

## THE BIOLOGY OF *FISSURELLA MAXIMA* SOWERBY (MOLLUSCA: ARCHAEOGASTROPODA) IN NORTHERN CHILE. 2. NOTES ON ITS REPRODUCTION

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### ABSTRACT

For 14 months, monthly samples were collected to study reproduction in *Fissurella maxima* at Huayquique. Results indicate that *F. maxima* is a dioecious species; no sign of hermaphroditism has been observed. The sex ratio is 1:1 in the different size classes analyzed. Ovaries are green and testis are median brown to yellowish white. Eggs in the ovary measure from 120–280  $\mu$  without envelopes. The gonads are parasitized by adult digenea trematods of the genus *Proctoeces*. Some effects of parasitism are discussed.

Variations in mean monthly gonadosomatic index suggest that there is a main spawning period in late November–December (late spring-early summer) and a secondary period in July–August (winter). Fluctuations in mean gonad index show a close correlation with sea water temperature variations.

The youngest mature specimens detected were about 5 cm in shell length (over two years old), but the majority of mature animals were over 6.5 cm.

### INTRODUCTION

*Fissurella maxima* Sowerby, 1835, is the most conspicuous of the Chilean *Fissurella* species, reaching sizes of about 12 cm in shell length at the Iquique region (20°14'S, 70°10'W) and 14 cm at Los Vilos (31°55'S, 71°32'W). It lives throughout the low intertidal and high subtidal levels, under *Lessonia* sp. leaves on exposed rocky shores. *F. maxima* is a species with a life span of about 7–10 years (Bretos, 1982), and like *F. crassa* it seems to form two shell growth rings per year (Bretos, 1980). Typical commercial sizes vary from 60–85 mm in shell length at Iquique; these animals are usually between 2 and 4 years old.

Although keyhole limpets of the genus *Fissurella* are abundant on Chilean coasts, little information is available on their biology and there appears to be no published studies on their reproduction. Some data has been found on reproduction of *Fissurella* from other regions. The breeding cycle of a small sized Caribbean species, *F. barbadensis* Gmelin has been described by Ward (1966). This research was based on collections, made at bimonthly intervals, analyzed by using histological study of the gonads. Two principal spawning periods were recorded: from September to November and from March to June. Spawning specimens were present in all but two samples throughout the collecting period (early January and early April). The results of this study indicate that there is no resting phase in *F. barbadensis* along the coasts of Barbados.

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Concerning European species, Boutan (1885) reported that *F. reticulata* spawns from May to early July at Port Vendres. In *F. (Cremides) nubecula* (L.) spawning occurs in May at Naples Port (Bacci, 1947).

The present study was undertaken as the first step in the analysis of reproduction of *F. maxima* in Northern Chile.

## MATERIALS AND METHODS

*F. maxima* samples were collected at Huayquique (20°17'S, 70°08'W), in northern Chile. Sampling took place at approximately monthly intervals, from July 1979 to August 1980. The animals were collected by diving in shallow waters, from 0–2 m below low-water mark, and intertidally. The sampling area was a rocky shore, open coast habitat.

In the laboratory, each animal was weighed (wet weight) and removed from its shell. Wet weight of gonad and soft parts were also determined by using a digital Sauter balance to the nearest 0.1 g. Shell length was measured by using vernier calipers to the nearest 0.1 mm. Sampling covered the available size range. Size of the specimens was not selected in order to determine the size at which *F. maxima* attains first sexual maturity.

The sex of the animal was determined when the gonad was exposed by gross dissection. Gonads were observed under a stereo-microscope and notes were made on their appearance. Egg diameters in the ovary were measured by using micrometric eye lens.

Data were grouped in size classes of 5 or 10 mm. The general reproductive condition of each sample was assessed by calculating the gonadosomatic index (GSI). This was calculated by expressing the ratio of gonad wet weight to total wet body weight as a percentage. Sexually undetermined animals were numerous in size classes up to 60 mm in shell length. For this reason, data were analyzed mainly in animals whose shell length was greater than 60.0 mm. Separate monthly GSI means were calculated for each sex in animals over 60.0 mm in shell length.

Sexual maturity of each animal was estimated by considering its GSI, and the size and appearance of the gonad. Sexual maturity of animals in each sample was estimated.

Trematods were present in the gonadas of *F. maxima*. The percentage of infection was analyzed in sexually undetermined specimens.

Variations in monthly GSI means were related to sea surface temperature. It was measured daily at 9:00 hours at the sampling locality.

## RESULTS

A total of 1602 animals were examined whose sizes ranged from 21.5 to 98.6 mm in shell length (Table I). Only 24 animals were captured in May 1980 because of strong seas.

### *The gonads*

*F. maxima* is a dioecious species; no hermaphrodites were detected among the animals studied. The sexes cannot be distinguished externally.

Animals classified as sexually undetermined had inconspicuous or no discernible gonads, whitish or transparent, sometimes pinkish colored. The pinkish color was due to parasites in the gonad. These parasites were identified as adult specimens of the digenetic trematods *Proctoeces* Odhner, 1911 (Bretos and Jirón, 1980). Many



TABLE I

*Material of F. maxima collected at Huayquique*

Date	Total N	Sexed animals				Sexually undet. animals
		Females		Males		
		N	%	N	%	
4-7-79	136	69	52.3	63	47.7	4
27-8-79	139	72	57.1	54	42.9	13
25-9-79	150	61	44.9	75	55.1	14
22-10-79	137	50	46.3	58	53.7	29
19-11-79	130	35	44.3	44	55.7	51
20-12-79	116	34	44.7	42	55.3	40
2-1-80	105	21	39.6	32	60.4	52
11-2-80	70	30	46.9	34	53.1	6
10-3-80	78	38	50.0	38	50.0	2
14-4-80	138	60	44.8	74	55.2	4
29-5-80	24	13	54.2	11	45.8	0
9-6-80	105	48	48.0	52	52.0	5
29-7-80	148	72	51.4	68	48.6	8
26-8-80	126	68	57.6	50	42.4	8
Total	1.602	671		695		236

Percentages of females and males are given for sexed animals.

young specimens of sexually undetermined *F. maxima* (73.7%) had as many as 17 adult trematods in their gonads (Table II).

The gonad is single. When developed or mature, the female gonad is green and the male gonad varies from median brown to yellowish white.

In young specimens the small gonad is found next to the digestive gland; its weight was under 0.1 g. The smallest female with a detectable gonad was 27.3 mm

TABLE II

*Numbers of sexually undetermined specimens of F. maxima from Huayquique, and quantity of parasites in their gonads*

Shell length (mm)	Infected specimens	Intensity		Not infected specimens	Total specimens examined
		Mean $\pm$ SD	Range		
20.1-25.0	1	2	2	0	1
25.1-30.0	1	1	1	0	1
30.1-35.0	1	2	2	4	5
35.1-40.0	8	4.0 $\pm$ 3.0	1-10	10	18
40.1-45.0	25	3.6 $\pm$ 2.4	1-9	10	35
45.1-50.0	45	4.4 $\pm$ 2.9	1-17	17	62
50.1-55.0	63	4.1 $\pm$ 2.2	1-11	17	80
55.1-60.0	25	4.9 $\pm$ 3.4	1-16	2	27
60.1-65.0	3	5.0 $\pm$ 2.7	3-8	1	4
65.1-70.0	2	4.5	4-5	0	2
70.1-75.0	0	—	—	1	1
Total	174			62	236
%	73.73			26.27	100.00



long, the smallest male 30.7 mm. Both sizes correspond to one-year-old animals (Bretos, 1982).

As the gonad grows, it remains attached to the digestive gland by the connective tissue envelope sheet. When the gonad is separated from the digestive gland by dissection, the gonad sac opens. Both gonads consist of a sac with a large lumen. Trabeculae occur within the gonads. At mature stage, the gonads are filled with the gametes. Eggs found in the lumen of the ovary measured from 120–280  $\mu$  in diameter without envelopes. At least two sheets of a gelatinous matrix have been detected around the eggs at observation under the microscope.

The mature gonad can attain a wet weight of 30.6 g in females and 17.4 g in the reproductive season (November). The gonad is turgent and gametes can easily emerge when the gonad is dissected. The sex cells are discharged into the sea water through the right nephridiopore. It has been observed in males placed in aquaria, that the sperms are liberated through the apical hole as a white jet.

### Sex ratio

Sex ratio was calculated as a percentage in size classes without considering sexually undetermined individuals (Fig. 1). Sexes were similarly represented in classes over 60 mm. Among sexed animals, 49.12% corresponds to females and 50.88% to males (Table III).

Sexually undetermined specimens are abundant in size classes up to 60 mm in shell length (two year old animals). Among 236 undetermined animals examined, only 7 were longer than 60.0 mm (Table II).

Most of the sexually undetermined animals occurred in samples from October to January (Table I). The lowest mean shell lengths (Table IV) were observed during this period because many small animals were found in these months.

### Estimated maturity

Some gonad characteristics and the gonadosomatic index were used as criteria to classify sexed animals as mature or immature.

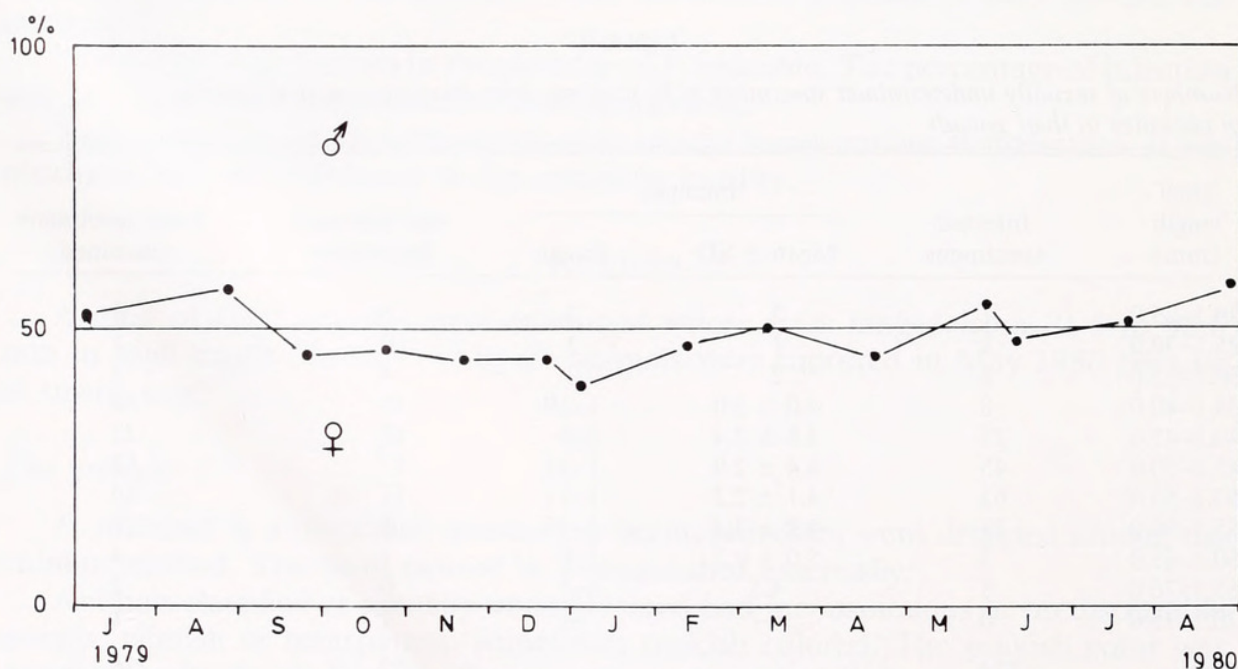


FIGURE 1. Sex ratio in *F. maxima* from Huayquique.



TABLE III

*Sex distribution of F. maxima in size classes from all samples*

Shell length (mm)	Sexed animals				Females + males	Sexually undet.	Total
	Females		Males				
	N	%	N	%			
20.1–30.0	1	—	0	—	1	2	3
30.1–40.0	1	—	2	—	3	23	26
40.1–50.0	25	48.1	27	51.9	52	97	149
50.1–60.0	110	50.9	106	49.1	216	107	323
60.1–70.0	177	48.9	185	51.1	362	6	368
70.1–80.0	228	48.7	240	51.3	468	1	469
80.1–90.0	114	48.5	121	51.5	235	0	235
90.1–100.0	15	51.7	14	48.3	29	0	29
Total	671		695		1366	236	1602
% in total		49.1		50.9	100		

Two aspects were studied in male gonads: the color variation and the relative abundance of ripe spermatozoa. An attempt was made to find a correlation between the color and the maturity stage in male gonads. Testis with high GSI were creamy or light olive green, but there was no clear color graduation nor a constant relationship between the color and the GSI. In addition, male gonads were classified as milky, semi-milky, or not-milky, according to the quantity of semen observed among the testis trabeculae after dissection. Milky and semi-milky testis were usually present in specimens with high or medium GSI values.

No color differences were observed in female gonads of animals with different GSI values. Only very small ovaries had a lighter green color. Female specimens with

TABLE IV

*Size of F. maxima*

Samples	N	Shell length (mm)	
		Mean $\pm$ S.D.	Range
Jul 79	136	74.9 $\pm$ 9.2	35.2–96.5
Aug 79	139	74.2 $\pm$ 13.1	35.5–98.6
Sep 79	150	72.7 $\pm$ 13.8	27.3–96.1
Oct 79	137	61.7 $\pm$ 10.7	34.3–86.3
Nov 79	130	60.9 $\pm$ 13.0	36.8–90.3
Dec 79	116	61.2 $\pm$ 9.4	41.4–89.9
Jan 80	105	56.3 $\pm$ 11.1	21.5–87.2
Feb 80	70	63.0 $\pm$ 11.6	30.7–95.7
Mar 80	78	72.5 $\pm$ 12.7	37.2–94.2
Apr 80	138	68.3 $\pm$ 11.2	37.4–98.5
May 80	24	78.7 $\pm$ 7.8	61.5–92.9
Jun 80	107	67.0 $\pm$ 12.5	25.9–97.9
Jul 80	148	66.0 $\pm$ 11.3	39.5–90.9
Aug 80	126	67.0 $\pm$ 12.2	32.2–91.6

Collected at Huayquique. N = number of specimens. S.D. = standard deviation.



high or medium GSI values had ovaries of friable consistency and in which eggs detached easily from the trabeculae at the time of dissection.

Assuming that an increase in GSI may be interpreted as a buildup of gametogenic cells and gametes, while a decrease indicates spawning, GSI was used to estimate the reproductive activity in both sexes in the present study.

After analyzing the data obtained, we concluded that the GSI was the most reliable method for classifying *F. maxima* animals as "mature" or "immature".

Animals with spent and recovering gonads were grouped together as "immature" specimens. Only fully mature animals, with high GSI were considered "mature".

Sexual maturity estimations are summarized in Table V. The highest number of mature animals was detected in late July, 1980. Many mature specimens were also found in October and November, 1979. Mature animals were present throughout the year, although they were scarce in some months (Table V).

### *The onset of sexual maturity*

The size at which *F. maxima* may first spawn is considered as the minimum size at which estimated mature animals have been found.

The youngest mature female detected measured 49.8 mm and the youngest mature male 47.7 mm in shell length (1.5-year-old animals). Nevertheless, numerous mature specimens were usually observed in size classes over 65 mm (animals two or more years old) (Bretos, 1982).

The highest GSI, meaning fully developed gonads, were detected in animals ranging from 70 to 90 mm in shell length, with GSI values from 21.3 (males) to 32.2 (females).

### *Spawning*

Mean GSI were calculated separately per month for females and males over 60.0 mm shell length (Fig. 3). Mean GSI values exhibit the same tendencies in both sexes although the highest values were observed in females in November.

TABLE V

*Estimated maturity of F. maxima over 60.0 mm in shell length, at Huayquique.*

Date	Females			Males		
	Mature	Immature	Total	Mature	Immature	Total
Jul 79	13	52	65	27	34	61
Aug 79	25	39	64	25	27	52
Sep 79	1	54	55	13	52	65
Oct 79	13*	13	26	23*	22	45
Nov 79	14*	8	22	19*	14	33
Dec 79	1	27	28	3	26	29
Jan 80	0	16	16	1	21	22
Feb 80	6	16	22	8	15	23
Mar 80	10	24	34	5	27	32
Apr 80	21	32	53	28	33	61
May 80	0	13	13	2	9	11
Jun 80	6	29	35	21*	18	39
Jul 80	39*	12	51	40*	8	48
Aug 80	17	33	50	23*	16	39

\* Mature animals are equal or more numerous than immature animals.



Two noticeable peaks appeared in November, 1979 and late July, 1980. The peak of early July, 1979 is less conspicuous.

The lowest mean GSI value was observed in early January; only one male was estimated as sexually mature in this sample (Table V). These facts strongly support the idea that a massive spawning period is complete in December. Mean GSI also decreased in August-September suggesting the occurrence of a winter spawning. Mean GSI also decreased in May, but the sample obtained included few animals, indicating that it may not be a representative sample of the population.

According to the GSI fluctuations, we assume that there are two spawning seasons per year in the *F. maxima* population under study: a main reproductive period occurring in late spring-early summer (November-December) and a secondary period occurring in winter (July-August).

A close relationship appears to exist between the GSI cycle and the sea temperature cycle (Fig. 2).

Surface sea water temperature at Huayquique exhibits two rises each year (see Fig. 2, and Bretos, 1978). A little peak is found in winter; temperatures may reach more than 17°C. The long and conspicuous rise of temperature begins in October-November, *i.e.*, in spring, and its highest values are observed in January-February (summer). Sea water temperatures are particularly high, up to 25°C, in years in which El Niño current descends to northern Chile from Peru, as observed in the summer of 1977 (Bretos, 1978).

#### DISCUSSION

According to Bacci (1947) there is "a certain degree of hermaphroditism" in *F. nubecula* from the Gulf of Naples, detectable by statistical methods. This means that sex reversal of the protandric type would occur in about 12% in this species of limpet. On the contrary, other papers concerning the anatomy of reproductive organs in *Fissurella* have described normal ovaries and testis and no signs of hermaphroditism (Boutan, 1885; Ziegenhorn and Thiem, 1925). Ward (1966) reported that there was no indication of hermaphroditism nor of change of sex at any shell length in *F. barbadensis*. The results of the present study indicate that *F. maxima* is a dioecious species in which sex reversal has not been detected. No significant differences were found in size classes over 60 mm in shell length (Table III). Personal unpublished observations on the gonads of the eight other *Fissurella* species from northern Chile (Bretos, 1976) supports this.

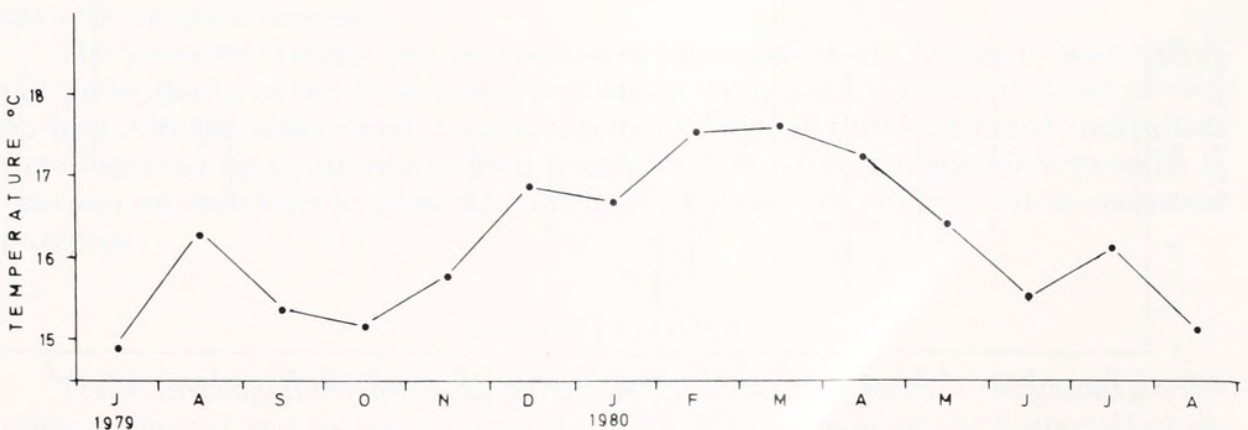


FIGURE 2. Mean sea surface temperatures at Huayquique.



Not all of the individuals of the same species or population develop their gonads at the same time, age, or shell length, since growth is variable from one animal to another, and growth rate depends partly on endogenous factors (Wilbur and Owen, 1964; Bretos, 1978). Nevertheless, it is surprising to find sexually undetermined *F. maxima* animals measuring as much as 72 mm in shell length (Table II). These are juveniles in which development of the gonad has not yet, or only partially, begun. Considering that it is possible to identify clearly ovaries and testis in small *F. maxima* individuals (27.3 mm and 30.7 mm in shell length respectively), it may be assumed that exogenous factors could be acting to retard gonad development. The main exogenous factor is the high incidence of trematod parasites in the gonad, attaining an infection rate of up to 73.73% in sexually undetermined animals (Table II). Gastropods

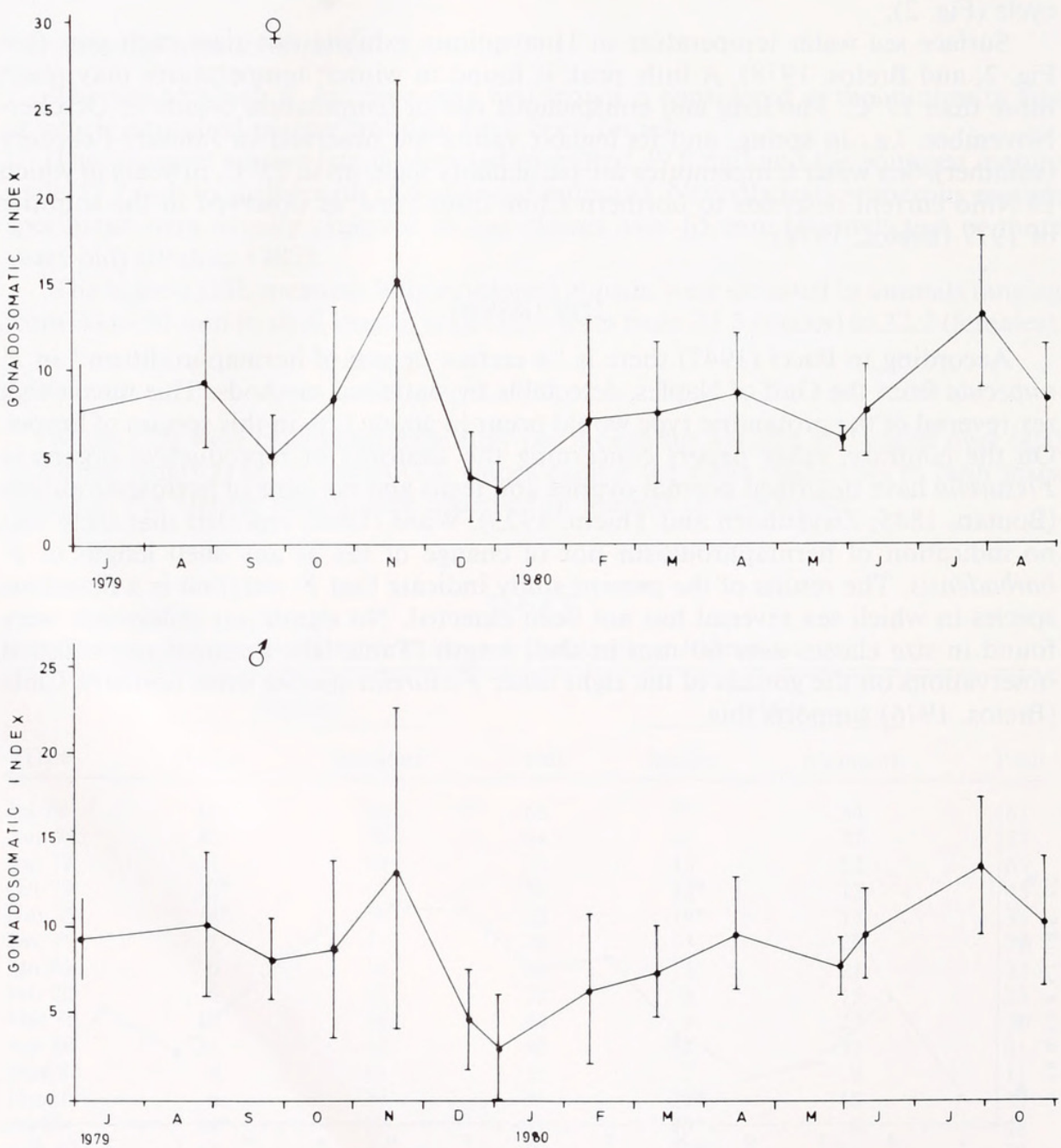


FIGURE 3. Monthly changes in gonadosomatic index in females and males. Mean values and standard deviation.



are often part of parasitic life cycles, particularly those of digenetic trematodes. The gametogenic activity of the mollusc is either curtailed or even completely suppressed by parasitism (Webber, 1977), or the invaded gonad may be destroyed (McArthur and Featherston, 1976), causing parasitic castration. It should be noted that the gonads or other organs of molluscs are usually invaded by trematode cercaria, *i.e.*, by trematode larval forms. In *F. maxima* the digenetic trematodes that parasitize the gonads are adult specimens (Bretos and Jirón, 1980), not larval stages which may cause considerable damage. Gametogenetic activity does not seem to be suppressed in *F. maxima* nor is the gonad destroyed by adult *Proctoeces* trematods.

*F. maxima* eggs are much larger (0.12–0.28 mm without envelopes) than those of *F. reticulata* that measure 0.1 mm (Boutan, 1885) or those of *F. barbadensis* [0.08–0.18 mm including the gelatinous coat (Ward, 1966)]. This may be related to the size that each species attains: *F. barbadensis* can measure up to 3.3 cm in shell length and *F. reticulata* is also small, but *F. maxima* can reach a shell length of 12 or more centimeters.

The data obtained suggest that reproduction occurs rhythmically in the *F. maxima* study population, showing a semiannual pattern of breeding. According to the GSI calculated, two spawning periods per year were detected: one in winter and the other in late spring-early summer (Fig. 3). A similar reproductive pattern is found in *F. barbadensis* (Ward, 1966), which has two breeding seasons.

Semiannual breeding seasons occur in a number of tropical and temperate species. One of the major environmental parameters affecting or influencing the reproductive state of a population is temperature. Seasonally changing sea temperatures may influence reproductive activities and may serve to promote and synchronize spawning (Webber, 1977). Mean sea surface temperature shows a bimodal cycle at Huayquique (Fig. 2), presenting the main peak in summer and a little one in winter. There appears to be a close correlation between sea temperature fluctuations and *F. maxima* gonadosomatic index variations (Fig. 3), thus, its reproductive activity. *F. maxima* seems to be one of the mollusc species whose spawning is influenced by sea temperature changes.

*F. gibba* individuals at Banyuls, and *F. reticulata* individuals at Port-Vendres seemed to be more numerous during the breeding season (Boutan, 1885). The samples of *F. maxima* obtained in winter and summer seem to be more numerous (Table I), but this may be a coincidence. Nevertheless, more females than males were detected in July-August (winter months). This period coincides with the secondary spawning season of *F. maxima* at Huayquique. During the main spawning period (November-December) however, males were more numerous than females (Table I), but there was also a large number of sexually undetermined specimens which altered the real sex ratio in these samples.

The youngest sexually mature specimens measured about 50 mm in shell length, but the majority of the *F. maxima* population were mature at shell lengths of over 65 mm. On the other hand, a good number of sexually undetermined individuals were observed up to 60 mm in shell length. It is therefore not advisable to catch *F. maxima* animals smaller than 65 mm in shell length for commercial or industrial purposes.

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