

Abstract.—The reproductive biology of Brazilian menhaden, *Brevoortia aurea*, inhabiting the estuarine waters of the Río de la Plata (Argentina–Uruguay), was studied by using histological analysis of the ovaries. The samples were collected during the peak of the spawning period of this species (November) during 1994, 1995, and 1997. *Brevoortia aurea* is a multiple spawner with indeterminate annual fecundity. Spawning frequency, determined from the percentage of females with postovulatory follicles, was about 12% during November 1995. At this frequency, each female on average spawned a new batch of eggs about every 8 days. Batch fecundity, estimated from counts of hydrated oocytes, was fitted to a power function of length and a linear function of ovary-free female weight. Batch fecundity estimates ranged from 20,000 (27 cm fork length) to 130,000 (34 cm fork length) hydrated oocytes. Annual differences in the size-fecundity relationship were observed. Relative fecundity estimates obtained from the different years sampled ranged from 60 to 212 hydrated oocytes/g of female (ovary-free weight).

Spawning frequency and batch fecundity of Brazilian menhaden, *Brevoortia aurea*, in the Río de la Plata estuary off Argentina and Uruguay*

Gustavo J. Macchi

Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
Rivadavia 1917

(1033) Buenos Aires, Argentina

Present address: Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP)
Paseo Victoria Ocampo No. 1
Mar del Plata (7600), Argentina

E-mail address: gmacchi@inidep.edu.ar

Eduardo M. Acha

Dto. Ciencias Marinas

Universidad Nacional de Mar del Plata

Funes 3350

(7600) Mar del Plata, Argentina

The Brazilian menhaden (*Brevoortia aurea*) is a pelagic coastal species of the southwest Atlantic ranging from Salvador de Bahía, Brazil (13°S), to the south of Buenos Aires province, Argentina (40°S) (Cousseau and Díaz de Astarloa, 1993). It is abundant in the estuarine waters of the Río de la Plata area, where it is caught by the commercial fleets of Argentina and Uruguay, although it is a resource of little economic value (Argentine landings in 1997 were 893 metric tons (t); Anonymous, 1998). Maximum body size is about 41 cm total length (36 cm fork length), which corresponds to an age of 11 years (López Cazorla, 1985). These attributes are similar to those for Atlantic menhaden (*Brevoortia tyrannus*) from the Atlantic coast of the United States, which attains a maximum body size of 36–37 cm fork length and a maximum age of about 10 years (Powell, 1994). Estimation of size at sexual maturity of Brazilian menhaden has not been documented, but the minimum length at maturity for female

is 22.5 cm TL (Cassia et al., 1979). Previous reports on life history attributes include descriptions of embryonic and larval development (de Ciechowski, 1968; Weiss and Krug, 1977), growth (López Cazorla, 1985), taxonomy (Cousseau and Díaz de Astarloa, 1993; Díaz de Astarloa and Cousseau, 1993), and feeding (Sánchez, 1989; Giangioffe and Sánchez, 1993). Comprehensive information on reproduction of Brazilian menhaden does not exist. Recently, we reported that major spawning in the Río de la Plata area occurs from September to December, in estuarine waters across a wide range of salinities (Acha and Macchi, 2000). Cassia et al. (1979) reported total fecundity estimates based on the assumption that the number of eggs spawned by a female is fixed prior to the onset of spawning. Brazilian menhaden may be a multiple spawner, in which case

* Contribution 1128 of the Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata, Argentina.

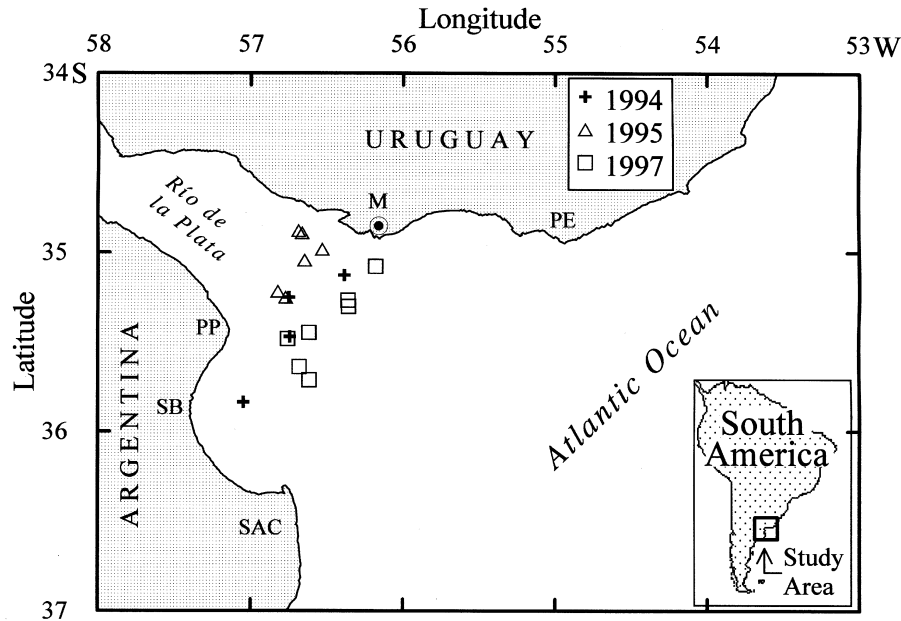


Figure 1

Sample locations for female Brazilian menhaden taken in the Río de la Plata estuary, during November 1994, 1995, and 1997. PE = Punta del Este, M = Montevideo, PP = Piedras Point, SB = Samborombón Bay, SAC = San Antonio Cape.

batch fecundity and spawning frequency must be estimated to determine annual fecundity (Hunter and Goldberg, 1980).

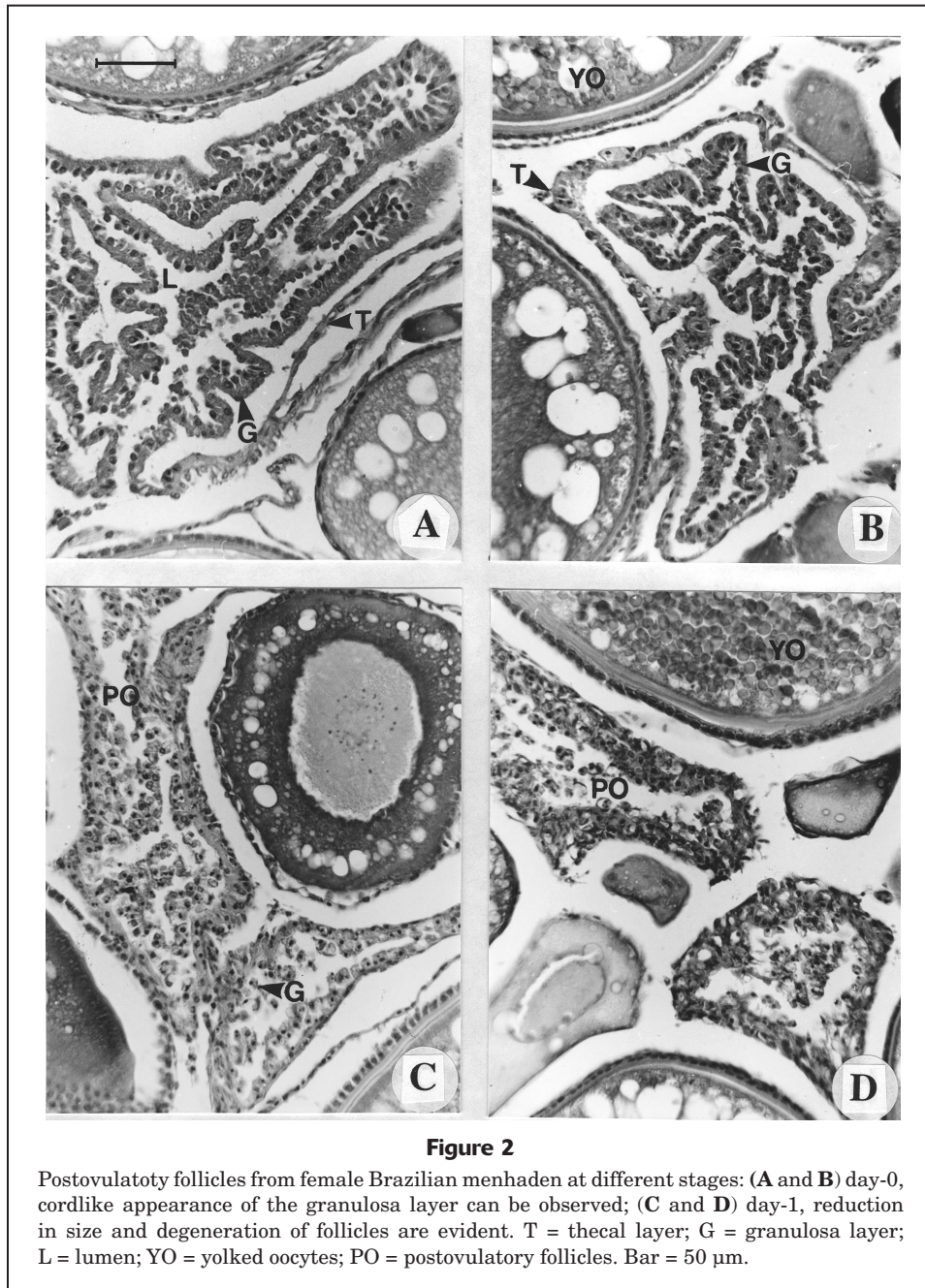
Through histological analysis of the ovaries, we investigated the reproductive biology of *B. aurea* from the Río de la Plata estuary to determine the pattern of spawning and estimate spawning frequency and batch fecundity during the main spawning peak.

Materials and methods

Brevoortia aurea females were collected from the Río de la Plata estuary during three fortnightly research cruises in November 1994, 1995, and 1997 (Fig. 1). Fish samples and oceanographic data (temperature and salinity) were taken for each trawl station from 6 to 20 m depths. Fork length (FL, cm) and total weight (TW, g) were recorded for each fish sampled. Length distributions obtained for the three years were compared with a Kolmogorov-Smirnov Test (KS) (Sokal and Rohlf, 1969). During 1994, only gravid females with hydrated oocytes ($n=54$) for fecundity estimation were sampled. Females collected in 1995 ($n=169$) and 1997 ($n=92$) were randomly selected including different maturity stages. The ovaries were removed and fixed in 10% neutral-buffered formalin for one week. In the laboratory, the gonads were weighed, and a portion of tissue (about 2.0 g) was removed

from the center of each ovary, dehydrated in methanol, cleared in benzol, and embedded in paraffin. Tissues were cut into 4- μ m sections, and stained with Harris's hematoxylin followed by eosin counterstain. Classification of ovaries was based on the stage of oocyte development and on the occurrence of post-ovulatory follicles (POF) according to Hunter and Goldberg (1980). Our description of the stages of POF degeneration was adapted from that given by Fitzhugh and Hettler (1995) for *B. tyrannus* and these stages were classified as day-0 and day-1, according to the elapsed time from spawning. A day-0 POF (elapsed time from spawning <24 h) has an irregular, convoluted shape; the granulosa cells are aligned, and many folds and the lumen are clearly visible (Fig. 2, A and B). A Day-1 POF (elapsed time from spawning >24 h) shows degenerative process, the linear appearance of the granulosa cells is not distinct and the lumen becomes reduced (Fig. 2, C and D).

Oocyte diameters for five gravid ovaries were measured after fixation ($40 \pm \mu$ m) with an ocular micrometer ($n=749$). Spawning frequency was estimated from samples ($n=169$) collected during November 1995. Daily fraction of spawning females was estimated by the incidence of fish with day-0 and day-1 POFs (Hunter and Goldberg, 1980) and spawning frequency was determined from the average of the percentages of day-0 and day-1 spawning females (Fitzhugh et al., 1993; Macchi, 1998).



Batch fecundity (BF; number of oocytes released per spawning) was estimated gravimetrically by the hydrated oocyte method (Hunter et al., 1985) for 112 females (44 from 1994, 38 from 1995, and 30 from 1997). The hydrated ovaries showed no evidence of recent spawning (no POFs). Three pieces of ovary, approximately 0.1–0.2 g each, were removed from the anterior, middle, and posterior parts of one gonad, weighed (± 0.1 mg), and the number of hydrated oocytes were counted. Batch fecundity for each female was the product of the mean number

of hydrated oocytes per unit of weight and the total weight of the ovaries. Relative fecundity (RF; hydrated oocytes per gram of body weight) was determined as the batch fecundity divided by female weight (without ovary). The relationships of batch fecundity to fork length and to total weight (ovary free) were described by regression analysis (Draper and Smith, 1981), and the significance evaluated by testing whether the slope of the regression was significantly different from zero. Interannual comparisons were based on coincident length ranges in the

three years, and an analysis of covariance to log-transformed data was applied (Draper and Smith, 1981).

Results

Length distributions for Brazilian menhaden ranged from 26 to 36 cm FL and did not differ among the years sampled ($P>20$). All individuals collected were adult mature females with yolked or hydrated oocytes; no immature ovaries were observed.

The oocyte diameter distribution of gravid *B. aurea* females (with hydrated oocytes) showed four groups of oocytes (Fig. 3). The smallest group was composed mainly of primary growth oocytes (smaller than 120 μm). The next larger group was composed of cortical alveolus stage and partially yolked oocytes (primary yolk stage) ranging from 120 to 500 μm . A third group included advanced yolked oocytes (secondary yolk stage) ranging from 500 to 700 μm . The fourth group was the largest and corresponded to the hydrated oocytes measuring 1100 to 1500 μm . The continuous distribution from primary growth oocytes to advanced yolked oocytes is a characteristic pattern in multiple spawning fishes.

Spawning frequency was estimated by examining ovarian tissue from 169 mature females sampled between 21 and 23 of November 1995 during the main spawning peak. Of all specimens examined,

about 14% had new postovulatory follicles (day-0) and 10% had day-1 postovulatory follicles (Table 1), the average was 11.98% (SD=7.53%).

Batch fecundity estimates ranged from 20,000 hydrated oocytes for a 27-cm-FL female to 130,000 hydrated oocytes for a 34-cm-FL female. A power model and a linear model were fitted to the relationships BF versus FL and BF versus TW, respectively (Fig. 4). Analysis of covariance indicated that the slope of the regression of fecundity on length did not differ between years, but the intercepts were significantly different (1994–95, $F_{(1,74)}=6.68$, $P<0.05$; 1994–97, $F_{(1,68)}=26.02$, $P<0.01$; 1995–97, $F_{(1,61)}=4.53$, $P<0.05$). Relative fecundity ranged from 60 to 212 hydrated oocytes per gram of female (ovary free). These values were different ($P<0.05$) between the years 1994 (107 ± 29 hydrated oocytes) and 1995 (135 ± 34 hydrated oocytes), and 1994 versus 1997 (149 ± 30 hydrated oocytes), but not between 1995 and 1997.

Discussion

Brevoortia aurea is a multiple spawner with indeterminate annual fecundity, according to our observations of maturing ovaries with postovulatory follicles and yolked oocytes (partially spent stage). These observations suggest that after one batch of eggs is spawned, a new batch develops and is released (Hunter et al., 1992). Further, the oocyte size-frequency distribution of gravid females shows different batches of growing oocytes, including hydrated eggs. Fractional spawning has been suggested for other congeners, such as Atlantic menhaden and gulf menhaden (*Brevoortia patronus*), on the basis of oocyte diameter distributions (Lewis and Roithmayr, 1980; Lewis et al., 1987). These species have a long reproductive season, 6–8 mo. (Powell, 1994), similar to that of *B. aurea*. Atlantic and gulf menhaden spawn during the fall and winter (Powell, 1994), whereas Brazilian menhaden spawn in spring–summer, as do most of the fishes inhabiting the Río de la Plata estuary (Macchi and Acha, 1998).

During November the temperature ranges from 19° to 21°C in the spawning area of Brazilian menhaden. Because duration of the POF stage varies at different temperatures, disappearance of the lumen

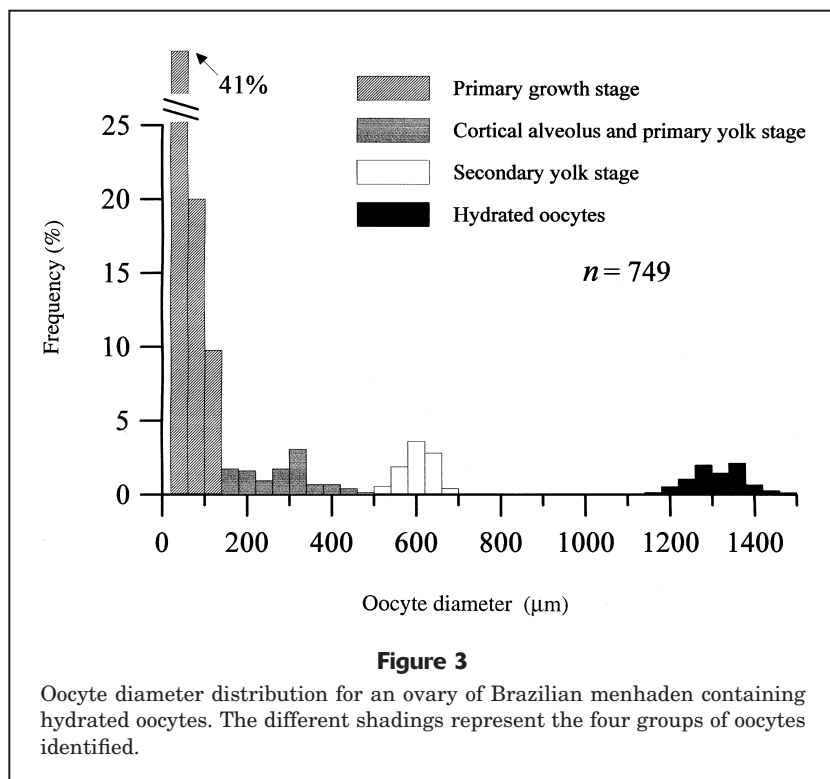


Table 1

Number of Brazilian menhaden females in reproductive activity from the Río de la Plata estuary that were histologically staged for estimation of spawning frequency. POF = postovulatory follicles; CI = confidence interval.

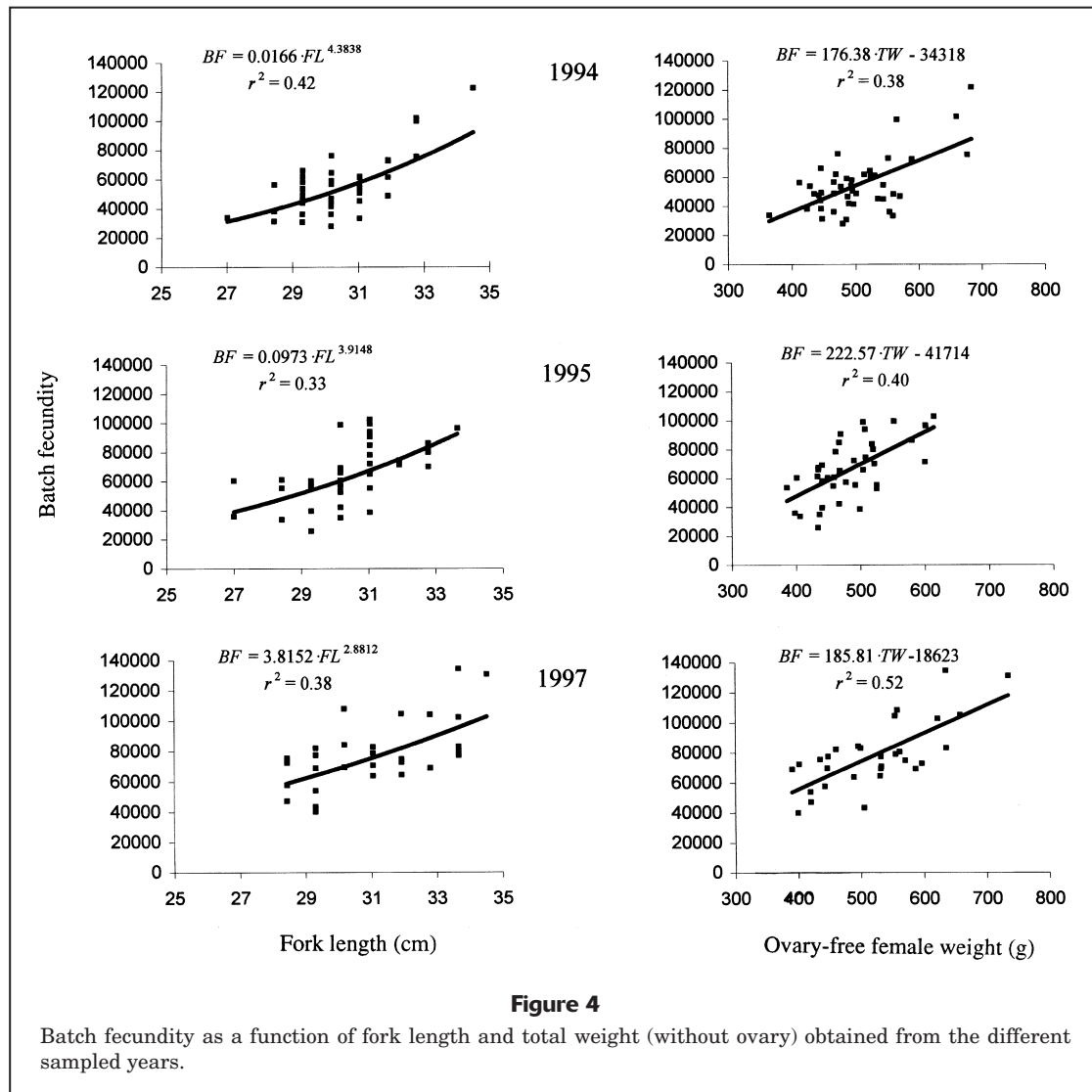
Day of month (November 1995)	Yolked oocytes and no POF	Hydrated oocytes	Day-0 POF	Day-1 POF	Total mature females
21	2	13	3	2	20
21	1	11	1	2	15
22	0	19	1	0	20
22	4	13	0	3	20
22	4	0	9	7	20
22	8	9	3	0	20
23	1	18	1	0	20
23	5	12	1	2	20
23	9	0	4	1	14
Total	34	95	23	17	169
% (average)			13.7	10.1	
95% CI			±9.43	±7.19	

after the loss of the alignment of the granulosa layer was used as the main characteristic to distinguish day-0 from day-1 postspawning ovaries, according to Fitzhugh and Hettler (1995). They concluded that this is the most important feature for classification of POFs in Atlantic menhaden spawning at about 20°C. Spawning frequency estimated from the average between day-0 and day-1 POF percentages (11.98%, SD=7.53%) indicated that *B. aurea* spawn once every eight days during the peak of the reproductive season (November). This value should be considered a preliminary estimate because we did not have samples during other months in the spawning period. Daily spawning fraction of Brazilian menhaden was similar to that of other clupeoids inhabiting temperate waters, such as *Engraulis mordax* (Hunter and Goldberg, 1980), *E. ringens* (Alheith et al., 1984), *Sardinops sagax* (Herrera and Claramunt, 1990), *Sardina pilchardus* (Pérez et al., 1992), *E. capensis* (Melo, 1994), and *E. anchoita* (Pájaro et al., 1997).

Cassia et al. (1979) reported Brazilian menhaden fecundity by counts of yolked oocytes (diameter >540 µm) from ovaries in an advanced maturity stage. They estimated a potential fecundity of 120,000 oocytes for one 30-cm-FL female but did not consider a multiple spawning pattern. Our estimated batch fecundity for females of this length was about 60,000 hydrated oocytes. During the main reproductive period (September–December), a female Brazilian menhaden with a spawning frequency of 8 days would spawn 15 times. Although total fecundity has been calculated for Atlantic menhaden (Higham and Nicholson, 1964; Dietrich, 1979; Lewis et al., 1987) and gulf menhaden (Lewis and Roithmayr, 1980),

batch fecundity estimates have not been reported, and gravid females in wild population have rarely been observed (Ahrenholz, 1991).

Batch fecundity was fitted to a power function of fork length and a linear function of ovary-free body weight. Analysis of covariance showed significant differences ($P < 0.05$) in regression coefficients among the three years considered (1994, 1995, and 1997). The low coefficients of determination in regressions were similar to those obtained for Atlantic menhaden (Lewis et al., 1987), possibly as a consequence of different ages occurring within length classes after sexual maturity (Lopez Cazorla, 1985). Mean relative fecundities for *B. aurea* (107 [in 1994], 135 [in 1995], and 149 [in 1997] oocytes/g ovary-free body weight) were much lower than those for *Engraulis anchoita* (about 600 oocytes/g ovary-free body weight), the most abundant clupeoid of the Argentine Sea (Pájaro et al., 1997). The difference may reflect the larger egg size of *B. aurea* (1500–1600 µm), compared with *E. anchoita* (about 900 µm; de Ciechowski and Weiss, 1973). The reproductive strategy of some species indicates that egg size takes priority over fecundity (Kjesbu et al., 1996). The larger eggs may be advantageous during the first days of life because hatchlings have larger yolk reserves, contributing to higher growth rates, and may avoid predation more effectively (Hinckley, 1990; Wootton, 1994). Our paper provides the first estimation of spawning frequency and batch fecundity of Brazilian menhaden during the spawning peak. These parameters should also be estimated at the end of the reproductive season to evaluate possible variations and their effect over the annual egg production. Future stud-



ies about egg quality of *B. aurea* compared with that of *E. anchoita* should include determination of oocyte dry weight, ash dry weight, and total lipid and fatty acid composition.

Acknowledgments

This research was conducted within the INIDEP's Coastal Project. We thank Teresa Carlé and Haraldo E. Christiansen for the preparation of histological sections and Marcela Tobio who was responsible for the photographic procedures. We would also like to thank Norberto Scarlato and two anonymous reviewers for a critical review of our manuscript.

Literature cited

- Acha, E. M., and G. J. Macchi.
2000. Spawning of Brazilian menhaden, *Brevoortia aurea*, in the Río de la Plata estuary off Argentina and Uruguay. Fish. Bull. 98:227-235.
- Ahrenholz, D. W.
1991. Population biology and life history of the North American menhadens, *Brevoortia* spp. Mar. Fish. Rev. 53(4): 3-19.
- Alheith, J., V. H. Alarcón, and B. J. Macewicz.
1984. Spawning frequency and sex ratio in the Peruvian anchovy *Engraulis ringens*. CalCOFI Rep. 25:43-52.
- Anonymous.
1998. Capturas marítimas totales 1997. Ministerio de Economía y Obras y Servicios Públicos. Secretaría de Agricultura, Ganadería, Pesca y Alimentación. Subsecretaría de

- Pesca, Dirección Nacional de Pesca y Acuicultura, Buenos Aires, 45 p.
- Cassia, M. C., J. D. de Ciechowski, and H. E. Christiansen.**
1979. Estudios sobre la reproducción y fecundidad de la saraca, *Brevoortia aurea* (Agassiz, 1829) Hildebrand, 1948 (Pisces. Clupeidae) y la comparación de dos métodos utilizados. *Physis* 94:39–46.
- Cousseau, M. B., and J. M. Díaz de Astarloa.**
1993. El género *Brevoortia* en la costa atlántica sudamericana. *Frente Marítimo* 14:49–58.
- de Ciechowski, J. D.**
1968. Huevos y larvas de tres peces marinos: anchoa marini, *Brevoortia aurea* y *Prionotus nudigula* de la zona de Mar del Plata. *Boletín Instituto Biología Marina* 17:1–27.
- de Ciechowski, J. D., and G. Weiss.**
1973. Desove y fecundidad de la anchoíta argentina, *Engraulis anchoita*, Hubbs & Marini. *Physis* 32 (84):137–153.
- Díaz de Astarloa, J. M., and M. B. Cousseau.**
1993. Características regionales de la saraca (*Brevoortia aurea*) dentro del área costera bonaerense. *Frente Marítimo* 14:59–62.
- Dietrich, Ch. S., Jr.**
1979. Fecundity of the Atlantic menhaden, *Brevoortia tyrannus*. *Fish. Bull.* 77(1):308–311.
- Draper, N., and H. Smith.**
1981. Applied regression analysis, second edition. J. Wiley and Sons, New York, NY, 709 p.
- Fitzhugh, G. R., and W. F. Hettler.**
1995. Temperature influence on postovulatory follicle degeneration in Atlantic menhaden, *Brevoortia tyrannus*. *Fish. Bull.* 93:568–572.
- Fitzhugh, G. R., B. A. Thompson, and T. G. Snider III.**
1993. Ovarian development, fecundity, and spawning frequency of black drum *Pogonia cromis* in Louisiana. *Fish. Bull.* 91:244–253.
- Giangiobbe, A., and F. Sánchez.**
1993. Alimentación de la saraca (*Brevoortia aurea*). *Frente Marítimo* 14:71–80.
- Herrera, G., and G. Claramunt.**
1990. Estimaciones de la fecundidad parcial y frecuencia de desove de *Sardinops sagax* durante 1987 y 1988, en el norte de Chile. *Invest. Cient. y Tec., Serie: Ciencias de Mar* 1:55–68.
- Higham, J., and W. R. Nicholson.**
1964. Sexual maturation and spawning of Atlantic menhaden. *Fish. Bull.* 63(2):255–271.
- Hinckley, S.**
1990. Variation of egg size of walleye pollack *Theragra chalcogramma* with a preliminary examination of the effect of egg size on larval size. *Fish. Bull.* 88:471–483.
- Hunter, J. R., and S. R. Goldberg.**
1980. Spawning incidence and batch fecundity in northern anchovy, *Engraulis mordax*. *Fish. Bull.* 77(3):641–652.
- Hunter, J. R., N. C. H. Lo, and R. J. H. Leong.**
1985. Batch fecundity in multiple spawning fishes. In An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy, *Engraulis mordax* (R. M. Lasker, ed.), p. 67–77. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36.
- Hunter, J. R., B. J. Macewicz, N. C. H. Lo, and C. A. Kimbrell.**
1992. Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. *Fish. Bull.* 90:101–128.
- Kjesbu, O. S., P. Solemdal, P. Bratland, and M. Fonn.**
1996. Variation in annual egg production in individual captive atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* 53:610–620.
- Lewis, R. M., D. W. Ahrenholz, and S. P. Epperly.**
1987. Fecundity of Atlantic menhaden, *Brevoortia tyrannus*. *Estuaries* 10(4):347–350.
- Lewis, R. M., and Ch. M. Roithmayr.**
1980. Spawning and sexual maturity of Gulf menhaden, *Brevoortia patronus*. *Fish. Bull.* 78(4):947–951.
- López Cazorla, A.**
1985. Edad, crecimiento y comportamiento migratorio de *Brevoortia aurea* (Agassiz, 1829) (Osteichthyes, Clupeidae) de Bahía Blanca (Argentina). *Inv. Pesq.* 49(2):297–313.
- Macchi, G. J.**
1998. Preliminary estimate of spawning frequency and batch fecundity of striped weakfish, *Cynoscion striatus*, in coastal waters off Buenos Aires province. *Fish. Bull.* 96:375–381.
- Macchi, G. J., and E. M. Acha.**
1998. Aspectos reproductivos de las principales especies de peces muestreadas durante la campaña costera H-13/94. *INIDEP Inf. Téc.* 21:67–89.
- Melo, Y. C.**
1994. Spawning frequency of the anchovy *Engraulis capensis*. *S. Afr. J. Mar. Sci.* 14: 321–331.
- Pájaro, M., G. J. Macchi, and R. P. Sánchez.**
1997. Fecundidad y frecuencia reproductiva de las poblaciones bonaerense y patagónica de anchoíta argentina (*Engraulis anchoita*). *Rev. Invest. Des. Pesq.* 11:19–38.
- Pérez, N., I. Figueiredo, and B. J. Macewicz.**
1992. The spawning frequency of sardine, *Sardina pilchardus* (Walb.), off the Atlantic Iberian coast. *Bol. Inst. Esp. Oceanogr.* 8 (1):175–189.
- Powell, A. B.**
1994. Life history traits of two allopatric clupeids, Atlantic menhaden and Gulf menhaden, and the effects of harvesting on these traits. *N. Am. J. Fish. Manage.* 14:53–64.
- Sánchez, M. F.**
1989. Características morfológicas del aparato digestivo y espectro trófico de la saraca (*Brevoortia aurea*, Clupeiformes, Pisces). *Physis* 47(112):21–33.
- Sokal, R. R., and F. J. Rohlf.**
1969. Biometry: the principles and practice of statistic in biological research. W. H. Freeman, San Francisco, CA, 776 p.
- Weiss, G., and L. C. Krug.**
1977. Características do desenvolvimento e metamorfose de *Lycengraulis olidus* (Engraulidae) e *Brevoortia pectinata* (Clupeidae) no estuário da Lagoa dos Patos, Brasil. *Atlântica* 2(1):83–117.
- Wootton, R. J.**
1994. Life histories as sampling devices: optimum egg size in pelagic fishes. *J. Fish Biol.* 45:1067–1077.