulin and Hure [7] described the length of embryonic developmental stage of pilchard in the Central Adriatic, while in 1983 [8] presented the data on spawning and spawning sites for a number of pelagic fish species (Sardina pilchardus, Engraulis encrasicolus, Scomber scombrus, Sardinella aurita and Sprattus sprattus sprattus) in the Adriatic. Karlovac [9–11] presented the data on planktonic stages of pilchard but also on the composition of larval stages of fish of the total ichthyoplankton for the Central Adriatic. Varagnolo [12] provided a "comparative calendar" of the occurrence of pelagic eggs of Teleost eggs in the plankton of Chioggia (north Italy), and the same year [13] presented the data on daily variations in the presence of different developmental stages of a number of marine Teleosts in the same region. In 1965 he described the distribution of pelagic eggs of Teleosts in the North Adriatic [14].

Data on long-term changes of the total ichthyoplankton composition for the Central Adriatic were presented by Vučetić [15] who also analysed long-term changes in the total number of larval stages of fish in the open sea and in the coastal waters, but

without determining the species. Regner [16] presented the data on anchovy postlarvae nutrition in the Central Adriatic, while in 1977 he presented the data for postlarvae of the species *Serranus hepatus* (L.) and *Cepola macrophthalma* (L.), as well as for planktonic stages of the round sardinella (*Sardinella aurita*) [17, 18]. The data on the larval stages of certain fish species in the coastal area of the Kaštela Bay, as well as description of zooplanktonic predators of planktonic fish stages, were presented by the same author [19, 20], while in 1982, he presented the data on changes in quantitative and qualitative composition of larvae and post-larvae of fish in the open sea of the Central Adriatic [21]. In 1985, the same author presented the data on ecology of planktonic stages of anchovy for the Central Adriatic region [22]

Regner et al. [23] presented the data on spawning of sardine (*Sardina pilchardus* Walb.) in the Adriatic under the upwelling conditions. Regner and Dulčić [24] estimated growth parameters for anchovy post-larvae based on the number of otolith annuli, while [25] presented the data on anchovy spawning in the Central Adriatic in the course of 1989, which was the year of intensive blooming of phytoplankton and benthic diatoms.

In 1992, Dulčić [26], in his master thesis, presented the data on growth parameters of pilchard post-larvae (*Sardina pilchardus* Walb.) based on otolith annuli, while in 1997 he estimated the anchovy post-larvae (*Engraulis encrasicolus* L.) growth parameters in the North Adriatic, also on the basis of otolith annuli. The same author, in 1993, presented the data on embryonal and larval development of the species *Scorpaena porcus* L. grown under laboratory conditions by artificial insemination of females. In 1994, a group of authors led by Dulčić described early developmental stages of the species *Serranus hepatus* in the Central Adriatic, also grown under artificial conditions [26–28]. In 1990s, Dulčić presented the data on embryonal development of a number of species from the following families Scombridae, Carangidae and Centrolophidae [29–31].

The first ichthyoplankton research in the South Adriatic and the area of the Boka Kotorska Bay dates back to 1966 [32], when spatio-temporal dynamics of pilchard spawning (*Sardina pilchardus*) was determined, and soon after, a research on distribution and density of anchovy eggs in the area of the Boka Kotorska Bay [33] was conducted. No research was conducted on ichthyoplankton for a long time afterwards, and only in 2006, the research on qualitative and quantitative composition of ichthyoplankton in the area of the Boka Kotorska Bay was resumed [34–38]. This same year, regular annual analysis of abundance of early developmental stages of anchovy (*E. encrasicolus*) in the open sea of Montenegro's coast began with the objective of biomass estimation by DEP method [38].

2 Study Area

The Boka Kotorska Bay is the most indented bay the Adriatic Sea. It is situated in the southernmost part of the eastern Adriatic coast, in the contact zone between Montenegro and Croatia. At the entry of the bay, Cape Oštra is to the west and Cape Mirišta to the east, with the passage between leading into the Herceg Novi Bay – the first of the four bays of the Boka Kotorska. The Herceg Novi Bay then continues to the Tivat Bay through the Kumbor Strait, further to the bays of Risan and Kotor, through the Verige Strait. These bays form an enclosed basin connected with the open seas of the Adriatic by the passage Cape Oštra – Cape Mirišta. The specific position results in specific properties of each of the bays but also of the Boka Kotorska Bay as a whole.

The relief of the Boka Kotorska Bay is quite complex. Two areas are distinct in the seabed relief: coastal plain and continental shelf, while other areas – continental slope and abyssal plain – are not present due to small area and limited depths.

Taking into account the structure and vertical stretch of the coastal zone, it can be said that there is not even a coastal plain in the entire Bay of Kotor (except for a small, narrow strip on the eastern side), Risan and Tivat (except for a part on the eastern side – coves Kukuljina and Krtole) and Herceg Novi (except for a small part of the northern side) as rocky steep slopes descend to the very surface of the sea and shoreline, and in these parts, the steep continental area spreads to the very bottom of the bay.

In all of the bays, depths increase towards their central part, although in the Kotor Bay, the maximum depth is not in the central part of the bay, but along its northern coast (Dražin Vrt). A depression, more than 50 m in depth, was noted in the Kotor Bay and in the bays of Tivat and Herceg Novi, another one, also more than 40 m in depth. In a recent exploration of the Boka Kotorska Bay by the Hydrographical Institute of Montenegro's Navy, the maximum depth of 64 m was determined as a narrow indentation in the Kotor Bay [39].

3 Trophic Status

Results of a long-term research of coastal waters of the Montenegro's coast, where more than 20 different parameters indicating the seawater quality were analysed (transparency, colour, chemical parameters and biological characteristics), show that coastal waters of Montenegro are eutrophic, with particularly pronounced eutrophication in the Boka Kotorska Bay [40]. Phytoplankton biomass expressed as chlorophyll *a* in the Boka Kotorska Bay has 2 peaks – spring and autumn – when maximum microphytoplankton values were determined, particularly in the Kotor Bay. In the period March–April and September–December, the Kotor Bay becomes hypereutrophic [41]. Research activities conducted in the winter and early spring (March) showed notable water column stratification, with high microphytoplankton values on the seawater surface, which is typical of extremely eutrophic waters [42].

4 Materials and Methods

The exploration of the qualitative and quantitative ichthyoplankton composition in the area of the Boka Kotorska Bay were done by vertical tows using two plankton net types – PairOVET (modified CalVet) net with cylinder diameter of 25 cm, total mouth opening of 0.098 m² and mesh size of 1.6×10^{-4} mm. This net was used during explorations conducted by seasonal dynamics in the total of 18 stations in the period July 2006 to January 2009. Surface temperature and salinity values were recorded only for four seasons (July 2006, December 2006, April 2007 and August 2007); there are no data on abiotic factors for other seasons due to a probe failure.

WP2 plankton net, with a mesh size of 0.200 mm and mouth opening of 57 cm, was used in the investigation of ichthyoplankton abundance and composition in a part of the aquatorium of the Tivat Bay, where in the period March 2015 to February 2016, ichthyoplankton was sampled in three stations, on monthly basis (Fig. 1). Data on surface temperature and salinity were recorded during the investigation.

Nets were towed vertically at the speed of 0.5–1 m/s, 5 m above the maximum bottom depth to the surface. Temperature and salinity were recorded from each of the investigated locations. The ichthyoplankton samples collected were kept in a 2.5% formaldehyde solution in seawater and analysed using magnifier NIKON SMZ 800 with a MOTIC camera attached. Ichthyoplankton was determined to the level of the species where possible, and where not, only to the level of genus.

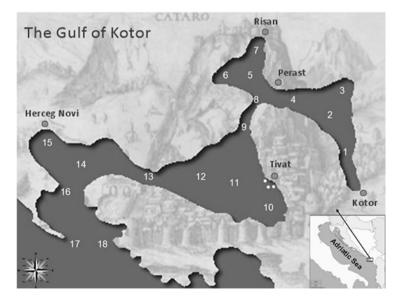


Fig. 1 The Boka Kotorska Bay with locations where ichthyoplankton was investigated (2006-2009 - numbers; 2015-2016 - dots)

Ichthyoplanktonic communities' diversity was analysed by two diversity indices: Shannon's diversity index (H') and Simpson's index (D). Diversity analysis was done for each location under research separately, by seasons, and the average value of total diversity for all locations was analysed as well, by seasons. The diversity in species for the area of the Tivat Bay was calculated by months, due to a very small distance between the locations surveyed. Shannon's diversity index is one of the most frequently used diversity indices as it includes both the species richness and components of the evenness with which individuals are distributed among species. It is also the index that is the most sensitive to changes in presence of rare species in the sample. Calculations are done with the following formula [43]:

$$H^{'} = -\sum_{i=1}^{s} (p_i)(\log_2 p_i)$$

where p_i is the proportion of the *i*th species in the sample and *S* is the total number of species in the sample. Shannon's index increases as the species number increases. In practice, it proved that for biological communities *H'* value does not exceed 5.0 [43].

Simpson's index gives the probability of any two individuals randomly drawn belonging to the same species. When expressed as reciprocal value (D = 1/C), the index value grows as the evenness of the community grows [44]. Reciprocal Simpson's index is calculated using the following formula [43]:

$$D = 1/C \quad C = \sum_{i=1}^{s} p_i^{2}$$

where p_i is the proportion of the *i*th species in the sample and *s* number of species in the sample. Values of this index range from 1 to *s*.

5 Results

In the entire investigated area, the total of 1400 fish eggs and 354 larvae and postlarvae was found (Table 1), and 40 different species, 7 genera and 20 families were identified:

Family: Sparidae

Diplodus puntazzo (Cetti, 1777) – Sharpsnout seabream Diplodus annularis (Linnaeus, 1758) – Annular seabream Diplodus sargus (Linnaeus, 1758) – White seabream, Sargo Pagellus acarne (Risso, 1827) – Axillary seabream

No.	Species	Summer	Autumn	Winter	Spring
1	Diplodus puntazzo	+	+		
2	Diplodus annularis	+			+
3	Diplodus sargus	+			+
4	Pagellus acarne	+			
5	Boops boops				+
6	Spondyliosoma cantharus				+
7	Pagrus pagrus	+			
8	Lithognathus mormyrus	+	+		+
9	Engraulis encrasicolus	+	+	+	+
10	Sardina pilchardus			+	+
11	Sardinella aurita	+			
12	Coris julis	+			+
13	Labrus merula				+
14	Arnoglossus laterna	+		+	
15	Arnoglossus thori			+	+
16	Trachurus mediterraneus	+			
17	Trachurus trachurus				+
18	Seriola dumerili	+			
19	Gobius sp.	+	+	+	+
20	Trigla sp.			+	+
21	Labrus sp.	+			
22	Mugil sp.	+			
23	Arnoglossus sp.		+		
24	Trachinus sp.			+	
25	Serranus hepatus	+			+
26	Serranus scriba				+
27	Serranus cabrilla				+
28	Callionymus risso	+			+
29	Callionymus lyra	+			+
30	Callionymus maculatus			+	
31	Callionymus festivus	+	+		
32	Callionymus pusillus	+			
33	Auxis rochei				+
34	Gadiculus argenteus	+			+
35	Trisopterus minutus			+	
36	Scomber scombrus		+	+	+
37	Scomber japonicus	+			
38	Sarda sarda	+			
39	Trachinus draco	+			
40	Dicentrarchus labrax			+	
41	Ophisurus serpens				+
42	Gaidropsarus mediterraneus	+			

 Table 1
 Presence of planktonic fish stages, by seasons (+ - presence)

(continued)

No.	Species	Summer	Autumn	Winter	Spring
43	Mugil cephalus	+			
44	Scorpaena porcus	+			
45	Sparisoma cretense	+	+		
46	Sciaena umbra	+			

Table 1 (continued)

Boops boops (Linnaeus, 1758) - Bogue

Spondyliosoma cantharus (Linnaeus, 1758) - Black seabream

Pagrus pagrus (Linnaeus, 1758) - Red porgy

Lithognathus mormyrus (Linnaeus, 1758) - Striped seabream

Family: Engraulidae

Engraulis encrasicolus (Linnaeus, 1758) - European anchovy

Family: Clupeidae

Sardina pilchardus (Walbaum, 1792) - European pilchard

Sardinella aurita (Valenciennes, 1847) - Round sardinella

Family: Labridae

Coris julis (Linnaeus, 1758) - Mediterranean rainbow wrasse

Labrus merula (Linnaeus, 1758) - Brown wrasse

Labrus sp.

Family: Bothidae

Arnoglossus laterna (Walbaum, 1792) - Mediterranean scaldfish

Arnoglossus thori (Kyle, 1913) - Thor's scaldfish

Arnoglossus sp.

Family: Carangidae

Trachurus mediterraneus (Steindachner, 1868) - Mediterranean horse mackerel

Trachurus trachurus (Linnaeus, 1758) - Atlantic horse mackerel

Seriola dumerili (Risso, 1810) - Greater amberjack

Family: Gobiidae

Gobius spp.

Family: Triglidae Trigla spp. Family: Serranidae Serranus hepatus (Linnaeus, 1758) – Brown comber Serranus scriba (Linnaeus, 1758) - Painted comber Serranus cabrilla (Linnaeus, 1758)n – Comber Family: Callionymidae Callionymus risso (Lesueur, 1814) - Risso's dragonet Callionymus lyra (Linnaeus, 1758) - Common dragonet Callionymus maculatus (Rafinesque, 1810) - Spotted dragonet Callionymus festivus (Pallas, 1814) - Festive dragonet Callionymus pusillus (Delaroche, 1809) - Sailfin dragonet Family: Gadidae Gadiculus argenteus (Guichenot, 1850) - Silvery pout Trisopterus minutus (Linnaeus, 1758) - Poor cod Family: Scombridae Scomber scombrus (Linnaeus, 1758) – Atlantic mackerel Scomber japonicus (Houttuyn, 1782) - Chub mackerel Sarda sarda (Bloch, 1793) - Atlantic bonito Auxis rochei (Risso, 1810) - Bullet tuna Family: Scaridae Sparisoma cretense (Linnaeus, 1758) – Mediterranean parrotfish Family: Sciaenidae Sciaena umbra (Linnaeus, 1758) - Brown meagre Family: Trachinidae Trachinus draco (Linnaeus, 1758) - Greater weaver Trachinus sp. Family: Moronidae Dicentrarchus labrax (Linnaeus, 1758) - European sea bass

Family: Ophichthidae
Ophisurus serpens (Linnaeus, 1758) – Serpent eel
Family: Lotidae
Gaidropsarus mediterraneus (Linnaeus, 1758) – Shore rockling
Family: Mugilidae
Mugil cephalus (Linnaeus, 1758) – Flathead grey mullet
Mugil sp.
Family: Scorpaenidae
Scorpaena porcus (Linnaeus, 1758) – Black scorpionfish

6 Ichthyoplankton Diversity

Average value of the Shannon's diversity index in July 2006 was 0.85, while Simpson's reciprocal index was 2.38. July 2006 was the season with the highest diversity of all the seasons covered by the research, whereby average diversity value was H = 0.85 and D = 2.38, i.e. diversity values ranged from H = 0-2.35 and D = 1-7.74. In December 2006, the average value of the Shannon's index was 0.38, while the average value of the Simpson's index was 1.26 (S = 0-1.01, D = 1-2.57). April 2007 had significant diversity of species, although values were somewhat lower than in July 2006 (average values H' = 1.09, D = 2.86, total S = 0-1.99, (average values H' = 1.32, D = 3.36, total August 2007 D = 1-5.77). S = 0.5 - 1.99, D = 1.47 - 5.77) and April 2008 (average values H' = 0.96, D = 2.55, total S = 0.2 - 1.83, D = 1.1 - 5.76) had quite similar diversity values, while in July 2008 average values were H' = 0.84 and D = 2.21, with total S = 0-1.33, D = 1-3.57 (Figs. 2 and 3).

The highest value of species richness by stations was 5 (December 2006), 7 (April and July 2008) and 9 (April and August 2009), with the highest number of species in a single station recorded in July 2006.

Diversity index analyses were not done for seasons October 2008 and January 2009 due to very poor qualitative and quantitative composition of species.

Average surface temperature in July was 22.9° C, with the maximum temperature recorded 25.8° C at the station No. 5 (the Risan Bay) and 25° C at the station No. 2 (the Kotor Bay). In August 2007, the maximum surface temperature was recorded in the Kotor Bay (Station No. 3) reaching 25.2° C (Figs. 4 and 5).

The lowest surface temperature values were recorded in April 2007, while values measured in December 2006 were by approximately 1.5°C higher than those in April 2007. The lowest temperature in April 2007 was recorded at the

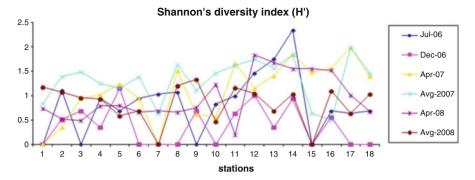


Fig. 2 Shannon's diversity index analysed by seasons [37]. See station location in Fig. 1

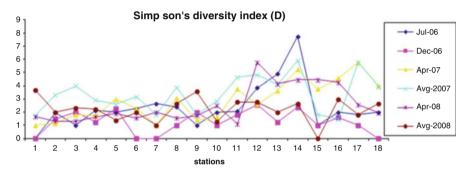


Fig. 3 Simpson's reciprocal index analysed by seasons [37]. See station location in Fig. 1

station No. 8 (Verige) 14.6°C, while the lowest temperature in December was 16.3°C at the station No. 17 (the Herceg Novi Bay).

The average surface salinity value in all stations under survey in July was 33.6‰. The lowest value of 30.36‰ was recorded at the station No. 6 (the Risan Bay), while the maximum value measured at the very exit of the Bay, at the station No. 17, reaching 36.7‰.

In December 2006, average salinity value was 34.99‰. The lowest value of 33.54‰ was recorded at the station No. 3 (the Kotor Bay), while the highest value of 37.85‰ was noted at the station No. 18 (Mamula).

In April 2007, average value was somewhat higher than for previous seasons, reaching 35.21‰. The lowest value of 34.06‰ was recorded at the station No. 7 (the Risan Bay), while the highest was 38.2‰, at the station No. 18.

In August 2007, average value was 36.25‰, the lowest was 33.4‰ at the station No. 7, and the highest value of 38.8‰ was recorded at the station No. 18.

The research conducted for the area of the Tivat Bay (in the period from March 2015 to March 2016) showed diversity rates of S = 0-1.88 and D = 1-4.28, with

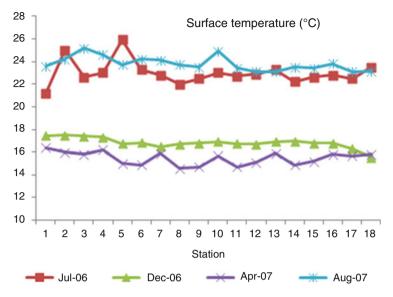


Fig. 4 Sea surface temperature, presented by seasons. See station location in Fig. 1

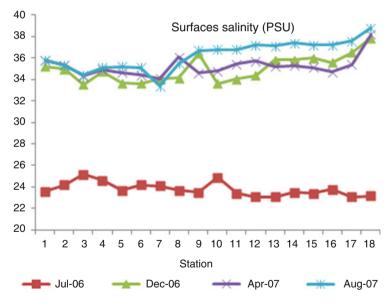


Fig. 5 Sea surface salinity, presented by seasons. See station location in Fig. 1

higher diversity rates during July and September 2015, while diversity of species in other months was at a relatively low level (Fig. 6).

The highest number of species per station was found in the area of the Tivat Bay -13 in July 2015 – while in other months, the species richness by stations was

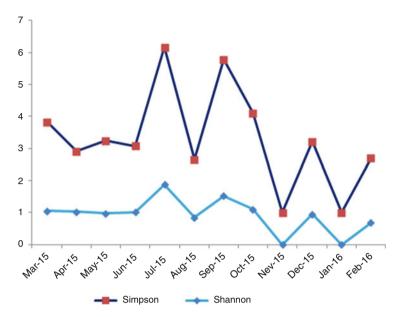


Fig. 6 Diversity indices in a part of the Tivat Bay aquatorium (March 2015–February 2016)

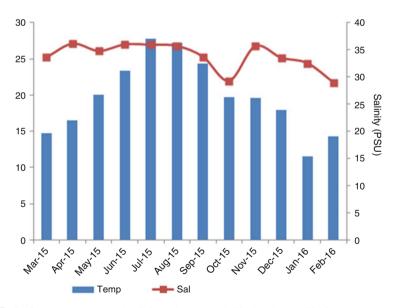


Fig. 7 Surface temperature and salinity values (March 2015–February 2016)

7 (June), 6 (April, May and August) and 5 (September), while in their months, the species diversity ranged from 1 to 3 species per station. After each tow of the pelagic net, data on surface temperature and salinity were recorded (Fig. 7).

7 Discussion

The investigation on ichthyoplankton composition and abundance in the Boka Kotorska Bay has shown presence of a significant number of pelagic species spawning within the Bay. After decades of long pause in analysing abundance of early developmental stages of anchovy in the Bay [33], this is, at the same time, the first investigation on diversity of ichthyoplankton species in this part of the south Adriatic.

The investigation showed dominance of certain species, such as anchovy (*Engraulis encrasicolus*), rainbow wrasse (*Coris julis*), annual seabream (*Diplodus annularis*), white seabream (*Diplodus sargus*), pilchard (*Sardina pilchardus*) and mackerel (*Scomber scombrus*) (Fig. 8).

Considering that during the entire investigation period the same species dominated the same seasons, such situation leads to conclusion that the Boka Kotorska Bay is a very important feeding zone and/or spawning zone for these species. However, this cannot be applied to pilchard, since plankton stages of this species were found in larger numbers only in December 2006 [37].

The highest diversity values were recorded in summer, which points to the fact that majority of species spawned in that season. These findings are in concordance with earlier surveys of ichthyoplankton abundance and diversity, showing that late spring and early summer are transition periods for spawning of Mediterranean fish species, when diversity and richness of species reach their maximum [45–48]. Furthermore, it was noted that the highest level of species diversity was recorded in stations with intensive seawater circulation (the Kumbor Strait and Verige), and under the influence of the open sea (Mamula). Relatively low diversity values in most of the stations included in the investigation are most probably the result of the dominance of the species, but they can also be the result of anthropogenic influence, particularly notable in summer months, due to intensive influx of tourists. Considering that diversity indices are mathematical expression of the relationship between qualitative and quantitative composition of the community, their value will be

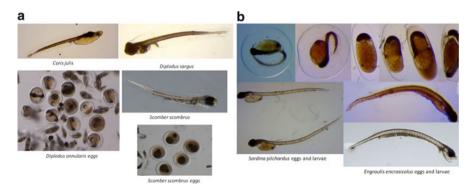


Fig. 8 Some of the most dominant species of ichthyoplankton

significantly higher in a station that has no dominant species, even though the qualitative composition is the same.

All determined species have pelagic eggs and the general pattern of ichthyoplankton assemblages of the area seems to be closely related to the adult fish assemblage and the spawning locations of adult populations [49].

Total number of determined species in a relatively small area of the Boka Kotorska Bay indicates significant species diversity, especially when compared to similar studies carried out in the Northern Adriatic Sea and other parts of the Mediterranean. During the investigation carried out in Northeast Adriatic, in the Kornati Archipelago and Murter Sea during a 12-month cycle, the total of 28 families and 52 species were identified [50]. Investigations of qualitative and quantitative composition of the larval fish stages in the plankton at the open sea of the Central Adriatic Sea during 6-year cycle (1971–1977) showed presence of 56 different species and 14 genera [21]. In Northern Ionian Sea, the study of spatial distribution, abundance and composition of fish larvae carried in March 2000 showed the presence of 46 different species of Teleost early stages, belonging to 38 genera and 22 families [51]. Survey carried out in the Central Cantabrian Sea shelf (southern Bay of Biscay) showed presence of 34 taxa of fish larvae during the summer cruise [52]. Composition of ichthyoplankton from the Mar Menor lagoon (south East Spain) during the investigation carried out from February to December 1997 showed the presence of 14 families, 22 genera and 36 different species [53]. Survey of larval fish assemblages in the coastal waters of central Greece (Ionian and Aegean Seas) during 1998 and 1999 showed presence of 74 larval taxa [54]. The results of this study could have implications for the management of marine resources as well as indicate the importance of Boka Kotorska Bay as a nursery and spawning ground for significant number of economically important fish species, due to the fact that this investigation confirmed our assumption that Boka Kotorska Bay is the nursery and spawning ground for most pelagic fish species [37].

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