Biochemical composition of body compartments during the reproductive cycle of the starfish *Allostichaster capensis* in Patagonia, Argentina

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Abstract: *Allostichaster capensis* (Perrier, 1875) (= *inaequalis*) is a fissiparous starfish inhabiting the South Atlantic Ocean coasts. On the Patagonian coast, it displays an annual sexual and asexual reproductive cycle. Fission is most frequent during the austral summer (November-January) and arm regeneration takes place during the rest of the year. In this article, seasonal changes in biochemical composition of body compartments are presented for a population of this fissiparous starfish in Bahia Kaiser, Golfo Nuevo, Chubut, Argentina, from June 2000 to May 2002. During the annual cycle, an inverse relationship between gonad and pyloric caeca indices suggests nutrient transfer. Biochemical analysis revealed that only lipids are translocated from pyloric caeca to gonads at the beginning of gametogenesis. Rev. Biol. Trop. 56 (Suppl. 3): 351-360. Epub 2009 January 05.

Key words: biochemical composition, reproductive cycle, fissiparous starfish, *Allostichaster capensis*, Northern Patagonia.

Allostichaster capensis (Perrier, 1875) (= *inaequalis*) is a fissiparous starfish inhabiting the South Atlantic Ocean coasts (Clark & Downey 1992). On the Patagonian coast, it displays an annual sexual and asexual reproductive cycle. Fission is most frequent during the austral summer (November-January) and arm regeneration takes place during the rest of the year. Gametogenesis starts in March, when gonads appear in the regenerating arms. Sexual maturity is achieved in August (austral winter), spawning occurs from September to October (spring) and there is a long recovery phase from November to March, while regenerating arms are in the first stages of regeneration. Shedding of spermatozoa takes place when the arms are almost fully regenerated and just prior to fission (Rubilar *et al.* 2005).

Most of the approximately 21 species of starfish that reproduce asexually have both sexual and asexual reproduction (Emson & Wilkie 1980, Mladenov & Burke 1994, Karako *et al.* 2002). Thus, nutrients must be shared between both reproductive strategies as they are energy demanding. Concentrations of lipid, carbohydrate and protein of gonads, pyloric caeca, and also body wall when food availability is low, change during the reproductive cycle (Boolootian 1966, Oudejans & van der Sluis 1979, Barker & Xu 1991). The complexity of the situation produced by two reproductive strategies found in *A. capensis,* requires close examination of biochemical cycles of the body compartments since they might indicate nutrient pathways for both processes. In addition, the changes in levels of the biochemical constituents may indicate the nutritional condition of the population as these cycles are controlled by physical and environmental factors such as temperature and food availability (Boolootian 1966, Giese 1966).

We know of no study describing biochemical analyses on a fissiparous starfish following both its sexual and asexual reproductive cycles. Most studies refer to the biochemical aspects of specimens which have had induced arms autotomy (Lawrence *et al.* 1986, Lawrence & Ellwood 1991, Lares & Lawrence 1994, Lawrence & Larrain 1994, Pomory & Lawrence 1999). Thus, the purpose of the present work is to describe the biochemical changes in body compartments of *A. capensis* during the reproductive cycles.

MATERIALS AND METHODS

Thirty-five *Allostichaster capensis* were randomly collected monthly from June 2000 to May 2002 from Bahía Kaiser (46º45'S-65º0'W), Golfo Nuevo, at 10 m depth on hard substratum by SCUBA diving. See Rubilar *et al.* (2005) for details regarding environmental factors, sample area and organ indices.

As less than three percent of the population were females, proximate analysis involved only males. Eighty-three percent of the individuals sampled had arms between 20–30 mm long and weight varied from 1g to 24 g with a mean of 10 g and were sexually mature (Rubilar *et al.* 2005). Due to the small size of the individuals, each monthly sample for biochemical analyses, body compartments (gonads, pyloric caeca, and body walls) from all individuals sampled each month were pooled to have enough material for all analyses. The pooled samples of body compartment were weighed and dried at 60°C to constant weight. The dried material was ground in a mortar to homogeneity. Total lipid was measured gravimetrically by the method of Bligh and Dyer (1959). Soluble carbohydrate was measured by the colorimetric method of Dubois *et al.* (1956) with glycogen as the standard. Soluble protein was measured by the Folin method of Lowry *et al.* (1951) with bovine serum albumin as the standard. Ash content was measured by combustion in a muffle furnace at 600°C for 6 hours (Barker and Xu 1991).

All biochemical analyses were performed by triplicate. Levels of biochemical components were expressed in % g dry tissue and amounts (calculated by multiplying the concentration by weight of the compartment) were expressed for a standard individual in mg. Given that regenerating arms are continuously growing, their contribution varies from month to month, therefore proximate composition was expressed as absolute values (amounts) for a standard individual with a specific configuration of arms for each month based on the number of arms and the presence of pyloric caeca and gonads in the arms (six arms from May to September; five, in October and April; four, in February and March and three from November to January), using the following equation: Amount = wet weight x number of arms x level value x (dry weight /100). The degree of association of the biochemical composition among different organs was estimated by the Spearman rank-order correlation coefficient (r_s) (Zar 1984).

RESULTS

Lipid, carbohydrate and protein of gonads: lipid levels in gonads showed a increase from 1.8% in February to 6.9% in March, coincident with the beginning of the growing phase. Values remained high during the entire phase and maturation around 8.8%, in September they started to decrease, falling to 2.9% in October, coincident with the shedding of spermatozoa. During recovery phase lipid levels constantly diminished to their minimum in January with an average value of 1.2% (Fig. 1, Table 1). Amounts also increased with proliferation of gametes from 0.8 mg in February to 16 mg in March, and continuously increased until maturity reaching 42.8 mg. Values diminished with the shedding of spermatozoa. During recovery phase amounts remained very low around 1.22 mg (Table 2). The low values

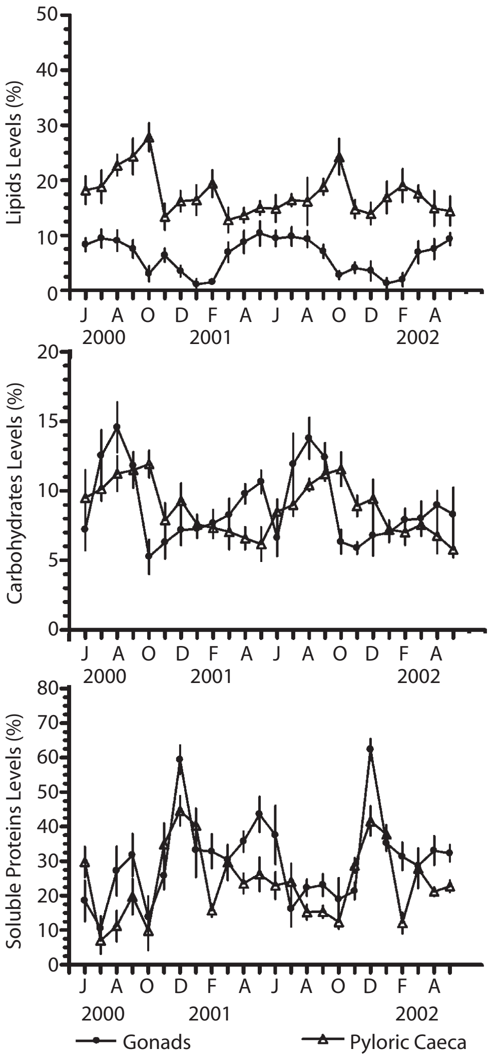

Fig. 1. *Allostichaster capensis* monthly variation of lipid level (% of dry weight), carbohydrate and soluble protein in pyloric caeca and gonads. Mean ± SE (N=3 analytical replicates of the pooled gonads and pyloric caeca of 35 individuals collected every month).

found both in levels and amounts, were coincident with the highest frequency of fission (November - January) and the first stages of regeneration (January - March).

Carbohydrate levels continuously increased after the spawning, from 5.8% in October to 14.1% in August. Values were not apparently

TABLE 1

Allostichaster capensis *sexual and asexual reproductive stages*

	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M
Asexual						▨	▨	▨	🟨	🟨								▨	▨	▨	🟨	🟨		
Sexual	🟥	🟥	🟦	🟩	🟩					🟥	🟥	🟥	🟥	🟥	🟦	🟩	🟩					🟥	🟥	🟥
PCI	-	-	-	-	-	-	-	-	-	-	4.8	4.1	3.7	2.2	2.9	3.3	4.4	4.5	4.9	5.0	5.1	4.7	4.5	4.3
GI	2.0	3.4	4.8	2.2	0.6	0.7	0.4	0.6	0.6	3.3	3.2	3.0	3.9	4.3	4.7	1.6	0.4	0.6	0.4	0.5	0.6	3.0	3.3	3.7

Pyloric caeca index (PCI) and gonadal index (GI) during the reproductive cycle. Asexual: Grey= Fission, yellow = First stages of regeneration, white = Regeneration. Sexual: White= Recovery stage, red= Growing stage, blue= Mature stage, green = Spawning stage.

TABLE 2

Allostichaster capensis *monthly variation of lipid amounts (mg* ind^{-1}*), carbohydrate and soluble protein in pyloric caeca and gonads*

	Lipid Amounts (mg)		Carbohydrate amounts (mg)		Soluble protein amounts (mg)	
Date	Gonad	Pyloric Caeca	Gonad	Pyloric Caeca	Gonad	Pyloric Caeca
Jun 00	17.61±4.62	-	15.24±3.49	-	80.62±12.48	-
Jul 00	35.68±4.57	-	47.19±8.16	-	86.66±14.57	-
Aug 00	58.41±5.25	-	30.98±6.16	-	133.18±12.47	-
Sep 00	17.83±3.16	-	28.77±5.49	-	77.28±16.26	-
Oct 00	1.79±0.26	-	2.36±0.96	-	6.23±2.70	-
Nov 00	2.91±1.82	-	2.78±0.13	-	11.70±1.78	-
Dec 00	1.09±0.08	-	2.22±0.34	-	18.41±0.50	-
Jan 01	0.84±0.13	-	4.19±0.13	-	19.16±4.54	-
Feb 01	0.82±0.20	-	4.10±0.07	-	17.55±2.66	-
Mar 01	16.03±4.49	-	12.87±2.46	-	57.61±9.47	-
Apr 01	41.96±4.09	66.02±6.84	29.37±5.49	34.77±6.88	122.18±10.28	119.00±14.62
May 01	35.45±3.49	63.60±4.74	30.33±5.94	27.81±6.40	124.91±12.32	112.16±21.08
Jun 01	64.48±5.90	77.51±3.45	45.23±5.49	39.72±3.46	159.95±18.21	154.51±18.87
Jul 01	53.73±5.83	61.22±4.05	63.41±2.46	22.07±2.49	143.84±18.65	60.95±10.25
Aug 01	55.13±4.78	72.26±6.18	78.97±10.16	45.35±5.55	133.18±10.25	67.97±8.49
Sep 01	5.51±1.06	96.78±9.46	10.64±1.19	47.96±3.44	18.46±0.75	68.78±9.58
Oct 01	0.77±0.13	58.76±8.16	2.31±0.16	48.95±5.20	10.40±4.48	69.18±10.25
Nov 01	1.34±0.19	37.72±4.16	2.99±0.07	30.29±5.20	10.40±0.35	68.85±12.49
Dec 01	0.69±0.03	31.79±5.66	1.40±0.15	28.90±4.49	11.91±1.04	77.95±8.01
Jan 02	0.45±0.14	13.30±8.70	1.84±0.13	5.41±1.16	9.07±0.58	21.21±5.46
Feb 02	1.53±0.22	10.6.65±12.38	6.18±1.05	26.81±3.59	24.10±3.11	110.61±15.55
Mar 02	18.38±3.16	100.28±9.15	14.29±2.49	27.32±3.46	66.70±1.07	110.10±16.17
Apr 02	26.20±4.27	83.60±4.31	11.81±2.00	31.75±5.94	59.86±0.28	99.01±6.41
May 02	39.81±6.01	74.78±2.71	28.46±6.49	34.71±1.51	114.78±8.46	109.57±8.03

Mean ± SE (N=3 analytical replicates of the pooled gonads and pyloric caeca of 35 individuals collected every month).

affected by recovery phase or fission, since during this period the levels were increasing (Fig. 1, Table 1). Amounts increased with gametogenesis from 5.3 mg in February to 13.6 mg in March. Values continuously increased until maturity in August reaching 55 mg. After spawning, amounts started to decrease and remained low during recovery phase with a value of approximately 3.2 mg which was coincident with fission and the first stages of regeneration (Table 2).

Soluble protein levels did not vary from January to June with a value about 36.3%. Values started to decrease with maturation and continued to fall during spawning when the levels decreased until 16.4%. The maximum level (60.9%) was reached in December (Fig. 1, Table 1). Soluble protein amounts continuously increased from 62.1 mg in March to 133.2 mg in August when maturation was achieved. Values markedly decreased during spawning, to reach 8.3 mg in October. Amounts remained constantly low during recovery phase (15.3 mg) (Table 2).

Lipids, carbohydrates and proteins of pyloric caeca: lipid levels were constantly low during proliferation of gametes and maturation with values around 15.6%. Spawning did not affect the lipid levels in the pyloric caeca, since values increased to their maximum 23.8%. Levels diminished during fission to 15.2% (Fig. 1, Table 1). Amounts slightly decreased from March (100.3 mg) to May 2002 (74.7 mg). In this period, the change observed in lipid amounts in the pyloric caeca was similar to that of gonads (25.5 mg and 21.4 mg respectively).This change shows an inverse relationship between the lipid amounts of the pyloric caeca and those of gonads (r_s= -0.990; *P*<0.05). During maturation, lipid amounts were high and started to diminish in October, reaching the minimum value in January (13.3 mg), coincident with fission. Values rose again in February to 106.6 mg when regeneration started (Table 2).

Carbohydrate levels decreased at the beginning of fission reaching 8.4% in November. Levels continuously decreased during the first stages of regeneration and gametogenesis until May reaching 6.0%. Values increased again in June (9.0%) to continuously rise until October (Fig. 1, Table 1). Apparently maturation and spawning did not have an effect on the carbohydrate levels of the pyloric caeca. Amounts were maximum during maturation and spawning (47.4 mg), started to decrease in November to their minimum in January (5.4mg), coincident with fission. Values increased in February reaching 26.8 mg and remained nearly constant during growing phase (Table 2).

Soluble protein levels increased markedly during fission from 11.1% in October to 31.8% in November, the maximum value was observed in December (43.1%) reflecting the fall in the other constituents. Then, they diminished after the maximum value, to increase again in March (28.7%). Levels were nearly constant during growing phase with a value around 25.5%, to slightly decrease during maturation and spawning (13.9%) (Fig. 1, Table 1). Amounts were high from February to June with values around 116.8 mg but fell with maturity and finally remained approximately 68.9 mg during spawning and fission. The minimum was found in January (21.2 mg) (Table 2).

Lipids, carbohydrates and proteins of body walls: lipid levels in body walls were very low during fission (0.12%) and during the first stages of regeneration (0.38%). Carbohydrate levels continuously decreased from November (2.2%) to January (1.5%) and reached their minimum in February (1.2%). Soluble protein showed the maximum value in December (70.6%) and fell again in February (37.7%) to increase again to 29.4% in May (Fig. 2).

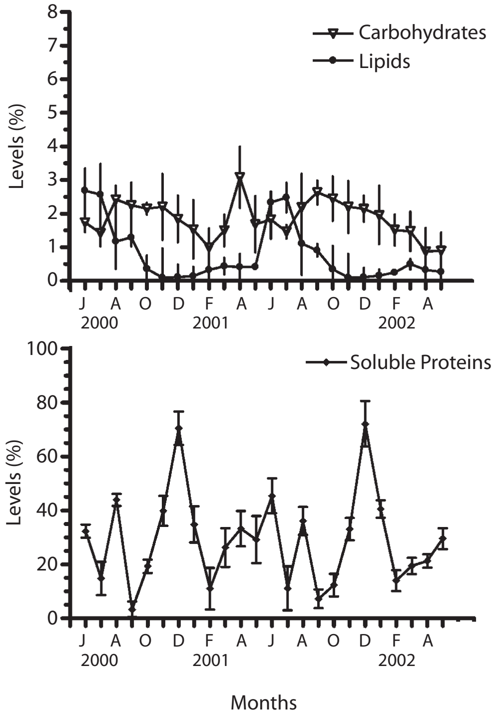

Fig. 2. *Allostichaster capensis* monthly variation of lipid level (% of dry weight), carbohydrate and soluble protein in body walls. Mean ± SE (N=3 analytical replicates of the pooled gonads and pyloric caeca of 35 individuals collected every month).

Ash and water content: ash level remained nearly constant in gonads with values around 10.7%. Pyloric caeca also had a content approximately constant (7.9%) during the reproductive cycle. Pyloric caeca presented lower ash level than gonads. Ash level in body walls remained nearly constant during the reproductive cycle (62.5%) (Table 3).

Water content kept nearly constant in gonads with values around 78.0%. Pyloric caeca showed the same scenario with a content around 72.9% during the reproductive stages. Pyloric caeca had 7.9% water content while gonads had 10.7%.Water content remained nearly constant in body walls (62.5%) during the reproductive cycle (Table 4).

DISCUSSION

Allostichaster capensis displays sexual and asexual reproductive cycles (Rubilar *et al.* 2005), hence resources might split into both reproductive strategies. Spermatogenesis is an energy demanding process and it is usually

TABLE 3

Allostichaster capensis *monthly variation of water level (% of dry weight) in gonads, pyloric caeca and body walls*

Ashes (%)			
Date	Gonad	Pyloric Caeca	Body Walls
Jun 00	10.67±0.32	8.79±0.48	59.83±2.32
Jul 00	12.75±0.58	8.29±0.52	65.62±2.85
Aug 00	15.12±0.94	7.64±0.39	66.58±2.68
Sep 00	10.50±0.25	6.91±0.26	59.65±2.02
Oct 00	10.27±0.33	6.66±0.33	61.26±2.74
Nov 00	9.38±.12	5.91±0.35	59.47±2.11
Dec 00	9.31±0.15	7.26±0.42	60.80±2.85
Jan 01	9.29±0.19	6.90±0.32	63.13±2.79
Feb 01	9.42±0.32	9.02±0.47	64.08±2.95
Mar 01	9.59±0.22	8.45±0.45	56.33±2.04
Apr 01	9.85±0.65	11.66±0.68	62.38±1.99
May 01	11.26±1.12	6.97±0.32	68.30±1.89
Jun 01	11.22±0.98	8.51±0.45	60.23±2.66
Jul 01	13.54±0.86	8.12±0.47	64.42±2.57
Aug 01	14.52±1.32	7.85±0.39	68.54±2.98
Sep 01	11.22±0.87	7.01±0.34	61.32±2.04
Oct 01	10.32±0.97	6.54±0.33	58.57±2.33
Nov 01	10.12±0.84	6.12±0.35	61.23±2.55
Dec 01	9.58±0.33	7.12±0.44	62.25±2.34
Jan 02	9.42±0.28	7.25±0.45	65.12±2.26
Feb 02	9.32±0.35	9.21±0.68	65.45±2.04
Mar 02	9.51±0.37	8.95±0.35	57.68±1.98
Apr 02	10.21±0.45	10.85±0.75	60.54±2.36
May 02	10.85±0.56	7.10±0.22	67.21±2.57

Mean ± SE (N=3 analytical replicates of the pooled gonads and pyloric caeca of 35 individuals collected every month).

TABLE 4

Allostichaster capensis *monthly variation of ashes level (% of dry weight) in gonads, pyloric caeca and body walls*

	Water (%)		
Date	Gonad	Pyloric Caeca	Body Walls
Jun 00	78.59±2.30	69.78±0.68	57.16±1.32
Jul 00	80.86±1.58	77.61±1.15	60.68±1.08
Aug 00	79.13±0.93	72.86±0.98	60.78±1.52
Sep 00	76.65±1.68	72.40±1.22	63.10±1.53
Oct 00	74.82±2.65	71.82±0.85	58.31±1.35
Nov 00	76.98±1.11	71.63±1.32	59.80±1.25
Dec 00	78.21±1.24	74.92±1.85	57.91±1.34
Jan 01	77.92±2.12	72.74±1.74	60.81±1.18
Feb 01	79.91±1.55	71.67±0.85	58.20±1.65
Mar 01	76.15±0.98	69.16±0.47	60.71±0.98
Apr 01	78.20±1.05	74.92±0.96	57.91±1.47
May 01	80.12±2.68	76.51±2.10	59.76±1.85
Jun 01	77.54±0.88	73.44±1.58	58.91±1.32
Jul 01	78.78±1.57	79.65±1.67	59.55±1.12
Aug 01	80.54±2.35	74.33±1.95	61.41±1.24
Sep 01	78.32±1.95	71.93±0.85	62.34±0.88
Oct 01	77.64±1.68	70.22±0.79	60.32±1.71
Nov 01	75.53±1.74	69.81±0.65	61.64±1.53
Dec 01	79.63±1.65	73.15±1.11	60.93±1.42
Jan 02	77.42±1.25	71.38±1.16	58.38±1.11
Feb 02	74.00±0.98	70.92±1.32	59.83±1.34
Mar 02	79.92±1.49	72.15±1.08	61.66±1.21
Apr 02	80.22±1.38	73.91±1.34	62.73±1.32
May 02	76.94±0.98	74.14±1.51	60.61±1.24

Mean ± SE (N=3 analytical replicates of the pooled gonads or pyloric caeca of 35 individuals collected every month).

accepted that the energy supply for gonad development comes from pyloric caeca nutrient reserves (Giese 1966, Giese and Pearse 1975, Lawrence and Lane 1982). In *A. capensis*, during growing phase, lipid amounts diminished in pyloric caeca and this change was reciprocal to a simultaneous increase in the gonads. Thus, a net lipid translocation from the pyloric caeca to the testis can be suggested, with an energy input of 846.5 kJ for gonad development. During the same period carbohydrate amounts increased almost five times in gonads but no change was evident in the pyloric caeca. Apparently there was no transfer for carbohydrates. There were higher levels of carbohydrates than lipids in the testis of *A. capensis* as well as in other echinoderms (Miller and Lawrence 1999, Brockington *et al.* 2001, Montero-Torreiro and García-Martínez 2003, Galley *et al.* 2005). High carbohydrate content appears to be necessary in male gonads since glycogen serves as energy resources for the spermatozoa once they are shedded from the testis (Anderson and Personne 1970). Soluble protein amounts increased in gonads with the beginning of gametogenesis, while they remained constant in pyloric caeca. According to Ferguson (1975) spermatogenesis involves relatively rapid protein synthesis, which would explain the rise in soluble protein amounts. Therefore, apparently pyloric caeca provided only stored lipids for the first stages of spermatogenesis.

Lipid and carbohydrate amounts diminished in pyloric caeca during fission. This decrease could be caused by the loss of tissue due to fission. The minimum values were found in January when more than 90% of the individuals had undergone fission and there were no pyloric caeca in the regenerating arms (Rubilar *et al.*2005). The rise in the amounts of the three biochemical constituents visualized in February is probably due to the presence of pyloric caeca in the regenerating arms which produced an increase in the mass of the organ and biochemical components of the individuals.

Arm loss during fission could reduce predatory efficiency and fitness, since arms are used to locomotion, feeding, nutrient storage and reproductive output (Lawrence 1992, Lawrence *et al.* 1986, Díaz-Guisado *et al.* 2006). Ramsay *et al.* (2001) demonstrated that *Asterias rubens* that had multiple arms autotomized had diminished the ability of tackling preys. However, *A. capensis* is able to feed with three arms, although with reduced efficiency (Villares 2008). Loss of tissue caused by fission implies costs of regeneration of the lost structures as well as reduced ability to obtain resources (Lawrence and Larrain 1994). According to Goss′ paradigm (1969) if regeneration is critical for survival of an individual or the organs are needed to increase fitness, the available energy might be expected to be used in regeneration. In *Luidia clathrata,* regeneration occurs even under starvation conditions, indicating that the process is important for the individual (Lawrence *et al.* 1986). The reduced predatory efficiency caused by fission could make arm regeneration crucial to increase fitness. Somatic growth typically diminishes or ceases during gonad development in most echinoderms (Lawrence 1987) and it is usually related to the amount of energy supplied by feeding (Lawrence and Ellwood 1991). Therefore, the decrease in predatory efficiency could be responsible of diversion of energy from sexual reproduction to regeneration of the arms and could have caused the long five months' recovery period observed in the gonads of *A. capensis*. In this way, regeneration would not only imply increased feeding efficiency and hence, more nutrients supply, but also synchronous gametogenesis within and between individuals and a larger reproductive output.

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RESUMEN

Se estudiaron los cambios estacionales en la composición bioquímica de las gónadas, el ciego pilórico y las paredes de la estrella de mar fisípara *Allostichaster capensis* desde junio 2000 hasta mayo 2002 en una población de Bahía Kaiser, Golfo Nuevo, Chubut, Argentina. *A. capensis* es una estrella de mar fisípara que habita las costas del Atlántico Sur. En Bahía Kaiser, la población presenta un desbalance sexual muy marcado, así como también reproducción sexual y asexual. La población presenta el mayor pico de fisión durante el verano, luego del desove y durante el resto del año los individuos regeneran los brazos. Se observó una relación inversa entre los índices gonadales y el del ciego pilórico, lo que sugiere una transferencia de nutrimentos. Sin embargo, análisis bioquímicos revelan que únicamente existe una transferencia de lípidos al comienzo de la gametogénesis.

Palabras clave: composición bioquímica, ciclo reproductivo, estrella de mar fisípira, *Allostichaster capensis*, Patagonia norte.

REFERENCES

Anderson, W.A. & P. Personne. 1970. The localization of glycogen in the spermatozoa of various invertebrate and vertebrate species. J. Cel. Biol. 44: 29-51.

Barker, M.F. & R.A. Xu. 1991. Seasonal changes in biochemical composition of body walls, gonads and pyloric caeca in two populations of *Sclerasterias mollis* (Echinodermata: Asteroidea) during the annual reproductive cycle. Mar. Biol. 109; 27-34.

Bligh, E.G. & W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

Boolootian, R.A. 1966. Reproductive biology, p. 561-613. *In*: R.A. Boolootian (ed.). Physiology of echinodermata. Interscience, New York.

Brockington, S., A. Clarke & A.L.G. Chapman. 2001. Seasonality of feeding and nutritional status during the austral winter in the Antarctic sea urchin *Sterechinus neumayeri*. Mar. Biol. 139:127–138.

Clark, A. & M. Downey. 1992. Starfishes of the Atlantic. New York: Chapman and Hall. 793 p.

Diaz Guisado, D., C.F. Gaymer, K.B. Brokordt & J.M. Lawrence. 2006. Autotomy reduces feeding, energy storage and growth of the sea star *Stichaster striatus*. J. Exp. Mar. Biol.Ecol. 338:73-80.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers & F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350-356.

Emson, R.H. & I.C. Wilkie. 1980. Fission and autotomy in echinoderms. Ocean Mar. Biol. Ann. Rev. 18: 155-250.

Ferguson, J.C.1975. The role of free amino acids in nitrogen storage during the annual cycle of a starfish. Comp. Biochem. Physiol. 51 A: 341-350.

Galley, E.A, P.A. Tyler, A. Clarke & C.R. Smith. 2005. Reproductive biology and biochemical composition of the brooding echinoid *Amphipneustes lorioli* on the Antartic continental shelf. Mar. Biol. 148: 59-71.

Giese, A. C. 1966. On the biochemical constitution of some echinoderm, p. 757-796. *In*: R. A. Boolootian (ed). Physiology of echinodermata. Interscience, New York.

Giese, A.C. & J.S. Pearse. 1975. Reproduction of Marine Invertebrates. Academic, New York. 808.

Goss, R.J.1969. Principles of Regeneration. Academic, New York. 292 p.

Karako, S., Y. Achituv, R. Perl-Treves & D. Katcoff, D. 2002. *Asterina burtoni* (Asteroidea; Echinodermata) in the Mediterranean and the Red Sea: Does asexual reproduction facilitate colonization?. Mar. Ecol. Prog. Ser. 234: 139-145.

Lares, M.T & J.M. Lawrence. 1994. Nutrient and energy allocation during arm regeneration in *Echinaster paucispinus* (Clark) (Echinodermata: Asteroidea). J. Exp. Mar. Biol. Ecol. 180: 49-58.

Lawrence, J.M. 1987. Echinodermata, p. 229-321. *In* T.J. Pandian & F.J. Vernberg (eds.). Animal Energetics. Academic, New York.

Lawrence, J.M. 1992. Arm loss and regeneration in Asteroidea (Echinodermata), p. 39-52. *In* L. Scalera-Liaci and C.Canicatti (eds.). Echinoderm Research. Balkema, Rotterdam.

Lawrence, J.M. & A. Ellwood. 1991. Simultaneous allocation of resources to arm regeneration and somatic and gonadal production in *Luidia clathrata* (Say) Echinodermata: Asteroidea), p. 543-548. *In*: T. Yanagisawa, L. Yasumasu, C. Oguro, N. Suzuki & T. Motokama (eds.). Biology of Echinodermata. Balkema, Rotterdam.

Lawrence, J.M. & P. Lane. 1982. The utilization of nutrients by post-metamorphic echinoderms, p. 331-372. *In* M. Jangoux & J.M. Lawrence (eds.). Echinoderm Nutrition. Balkema, Rotterdam.

Lawrence, J.M. & A. Larrain. 1994. The cost of arm autotomy in the starfish *Stichaster striatus*. Mar. Ecol. Prog. Ser. 109: 311-313.

Lawrence, J.M., T.S. Klinger, J.B. McClintock, S.A. Watts, C.P. Chen, A. Marsh & L. Smith. 1986. Allocation of nutrient resources to body components by regenerating *Luidia clathrata* (Say) (Echinodermata: Asteroidea). J, Exp. Mar. Biol. Ecol. 102: 47-53.

Lowry, O.H., N.J. Rosebrough, A.L. Farr & R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol.Chem.193: 265-275.

Miller, S.R. & J.M. Lawrence. 1999. Gonad and pyloric caeca production in the nine-armed starfish *Luidia senegalensis* off the southwest Florida gulf coast during the annual reproductive cycle. Bull.Mar. Sci. 65: 174-184.

Mladenov, P.V. & R.D. Burke. 1994. Asexual propagation, p. 339-383. *In* K.G. Adiyodi & R.G. Adiyodi (eds.).

Reproductive biology of invertebrates. Oxford & IBH, New Delhi.

Montero-Torreriro, M.F. & P. Garcia-Martinez. 2003. Seasonal changes in the biochemical composition of body components of the sea urchin, *Paracentrotus lividus,* in Lorbé (Galicia, norh-western Spain). J. Mar. Biol. Assoc. U. K. 83: 575-581.

Oudejans, R.C. & I. van der Sluis. 1979. Changes in the biochemical composition of the ovaries of the seastar *Asterias rubens* during its annual reproductive cycle. Mar. Biol. 50: 255-261.

Pomory, C.M. & J.M. Lawrence. 1999. Effect of arm regeneration on energy storage and gonad production in *Ophiocoma echinata* (Echinodermata: Ophiuroidea). Mar. Biol. 135: 57-63.

Ramsay, K., M.J. Kaiser & C.A. Richardson. 2001. Invest in arms: behavioural and energetic implications of multiple autotomy in starfish (*Asterias rubens*). Behav. Ecol. Sociobiol. 50: 360-365.

Rubilar, T., C.T. Pastor de Ward & M.E. Díaz de Vivar. 2005. Sexual and asexual reproduction of *Allostichaster capensis* (Echinodermata: Asteroidea) in Golfo Nuevo. Mar. Biol. 146: 1083-1090.

Villares, G. 2008. Variación de la eficiencia alimenticia con la abundancia de alimento y la regeneración del asteroideo *Allostichaster capensis* (Echinodermata: Asteroidea). Licenciate Thesis, Universidad Nacional de la Patagonia San Juan Bosco, Trelew, Argentina.

Zar, J.H. 1984. Bioestatistical analysis. Prentice Hall, New York. 880 p.