Abstract.—*Callorhynchus callorhynchus* Linnaeus ("cockfish" or "pez gallo"), the only holocephalan fish species found in the coastal waters of the southwestern Atlantic Ocean, has been caught off Argentina as bycatch of the hake, *Merluccius hubbsi*, fishery since 1920. Here we describe the morphology of its reproductive system and report on several aspects of its reproductive biology. This study is based on survey data and sampling of commercial landings from San Matías Gulf (41–42°S; 64–65°W), conducted from 1984 to 1986.

The data suggest that reproductive activities extend nearly throughout the year; mating and spawning occur in spring and early summer, followed by a short period (ca. one month) of gonadal recovery. Average size at sexual maturity (standard length, measured from the tip of the mouth to the origin of the superior caudal lobe) is 40 cm for males and 49 cm for females. Male gonadal and green gland indices peak asynchronously. During the mating season the green gland forms spermatophores that are transferred to the female at the time of copulation. The cloaca of adult females has a seminal receptacle where the mass of spermatophores is stored after copulation. Female gonadal and nidamental gland indices peak synchronously. After fertilization the oocytes are encapsulated before spawning. Oocyte diameter increases with the size of females up to a maximum of 48 mm.

Reproductive biology of the cockfish, *Callorhynchus callorhynchus* (Holocephali: Callorhynchidae), in Patagonian waters (Argentina)

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The cockfish, *Callorhynchus callorhynchus* Linnaeus, 1758, is the only holocephalan fish species found in the coastal waters of the southwestern Atlantic Ocean (Norman, 1937; Hart, 1946; Menni and Gostonyi, 1982). Adults are caught as bycatch of the hake, *Merluccius hubbsi*, fishery that operates off Argentina (Di Giácomo and Perier, 1991). Although there is considerable concern with regard to harvesting fish species with comparatively low reproductive potential (such as many elasmobranch and holocephalan species), the reproductive biology and life history of the cockfish are poorly known. In this study we describe the morphology of the reproductive system of *C. callorhynchus* and present information on its reproductive biology.

Materials and methods

Bottom trawl surveys

A survey off the north coast of San Matías Gulf (northern Patagonia; 41–42°S, 64–65°W; Fig. 1) was conducted from 15 to 19 October, 1986, aboard the FRV *C. Cánepa*. Thirty three 30-minute hauls were made at depths ranging from 20 m to 130 m, by using a 96-foot commercial otter trawl with rectangular doors (Di Giácomo and Perier, 1991). All specimens of *C. callorhynchus* were processed following laboratory procedures described below.

Sampling of the commercial landings

The commercial catch of the bottom trawl fishery landed in San Antonio Oeste (40°43'04"S, 64°56'W) was sampled monthly between February 1984 and July 1986. On each sampling date three boxes (N=20 fish per box) weighing 37 kg each were randomly selected. Samples of the commercial catch were not available during April, September, June, and December because either the fishery was inactive or no cockfish were landed.

Laboratory procedures

Standard length (SL, distance from the tip of the snout to the origin of the upper caudal lobe, Fig. 2A), total weight and liver weight were obtained from each fish sampled. Specimens were dissected to expose the reproductive system; testes and green glands of males, and ovaries and nidamental glands of females were individually weighed. The number of mature yolk oocytes and immature oocytes, maximum diam-

eter of the oocytes, number of individuals with egg cases in formation or ready for extrusion, and presence of spermatophores were recorded for females. Length of the myxopterygia (males) was measured from the insertion of the pelvic fin to the distal end (Fig. 2B).
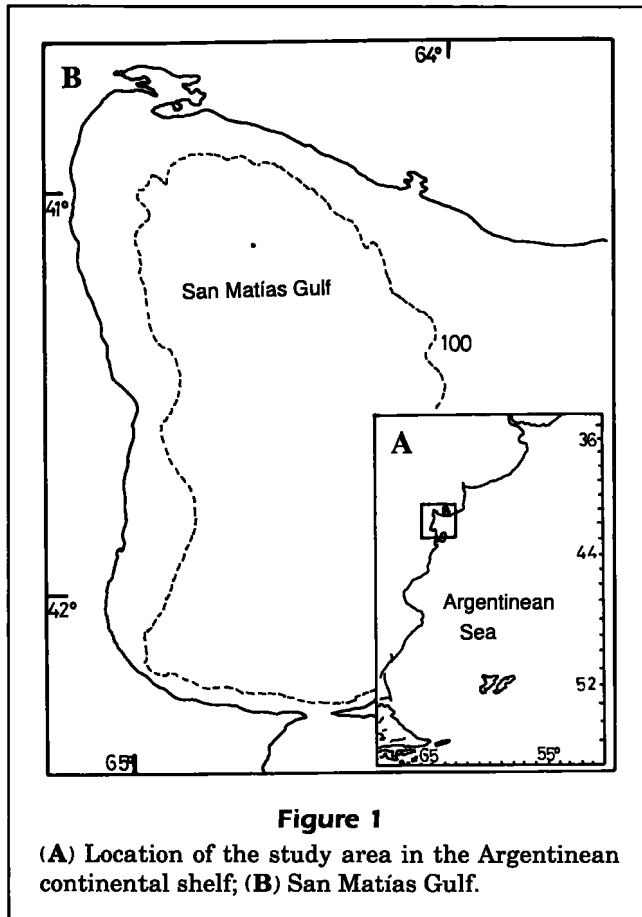

Figure 1

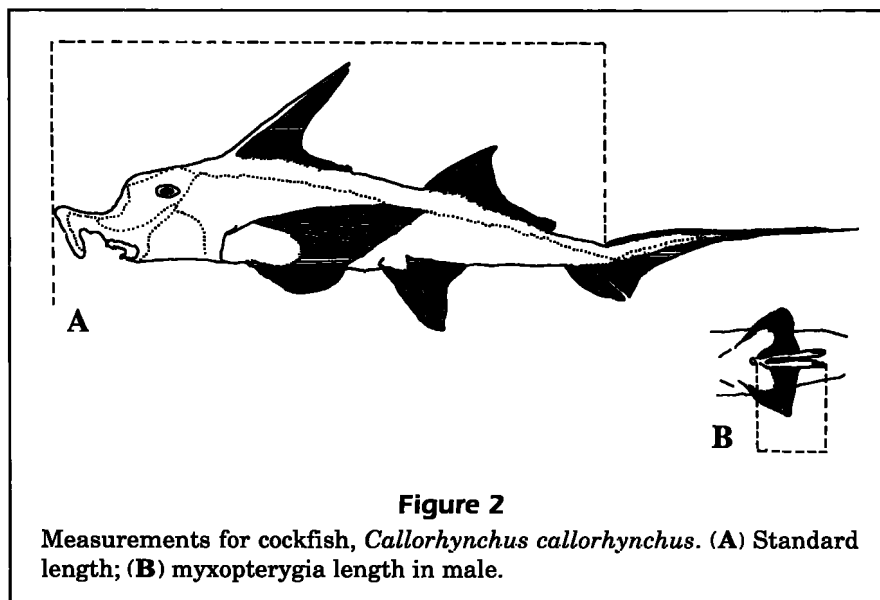(A) Location of the study area in the Argentinean continental shelf; (B) San Matías Gulf.

Indices of reproductive activity

The annual reproductive cycle was assessed by using the monthly samples of the commercial catch (1984–86). The gonadal index (GI), hepatic index (HI), nidamental gland index (NGI, females only) and green gland index (GGI, males only) were calculated as

$$GI = \frac{\text{weight of the gonad}}{\text{total weight}} \times 100$$

$$HI = \frac{\text{weight of the liver}}{\text{total weight}} \times 100$$

$$NGI = \frac{\text{weight of the nidamental gland}}{\text{total weight}} \times 100$$

$$GGI = \frac{\text{weight of the green gland}}{\text{total weight}} \times 100.$$

The duration of the mating season was estimated from the presence of spermatophore masses in the female cloaca and the annual cycle of the green gland index.

Regression analysis was performed between the indices of reproductive activity during the periods of maximum activity and SL, and between the diameter of mature oocytes and the number of immature oocytes and SL. A Wilcoxon test was used to assess the difference in the average number of mature ova between right and left ovaries of all mature females.

Sexual maturity

The degree of development of the myxopterygia (male claspers) relative to SL was used as an indicator of sexual maturity for males (Holden and Raitt, 1975). The size at first maturity in females was determined by examining 1) the percentage of mature females in each 10-mm size class, 2) the maximum diameter of mature ovarian eggs for each size class, and 3) the smallest size class with egg cases in formation. Females with yellow ovarian eggs were considered mature.

Results

Morphology of the reproductive system

The male reproductive system consists of the following paired struc-

Figure 2

Measurements for cockfish, *Callorhynchus callorhynchus*. (A) Standard length; (B) myxopterygia length in male.

tures: testes, epididymis (efferent ducts), and deferent ducts (Leigh-Sharpe, 1922). In sexually mature individuals, the deferent ducts are differentiated into two fusiform structures called green glands (Fig. 3A). These structures agglutinate the spermatozoa into spermatophores and secrete a gelatinous, green fluid. The reproductive system of the female is composed of the following paired structures: ovaries, oviducts, nidamental or shell glands, uteri, and vaginal openings that end in a cloaca (Fig. 3B).

Holocephalans are sexually dimorphic. Male secondary sexual structures (frontal tenaculum, and prepelvic and pelvic claspers) are utilized during mating (Fig. 4); the male apparently inserts the mass of spermatophores into the seminal receptacle of the female cloaca. Upon macroscopic examination (October), a mass of spermatophores was found at the female genital opening. Spermatozoa are liberated progressively by an unknown mechanism, before fertilization.

Females extrude fertilized eggs enclosed within a leathery case (Dean, 1906) that acts as protection for the embryo during development. At hatching the fry resembles the adult.

Indices of reproductive activity

The GI of males reached a maximum in March (3.0) and was low from July to October (range seen in Fig. 5A). The highest mean values of the GGI were found between July and October (Fig. 5B), although the index was also high during January and February. In females, the GI reached its highest values from July to October, indicating low reproductive activity during the rest of the year (Fig. 6A). The NGI (females) has two periods of activity: July to November (highest) and January to June (lowest) (Fig. 6B). The HI for both males and females (Fig. 5C, 6C) did not show significant annual fluctuations; maximum values occurred simultaneously with peaks in the other reproductive indices.

Mature and immature females could be differentiated based on GI values (range: 0.13 to 4.55); a GI value larger than 1.0 was characteristic of mature females. The presence in March of some females smaller than 50 cm SL with a GI higher than 1.0 and ovaries with translucent oocytes was suggestive of oocyte resorption. Oocyte diameter varied between 10 and 35 mm.
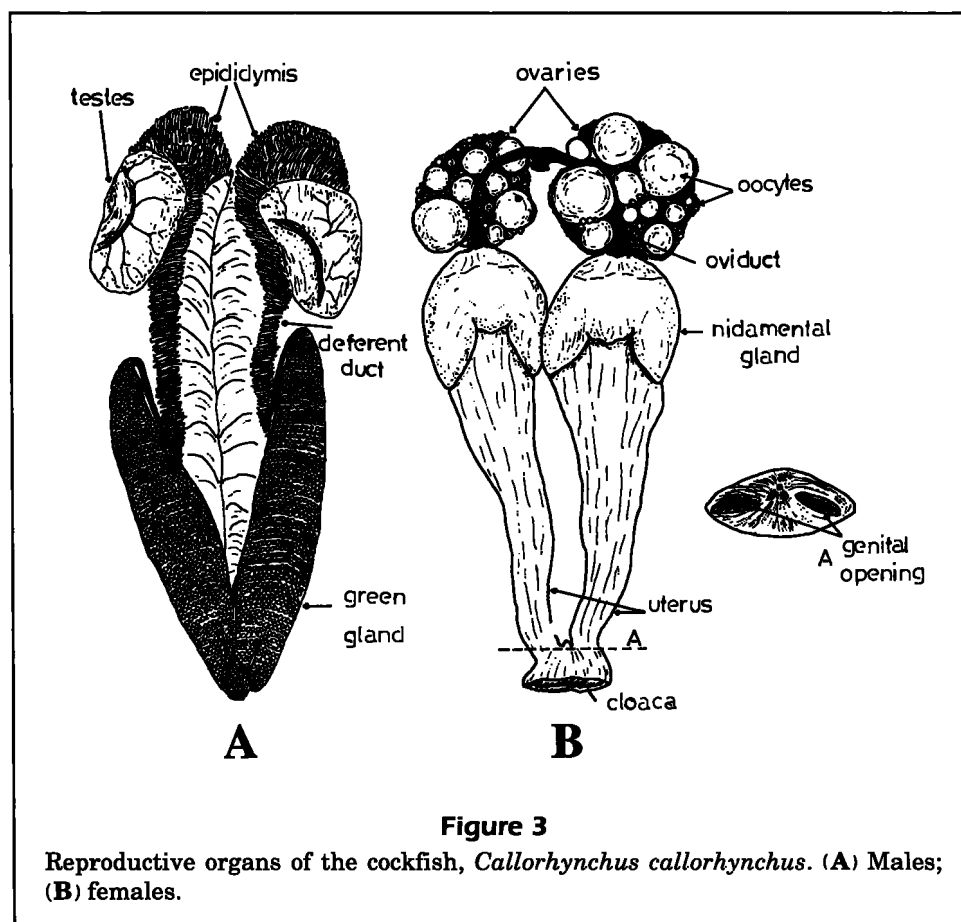

Figure 3
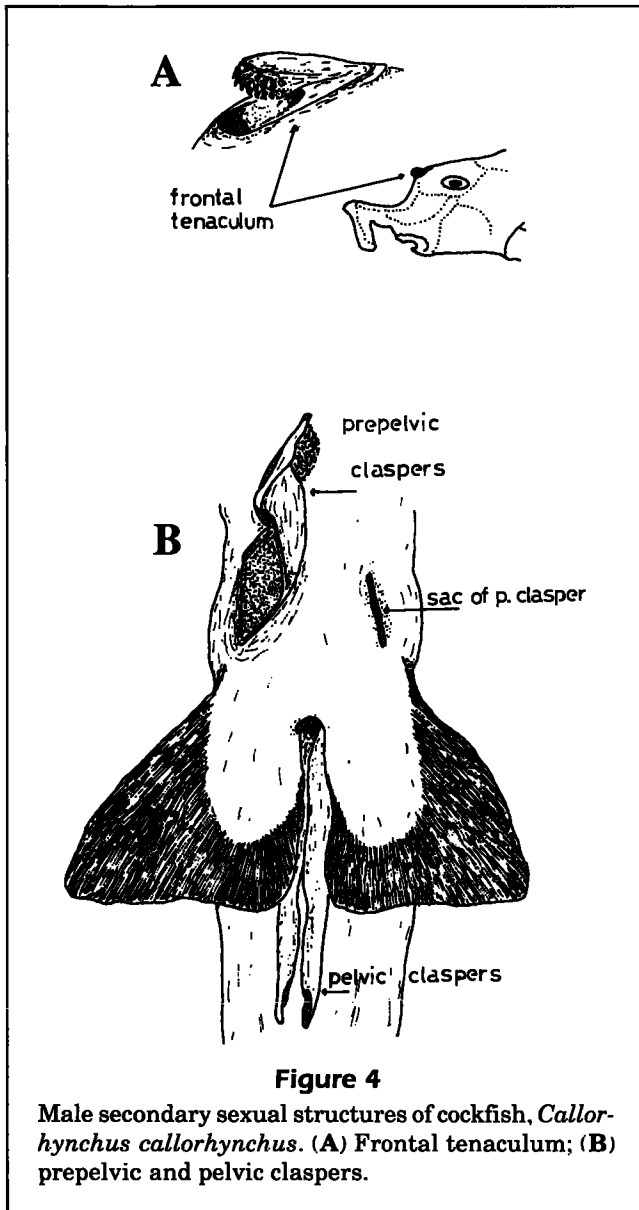Reproductive organs of the cockfish, *Callorhynchus callorhynchus*. (A) Males; (B) females.

Figure 4
Male secondary sexual structures of cockfish, *Callorhynchus callorhynchus*. (A) Frontal tenaculum; (B) prepelvic and pelvic claspers.

Gonad and green gland indices were highly variable within size classes during the period of maximum activity. There was no relationship between SL and GI (r^2=0.001; P>0.05; N=121), or between SL and GGI (r^2=0.021; P>0.05; N=101). Similarly, no relationship was found between female SL and GI (r^2=0.18; P>0.05; N=58) or NGI (r^2=0.012; P>0.05; N=48).

Size at sexual maturity

Immature and mature males could be identified from the relationship between SL and length of the myxopterygia. The myxopterygia did not exceed the posterior margin of the pelvic fin in immature individuals, whereas in mature individuals it consisted of partially calcified structures that exceeded the pos-

terior margin of the pelvic fin. The length of fish measured (N=123) ranged from 26 cm to 55 cm. Length at first maturity of males was estimated to be 40 cm, corresponding to a length of the myxopterygia of 45 mm. No individuals between juvenile and mature stages were observed. Juvenile stages were found in shallow waters (depth range 15–25 m) whereas adults were found in waters deeper than 25 m (Di Giácomo, 1992). Most mature females were larger than 50 cm (SL), whereas all females smaller than 48 cm were immature.
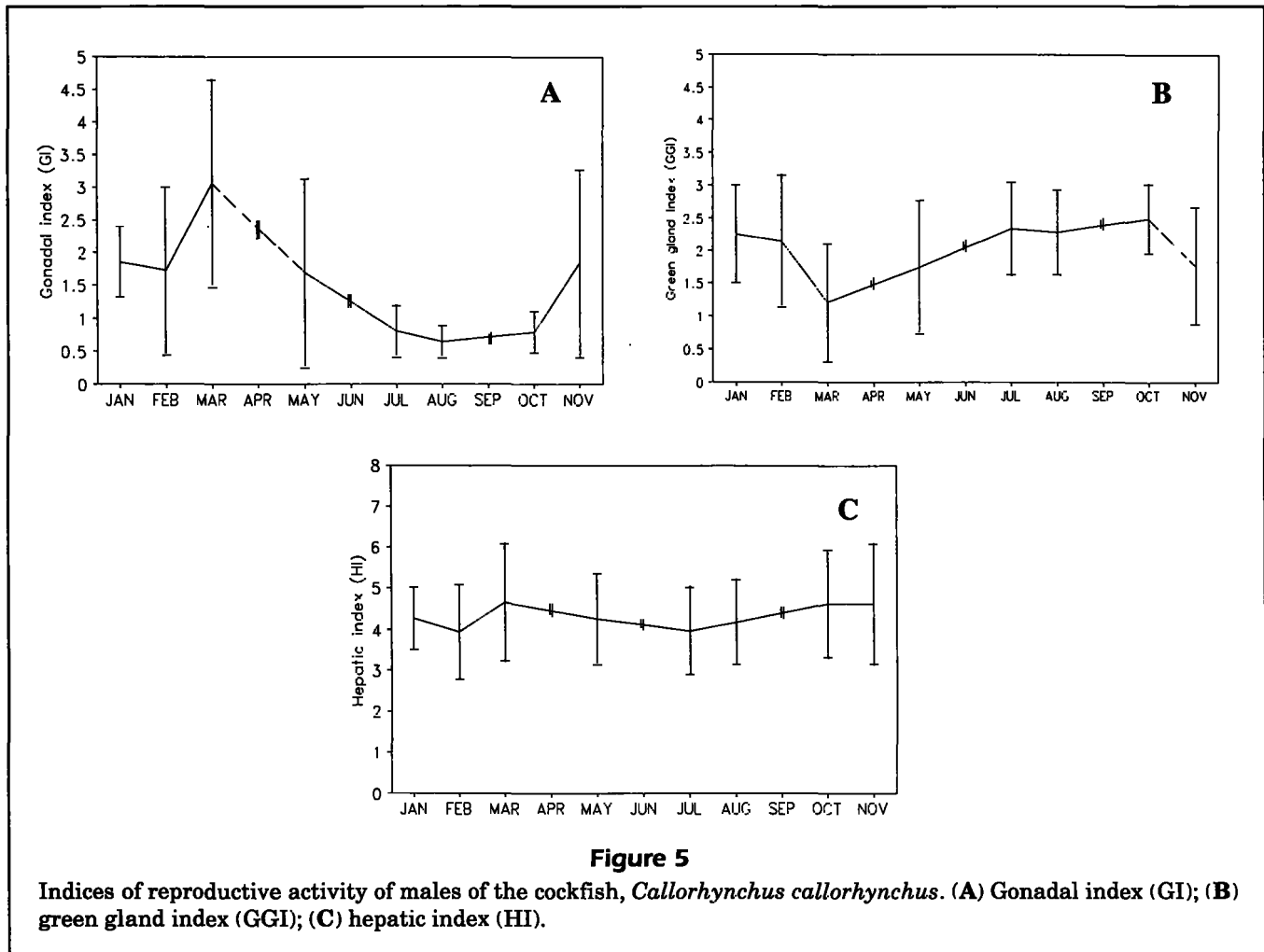
Ovarian maturation

The percentage of mature oocytes was greatest in August in both ovaries, coinciding with maxima in the indices of reproductive activity. Nevertheless, some mature oocytes were observed throughout the year (Fig. 7A). Mean number of mature oocytes was highest from July to October. There was a significant difference between the average number of mature oocytes in the right and left ovaries of individual mature females (Wilcoxon test, P<0.05; Fig. 7B).

Oocyte diameter increased with female size (r^2=0.083; P<0.01; N=90). Maximum oocyte diameter (48 mm) was recorded for a female of 71 cm SL. The regression of number of immature oocytes on SL showed a similar trend, indicating that average reproductive potential increases with size (r^2=0.024; P<0.01; N=101).

Oocytes smaller than 10 mm (diameter) were translucent to lightly whitish. Coloration of oocytes larger than 10 mm changed gradually. Yolk was incorporated until the oocytes reached complete development and passed to the nidamental gland. Production of egg cases began in the nidamental gland, when the oocytes reached maturity. The closed end of the egg case was caudally oriented; the cephalic portion remained open until the case was completely formed. When the egg case was about two-thirds of its final size, ovulation occurred and the egg (with a variable diameter that ranged from 40 to 48 mm) moved from the ovaries, through the oviduct, to the case.

Mating season

Spermatophores were found in the vaginal receptacles associated with the female cloaca. Mating occurred primarily from July to February, when maxima in the GGI were indicative of active production of spermatophores. The highest percentage of females with spermatophores in the cloaca was also observed during these months (Fig. 8). In October, recently deposited spermatophores in the female cloaca, characterized macroscopically by an intense green color, resembled green gland products. Yellowish spermatophores found in March and May (when

Figure 5

Indices of reproductive activity of males of the cockfish, *Callorhynchus callorhynchus*. (A) Gonadal index (GI); (B) green gland index (GGI); (C) hepatic index (HI).

the green gland index was low) probably remained from copulation during the previous year. After insemination, the spermatophores were apparently mobilized by the female in coordination with the production of egg cases in order to fertilize the oocytes.

Spawning season

The primary spawning season extended from July to November. The mean size of females with egg cases in formation increased from July to November (Fig. 8). Egg cases in an early stage of development were found in bottom trawl catches at 105 m, whereas females with egg cases with embryos almost fully developed were found in bottom dredge catches at depths ranging from 20 to 40 m.

Discussion

Morphology

The morphology of the reproductive system of holocephalan fishes has been described for *Chimaera*

monstrosa and *Callorhynchus antarcticus* (Leigh-Sharpe, 1922; Legendre, 1944), and *Hydrolagus colliei* (Cox, 1963). Species of living holocephalans are generally reported to lack a cloaca (Grasse, 1958; Lagler et al., 1977); Malagrino et al. (1981) mentioned the absence of a cloaca in females of *Chimaera phantasma* as characteristic of the group. This contrasts with the elasmobranchs, in which the female genital ducts terminate in the cloaca. Stanley (1963) noted the presence of a cloaca in newly hatched males and females of *H. colliei*, which is lost as they grow. *Callorhynchus callorhynchus*, by contrast, has a cloaca with a seminal receptacle where the spermatophores are stored.

Reproductive cycle

The peak of the male GI indicates the season when testicular activity is at a maximum. The delay between maxima in GGI and GI may reflect a sequence of phenomena: 1) production of spermatozoa in the testes, 2) movement of spermatozoa through the ef-

ferent ducts and the first part of the deferent ducts, and 3) accumulation in the green gland during the formation of spermatophores. Activity of the female gonads and nidamental glands is synchronous because as the oocytes mature they are ovulated and immediately encapsulated in the nidamental glands.

Unlike most elasmobranch species in which one ovary is functional and the other is vestigial (Mellinger, 1972; Menni et al., 1979; Teshima, 1981; Natanson and Cailliet, 1986; Smith and Merriner, 1986), the left and right ovaries of *C. callorhynchus* are both functional. However, the average number of ova produced in each ovary is different. As in *H. colliei*, the egg cases of *C. callorhynchus* are produced simultaneously by both of the nidamental glands (Dean, 1906).
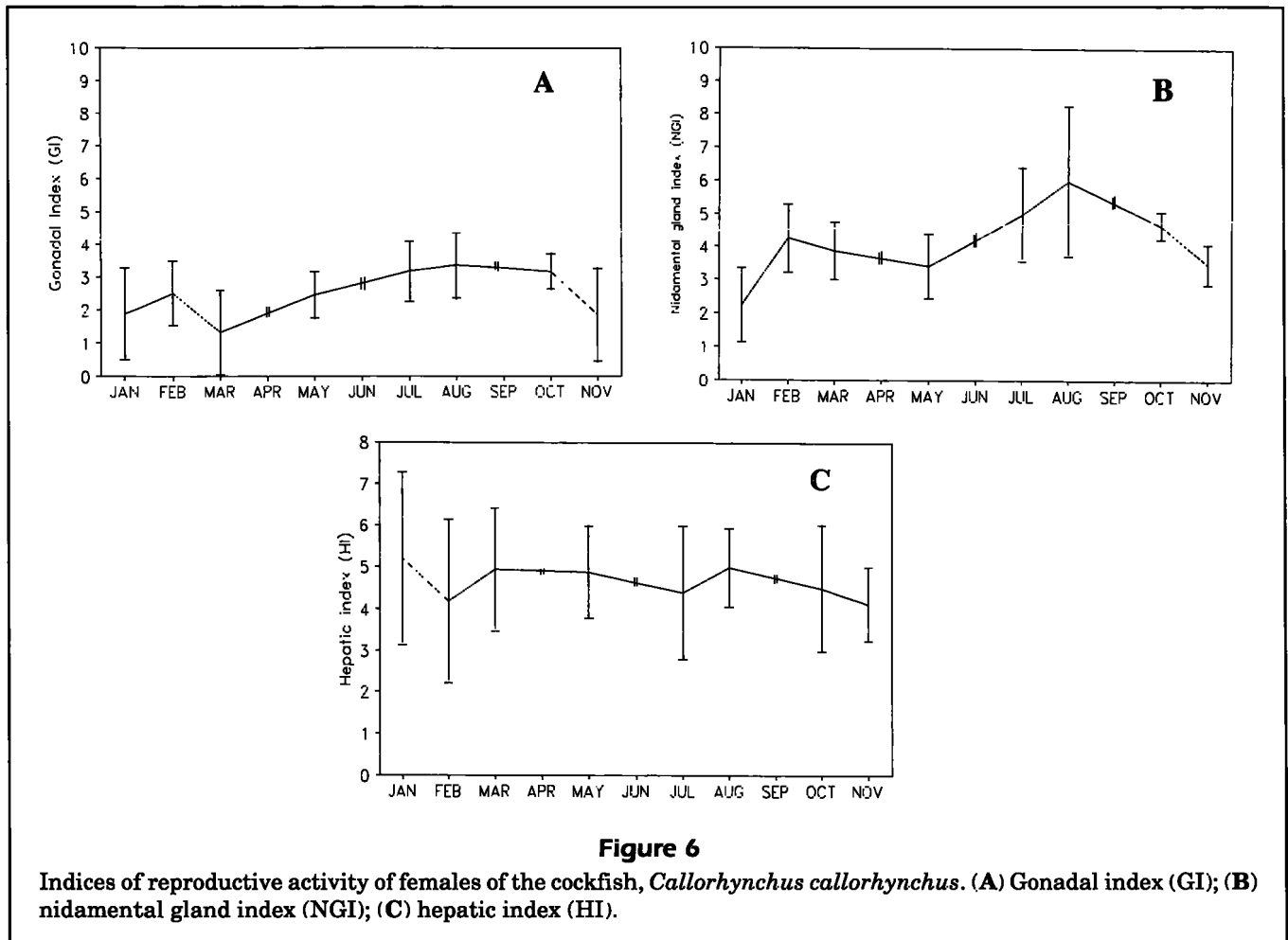
Changes taking place in the development of the gonad and the green gland of males suggest that reproductive activity is continuous, with a period of rest or recovery around March. Relatively high female index values in February can be considered a continuation of the main period of reproductive ac-

tivity. The sample of females analyzed in January was probably not representative because of their scarcity in commercial landings. Resting or recovery may last one or two months in *C. callorhynchus*, while males and females of *C. monstrosa* and *C. phantasma* have a resting period of six months (Vu Tan Tue, 1972; Malagrino et al., 1981).

Sexual maturity

A threshold value in the GI has been utilized to separate immature and mature females in some teleost species in which oocytic resorption has not been observed. In *C. callorhynchus* such a GI threshold criterion must be coupled with macroscopic observation of the ova in the ovary; in the case of low GI values, presence or absence of atrophied ova is indicative of resting or immature conditions, respectively.

Development of the myxopterygia is diagnostic of male maturity in *C. callorhynchus*, allowing the identification of the length at first maturity. Transition between stages can be related to an offshore migra-
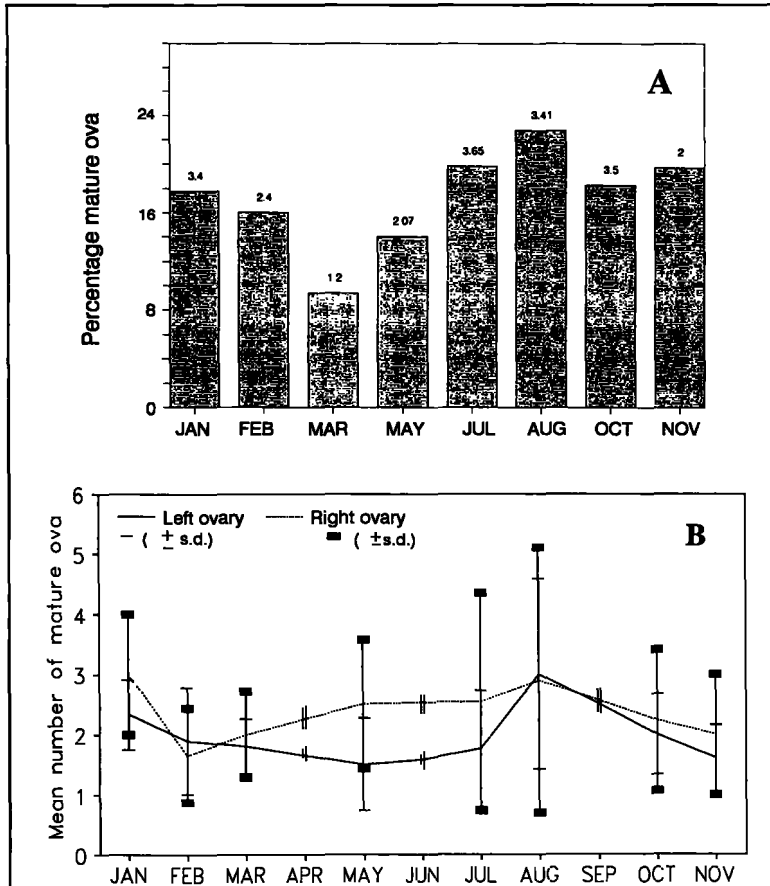

Figure 6

Indices of reproductive activity of females of the cockfish, *Callorhynchus callorhynchus*. (A) Gonadal index (GI); (B) nidamental gland index (NGI); (C) hepatic index (HI).

Figure 7

Females of the cockfish, *Callorhynchus callorhynchus*. (A) Percentage and average number (numbers above bars) of mature ova by month; (B) mean number of mature ova per ovary by month.
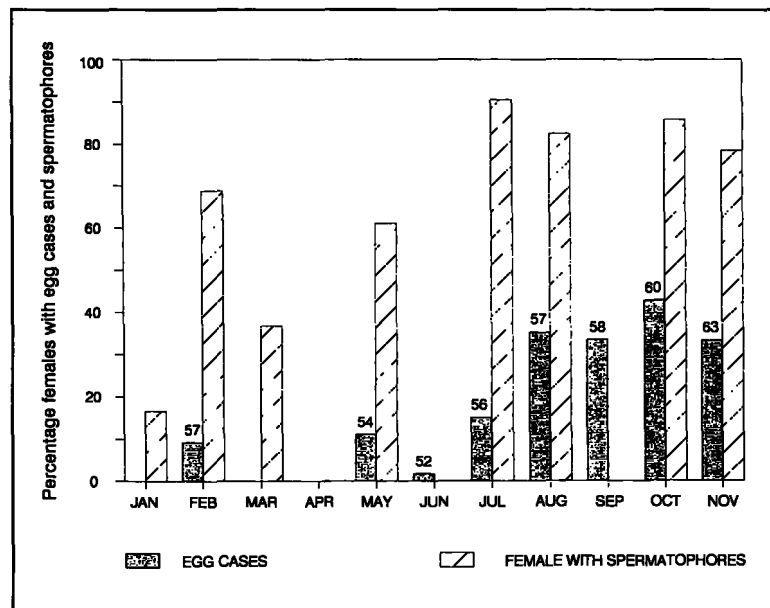

tion. The absence of intermediate stages in our materials can be explained by the fact that sampling was conducted mostly between 90 and 130 meters, where adults prevail (Di Giácomo, 1992).

Mature females cannot be clearly defined. Holden (1975) defined mature females of the ray *Raja clavata* as those individuals that had large, yellow oocytes in the ovaries. Smith and Merriner (1986) distinguished three stages of sexual maturity in females of the cownose ray, *Rhinoptera bonasus*: immature, maturing, and mature. The mature stage was characterized by the presence of large, yellow oocytes greater than one cm in diameter in the ovaries. The authors accounted for the minimum length of gravid females observed (*R. bonasus* is viviparous). We could not identify which oocytes were ready to be spawned in *C. callorhynchus*. Although all yellow oocytes were viable, resorption may lead to false conclusions regarding spawning state. Therefore, the minimum size fish that had egg cases in formation in the nidamental gland was considered diagnostic, since at that stage resorption is no longer possible. The smallest female with egg cases in formation measured 50 cm (SL), and the smallest female with mature oocytes was 48 cm SL. In females size at first maturity was coincident with the minimum size of fish showing yolked oocytes and egg cases in formation.

McEachran (1970) found that in females of the ray *Raja garmani* the oocytes in the ovaries were half the size of those found in the egg cases. In *C. callorhynchus*, the difference between the maximum size attained by the oocytes in the ovaries and the eggs in the case was about 25%. Assuming a similar speed of yolk incorporation into the oocytes for both species, we estimate that the females of *C. callorhynchus* may have a higher spawning rate than the ray, indicating that maturing oocytes may attain ovulation size faster. The maximum size of mature ova was larger in *C. callorhynchus* than in other holocephalan species such as *Chimaera phantasma* (Malagrino et al., 1981), *C. monstrosa*

Figure 8

Percentage of spermatophores and developing egg cases in females of the cockfish, *Callorhynchus callorhynchus*, by month.

(Legendre, 1944), *Hydrolagus colliei* (Dean, 1906), and *Callorhynchus milii* (Gorman, 1963).

Spermatophores

The presence of a "seminal plug" has been reported in *C. milii* (Norman, 1937). Males of *C. monstrosa* form an emerald green substance in the terminal portion of the deferent ducts, but seminal plugs in females have not been reported (Legendre, 1944). There is no seminal plug in *H. colliei*, in which copulation occurs just before eggs are laid (Dean, 1906).

On the basis of our examination of *C. callorhynchus*, we suggest that the term "plug" is improper. It is a seminal mass located in the female's seminal receptacle that does not obstruct the genital ducts and remains in the receptacle during the entire reproductive cycle. The coloration of the spermatophores in the cloaca changes progressively from an intense green to yellow as the spermatophores are mobilized.

Spawning ground

Although we demonstrated seasonality in the mating activity of *C. callorhynchus*, it was not possible to establish where spawning takes place. Quinn et al. (1980) suggested that adults of *H. colliei* migrate to shallow waters during the night. Eighty-five egg cases of *H. colliei* were found at a depth of 11 m, but one capsule was found at 120 m (Dean, 1906). *Callorhynchus monstrosa* spawns at about 100 m (Legendre, 1944); based on its bathymetric distribution (100 to 1700 m), it can be assumed that this species also migrates to the relatively shallow areas to spawn. Females of *C. milii* lay eggs between the surf zone and 37 m, in sand or mud substrata, and move to deeper water after spawning (Gorman, 1963).

Callorhynchus callorhynchus was caught at depths shallower than 170 m in the San Matías Gulf (Di Giácomo, 1992). Egg cases were found between 20–40 m and at 104 m, suggesting that this species may also migrate to shallow water to spawn. Further studies are required to document more conclusively the spawning behavior of females.

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