SPAWNING HABITAT AND BIOMASS ESTIMATION OF ANCHOVY (*Engraulis encrasicolus*, L.) IN BOKA KOTORSKA BAY

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ABSTRACT

In the present study, daily egg production method (DEPM) was used to estimate spawning stock biomass (SSB) of anchovy (*Egraulis encrasicolus*, L.) in the Boka Kotorska Bay. DEPM surveys were carried out in July 2006, April 2007, and August 2007. Data from CalVet net egg together with reproductive parameters of adult specimens from purse seine nets were used for this assessment. Estimated spawning stock biomass was 1336.97 t. in July 2006, 1013.57 in April 2007, and 1030.59 tons in August 2007.

Key words: Engraulis encrasicolus, Daily Egg Production Method, SSB, Boka Kotorska Bay

INTRODUCTION

Anchovy (*Engraulis encrasicolus*, L.) is one of the economically most important Mediterranean small pelagic species with very wide distribution all over the Adriatic Sea. It is found all over the Mediterranean and the Black sea. Anchovy approaches the coast in spring when the temperature rises, and reaches sexual maturity at the end of the first year of life. Reproductive period of anchovy in the Adriatic Sea lasts from spring to autumn, usually from April to October, and sometimes from March to November (Zavodnik, 1970; Merker & Vujošević, 1972; Regner, 1972, 1985, Piccinetti *et al.*, 1979).

Main spawning area of anchovy is in the eutrophic waters of the western part of the shallow northern Adriatic and along the Italian coast to the peninsula of Gargano (Regner, 1996).

Anchovy stock assessment using eggs and/or larvae surveys are based on the fact that the number of eggs produced by the fish population during the spawning season is proportional, to some extent, to the weight of the mature part of population (Beverton & Holt, 1957). The first attempt of stock assessment using egg survey in the Adriatic Sea was made by Štirn (1969) who estimated anchovy stock biomass in the northern Adriatic Sea. After late 70's sampling methods were standardized and number of surveys was performed mostly in northern and central Adriatic (Piccinetti et al., 1979, 1980, 1981; Regner et al., 1983; Regner, S. 1985, Dulčić & Cetinić, 1993).

In southern Adriatic Sea, DEPM was applied for the first time in June-July of 1993 in the south-western part of Adriatic sea (Casavola *et al.*, 1996), while in 1999 the same method was applied also in southern Adriatic, in the area near Bari (Italy) (Melià *et al.*, 2002).

In Montenegrin continental shelf DEP method was applied for the first time in August of 2005 in the scope of AdriaMed project.

The aim of this paper is to give information about spawning stock biomass of anchovy, in one of the ecologically most sensitive areas of Montenegrin coast.

MATERIAL AND METHODS

Three ichthyoplankton surveys were carried out in July 2006, April 2007, and August 2007. Plankton samples were taken with PairOVET (modified CalVet) plankton net on 18 stations in the Bay (Figure 1, Table 1). Diameters of net cylinders were 25 cm each, and the total mouth area was 0.098 m^2, with mesh size of 0.160 mm. Net was towed vertically at a speed of 0.5 m*sec^{-1}, from the depth of 5 m above the bottom, as the maximal depth in the Bay is 60 m. Temperature and salinity were measured from the sea surface to the maximal depth attained. Plankton material was preserved immediately on board in 2.5% solution of buffered formaldehyde. Anchovy eggs were counted, staged and aged after methodology developed by Regner, (1985) in the laboratory of the Institute of Marine Biology, Kotor, and data were further processed using the Surfer Golden Software 8 and applying the kriging interpolation method.

Figure 1: Geographical position of Montenegro and Boka Kotorska Bay (Investigated stations 1-18)

Adult samples were collected by purse seine nets from commercial catches. On July 22nd 2006 total of 48 specimens were collected, from which 18 females and 30 males. Next sampling was performed on April 13th 2007 (24 females and 21 males) and last sampling was done on July 29th 2007 when total of 43 specimens were analyzed, from which 23 females and 20 males.

Adult individuals were processed immediately after catch in order to get the best values of reproductive parameters. Total length (TL) and weight and gonad free weight of anchovy females was measured, extracted ovaries weighed and thereafter preserved in 10% solution of buffered formaldehyde for histological analysis of ovaries.

Table 1: Geographical position of investigated stations in Boka Kotorska Bay

STATION	COORDINATES						DEPTH (m)	DEPTH OF SAMPLING (m)
	° N			° E				
	Degrees	Minutes	Seconds	Degrees	Minutes	Seconds		
1. Kotor Bay	42	25	82	18	46	11	19	14
2. Kotor Bay	42	28	49	18	45	0	34	29
3. Kotor Bay	42	29	3	18	45	73	29	24
4. Kotor Bay	42	28	79	18	42	52	45	40
5. Risan Bay	42	29	77	18	41	0	30	25
6. Risan Bay	42	29	49	18	39	85	20	15
7. Risan Bay	42	30	49	18	41	50	18	13
8. Verige	42	28	57	18	41	50	44	39
9. Tivat Bay	42	25	76	18	40	11	37	32
10. Tivat Bay	42	25	22	18	41	69	20	15
11. Verige	42	27	12	18	40	82	37	32
12. Tivat Bay	42	25	67	18	37	86	41	36
13. Kumbor Strate	42	25	88	18	35	86	36	31
14. Herceg Novi Bay	42	26	26	18	32	72	42	37
15. Topla	42	26	84	18	31	43	12	7
16. Herceg Novi Bay	42	24	89	18	32	53	40	35
17. Herceg Novi Bay	42	23	62	18	33	13	57	52
18. Cape Mirišta	42	23	57	18	34	22	50	45

Anchovy eggs were classified under ten developmental stages (Regner, 1985). Temperature of the sea surface was used for calculation of developmental time from fertilization to hatching (in days). The following function (1) was used for all estimates of developmental times of anchovy eggs since 1979 (Regner, 1985):

$$D = 1/1.012896\left[1 + e^{(4.914322-(0.257451*T))}\right] \qquad (1)$$

where D is developmental time of eggs in days, and T is the temperature in °C.

Numbers of eggs were adjusted as a number of individuals per unit of the surface (1m²) using the function given by Tanaka (1973).

DEPM (Daily Egg production Method) is the method developed by Coastal research division of the Southwest Fisheries Center, La Jolla (SWFC, California). Method is based on ichthyoplankton investigation for estimating spawning stock biomass (SSB) of batch spawners with indeterminate annual fecundity, in particular Clupeidae and Engraulidae families.. It was developed in the late 1970's and since then, it has been applied to a variety of small pelagic stocks. Application of this method requires knowledge of the boundaries of the spawning areas.

The spawning stock biomass estimation is based on Parker's (1980) model:

$$B = \frac{E}{k * Frb * f * R} \qquad (2)$$

Where:

B = spawning biomass in metric tons;

E = number of eggs produced per day over the surveyed area (daily production);

k = conversion factor from grams to metric tons;

F_{rb} = relative batch fecundity;

f = spawning frequency;

R = sex ratio (fraction of mature females by weight).

Spawning frequency (fraction of mature females spawning per day was estimated using postovulatory follicles (POF's) method (Hunter & Macewicz 1985).

Determination of spawning frequency (f) by this method is based on frequency of females which have postovulatory follicles (POF's) compared to all sample of mature females (Hunter & Macewicz, 1985). Spawning frequency is determinated by number of D-1 POF's (those postovulatory follicles that appear between the 19 and 28 h after spawning). Those follicles are shrunken and have few folds, and less irregular form than new POF's. Lumen is reduced and may contain some granular material (Fig 2).

In order to determinate exact day of the spawning by POF's, morphology was examined in detail.

Figure 2: Histological slide of gonad (POF's)

Relative batch fecundity was calculated as a number of eggs produced per unit of female weight (ovary-free weight). It was calculated based on a random sample in which we found two hydrated females. Spawning frequency was calculated using average value of measurements that were done during 2005 and 2008 for biomass estimates of anchovy in Montenegrin continental shelf.

RESULTS AND DISCUSSION

The distribution and abundance of anchovy eggs are presented in Figures 3, 4, and 5. During the cruise in July 2006, six centers of intensive spawning were observed (stations 2, 4, 8, 10, 11 and 14). In April 2007, also six centers of intensive spawning were found at stations 2, 3, 4, 8, 9 and 11, while the highest abundance was found inside the Bay of Kotor at station 2. In August of 2007 five centers of intensive spawning were found (at stations 1,2,6,9, and 10).
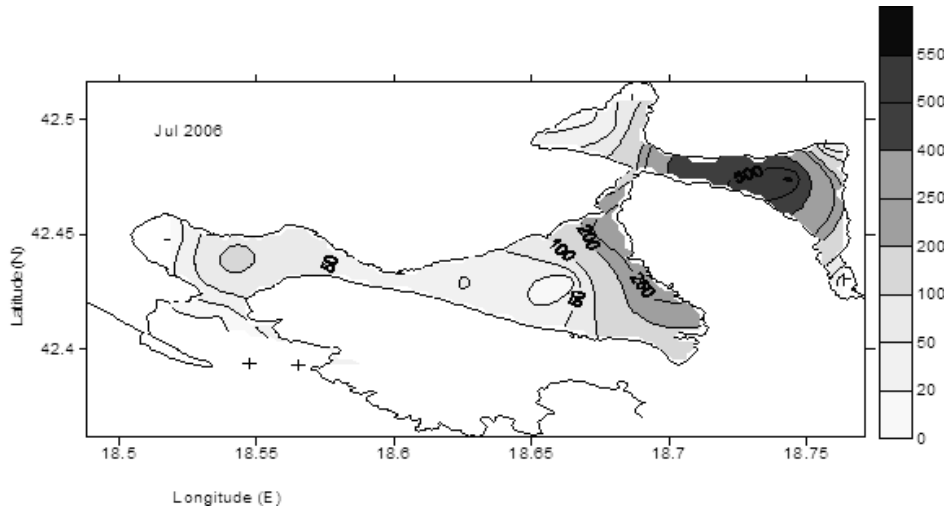
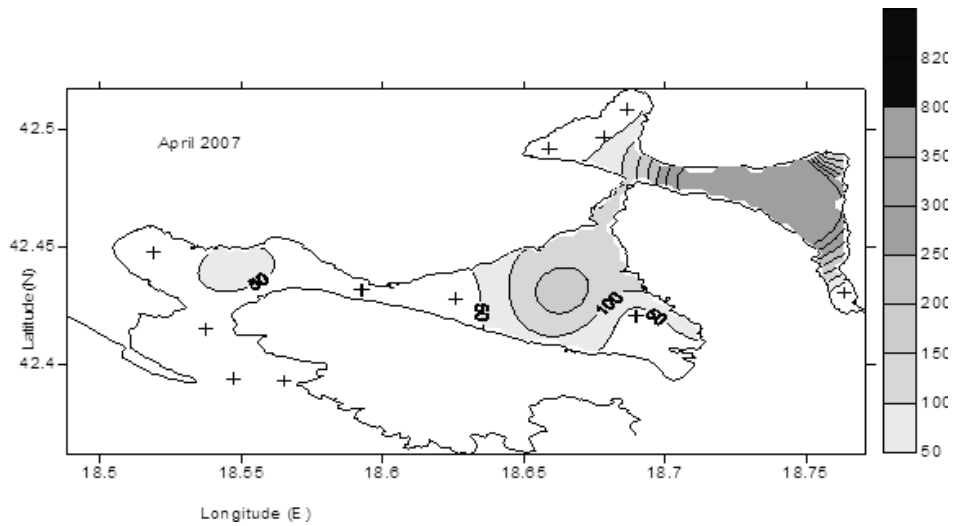Figure 3: Distribution of anchovy eggs in July of 2006 (N/ m^2 *day^{-1})

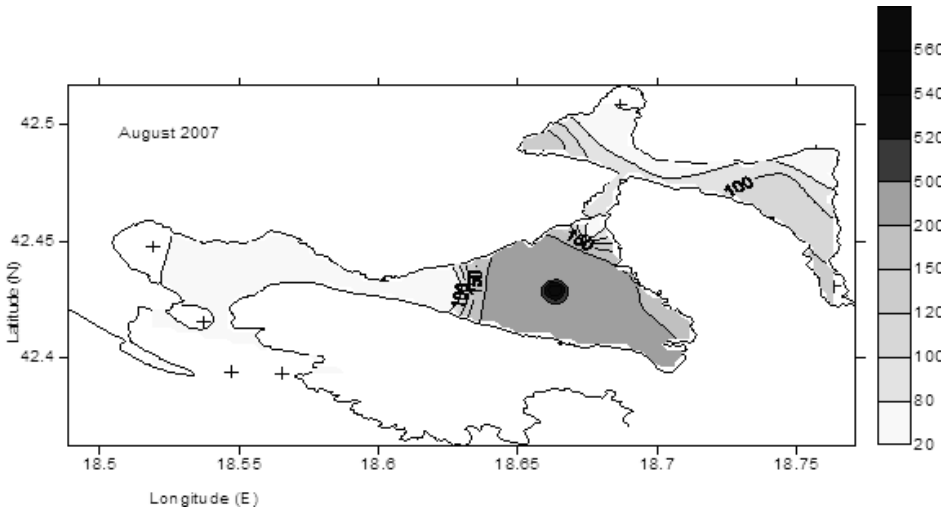Figure 4: Distribution of anchovy eggs in April of 2007 (N/ m^2 *day^{-1})

Figure 5: Distribution of anchovy eggs in August of 2007 (N/m^2 *day^{-1})

Total surface of surveyed area in the Boka Kotorska Bay was 87.334 km^2.

Estimates of anchovy's gonad – free spawning biomass, using Parker's (1980) equation for all investigated seasons is shown in Tables 2-7.

Table 2: Summary of parameters used and estimates of anchovy spawning biomass (July 2006)

Total surveyed area (km^2)	87.334
Daily egg production (N eggs/m^2 * day^{-1})	117.97
Total egg daily egg production in positive area	9.9E+09
Sex ratio	0.389
Spawning frequency (histological, POF)	0.053
Relative batch fecundity (N of eggs per gram of weight)	359.9
Spawning biomass (tons), postovulatory follicles (POF)	1336.97

Table 3: Anchovy biomass divided by bays (July 2006)

	Surface of the bay (km^2)	Biomass (tons)
Kotor Bay	16.262	582.216
Risan Bay	8.005	82.472
Tivat Bay	34.439	555.872
Hercegnovi Bay	28.628	116.414

Table 4: Summary of parameters used and estimates of anchovy spawning biomass

(April 2007)

Total surveyed area (km^2)	87.334
Daily egg production (N eggs/m^2 * day^{-1})	109.48
Total egg daily egg production in positive area	8.9E+09
Sex ratio	0.462
Spawning frequency (histological, POF)	0.053
Relative batch fecundity (N of eggs per gram of weight)	359.9
Spawning biomass (tons), postovulatory follicles (POF)	1013.573

Table 5: Anchovy biomass divided by bays (April 2007)

	Surface of the bay (km^2)	Biomass (tons)
Kotor Bay	16.262	593.2626
Risan Bay	8.005	40.426
Tivat Bay	34.439	306.687
Hercegnovi Bay	28.628	73.196

Table 6: Summary of parameters used and estimates of anchovy spawning biomass

(August 2007)

Total surveyed area (km^2)	87.334
Daily egg production (N eggs/m^2 * day^{-1})	99.19
Total egg daily egg production in positive area	9.5E+09
Sex ratio	0.483
Spawning frequency (histological, POF)	0.053
Relative batch fecundity (N of eggs per gram of weight)	359.9
Spawning biomass (tons), postovulatory follicles (POF)	1030.589

Table 7: Anchovy biomass divided by bays (August 2007)

	Surface of the bay (km^2)	Biomass (tons)
Kotor Bay	16.262	153.782
Risan Bay	8.005	71.487
Tivat Bay	34.439	695.657
Herceg Novi Bay	28.628	109.661

Based on the average number of anchovy eggs per m^2 per day in separate bays (Kotor Bay, Risan Bay, Tivat Bay and Herceg Novi Bay), biomass was estimated separately for each bay. Although, it is clear that one can not draw a clear line between the bays, however, the following table shows the values of anchovy biomass in all bays in order to compare the results.

The distribution of anchovy eggs is under the significant influence of environmental conditions (especially of temperature and salinity) as well as several other oceanographic conditions like sea currents, river runoffs, mixing, nutrients etc. These factors have a strong influence upon adult population, particularly upon place and timing of their reproduction. High abundance of anchovy eggs cannot be related only to the favorable values of temperature and salinity, but to the combination of several environmental and biological factors (Marano, 2001).

River run offs have strong influence on spawning and survival rate of early life stages and recruitment of anchovy (Lloret *et al.*, 2004).

Abundance of anchovy eggs was calculated as a number of individuals per m^2 per day of the sea surface for comparing results with earlier investigations (Merker & Vujošević, 1972). Also, daily production of anchovy eggs was calculated in order to determine daily intensity of spawning.

Although the main spawning areas are in accordance with earlier investigations (Merker, 1972), the abundance is different. Namely, Merker (1972) found eggs in April just at several stations, with the main spawning area

in the Bay of Tivat (73 eggs/m²), while the main spawning season was in June and July (max. 376 eggs/m²), in the central part of the Bay of Kotor.

This kind of distribution of anchovy eggs (with main spawning areas in bays of Kotor and Tivat, Figs. 4.5 and 6) could be explained by the facts that, beside very favourable values of temperature and salinity, these bays are eutrophic and represent favourable spawning and nursery areas for anchovy. High abundance of anchovy eggs in April could be also explained by high value of nutrients and mixing of water column as a whole.

Investigation of length frequencies of anchovy, which is part of the national project from 2004, shows that in Boka Kotorska Bay live mostly young anchovies. So, it can be concluded that after reaching first sexual maturity, young anchovies spawn in the bay and after spawning season goes towards the open waters were continues its life cycle.

Our data are in very good accordance with data from Zrmanja river (Sinovčić, G. and Zorica, B., 2006) where is the similar situation as in the Boka Kotorska Bay. Those data prove that juvenile anchovies spawn in the Bay, while adult anchovies spawn in the open part of the Adriatic Sea. Those investigations deny earlier assumptions that adults came inshore to spawn. (Fage,1935 ; Merker and Vujosevic, 1972).

Results of spawning biomass of anchovy in Boka Kotorska Bay shows relative stable biomass level during tree investigated seasons, what points to relatively stable ecological conditions in the Boka Kotorska Bay.

Having in mind that there are no long-term ichthyoplankton studies in the South Adriatic Sea, this investigation, which was continued after several decades of intermission, shows that the Boka Kotorska Bay is an important spawning area of anchovy.

Despite their importance, studies dealing with larval fish distribution and abundance in the south Adriatic (as well as on fish eggs) are very scarce.

Based on data obtained by the estimated biomass by DEP method MSY (Maximal Sustainable Yield) could be determined, or the maximum amount of fish that may be caught without compromising the dynamics of natural populations.

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