Abstract—Blacktail comber (Serranus atricauda Günther) is a commercially important species in the Canary Islands fisheries. A total of 425 individuals were collected and histological techniques were used to investigate the reproductive data. Results indicated that the spawning season occurred throughout the year, peaking between March and July. Individuals reached 50% maturity at 19.3 cm TL and 95% at 33.1 cm TL. Batch fecundity estimates ranged from 21,774 to 369,578 oocytes per spawning event for specimens from 22.2 to 39.8 cm TL. Blacktail comber was estimated to spawn 42 times/year and 26.5% of individuals spawned, on average, every 3.8 days. Estimates of potential annual fecundity for the species ranged from 0.91 to 15.5 million oocytes, and an average of 5.1 ±4.1 million.

Spawning season, maturity sizes, and fecundity in blacktail comber (Serranus atricauda) (Serranidae) from the eastern-central Atlantic

Mercedes García-Díaz
José A. González
Instituto Canario de Ciencias Marinas
Departamento de Biología Pesquera
P.O. Box 56, E-35200 Telde (Las Palmas), Spain

María J. Lorente
Departamento de Biología Animal (U. Morfológia Microscópica)
Universidad de Valencia
Moliner 50
Burjassot (Valencia), Spain

Victor M. Tuset
Instituto Canario de Ciencias Marinas
Departamento de Biología Pesquera
P.O. Box 56, E-35200 Telde (Las Palmas), Spain
E-mail address (for V. M. Tuset, contact author): victorta@iccm.rcanaria.es
Present address (for V. M. Tuset): Instituto Canario de Ciencias Marinas
P.O. Box 56, E-35200 Telde
Las Palmas, Spain

Fish of the genus Serranus are synchronous hermaphrodites; male and female tissues are simultaneously functional (Smith, 1965). The most common style of reproduction is serial monogamy, in which individuals are solitary during the day before pairing up and spawning in the late afternoon (Fischer, 1986). During spawning one fish in each pair functions as a male and the other as a female and cross-fertilization occurs. This special characteristic, and the possibility of self-fertilization, have encouraged detailed studies of the gonad structure of the genus Serranus (Atz, 1965; Fishelson, 1970; Reinboth, 1970; Febvre et al., 1975; Zanuy, 1977; Bruslé, 1983; Abd-el-Aziz and Ramadan, 1990; García-Díaz et al., 1997, 2002), although knowledge of other reproductive features is scarce.

Models of dynamic population used in the management of fishery resources and in biological studies of fish require knowledge of the reproductive system (Koslow et al., 1995). This includes gonad morphology (external and cellular description of the ovary and testis), reproductive pattern (hermaphroditism or gonocorism), reproductive behavior, reproductive cycle, spawning season duration, size at maturity, sex ratio, size at sexual transition, and fecundity. Of all these reproductive features, fecundity is the most difficult biological parameter to obtain, although it is of interest to fishery scientists both as a critical parameter for stock assessments based on egg production methods and as a basic aspect of fish biology and population dynamics. To calculate fecundity, fish are divided into determinate and indeterminate spawners (Hunter and Macewicz, 1985a; Hunter et al., 1992). With indeterminate spawners, multiple or serial batches are noted, and spawning may take place many times during a protracted spawning...
season. Therefore fecundity is very difficult to determine before the onset of the spawning season because 1) there is no way to differentiate between the oocytes that are going to be shed during the next season from those which will remain for future seasons, and 2) the number of reserve oocytes that will mature during the next spawning season cannot be predetermined (Hunter and Goldberg, 1980; Hunter et al., 1985). To estimate indeterminate annual fecundity, the mean number of eggs per batch and spawning frequency throughout the spawning season must be calculated. Owing to the complexity of obtaining these data, fecundity studies are normally limited to determinate spawners or pelagic species of substantial economical interest (Hunter and Goldberg, 1980; Hunter and Macewicz, 1985a; Kar lou-Riga and Economidis, 1997; Murua et al., 1998).

The blacktail comber (Serranus atricu da Günther, 1874), is a littoral (3–150 m) benthic species, ranging throughout the eastern central Atlantic (from the Bay of Biscay to Mauritania, the Azores, Madeira, and the Canary Islands) and in the western Mediterranean. It is, therefore, a species of wide distribution and commercial interest in many regions (Bauchot, 1987; Smith, 1990). In the Canary Islands, it is an economically important species for small-scale inshore fisheries (Pérez-Barroso et al., 1993). García-Díaz et al. (1999, 2002) determined that this species is a functional simultaneous hermaphrodite. The ovary is classified as asynchronous, i.e., various stages of oocyte development occur simultaneously (primary growth stage, yolk vesicle formation, vitellogenesis, oocyte maturation, and mature egg). The histological structure of the gonad and of the sperm indicates that this species is an externally fertilizing teleost.

The objective of this article is to increase our understanding of the reproductive biology of the blacktail comber in the Canary Islands by estimating its spawning season duration, size at maturity, and fecundity.

Materials and methods

Sampling

A total of 425 individuals of S. atricu da, ranging from 15.7 to 43.2 cm total length were sampled monthly from commercial catches off the Canary Islands between September 1992 and November 1994 (Fig. 1). Total length (TL), to the nearest centimeter, was measured for each specimen, together with the total and gutted weight (TW and GW, respectively), gonad weight (GNW), and liver weight (LW), with an accuracy of 0.1 g. Gonads were removed and fixed in 10% buffered formaldehyde. After 24–48 h, they were dehydrated, embedded in paraffin wax, sectioned in longitudinal or cross-sections (4 to 5 μm thick), and stained with Harris “hematoxylin-Puttis” eosin. Oocyte stages and spermatogenic cells were classified according to García-Díaz et al. (2002). Postovulatory follicles and atresia were also characterized according to the criteria used for Engraulis mordax by Hunter and Goldberg (1980) and Hunter and Macewicz (1985b). Maturity stages (MS) were determined from histological observations (the development of the ovary and testis and also the presence and absence of different types of the oocytes and spermatocytes) according to García-Díaz et al. (1997, 2002) (Table 1). The developmental stages of the oocytes were categorized according to Selman et al. (1993): primary growth (stage I); cortical alveoli formation (stage II); vitellogenesis (stage III); oocyte maturation (stage IV); and mature egg (stage V).

Seasonality of gonad development

Monthly changes in the five following variables were analyzed to determine the spawning season of blacktail comber (García-Díaz et al., 1997):

1. Percent frequency of the maturity stages; this indicates population changes in gonad development.
2. Gonadosomatic index (GSI=(gonad weight/gutted weight)×100): this shows differences in development of the gonads with respect to gutted body weight;
Table 1
Description of gonad stages according to García-Díaz et al. (1997, 2002).

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>Histological appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Immature</td>
<td>Ovary contains oogonia and oocytes from primary growth (stage I). Testis formed mainly by spermatogonia and primary spermatocytes. Ovary and testis joined by connective tissue.</td>
</tr>
<tr>
<td>II. Developing virgin, or recovering-spent</td>
<td>Ovary begins to acquire ovarian lamellae. Contains stage-I oocytes and yolk-vesicle-formation-stage oocytes (stage II) in advanced phases. Testis arranged in tubules with spermatogonia and primary and secondary spermatocytes.</td>
</tr>
<tr>
<td>III. Developing, maturing</td>
<td>Ovary with oocytes at all previous stages and oocytes in vitellogenic stage (stage III). Seminiferous tubules contain all spermatogenic cells.</td>
</tr>
<tr>
<td>IV. Ripe</td>
<td>Ovary with oocytes at all previous stages and with maturation oocytes (stage IV), mature and hydrated eggs (stage V). Atresic oocytes and postovulatory follicles appear. Testis completely mature, tubules filled with spermatozoa (which accumulate in deferent duct next to gonadal wall) to be expelled.</td>
</tr>
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</table>

3 Hepatosomatic index ($HSI=(liver\ weight/gutted\ weight)\times100$): this estimates the relative size of the liver to body weight:

4 Condition factor ($Kn=(total\ weight/TL^b)\times1000$): this is as an overall measurement of robustness of the fish, $b$ being the exponential of the regression $TW=\alpha TL^a$, which is 3.25 according to Tuset et al. (2004);

5 Oocyte diameter (DO): this gives information on the cell development of each individual. To obtain this value, of ten fish randomly chosen from the monthly samples, the diameters of the first 50 oocytes encountered were measured with an ocular micrometer. Measurements were taken only of oocytes sectioned through the nucleus.

Length at sexual maturity

Total length of all individuals was used to estimate the size at first maturity and size at mass maturity. These are defined as the sizes (TL) at which 50% (TL_{50\%}) and 95% (TL_{95\%}) of all fish sampled are at the relevant maturity stage (developing MS III, ripe MS IV, or spent MS V) (García-Díaz et al., 1997). The proportions were estimated at length classes of 1 cm, and the data were fitted to the logistic curve (Pope et al., 1983):

$$p=100/(1+\exp(a+bTL)),$$

where $p =$ percentage of mature individuals as a function of size class (TL); and

$a$ and $b$ = specific parameters that can change during the life cycle.

A logarithmic transformation was applied to the equation to calculate the parameters $a$ and $b$ by means of linear regression.

Reproductive potential

The pattern of annual fecundity (indeterminate or determine) was assessed by oocyte size-frequency distribution (Hunter and Macewicz, 1985a). Ten fish ranging between 18.1 and 31.5 cm TL in ripe stage were selected for analysis of oocyte size-frequency distribution—five fish in March and another five in July—to represent early and late gonad development (White et al., 2003). Because the mean size ($t$-test, $P>0.05$) was not significantly different between both samples, one frequency distribution was obtained for each month. The diameters of the first 500 oocytes encountered were measured with an ocular micrometer for each fish. Measurements were taken only of oocytes sectioned through the nucleus.

 Gonads of individuals in MS III (developing) and MS IV (ripe) were collected to estimate fecundity. One lobule was fixed in 10% buffered formaldehyde (24 h) for histological study, and the other was weighed and preserved in Gilson's fluid to estimate fecundity. Gonads with postovulatory follicles were omitted from batch fecundity estimates (Hunter et al., 1985).

The oocyte size-frequency method was applied to determine the batch fecundity (Hunter et al., 1985). This entails counting the number of oocytes in the most developed modal group of oocytes and plotting the size-frequency. In each lobule 200 oocytes were measured with the Nikon digital counter SC-112 and data processor DP-201 connected to the micrometer stage of a profile projec tor (V-12A, Nikon, Melville, NY). The routine NORMSEP (normal distribution separator) of FAO-ICLARM Stock Assessment Tools (FISAT II, vers. 1.0.0, FAO, Rome, Italy) program was used to separate the most developed modal group of oocytes based on Hasselblad's maximum likelihood method (Hasselblad, 1966).

Batch fecundity was estimated gravimetrically by using the oocyte size-frequency method (Hunter et
al., 1985; Yoneda et al., 2001). Samples were filtered through a 100-mm mesh sieve (approximately the minimum diameter of vitellogenic oocytes, Garcia-Diaz et al., 2002) and were carefully washed under running distilled water to eliminate tissue remains and fixer. We used a sieve made from a piece of nylon plankton net inserted between two sections of PVC pipe, 15 cm in diameter and 10 cm in depth (Lowerre-Barbieri and Barbieri, 1993). A compact mass of oocytes was dried for 24 hours on filter paper and weighed precisely to 0.001 g. Afterwards, a subsample of 0.2 g was selected and placed in the count dish, covered with 3–4 drops of glycerin, and the number of oocytes in the sample was tallied with a hand counter. Batch fecundity was considered to be the total number of oocytes within the most developed modal group of oocytes (Hunter et al., 1985).

Spawning frequency (the number of spawnings per year by an individual) was estimated by dividing the duration of the spawning season by the average number of days between spawning for all individuals (Hunter and Macewicz, 1985a). The duration of the spawning season was the number of days between the first (7th February) and last (16th July) occurrence of hydrated oocyte or postovulatory follicles. The average number of days between spawning was the inverse of the percent frequency of hydrated individuals, multiplied by 100 (Collins et al., 1998).

The potential annual fecundity estimates (PAFEs) were obtained by multiplying batch fecundity by spawning frequency, and relative fecundity was defined as PAFE divided by individual weight (Hunter et al., 1992). The relationships between PAFE and TL, PAFE and TW, and PAFE and gonad weight (GW) were calculated by the following compound equation:

$$PAFE = a \cdot (b^X),$$

where $X = TL, TW, or GW$; and $a$ and $b$ = specific parameters.

A logarithmic transformation was applied to the equation to calculate parameters $a$ and $b$ by using linear regression (Zar, 1996).

### Results

**Spawning season and maturity sizes**

The specimens revealed the presence of maturing and ripe individuals between December and October, excluding January because only one individual was sampled then. This finding may indicate that the population spawns throughout this period, peaking between February (37.5%) and June (89.8%) (Fig. 2).

The highest values for GSI (Fig. 3A) occurred between March (2.96) and July (3.37) and a peak of maximum activity occurred in June (3.93), decreasing over the following months. The HSI (Fig. 3B) and Kn (Fig. 3C) presented irregular values during the annual cycle. Consequently, no correlation was observed between either ovarian and liver growth with ovarian and fish growth. Oocyte diameter (DO) (Fig. 3D) values varied similarly to those from the GSI; highest values occurred from March (261.37 μm) to July (275.07 μm) and peaked in June (365.85 μm). However, the variability of oocyte diameter showed the presence of mature individuals (MS III + IV) in the months of September, October, and December, according to gonad classification.

The smallest individual with mature gonads was 16 cm TL. The maturity curve established a TL$_{50%}$ of 19.3 cm and TL$_{95%}$ of 33.1 cm (Fig. 4).

### Reproductive potential

From the oocyte size-frequency distributions, we concluded that the type of fecundity in this species is indeterminate evidenced by the lack of hiatus between advanced yolked oocytes and less mature oocytes (Fig. 5).

Batch fecundity estimates ($n=28$) varied between 21,774 and 369,578 oocytes. These estimates came from blacktail comber ranging in total length from 22.2 to 39.8 cm, total weight from 127.2 to 896.6 g, gutted weight from 122.9 to 834.6 g, and gonadal weight from 1.3 to 41.3 g.

The spawning-frequency estimate for individuals from 17.2 to 43.2 cm TL was 42 times/year, and 26.5% (45/170) of individuals spawned at an average of every 3.8 days.

Potential annual fecundity estimates ranged from 0.91 to 15.5 million oocytes, and an average of 5.1 ±4.1 million (Fig. 6). Relative fecundity varied between 5062 and 20,869 oocytes per gram of individual, and mean relative fecundity was 10,547 ±4148 oocytes.
Regression analysis showed significant positive correlation between annual fecundity and total length, total weight, and gutted weight. Total weight was the best predictor of annual fecundity ($PAFE=1,053,504.23\times 1.0^{TW}; r^2=0.764, P<0.01, n=28$); followed by total length ($PAFE=63,648\times 4351\times 1380^{TL}; r^2=0.752, P<0.01, n=28$); and gutted weight ($PAFE=1,055,580\times 1.00^{GW}; r^2=0.723, P<0.01, n=28$) (Fig. 6, A–C).

**Discussion**

The histological description of gonad structure is fundamental to understanding reproduction. In most reproductive fish studies, the histological technique is omitted because it is too expensive and time-consuming. Consequently, in many cases knowledge of reproduction is limited or biased (or is both) (García-Díaz et al., 2001).

The spawning period in teleosts is determined from changes occurring within the gonad throughout the year. The macroscopic or histological observations of the
gonad (qualitative methods), somatic indices (e.g., GSI, HIS, and Kn), or evolution of oocyte diameter (quantitative methods) (or all four methods) are commonly applied. Many authors have disagreed about their applications and biological interpretation (De Vlaming et al., 1982; Wootton, 1990; Shapiro et al., 1993; Sadovy, 1996; Karlou-Riga and Economidis, 1997). Our results demonstrate that the GSI indicates only the spawning peak and not the presence of batch or multiple spawning within the species; and the HSI and Kn do not exhibit a clear trend throughout the year. Normally, variations of these indices (HSI and Kn) imply energy storage for reproduction (Hoar et al., 1983; N’Da and Déniel, 1993). However, S. atricauda does not require such storage because feeding activity is not altered during the reproductive period (Morato et al., 2000). Oocyte diameter presents a trend similar to that of GSI, although its biological interpretation is different. The oocyte may begin the vitellogenesis phase and there may be no significant increase in gonad weight. Consequently, GSI values do not necessarily change significantly when the spawning period has begun. Thus, the quantitative indices (GSI, HSI, Kn) show the general trend of the population, whereas the oocyte diameter provides information about the population as a whole, as well as at the individual level.

The analysis of qualitative and quantitative data in S. atricauda seems to indicate that spawning takes place throughout the year, although the general population spawns between March and July. In the Canary Islands, other members of the genus spawn over long periods: eight months in S. cabrilla and nine months in S. scriba (García-Díaz et al., 1997; García-Díaz 2003). These three species have the longest spawning season of all fish studied in the Canaries, although they are also the only species whose reproductive patterns have been analyzed with histological procedures. Nevertheless, environmental or biological factors (or both) must be related to this extended spawning period.

To estimate spawning frequency, the hydrated oocyte method is less time consuming but the POF method is better because the spatial and temporal distribution of the postovulatory follicle structures is not continuous (Hunter and Goldberg, 1980). Reproductive potential measured as potential annual fecundity has not been addressed in any species of the genus Serranus with the POF method to date. It is true that this fecundity must be considered as a rough estimation because the spawning frequency was a preliminary estimation because of the scarce number of individuals in sampling months. Siiau and Bouian (1994) calculated the total fecundity in S. cabrilla and S. scriba, considering all the vitellogenesis oocytes. Their method has been widely rejected because it is valid only for

**Figure 5**

Oocyte size-frequency distributions (25-μm intervals) for blacktail comber (Serranus atricauda) ovaries from the Canary Islands.

**Figure 6**

Relationships between potential annual fecundity and total length (A), total weight (B), and gutted weight (C) for black-tail comber (Serranus atricauda) from the Canary Islands.
determinate spawners, and both the above-mentioned serranids are indeterminate spawners (García-Díaz et al., 1997; García-Díaz, 2003). The fact that the method was used for these two indeterminate spawners could explain why the values of maximum fecundity obtained by these authors for S. cabrilla (441,502 oocytes) and S. scriba (54,649 oocytes) were lower than that for S. atricauda (15.5 millions oocytes). Consequently, the present study enables us to provide new reproductive data for blacktail comber, where potential annual fecundity is essential for future egg production models and estimates of spawning stock biomass.

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