BIOLOGICAL STUDIES ON THE CHILEAN SEA-URCHIN LOXECHINUS ALBUS (MOLINA) (ECHINODERMATA; ECHINOIDEA) IV.- MATURATION CYCLE AND SEASONAL BIOCHEMICAL CHANGES IN THE GONAD

by

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ABSTRACT.

In the south Pacific in the Province of Valparaiso (Chile) the sea-urchin <u>Loxechinus</u> albus spawns in spring time when the sea water temperature is relatively stable. During the maturation process, the nitrogen and the water content of the gonads follow the decrease of the gonad index, while the ether extract increases.

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SUMMARY.

In one reproductive cycle of Loxechinus albus in Central Chile it is possible to recognize animals in spawning condition almost the year round. However, the main spawning season is a very restricted period beginning in August in late winter and lasting through to October in spring. During gamete release the protein and the water content of the gonad decreases; these compounds are possibly replaced by the ether extract. To delimit the spawning period, the developmental period of the larvae has also been taken into account, and this is the phase in which they reach metamorphosis which occurs when coastal water temperature are at their most stable. After the spawning season there is a period when some sea-urchins are still in spawning condition. This period is in reality a continuation of maturation which is interrupted by the onset of winter conditions. The accumulated energy is essentially employed to avoid starvation. The reproductive cycle is not a fixed process, and varies both geographically and from one breeding season to another.

RESUMEN.

En un ciclo reproductivo de Loxechinus albus en Chile Central es posible identificar animales en condiciones de desovar durante casi todo el año. Sin embargo el dosove propiamente tal es un período restringido que se extiende desde agosto hasta octubre. Durante la polución de los gametos las proteinas y el contenido de agua de las gónadas disminuye, compuestos que posiblemente son reemplazados por el extracto etéreo. Para delimitar el período de desove, también se consideró la fase en que las larvas de esta especie pueden alcanzar la metamorfosis, lo cuál sucede cuando las temperaturas del agua en el área son las más estables del año. Transcurrido el período de desove aun es posible encontrar animales en estas condiciones. Este es en realidad un intento de continuar la maduración de las gónadas, interrumpida por las condiciones ambientales del Invierno. La energía acumulada es empleada esencialmente para evitar la inanición. El ciclo reproductivo no es un proceso fijo, varía geográficamente y de un ciclo anual a otro.

INTRODUCTION.

In several regions of the world the sea-urchins are utilized as a human food resource: Tripneustes esculentus in Barbados constitutes an important fishery (Lewis, 1958); similarly many species of the genera Strongylocentrotidae are of commercial importance in Japan (Fuji, 1967). Edible sea-urchins have also been fished in the Mediterranean (Harvey, 1956). Loxechinus albus a species of seaurchin indigenous to coastal waters from Peru to the southern rocky shores of the Magellan strait (Larrain, 1975), has been consumed as food since the time of the earliest settlements along the Pacific Because of the high demand in the market for gonads of this coast. species, considered as a delicacy (Schwabe, 1936; Arrau, 1958), the commercial exploitation of the animals has virtually produced extinction of the natural populations along the north and central coasts of Chile. Since the L. albus fishery is the only income of large groups of fishermen there is now considerable risk of its depletion in the south of Chile. The laboratory of Aquaculture, Department of Oceanology, University of Chile in Valparaiso was granted support to carry out a research project on the biology of this species. This project was directed towards gathering basic information needed for the development of artificial culture methods.

Previous papers (Bückle et al., 1976) have described the results of mass culture of larvae and feeding experiments with animals reared in captivity (Bückle et al ., 1977b). In the present study the reproductive cycle, and its correlation with the seasonal biochemical changes in the gonad, are investigated. A similar approach to understanding the reproductive cycle of sea-urchins has been carried out in other species such as <u>Echinus</u> esculentus (Stott, 1931), <u>Strongylocentrotus</u> purpuratus and <u>S</u>. <u>franciscanus</u> (Bennet and Giese, 1955; Greenfield, et al. 1959), and <u>S</u>. intermedius (Fuji, 1967) among others.

MATERIALS AND METHODS.

A natural population of <u>Loxechinus albus</u> in the Province of Valparaiso was sampled bi-weekly from May 1968 till October 1970. Animals from two to fifteen metres depth were collected by diving along a five kilometre stretch of exposed coastline. In all, over 100 individuals were taken each month, with the exception of July 1968 and May 1969 when heavy seas hampered sampling. In the following years smaller samples were used to corroborate the maturation process and other biological observations.

From each individual were recorded: a) the total wet weight measured with a Sartorius balance to the nearest gram, b) the height and diameter of the test, measured with a sliding caliper to the nearest millimetre, c) the volume of the perivisceral fluid (after removal of the Aristotle's lantern) together with the colour of the fluid, classified according to a colour dictionary (Maesz and Paul, 1950), d) gonad total wet weight, e) the length and weight of the largest and smallest gonads, f) the colour of the gonads, g) the digestive tract contents which were preserved in indiluted formalin for later analysis as well as h) the ocurrence of the comensal crab <u>Pinnaxodes chilensis</u> which are usually found in the distal region of the digestive tract and i) samples of the spines of the sea-urchin.

The reproductive cycle was observed through both the gonad index and a histological analysis of gonad tissue. The monthly variation of the gonad index was calculated as the ratio of the wet gonad weight (g) to the diameter³ x 1000. For histological analysis, samples of the gonads of each sea-urchin were fixed in Bouin's fluid and in 10% formalin. From the gonads fixed in Bouin's fluid a random sample of 500 were embedded in paraffin wax, sectioned at 5 μ thickness (from the middle of the gonad), and stained with haematoxylin and eosin. All the gonads fixed in formalin (1164) were investigated by cryoscopic technique and stained with Delafield's haematoxylin. The detailed analysis of the gametogenic cycle of <u>L</u>. <u>albus</u> will be presented in a future paper.

For a period of one year, starting in November 1968, the content of lipids and other dissolved chemical compounds (Greenfield, <u>et.al.</u>, 1959) were analyzed by ether extraction in the Soxhlet apparatus (= 0.5% standard error). The total nitrogen content was measured with the micro-Kjeldahl method (=0.01% error).

The water content was measured and expressed as the percentage of the dry weight (=6% standard error) after 24 hours at $65-70^{\circ}$ C. The results are given by Guisado and Rojas, (1971).

All the animals analised in this study were adults with an average diameter of 7.07 cm (SD±1.59).

The perivisceral fluid index was calculated as the volume of the fluid (ml) / diameter³ x 10^3 .

RESULTS.

The monthly variations in sex ratio of the natural populations of L. <u>albus</u> from Valparaiso, from May 1968 until May 1969, are shown in Fig. 1. The average for this period was 50.2% for the females, 39.7% for the males and 10.1% for undifferentiated (neuter) animals which were either too young for sexual development, or were adults in a special gonadal condition. Only in July 1968 were no neuters present in the samples. Evidence for gametogenic activity was found in females starting at 3.8 cm diameter and in males starting at 4.7 cm. The largest animal with ripe gametes was a 12.9 cm female. Gutierrez and Otsu, (1975) studying the same species in the northern part of the country (22°54'50" S. Lat., 70°18'30" W. Long.) obtained very different results, reporting that in 1965, 95% of the population were females; they also noted that in January 1966 the proportion of males was slightly higher than 53%: no neuter animals were reported.

The seasonal changes in the gonadal condition are summarized in Fig. 2 only for mature animals and those in spawning condition. (These will be examined in greater detail in a later publication). L. albus went through the same pattern of development in the years sampled: a period of maturation followed by a spawning period and finally a resting period. Spawning apparently took place twice a year with spawning period in spring (September-November) and a second period in autumn (March-May). The highest level of reproductive activity occured in spring, and declined through the summer. This followed by a second spawning period, which however, was at a lower level. Late autumn and early winter was characterized by a resting period during which no reproductive activity occured. The two reproductive cycles observed are quite similar.

The gonadal index was in general lower for the females, with the exception of September 1969 when the female gonadal index was clearly higher than the male index (Table 1). After homogeneity test (d.f. =22; $x^2 = 2.24$; P=0.995) both indexes were pooled together. When the pooled gonad index is compared with the pooled mean percentage of spawning animals (Fig. 3), a decrease of the index is apparent from May till July 1968 and from May till June 1969, in spite of an absence of spawning animals. Afterwards the index recovered just before the first spawning animals were evident. The process continued with a marked decline in the gonad index accompanied by an increase in the number of spawning animals.

Certainly, determination of the spawning season by fluctuation in the gonadal index, and by the proportion of spawning animals, lends itself to subjective interpretation, but a more accurate appreciation of the fenomena is obtained when observations are included on the chemical changes during gonad maturation.

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Figure 1. Changes in sex ratio in the population of Loxechinus albus.

The annual mean percentage of ether extract from the gonads was 28.69% for the females and 26.55 for the males of the dry weight (Table 2). The ether extract from the gonads in the males and in the females separately have a relationship with the monthly gonad The correlation for the males is r=0.67 and for the females index. r=0.65 both significant at the 5% significance level (Fig. 4a). The total amount of ether extract has also a significant relationship with the pooled mean gonad index (Fig. 4b). After spawning, the ether extract decreases, as has been observed in other echinoderms Microscopic examination of the fresh gonads showed (Pearse, 1965). that the fat droplets at the beginning of the maturation process were very small and numerous, becoming fewer and bigger at the end of the season.

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Figure 2. Seasonal variation in gonad condition: S=mature, o=spawning.

The annual mean nitrogen content (proteic and non proteic) was 6.39% in the females and 7.12% in the males (Table 2). There was no significant correlation between the monthly mean nitrogen content and the monthly gonad index in either sex. Before the breeding season, the nitrogen content of the female gonad was more or less stable compared with the wider fluctuations observed in the males (Fig. 5). In July 1969 when spawning started, both sexes had approximately the same percentage of nitrogen. The breeding season in the females was characterized by a more or less steady decrease in nitrogen content until October. Guisado and Rojas, 1971 have observed that dark coloured gonads contain less nitrogen than those of light colour (Table 3). TABLE 1.

Temp.C.			Gonad Index			Pooled Gonad Index		
1968		<u> </u>	N	5 5	N	- x		
May	12.4	0.32	60	0.39	48	0.35		
J	12.6	0.29	6 9	0.40	44	0.35		
J	12.1	0.24	35	0.25	32	0.24		
А	11.8	0.29	55	0.33	47	0,31		
S	11.9	0.14	72	0.16	49	0.15		
0	12.2	0.13	64	0.13	54	0.13		
N	13.1	0.12	56	0.15	51	0.14		
D	13.4	0.16	61	0.15	43	0.16		
1969								
Jan	14.8	0.19	60	0.22	49	0.20		
F	13.8	0.22	43	0.25	46	0.24		
м	14.0	0.26	49	0.26	36	0.25		
Α	13.6	0.26	37	0.33	35	0.29		
M	13.5	0.29	30	0.40	15	0.34		
J	13.4	0.22	26	0.29	23	0,26		
J	12.1	0.32	3	0.30	3	0.31		
Α	11.9	0.20	10	0.20	5	0.20		
S	12.2	0.30	4	0.19	5	0.24		
0	12.2	0.16	5	0.16	8	0.16		
N	12.8	0.14	9	0.12	11	0.13		
D+J		0.20	7	0.25	13	0.23		
1970								
Feb.	14.3	0.22	7	0.21	2	0.21		
M	14.6	0,29	15	0.33	8	0.31		
A	12.9	0.20	17	0.30	4	0.25		
Μ	13.4	0.16	9	0.16	4	0.16		
J	12.5	0.18	5	0.26	9	0.22		
J	11.1	0.18	10	0.23	10	0.20		

Table 1. Monthly mean surface temperature, gonad index and pooled gonad index.

The annual mean water content in the males was 74.26% and in the females 75.06% (Table 2). The water content of the testes had a significant correlation with the gonad index (r=0.679) but in the ovaries this correlation was low (r=0.136) (Fig. 6). There seems to be a strong tendency among the males to lose water when the animals spawn. In the females, however, the water content is more or less stable during the annual cycle observed (Fig. 7).



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Figure 3. Seasonal variation of the pooled mean gonad index and the percentage of spawning individuals during two reproductive cycles.





a, in males (Δ ----) {Y=35.9295+(-33.4739 X); Syx=3.17}

and females (o- --) {Y=36.2208+(-32.8693 X); $S_{VX}=2.42$ }

b, the total ether extract contents of the gonads compared with the pooled mean gonad index $% \left({{{\left({{{\left({{{\left({{{c}} \right)}} \right)}_{{{\rm{c}}}}}}} \right)}_{{{\rm{c}}}}} \right)$

{ $Y=37.7614+(-39.3209 X); S_{vx}=2.29$ }.

The volume of the perivisceral fluid in both sexes tends to fluctuate greatly during the annual cycle (Fig. 8). There was no significant correlation with the gonad index, although the amount of fluid was lees in the spring of both 1968 and 1969, that is to say, during the first spawning period.

Figure 9 shows that there is a certain relationship between the pooled mean gonad index and the surface water temperature (Table 1, observed at 08:00 hours), if one of this two variables is rearranged forward or backward three months in time (r=0.85; 1% significant level) (Fig. 9).

Moore and Lopez (1972) found a relationship between seasonal variation in the gonad index and the rainfall, but we were not able to investigate this matter because rainfall figures from the sampling area were not available to us. TABLE 2.

	% ETHER EXTRACT			% NITROGEN			% WATER CONTENT		
1968	Total	x 99	ž 33	Total	ž 45	ž ôô x	Total	₹ 2 9	ž ŠŠ
Nov.	35.51 (4)	35.35 (2)	35.72 (2)	6.84 (6)	6.79 (4)	6.94 (2)	73.25 (4)	75.00 (2)	71.50 (2)
D	32.96 (1)	32.96 (1)	-	7.32 (4)	6.30 (2)	8.34 (2)	67.68 (5)	67.40 (2)	68.86 (2)
1969									
Jan.	29.00 (10)	31.19 (1)	27.55 (6)	7.09 (12)	6.51 (6)	7.67 (6)	73,85 (14)	71.71 (7)	72.61 (6)
F	27.94 (4)	26.73 (2)	-	6.74 (6)	6.64 (2)	6.20 (1)	76.04 (9)	76.47 (4)	77.50 (1)
м	24.70 (11)	27.17 (7)	21.28 (3)	6.76 (13)	6.52 (8)	7.01 (3)	74.88 (13)	74.03 (8)	72.20 (3)
A	26.66 (5)	28.21 (1)	26.28 (4)	6.99 (5)	7.47 (1)	6.87 (4)	75.32 (5)	74.00 (1)	75.50 (4)
м	27.03 (2)	28.04 (1)	26.02 (1)	6.94 (6)	6.69 (4)	6.55 (1)	84,08 (6)	80.37 (4)	98.00 (1)
J	23.06 (10)	23.34 (5)	22.77 (5)	6,94 (12)	6.57 (6)	7.30 (6)	77.83 (1 1)	77.31 (7)	78,83 (4)
J	25.46 (5)	27.64 (2)	23.98 (3)	6.63 (6)	6.59 (3)	6.67 (3)	72.56 (6)	72.06 (3)	72,96 (3)
A	29.51 (8)	28.36 (5)	31,45 (3)	6.64 (13)	6.07 (8)	7.54 (5)	76.05 (15)	76.32 (10)	75.48 (5)
s	29.15 (3)	26.28 (1)	30.56 (2)	6.25 (3)	5,29 (2)	8.18 (1)	69.06 (5)	75.35 (2)	65.36 (3)
0	32.85 (2)	-	31.15 (1)	5,17 (2)	5.17 (2)	-	69.36 (5)	71.25 (2)	72.10 (2)
N	29.21 (7)	29.18 (1)	29.22 (6)	6,26 (9)	6.19 (2)	6,28 (7)	77.19 (11)	79.36 (3)	76.40 (8)

Table 2. Monthly variation of the ether extract, nitrogen and water content of the sea-urchin gonads. In parenthesis are the numbers of individuals analyzed. If the totals exceed the sum of males and females, the excess represents neuter animals, (from Guisado and Rojas 1971).



Figure 5. Changes in the nitrogen content in the gonads of males $(\Delta - -)$ and females (o - -).

Colour	Colour classification	% Nitrogen
Ochre	Plate 13 G 8	6.48
Ochre-orange	Plate 10 J 10	6.66
Orange	Plate 10 F 7	6.67
Pale-orange	Plate 10 E 7	6.98
Orange-yellow	Plate 9 L 7	7.01

Table 3. The colour change of the sea-urchins gonads related to the nitrogen contents (form Guisado and Rojas, 1971).



(o- -) {Y=72.8762+7.6394 X; S_{YX}= 3.4853}.



Figure 7. Monthly variation in the water content of the sea-urchin gonads, males $(\Delta - - -)$, females (o - - -).



Figure 8. Graph showing the variation of the perivisceral index in males (Δ ---) and females (o- -).





 $\{Y = -0.5567 + 0.0603 X; S_{vx} = 0.0352\}.$

DISCUSSION.

During the reproductive cycle studied in 1968, the span of the breeding season of Loxechinus albus in the central region of Chile was from August in late winter of 1968 to April in autumn of the following year. In the ensuing cycle a displacement of one month in the spawning process was observed; it has also been reported for other species of sea-urchins (Lewis, 1958).

Before the first spawning animals were evident in the population, the gonad index decreased. This could be explained as a loss of weight due to starvation probably caused by the effect of colder water (Bückle, et al., in preparation).

The breeding pattern had two distinct spawning periods. The first one, which ocurred in spring time, is the most important. It is confined to a few months and has been reported also in other biological studies of this species dealing with its mass culture. Bückle, et al. (1976) reported that it was feasible to rear larvae in an open circulating system until October, which is the month when sea temperatures show leest fluctuation. Pizarro, (1973) analyzed the variations of this factor in the area of Valparaiso during a ten year period and showed that water temperatures were at their most stable during the span of the first spawning period. Successful larval development is enhanced by stable water temperature. Later, nowever, temperature changes as a result of the replacement of one

water mass by another (Pizarro, op. cit.) influenced the "stabilization and extinction interval" of the larval cultures (Bückle, et al, 1976). The results of experiments with larval development were negative during December and January. This suggests that although spawning may occur during and after this time, larval development in the wild is unlikely to be successful. However, the developmental range in time of the larvae is one problem in the reproduction biology of the sea-urchin, and the spawning range of the population another. Since the productivity of the species does not depend only on one of these variables, both of them should be taken into account in a analysis of its reproduction cycle. In this way the effective spawning period is a very restricted phenomenon compared to the large breeding season observed (Boolootian, 1963).

What appears to be, from the histological evidence, a second spawning period in the autumn may arise from a concentration of the energy needed to recover the gonads (which would explain the marked increase in gonad index at this time), and also from a resistance to the onset of starvation which is probably caused by the decreasing sea temperature (Bückle et al., in preparation). It is possible to assume that this "second spawning period" corresponds to a growth phase probably due to the storage of nutrients for the ensuing winter food shortages.

At the time of the first spawning period, the decreasing gonad index correlates also with a decrease in nitrogen content, especially for the females, where the high energy concentrated at the beginning of the breeding season (Fuji, 1967) is utilized during ovarian activity apparently without being replaced. The males, however, seem to concentrate more nitrogen to mantain sperm production. A reduction in the water content is very marked in the males and the water may be replaced by an increase in the ether extract in both sexes. The significant regressions with decrease of the gonad index, obtained with ether extract in males and females, and with water content in males, suggest that the first spawning peak is the most

important period for gametogenic production. On the other hand, the "second spawning period" is characterized by the greatest gonad index observed in the present study, which may explain the high water contents found in testes and ovaries, the low ether extracts and the normal level of protein in the animals at this period. Even if in this period there are still animals in spawning conditions, what appears to be an attempt to continue maturation is detained by the ensuing winter, and the energy accumulated is therefore in part utilized to maintain life.

* The change in the time of spawning is to be expected from the work of Bennet and Giese (1955) who have shown that maturation varies geographically in sea-urchins, particularly with respect to the onset of spawning. However, the two minor spawning in February and April reported in Antofagasta may in reality correspond to the "second spawning period" in Central Chile which we believe to be a growth phase rather than a spawning period. <u>L. albus</u> may therefore, have only one reproductive pattern in the subtropical region. BUCKLE - GUISADO - TARIFEÑO - ZULETA - CORDOVA - SERRANO

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BIBLIOGRAPHY.

- Arrau, L., 1958. Desarrollo del erizo comestible <u>Loxechinus</u> <u>albus</u> Mol. Rev. Biol. mar., Valparaiso 7 (1,2,3,): 39-61.
- Bennet, J. and Giese, A.C., 1955. The annual reproductive and nutritional cycles in two western sea urchins. Biol. Bull. 109: 226-237.
- Bückle, L.F., Guisado, Ch., Tarifeño, E., Zuleta, A., Cordova, L., Serrano, C., Maldonado, R., 1976. Estudios biológicos del erizo Loxechinus albus (Molina) (Echinoidea, Echinodermata).
 I., Investigaciones preliminares en cultivos masivos de larvas de erizo. Biol. Pesq. Chile 8: 31-64.
- Bückle, L.F., Guisado, Ch., Serrano, C., Cordova, L., Vasques, E., 1977a. Estudio del crecimiento en cautiverio del erizo <u>Loxechinus albus</u> (Molina) en las costas de Valparaiso y Chiloe, Chile. An. Centro Cienc. del Mar y Limnol. Univ. Nal. Auton. México 4 (1).
- Bückle, L.F., Guisado, Ch., Serrano, C., Vasques, E., 1977b. Estudios de la alimentación en cautiverio del erizo <u>Loxechinus albus</u> (Molina) en las costas de Valparaiso y Chiloe, Chile. An. Centro Cienc. del Mar y Limnol. Univ. Nal. Auton. México 4 (1).
- Bückle, L.F., Alveal, K., Tarifeño, E., Guisado, Ch., Cordova, L., Serrano, C., Valenzuela, J., in preparation. Biological studies on the Chilean sea-urchin <u>Loxechinus albus</u> (Molina) (Echinodermata, Echinoidea). V.- Food analysis and seasonal feeding rate.
- Boolootian, R.A., 1963. Reproductive physiology of echinoderms. Proceeding of the XVI International Congress on Zoology 3: 130-134.
- Fuji, A., 1967. Ecological studies on the growth and food consumption of japanese common littoral sea urchin, <u>Strogylocentrotus</u> <u>intermedius</u> (A. Agassiz). Mem. Fac. Fish., Hokkaido Univ., 15: 83-160.

- Giese, A.C., Greenfield, L., Huang, H., Farmanfarmaian, H., Boolootian, R. and Lasker, R., 1959. Organic productivity in the reproductive cycle of the purple sea urchin. Biol. Bull. mar. biol. Lab. Woods Hole 116: 49-58.
- Greenfield, L., Giese, A.C., Farmanfarmaian, A. and Boolootian, R. A., 1959. Cyclic biochemical changes in several echinoderms. J. Exp. Zool. 139: 507-524.
- Guisado, Ch. y Rojas, P., 1971. Estudios sobre la variación estacional de algunos contenidos químicos en <u>Loxechinus</u> albus (Molina) (Echinoidea) y de <u>Pinnaxodes</u> <u>chilensis</u> (M. Edwards)(Decapoda Brachyura), en relación a sus procesos biológicos. Tesis, Universidad Católica de Valparaiso, Chile.
- Gutiérrez, J. y Otsu, I., 1975. Periodicidad en las variaciones biométricas de Loxechinus albus Molina. Rev. Biol. mar., Valparaiso 15 (2): 179-199.
- Harvey, E.B., 1956. The American Arbacia and other Sea Urchins. Princeton University Press, New Jersey, 298 pp.
- Larrain, A., 1975. Los equinoideos regulares fosiles y recientes de Chile. Gayana 35: 1-189.
- Lewis, J.B., 1958. The biology of the tropical sea urchin <u>Tripneustes</u> esculentus Leske in Barbados, British West Indies, Can. J. Zool. <u>36: 607-621</u>.
- Maesz and Paul. Dictionary of colour. Mc. Grow Book Co. Inc. London 1950.
- Moore, H.B. and Lopez, N.N., 1972. Factors controlling variation in the seasonal spawning pattern of <u>Litechinus variegatus</u>. Mar. Biol. 14: 275-280.
- Pearse, J. S., 1965. Reproductive periodicities in several contrasting populations of <u>Odontaster validus</u> Koehler, a common antartic asteroid, Antartic Research series 15: 39-85.
- Pizarro, M., 1973. Estudios de ecología fitoplanctonica en la bahía de Valparaiso. Rev. Biol. mar., Valparaiso 15(1): 77-105.
- Schwabe, H., 1936. Investigaciones sobre Loxechinus albus Mol. y Pinnotheres chilensis Edw. Bol. Soc. Biol. Concepcion, X: 125-136.
- Stott, F. C., 1931. The spawning of <u>Echinus esculentus</u> and some changes in gonad composition. Jour. Exp. Biol. VIII (2): 133-150.

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* The results obtained by Gutierrez, J. and Otsu, I., 1975 for the same species in the northern region of the country suggest that the reproductive pattern are different. Sea-urchins in the province of Antofagasta, have a massive spawning which takes place in November and December, and two minor spawnings, one in February and the other in April (Gutierrez, J. and Otsu, I., op. cit.).