Population size structure, growth and reproduction of the European anchovy (*Engraulis encrasicolus*, L.) in the Lagoon of Lesina (south-western Adriatic Sea, Italy)

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Abstract

1 - The purpose of this study was to describe the basic characteristics of the European anchovy occurring in the Lagoon of Lesina in order to increase our knowledge of the ecology of the species in a lagoon ecosystem.

2 - Samples were collected between May and November 2012 using fyke nets. A hundred individuals per sample were measured and weighed. Sex was determined and the fish gonads were macroscopically classified. Length-frequency histograms were obtained, and the length-weight relationships and condition factors were estimated.

3 - The results of this study showed that the anchovies caught in the Lagoon of Lesina were relatively small. The growth of anchovies in the lagoon was often positively allometric \((b>3)\) and exponent \(b\) was frequently greater than the value estimated for specimens in the Adriatic Sea in the same months.

4 - The sex ratio of anchovies in this study was skewed towards females in almost all the sampling period. Macroscopic analysis of the gonads revealed that spawning took place during spring-summer, and it prolonged until October in the females. One peak of ripe females and males was observed in June and one in August-September.

5 - This study suggests that the anchovy population in the Lagoon of Lesina is probably composed of a large number of young individuals at the first maturity. Individual growth seems to be positively affected by the environmental conditions of the lagoon, which probably provide the anchovies with a substantial trophic advantage.

Keywords: *Engraulis encrasicolus*, population structure, length-weight relationship, condition factor, spawning period, coastal lagoon, Adriatic Sea.

Introduction

The European anchovy (*Engraulis encrasicolus*, Linnaeus 1758) is a short-lived coastal pelagic species which is distributed across the eastern Atlantic, from Norway to South Africa, and the Mediterranean, Black and Azov Seas. Anchovy is one of the most commercially important small pelagic fish species in the whole of the Mediterranean (Morello and Arneri, 2009). In the Adriatic Sea, anchovies, together with sardines, account for approximately 41% of the total marine catch (FAO, 2007). Adriatic anchovy stocks collapsed in 1987, and it is recently thought that the species is recovering. Researchers hypothesized that the collapse
was due to the combination of overfishing and low recruitment levels, the latter affected by adverse environmental conditions (Dulčić, 1995; Kla snsjeck and Legović, 2007; Regner, 1996; Santojanni et al., 2003). However, the reasons for the contraction of the Adriatic anchovy population were not definitively ascertained, because of a lack of specific knowledge of its ecology. Indeed, studies of the influence of environmental conditions and fluctuations on the anchovy populations and their recruitment dynamics in the Adriatic Sea are sporadic and fragmentary. In addition, ecological studies have focused on the north-western side of the Adriatic (Conway et al., 1998; Coombs et al., 1997, 2003; Dulčić, 1995, 1997), where the anchovy population has been observed to be closely linked to the significant freshwater river run-off and the associated stable weather conditions and nutrient enrichment during the summer months (Palomera, 1992). The literature on anchovy ecology in the south-western Adriatic is less abundant than what is available for the north-western Adriatic (Casavola et al., 1987; Casavola, 1998; Marano et al., 1998), despite the fact that this region hosts the only known large nursery area for anchovies in the Adriatic (Gulf of Manfredonia, South of the Gargano Promontory) (Morello and Arneri, 2009). In this area there are also two large coastal lagoons, Lesina and Varano, cyclically inhabited by juveniles, sub-adults and adult individuals of many euryhaline marine species. Coastal lagoons are important ecosystems, where marine species can take advantage of the abundant food resources and shelter from predation (Peterson and Whitfield, 2000; Sogard, 1994). The aims of the present study were: (1) to determine the anchovy population size structure; (2) to obtain the first estimates of growth parameters; (3) to gain information on the reproductive traits of anchovy in the Lagoon of Lesina.

Materials and Methods

Data on the European anchovy were collected during 11 surveys conducted in the Lagoon of Lesina, situated on the Adriatic coast of Central-Southern Italy (15°45′E, 41°88′N), from May to November 2012. This lagoon, part of the Gargano National Park, has an area of 51.36 km², an average depth of 0.7 m and a maximum depth of 1.5 m. Two channels, Acquarotta and Schiapparo, respectively connect the western and eastern sub-basins of the lagoon to the sea. Both channels are equipped with sluice gates to regulate water exchange between the lagoon and the sea, and lavorieri, complex permanent fish capture systems which are not currently used by the fishermen. Much of the annual freshwater budget is discharged into the eastern basin through two rivers and some intermittent streams, accounting for the east-west salinity gradient, which is more pronounced during summer (Manini et al., 2003). During the study period, the temperature at the sampling station ranged between 14.2 °C (15 November) and 26.2 °C (21 August). Salinity ranged from 18.9 psu (24 May) to 36.2 psu (4 September).

Samplings were carried out twice a month at a station located near Acquarotta (Fig. 1). Fish were caught by fyke nets with 6 mm mesh size at the end, set at night and removed the next morning. On each sampling date, a random sample of 100 individuals was taken and stored on ice until arrival in the laboratory.

In the laboratory, all specimens were measured (Total Length, TL) to the nearest 0.1 cm, and weighed (Wet Weight, WW) to the nearest 0.01 g. The sex of each fish was determined and the fish gonads were macroscopically classified. The maturity stage of testes was determined with reference to the universal scale, considering five stages in accordance with Holden and Raitt (1974), modified by authors for four stages. These stages are:
stage I – immature, stage II – developing virgin and ripening, stage III – ripening, stage IV – spent. The maturity stage of ovaries was determined with reference to six stages, as described in Ferreri et al. (2009). These stages are: stage I – immature or rest, stage II – developing, stage III - imminent spawning, stage IV – spawning, stage V – partial post-spawning, stage VI – spent.

The data were grouped into 0.5-cm length classes and length-frequency histograms were derived for each sampling date (Karacam and Düzgünes, 1990; Kraljević et al., 2011). Mean TL and WW were calculated for all specimens and for each sex. The length-weight relationship was estimated for males, females and both sexes combined in accordance with the exponential equation:

\[ WW = aTL^b \]

where WW = weight (g), TL = total length (cm) and \( a \) and \( b \) are the coefficients of the function. Parameters \( a \) and \( b \) were estimated by the ordinary least squares method based on the logarithmic form of the above equation. To examine for significant differences on the slope \( b \) of the TL-WW relationships between males and females, an analysis of covariance (ANCOVA, \( p < 0.05 \)) was applied. Fulton’s condition factor (Ricker, 1975) was calculated for each sex and for both sexes combined as follows:

\[ CF = \frac{(WW \times 100)}{TL^3} \]

The differences between mean TL, WW and CF in consecutive sampling dates, and between females and males within the same sample, were analyzed statistically using the permutation t-test.

Sex ratio, expressed as \( \frac{FF}{(FF+MM)} \), was calculated for each sampling date.

**Results**

A total of 1100 individuals were analyzed; 63% of them were females, 29% were males and 8% were undetermined (Table 1). The TL of the anchovies ranged between 6.8 and 11.2 cm, and the WW between 1.44 and 8.45 g. The length-frequency distributions exhibited a mode of 8 cm in May-July and 8.5 cm in August. The most frequent length classes in September were 8.5 and 9 cm. Moreover, a
secondary mode of 7.5 cm was found on 18 September. The length-frequency distribution was unimodal again on 2 October with a mode at 9 cm (Fig. 2).

Mean TL increased with time, showing the lowest values in May-July (about 8.4-8.5 cm) and the highest values in August-November (ranging between 8.8 and 9.3) (Fig. 3a). The differences between mean TL values on consecutive sampling dates were found to be significant (p<0.05) for most of the pairwise comparisons related to the July-October period (Table 2). The temporal variations in the mean TL of females (Fig. 3b) and males (Fig. 3c) showed the same trend as both sexes combined. Females exhibited significant differences for most of the pairwise comparisons of mean TL values on consecutive sampling dates in the July-November period. In contrast, males showed significant differences only in July-August (Table 2). Although mean TL values were usually greater for females than males, the t-test gave significant values only for the samples of 27 June and 4 September (Table 3).

Like mean TL, mean WW increased with time, from a range of 3.03-3.17 g in May-July to a range of 3.78-4.36 g in August-November, and showed the same temporal trend for females, males and both sexes combined (Fig. 4). Significant temporal differences were obtained for the same pairwise comparisons for which significant differences in mean TL were observed, for females, males and both sexes combined (Table 2). Significant differences between the mean weights of females and males were also found in the samples of 27 June and 4 September (Table 3).

Exponent b of the TL-WW relationship ranged between 2.775 and 3.759 (Table 4). The pattern of growth varied constantly between positive (b>3) and negative (b<3) allometries, with the highest b value seen in May and the lowest in July. Except for the 27 June and 4 September samplings, males showed greater b values than females. Except for the 2 October sampling, male and female b values did not differ significantly (ANCOVA; p<0.05).

The temporal variations in mean CF, calculated for each sex and for both sexes combined, and their standard errors are shown in Fig. 5. Mean CF decreased from 24 May (0.521 ± 0.005) to 13 June (0.496 ± 0.008), then it increased up to 4 September (0.586 ± 0.005), when it reached the highest value. Subsequently, mean CF decreased again till November (0.515 ± 0.004). Considering both sexes combined, the differences between mean CF in consecutive sampling dates were found to be significant for most of the pairwise comparisons (Table 2). Females and males showed the same temporal trends when superimposed on the graph obtained for both
Figure 2. Length-frequency distribution of anchovy. n = number of individuals examined.
sexes combined (Fig 5). Females showed significant differences for the same pairwise comparisons as both sexes combined, whereas males only for comparisons of samples taken in July, August and September. There were no significant differences between the CF values of females and males in the different sampling dates (Table 3).

Sex ratio deviated from the hypothetical value of 0.5 (50% females and 50% males) in almost all samples (Fig. 6). Females predominated in all sampling period, except on 4 September, when sex ratio was 0.5. This predominance of females was higher in October-November.

Macroscopic examination of the female and male gonads showed a temporal cycle of gonad maturity for both females and males (Fig. 7). Most of the female gonads identified were assigned to stages I (immature or rest) (31% of all females sampled) (Fig.7a). The occurrence of stage I females was high from 13 June to 1 August and subsequently at the end of the study period, with the highest percentages (>50%) recorded on 27 June (88%), 10 July (61%) and 15 November (62%). Stage II (developing) was more represented in the samples taken on 13 June (29%) and 18 September (17%). Ripe individuals (stage III, IV and V) were found from May to October, suggesting that this has been the spawning period for females. Imminent spawning (stage III) and spawning (stage IV) individuals were abundant during the summer months, the highest percentages being recorded on 21 August (61%) and 4 September (63%), indicating the peak spawning time. A large number of stage III females were found between July and September with the highest percentages recorded in the samples taken on 21 August.
Anchovy population in the Lagoon of Lesina

(44%) and 4 September (48%). The highest proportions of stage IV individuals were observed on 13 June (21%) and, subsequently, on 21 August (17%) and 4 September (15%). Partial post spawning (stage V) females were more abundant in May, representing the 83% of the sample. High percentages of stage V individuals were also recorded in the samples of October (32% and 76%), when this stage resulted predominant. Moreover, by October a high percentage of spent (stage VI) females were observed with the highest proportions observed on 2 October (29%) and 15 November (37%). Most of the male gonads examined (51% of all males sampled) were in developing/ripening stage (stage II) (Fig.7b). The highest proportions of stage II males were observed from July (64%) to September (61%) with a maximum value recorded on 1 August (84%). Mature males were found from June to October, indicating the spawning period. The highest proportions of ripe males (stage III) were found on 21 August (32%), 4 September (44%) and, in lesser extent, 13 June (25%). High percentages of spent (stage IV) males were observed in May (41%) and November (11%).

Table 2 - Permutation t-test scores achieved by testing differences between mean TL (Total Length), WW (Wet Weight) and CF (Condition Factor) of temporally consecutive samples: *, p<0.05; **, p<0.01; ***, p<0.001.

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>LT Both sexes</th>
<th>WW Both sexes</th>
<th>CF Both sexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>females</td>
<td>males</td>
<td>combined</td>
</tr>
<tr>
<td>24 May vs. 13 Jun</td>
<td>0.6105</td>
<td>0.9823</td>
<td>0.9116</td>
</tr>
<tr>
<td>13 Jun vs. 27 Jun</td>
<td>0.8881</td>
<td>0.1854</td>
<td>0.345</td>
</tr>
<tr>
<td>27 Jun vs. 10 Jul</td>
<td>0.8208</td>
<td>0.695</td>
<td>0.8512</td>
</tr>
<tr>
<td>10 Jul vs. 1 Aug</td>
<td>0.0001***</td>
<td>0.0014**</td>
<td>0.0001***</td>
</tr>
<tr>
<td>1 Aug vs. 21 Aug</td>
<td>0.0441*</td>
<td>0.0315*</td>
<td>0.0089**</td>
</tr>
<tr>
<td>21 Aug vs. 4 Sept</td>
<td>0.0375*</td>
<td>0.9095</td>
<td>0.2158</td>
</tr>
<tr>
<td>4 Sept vs. 18 Sept</td>
<td>0.0373*</td>
<td>0.6539</td>
<td>0.0077**</td>
</tr>
<tr>
<td>18 Sept vs. 2 Oct</td>
<td>0.004**</td>
<td>0.1749</td>
<td>0.0001***</td>
</tr>
<tr>
<td>2 Oct vs. 18 Oct</td>
<td>0.0966</td>
<td>0.3023</td>
<td>0.006**</td>
</tr>
<tr>
<td>18 Oct vs. 15 Nov</td>
<td>0.0141*</td>
<td>0.0943</td>
<td>0.1774</td>
</tr>
</tbody>
</table>

Figure 5. Temporal variation of mean condition factor values and standard errors during study period.
Discussion

Engraulis encrasicolus was constantly present in the Lagoon of Lesina during the spring and summer months, when the temperature and salinity of the lagoon rose to levels that were similar to those of the open sea. In terms of length and weight, the results of this study showed that the anchovies caught in the Lagoon of Lesina were small. Considering that fyke nets are non-selective fishing tools and the mesh size used captured adults and juveniles alike, the fishing method introduced no bias to the assessment of population size structure. The range of length values found in the Lagoon of Lesina agrees with data for young anchovies belonging to the 0+ age-group in other areas. For anchovy in the Black Sea, Erkoyuncu and Ozdamar (1989) and Karacam and Düzgünès (1990) estimated a mean length of 8.64 and 8.71 cm for 0+ age-group individuals respectively. Basilone et al. (2004) observed a mean length of 9.2 cm for the 0+ age-group anchovies in the Strait of Sicily. In addition, a high percentage of the individuals captured in the Lagoon of Lesina were ripe or ripening during this study. Anchovy attains sexual maturity at the end of the first year of life (Marano, 2001; Sinovčić, 2000). Length at first maturity is estimated to be 8-9 cm for the northern Adriatic (Varagnolo, 1967) and 9 cm for the South-Western Adriatic (Marano et al., 1998). These findings corroborate our results, including the fact that the anchovy population occurring in the Lagoon of Lesina was represented by young individuals at their first spawning.

The length-frequency distributions of anchovy were essentially unimodal, indicating that the catches are based on a single year-class. The occurrence of a secondary size mode on 18 September could be the result of new recruitment due to the prolonged spawning period of the anchovy, which usually extends from April to October in the Adriatic Sea (Marano et al., 1998; Regner et al., 1985; Sinovčić, 1978; Varagnolo, 1964).

Table 3 - Permutation t-test scores achieved by testing differences between mean TL (Total Length), WW (Wet Weight) and CF (Condition Factor) of males and females in each sample: *, p<0.05; **, p<0.01; ***, p<0.001.

<table>
<thead>
<tr>
<th>Date</th>
<th>TL</th>
<th>WW</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 May</td>
<td>0.1838</td>
<td>0.3888</td>
<td>0.7744</td>
</tr>
<tr>
<td>13 Jun</td>
<td>0.4376</td>
<td>0.814</td>
<td>0.8791</td>
</tr>
<tr>
<td>27 Jun</td>
<td>0.0147*</td>
<td>0.0387*</td>
<td>0.6564</td>
</tr>
<tr>
<td>10 Jul</td>
<td>0.091</td>
<td>0.0845</td>
<td>0.5524</td>
</tr>
<tr>
<td>1 Aug</td>
<td>0.0855</td>
<td>0.0616</td>
<td>0.2584</td>
</tr>
<tr>
<td>21 Aug</td>
<td>0.3724</td>
<td>0.8642</td>
<td>0.4045</td>
</tr>
<tr>
<td>4 Sept</td>
<td>0.0008***</td>
<td>0.002**</td>
<td>0.6919</td>
</tr>
<tr>
<td>18 Sept</td>
<td>0.9974</td>
<td>0.5241</td>
<td>0.0621</td>
</tr>
<tr>
<td>2 Oct</td>
<td>0.5296</td>
<td>0.3946</td>
<td>0.2556</td>
</tr>
<tr>
<td>15 Nov</td>
<td>0.8196</td>
<td>0.8634</td>
<td>0.8481</td>
</tr>
</tbody>
</table>

Figure 6. Temporal variation of anchovy sex ratios [FF/(FF+MM)] during study period.
Anchovy population in the Lagoon of Lesina (2009) found that water column Chla concentrations in the Lagoon of Lesina were greater in summer and October, and were positively correlated with temperature, salinity, TSM and POM, suggesting that the environmental conditions of the lagoon may have provided a substantial trophic advantage for anchovies during the study period. Similarly, it was found that condition factor values were very low between June and July and then increased significantly, reaching the highest values in the first sampling of September. The high water temperature and increased availability of nutrients during the summer months may also explain the results in this case.

Sex-ratio of the anchovy population in the Lagoon of Lesina essentially differed from the ratio of 1:1 found in the Adriatic Sea (Morello and Arneri, 2009), being generally skewed towards females. It was found that females dominated particularly during the inactivity period, in accordance with the findings of Mužinić (1956) and Sinovčić (2000) for the Eastern Adriatic and those of Padoan (1963) for the mouths of the River Po (Western Adriatic Sea). Anchovy, like other pelagic species, is a fast-growing species (Karacam and Düzgünes, 1990). The growth of anchovy in the Lagoon of Lesina was positively allometric (b>3) for most of the study period. Negative allometric growth (b<3) was observed in late June-July, when no increments in mean length and weight were recorded. Negative allometric growth was also observed in late September, when mean length, weight and condition factor values decreased significantly, probably due to a large number of new, smaller recruits (Arruda et al., 1991). Differences in the growth characteristics of specimens from sea to estuarine and lagoon ecosystems may be attributed to differences in trophic and environmental conditions (Weatherley and Grill, 1987). With respect to the available data on the length-weight relationships among anchovies of the same size range in the Adriatic Sea, the exponent b values frequently differed (see Table 3 in Morello and Arneri, 2009). Specifically, the b values obtained for the anchovies caught in the Lagoon of Lesina in May, June, August, October and November were greater than those estimated for the species in the sea in the same months. Interestingly, Roselli et al. (2009) found that water column Chla concentrations in the Lagoon of Lesina were greater in summer and October, and were positively correlated with temperature, salinity, TSM and POM, suggesting that the environmental conditions of the lagoon may have provided a substantial trophic advantage for anchovies during the study period. Similarly, it was found that condition factor values were very low between June and July and then increased significantly, reaching the highest values in the first sampling of September. The high water temperature and increased availability of nutrients during the summer months may also explain the results in this case.

Table 4 - Estimated parameters of the length-weight relationships [Ln(WW) = Ln(a) + bLn(TL)] (WW in g and TL in cm) for anchovies from the different sampling dates. a and b are the parameters of the relationship; SE is the standard error for the two parameters.

<table>
<thead>
<tr>
<th>Date</th>
<th>males</th>
<th>females</th>
<th>both sexes combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a (±SE)</td>
<td>b (±SE)</td>
<td>a (±SE)</td>
</tr>
<tr>
<td>24 May</td>
<td>-3.166 (±0.543)</td>
<td>3.962 (±0.588)</td>
<td>-2.740 (±0.249)</td>
</tr>
<tr>
<td>13 Jun</td>
<td>-2.993 (±0.408)</td>
<td>3.743 (±0.442)</td>
<td>-2.462 (±0.367)</td>
</tr>
<tr>
<td>27 Jun</td>
<td>-1.834 (±0.240)</td>
<td>2.501 (±0.262)</td>
<td>-2.376 (±0.241)</td>
</tr>
<tr>
<td>10 Jul</td>
<td>-2.263 (±0.217)</td>
<td>2.965 (±0.237)</td>
<td>-1.966 (±0.193)</td>
</tr>
<tr>
<td>1 Aug</td>
<td>-2.603 (±0.417)</td>
<td>3.363 (±0.445)</td>
<td>-2.055 (±0.201)</td>
</tr>
<tr>
<td>21 Aug</td>
<td>-2.651 (±0.230)</td>
<td>3.434 (±0.242)</td>
<td>-2.482 (±0.258)</td>
</tr>
<tr>
<td>4 Sept</td>
<td>-2.238 (±0.232)</td>
<td>3.005 (±0.245)</td>
<td>-2.298 (±0.277)</td>
</tr>
<tr>
<td>18 Sept</td>
<td>-2.290 (±0.158)</td>
<td>3.032 (±0.166)</td>
<td>-2.114 (±0.106)</td>
</tr>
<tr>
<td>2 Oct</td>
<td>-3.431 (±0.365)</td>
<td>4.212 (±0.380)</td>
<td>-2.563 (±0.109)</td>
</tr>
<tr>
<td>18 Oct</td>
<td>-2.365 (±0.097)</td>
<td>3.095 (±0.101)</td>
<td>-2.379 (±0.094)</td>
</tr>
</tbody>
</table>

E. encrasicolus is a fast-growing species (Karacam and Düzgünes, 1990). The growth of anchovy in the Lagoon of Lesina was positively allometric (b>3) for most of the study period. Negative allometric growth (b<3) was observed in late June-July, when no increments in mean length and weight were recorded. Negative allometric growth was also observed in late September, when mean length, weight and condition factor values decreased significantly, probably due to a large number of new, smaller recruits (Arruda et al., 1991). Differences in the growth characteristics of specimens from sea to estuarine and lagoon ecosystems may be attributed to differences in trophic and environmental conditions (Weatherley and Grill, 1987). With respect to the available data on the length-weight relationships among anchovies of the same size range in the Adriatic Sea, the exponent b values frequently differed (see Table 3 in Morello and Arneri, 2009). Specifically, the b values obtained for the anchovies caught in the Lagoon of Lesina in May, June, August, October and November were greater than those estimated for the species in the sea in the same months. Interestingly, Roselli et al. (2009) found that water column Chla concentrations in the Lagoon of Lesina were greater in summer and October, and were positively correlated with temperature, salinity, TSM and POM, suggesting that the environmental conditions of the lagoon may have provided a substantial trophic advantage for anchovies during the study period. Similarly, it was found that condition factor values were very low between June and July and then increased significantly, reaching the highest values in the first sampling of September. The high water temperature and increased availability of nutrients during the summer months may also explain the results in this case.

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multiple spawner, producing several discrete batches of oocytes within the reproductive period (Morello and Arneri, 2009). In agreement with the current literature, the macroscopic analysis of anchovy gonads indicated that spawning took place during the warmer part of the year, in May-October. However, anchovy eggs were not found in the Lagoon of Lesina, indicating that ripe specimens migrate from the lagoon to the sea for breeding (Brugnano et al., 2011). Two peaks of ripe females and males, in June and in August-September, were observed, suggesting the occurrence of two spawning peaks in the study period, as also found by Vučetic (1971) and Regner (1972) for the central Adriatic.

All of these aspects need to be investigated in detail, and future studies should attempt to investigate the relationships of temperature, salinity and Chla concentration to anchovy growth and reproduction (Morello and Arneri, 2009). In addition, it will be important to compare the growth parameters of anchovy in different transitional ecosystems and in the nearby open sea, in order to better understand how the environmental conditions of transitional ecosystems may modify the growth pattern of E. encrasicolus.

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