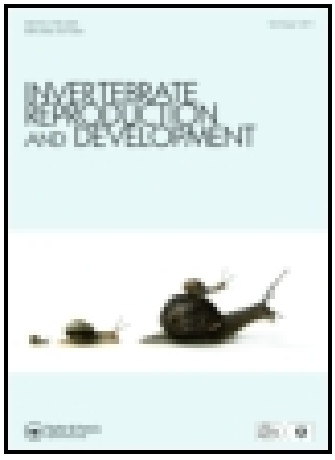

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Nutrient allocation in the gonads of the sea urchin Arbacia dufresnii in different stages of gonadal development

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Nutrient allocation in the gonads of the sea urchin *Arbacia dufresnii* in different stages of gonadal development

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This study reports nutrient allocation in different stages of gonadal development for two populations of the sea urchin *Arbacia dufresnii* off the Patagonian coast of Argentina (Nuevo Gulf and San Jorge Gulf). The biochemical composition of gonads was used to assess nutrient allocation by measuring ash, soluble protein, lipid and trichloroacetic acid-soluble carbohydrate concentrations, and absolute contents over a 24-month period. Reproductive output in terms of energy was calculated for females. Results were correlated with histological stage of the gonads. Soluble proteins were the main component for the Nuevo Gulf population while unmeasured organic material (i.e. insoluble proteins and nucleic acids, especially in testes) was prevalent in gonads from San Jorge Gulf. Soluble protein and lipid concentrations followed the gonadal cycle, while carbohydrate concentration was almost negligible, especially in the Nuevo Gulf population. The different patterns in the gonadal cycle in the two populations were reflected in the biochemical composition of gonads. Concentrations and contents of the biochemical components and reproductive output were higher in the population from San Jorge Gulf owing to the larger size of gonads and gametes. These findings contribute to the better understanding of the plasticity of the reproductive biology of *A. dufresnii* in different environments.

Keywords: Echinodermata; Echinoidea; biochemical composition; gonadal stages

Introduction

The changes in biochemical components of gonads, such as carbohydrates, lipids, and proteins during the reproductive cycle provide information regarding the deposition, allocation, and distribution of energy reserves in the organs of marine invertebrates, and also may be indicative of the nutritional condition of the population (Giese 1966; Clarke 1985; Lawrence et al. 1986). In sea urchins, the gonads play a dual role, not only in producing gametes but also in accumulating nutrients through the somatic cells called nutritive phagocytes (Walker et al. 2013). Therefore, the biochemical analysis of the gonads throughout the reproductive cycle can explain the nature and role of the nutritive phagocytes (Giese 1966; Lawrence & Guille 1982). In sea urchins, the reproductive cycle is regulated by a complex interaction of endogenous and exogenous factors (i.e. photoperiod, seawater temperature, nutritional condition) (Pearse & Cameron 1991; Walker et al. 2013). Gonad development is strongly correlated with food availability and hence, depends on the nutritional condition of the individuals (Walker & Lesser 1998). As exogenous factors vary with location, different populations may have distinct nutritional conditions reflected in the biochemical and cellular composition of the gonads. Changes in the biochemical composition of the gonads during the reproductive cycle have been studied in several species of sea urchins in different environments (see Walker et al. 2013 for details). However, few studies have been done on South Atlantic species (Tavares 2004; Pérez et al. 2008).

Arbacia dufresnii (Blainville, 1825) is a common sea urchin in the southwestern Atlantic Ocean (Brogger et al. 2013), and the only species of *Arbacia* inhabiting both coasts of South America (Lessios et al. 2012). There are large differences between body size and density of the population in Nuevo Gulf (NG) and the one in San Jorge Gulf (SJOG). The NG population is dense with small individuals while the San Jorge Gulf (SJOG) population is sparse with large individuals (Epherra et al. Forthcoming). Sea urchins from San Jorge Gulf have larger test diameter and heavier gonads than sea urchins from Nuevo Gulf. *A. dufresnii* has an annual reproductive cycle in both populations. Gametogenesis takes place mainly in autumn and winter. An extended spawning period occurs in spring and summer, with two different types of partial spawning. Gonads in spawning type-1 stage have a larger number of residual mature gametes, whereas few primary oocytes are present in ovaries and in testes, and there is no

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spermatogenic column layer. By contrast, gonads in spawning type-2 stage have few residual mature gametes whereas abundant primary oocytes are present in ovaries, and there is a spermatogenic column layer in testes. Sea urchins from Nuevo Gulf have a seasonal pattern of reproduction, with gonadal stages showing reabsorption (intergametic stage) and accumulation of reserves in nutritive cells (pregametic stage), while sea urchins from San Jorge Gulf have mature gametes during most of the year. The details of the gametogenic stages during the reproductive cycle for *A. dufresnii* at these two locations are described in Epherra et al. (Forthcoming). In this study, we focus on the biochemical composition and energy content related to the histological stage of each gonadal stage for the two populations. The aim is to evaluate whether the differences found in the reproductive cycle and body size of these two populations are reflected in the biochemical composition of the gonads.

Materials and methods

Sampling

Sampling was conducted at two sites located on the coast of Patagonia, Argentina: Punta Cuevas, Nuevo Gulf (NG, 42°46′44″S; 64°59′52″W), and La Tranquera beach, San Jorge Gulf (SJOG, 46°02′33″S; 67°35′52″W) (Figure 1). Both areas are shallow rocky reefs. However, the locations have different ecological environments (see Epherra et al. Forthcoming for details). At each site, collections were made at 5–10 m depth by scuba diving. In NG, 30 sea urchins with a test diameter over 25 mm were collected each month from September 2008 to August 2010. In SJOG, due to low abundance of the population, about 20 individuals were collected each month from February 2008 to March 2010. Live sea urchins were placed in seawater and transported to a wet laboratory. Each individual was blotted with filter paper to remove adhering water and then was weighed to the nearest 0.01 g. After narcotizing the sea urchins by immersion for 15 min in a 5% solution of $MgCl_2$ in filtered seawater, gonads were dissected out from each individual and weighed to the nearest 0.01 g. Sex was established by observing gonad coloration (♀: purple, ♂: pale brown). One gonad from each individual was fixed in Davidson solution for 24 h and then preserved in ethanol 70% for histological examinations. Gonadal stages were determined as described in Epherra et al. (Forthcoming), following the changes in the germinal cells and the nutritive phagocytes (NPs) as suggested by Walker et al. (2013). The populations did not have exactly the same stages (Table 1). Oocyte size frequency distribution was determined by measuring the maximum diameter (in micrometers) of at least 200 oocytes per gonadal stage using the image analysis software ImageJ. Only oocytes sectioned through the nucleus were measured. Thickness of the spermatogenic cell layer (spermatogonia, spermatocytes, spermatids), spermatozoa, and nutritive phagocytes layers were measured to the center of the lumen as described in Epherra et al. (Forthcoming).

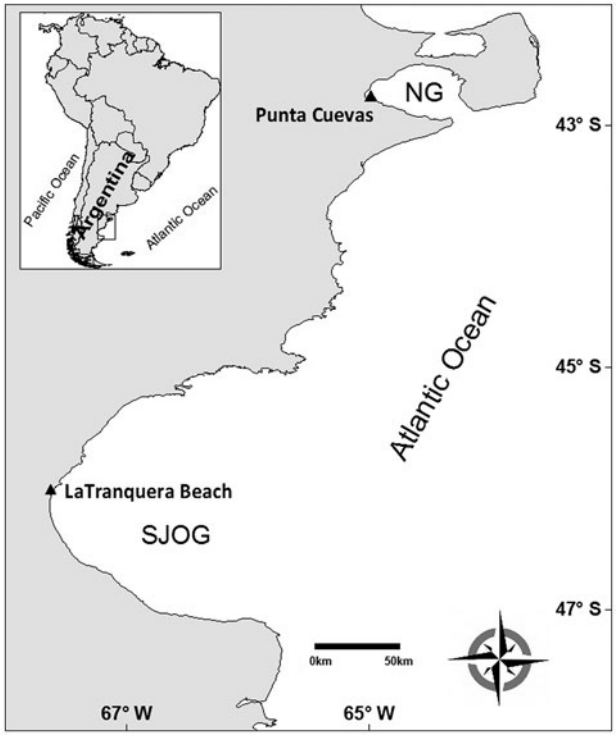

Figure 1. *A. dufresnii* Blainville, 1825, sampling areas off the east coast of Patagonia, Argentina.

Biochemical composition

Each month, gonads from six females and six males were individually dried at 60 °C to constant weight and ground to a powder in a mortar for biochemical analyses. When the small gonadal size limited the biochemical analyses on individual basis, gonads from several individuals at the same histological stage were pooled. Trichloroacetic acid-soluble carbohydrates were

Table 1. Gonadal stages in Nuevo Gulf and in San Jorge Gulf populations.

Nuevo Gulf population		San Jorge Gulf population	
Ovaries	Testes	Ovaries	Testes
Pregametic	Pregametic	Pregametic	Pregametic
Growth	Growth	–	Growth
Premature	Premature	Premature	Premature
Mature	–	Mature	Mature
Spawning-1	Spawning-1	Spawning-1	Spawning-1
Spawning-2	Spawning-2	Spawning-2	Spawning-2
Intergametic	Intergametic	–	–

measured according to Dubois et al. (1956), using glycogen as the standard, soluble proteins were measured by the method of Lowry et al. (1951), using bovine serum albumin as the standard, and total lipids were measured according to Zöllner and Kirsch (1962), with cholesterol as the standard. Ash concentration for each stage was determined for pooled gonads by combustion of dried samples for 5 h at 600 °C in a muffle furnace. Unmeasured organic material (UOM) (considered as refractive proteins and non-protein nitrogen, mainly, nucleic acids, in testes) was calculated by subtraction (Magniez 1983).

Samples were analyzed in duplicate. Results were expressed as percent dry weight of tissues. Contents of each biochemical component were calculated for a standard size individual and expressed as mg.individual^{-1}. The standard individual for each population was calculated according to Lawrence (1973). The standard individual wet weights were ♀: 10.4 g and ♂: 9.6 g for the NG population and ♀: 57.1 g and ♂: 58.7 g for the SJOG population.

Energy content was calculated by multiplying the content of each biochemical component by conversion coefficients in Brody (1945) and was expressed in kJ.individual^{-1}. The energy content was not calculated for males because DNA in the testes was not determined. Therefore, the reproductive output was calculated for females as the differences between the energy of mature and intergametic or spawned gonads (Pérez et al. 2010).

Data analysis

To evaluate changes in the gonad wet weight among different gonadal stages, the adjusted mean gonad weights (AGW) were obtained through a series of GLM ANCOVA, with test diameter as the covariate and gonadal stage as a factor for each sex and population. This statistical procedure was done as recommended by Grant and Tyler (1983) and Packard and Boardman (1999).

Post-hoc pairwise tests between gonadal stages were conducted using the Sidak approach to compare estimated adjusted means (Day & Quinn 1989). The assumption of homogeneity of slopes was tested prior to ANCOVA ($p > 0.10$ in all cases).

Changes in the concentrations of biochemical components in relation to the reproductive cycle were assessed using multivariate and univariate analyses. Redundancy analysis (RDA) was used as a constrained linear ordination technique to relate concentration of biochemical variables (protein, lipid, and carbohydrate concentration) to a set of potential explanatory variables (locations, reproductive stages, and years). A linear response of biochemical variables to explanatory factors is suitable for our data since a preliminary detrended correspondence analysis (DCA) showed a short gradient length (<1 SD) along the first ordination axis (Legendre & Legendre 1998; ter Braak & Smilauer 2002). Explanatory variables were all categorical variables: locations, gonadal stage, and year (Table 2) and were represented as centroids. The biochemical variables were centered. Then the scores were divided by the standard deviation using intervariable correlation scaling. No centering/standardization by samples were used. Monte Carlo permutations tests (with 9999 permutations, under the full model) were performed to examine which explanatory variables were significant ($p < 0.05$) regarding the biochemical composition (Legendre & Legendre 1998; Legendre et al. 2011). Since all explanatory variables were categorical, after including one category of the variable all concentrations were included as a whole set. To test for significant differences on each biochemical variable (concentration and content) among gonadal stages in each location and sex, a series of one-way ANOVAs were performed. ANOVA procedures were done using Type III sum of squares for unbalanced data. The univariate analyses were tested for normality using Shapiro–Wilks test and for homogeneity of variance using a Levene test. When assumptions were not met, a logit transformation was used (Warton & Hui 2011).

The frequency distributions of maximum oocyte diameter were shown among gonadal stages within each location. The effect of gonadal stage on spematogenic, spermatozoa, and nutritive layers were determined using ANCOVA, as described in Epherra et al. (Forthcoming). DCA/RDA and univariate analyses were performed using CANOCO v. 4.5 and INFOSTAT v2010, respectively. All values in the text are expressed as mean values ± standard error. A significance level of 5% was used for all analyses.

Results

Gonadal cycle

The adjusted gonad weight means (AGW) using test diameter as a covariate are summarized in Table 2. In the NG population, the AGW of the ovaries showed significant differences among stages ($F_{(6.91)} = 13.76$, $p < 0.001$). The AGW was high at the mature stage and at spawning, when ova had high frequencies. The lowest values were found at pregametic and intergametic stages. In testes, the AGW also showed significant differences among stages ($F_{(5.95)} = 10.82$, $p < 0.001$). The AGW was high at the spawning stage and the lowest mean values were found at the pregametic and the intergametic stages. In the SJOG population, the AGW of the ovaries showed significant differences among stages ($F_{(4.109)} = 13.42$, $p < 0.001$). The AGW was high at the premature and mature stages, the lowest mean value was found at the pregametic stage. In testes, the AGW also showed significant differences among stages ($F_{(5.93)} = 7.08$, $p < 0.001$). The mean value was high at premature and

Table 2. Adjusted gonad weight (AGW) (g) of ovaries and testes at each gonadal stage in both populations (Mean ± SE). Different letters (a, b) denote significant differences between adjusted means (Sidak test, $p < 0.05$).

	Nuevo Gulf population		San Jorge Gulf population	
Gonadal Stage	Ovaries	Testes	Ovaries	Testes
Pregametic	0.155 ± 0.030^{a}	0.223 ± 0.060^{b}	2.35 ± 0.46^{a}	3.72 ± 0.98^{a}
Growth	0.343 ± 0.050^{b}	0.540 ± 0.070^{a}	–	7.81 ± 1.23^{b}
Premature	0.497 ± 0.030^{b}	1.290 ± 0.450^{a}	7.95 ± 0.84^{b}	11.12 ± 1.25^{b}
Mature	0.826 ± 0.280^{b}	–	9.18 ± 0.79^{b}	13.34 ± 3.08^{b}
Spawning-1	0.776 ± 0.110^{b}	0.893 ± 0.130^{a}	5.37 ± 0.67^{b}	5.14 ± 1.05^{a}
Spawning-2	0.657 ± 0.110^{b}	0.723 ± 0.100^{a}	6.21 ± 1.05^{b}	6.08 ± 0.93^{a}
Intergametic	0.195 ± 0.030^{a}	0.239 ± 0.050^{b}	–	–

mature stages and the lowest mean value was found at the pregametic stage.

Biochemical composition

Concentration

RDA analysis showed that location and reproductive stages significantly influenced biochemical composition in both sexes ($p < 0.05$, forward model selection using a Monte Carlo permutation test). Years were removed from the model because of small marginal effects ($p > 0.40$) (Table 3; Figure 2). The biochemical composition differed with reproductive stages as shown in the ordination plot.

In ovaries, population and reproductive stages explained 37.7% of the total variation of the biochemical variables. Soluble protein and carbohydrate concentrations were strongly associated with each other, while lipid concentration was independent of the other biochemical components. TCA-soluble carbohydrate concentration had the highest variability and contributed to the discrimination of the stages and sites. Gonads of the NG population had a higher concentration of TCA-soluble carbohydrate and soluble protein than those from SJOG, while the latter had higher lipid concentration.

In testes, location and reproductive stages explained 37.3% of the total variation of the biochemical variables. Soluble protein and TCA-soluble carbohydrate concentrations were strongly associated with each other, while lipid concentration was independent of the other biochemical components. TCA-soluble carbohydrate and soluble protein concentrations had the highest variability and contributed to the discrimination of the stages and populations. Gonads of the NG population had a higher concentration of TCA-soluble carbohydrate and soluble protein than those from the SJOG population, while the latter had higher lipid concentrations.

Nuevo Gulf: The concentration values and the histological analyses are summarized in Figure 3. Soluble proteins were the main component of gonads in both sexes while TCA-soluble carbohydrate concentration was remarkably low. There were no significant differences in soluble protein concentration ($F_{(1.145)} = 3.67$, $p = 0.06$)

Table 3. Results of redundancy analysis (RDA) on biochemical variables and explanatory variables.

RDA Axes	1	2	3	4	Total
Ovaries					
Eigenvalues	0.366	0.011	0	0.567	1
Biochemical environment correlations	0.628	0.403	0.271	0	
Cumulative percentage variance of biochemical data	36.6	37.7	37.7	94.4	
Cumulative percentage variance of biochemical-environmental relation	97	99.9	100	0	
Sum of all eigenvalues					1
Sum of all canonical eigenvalues					0.377
Testes					
Eigenvalues	0.371	0.002	0.001	0.6	1
Biochemical environment correlations	0.621	0.23	0.265	0	
Cumulative percentage variance of biochemical data	37.1	37.3	37.3	97.3	
Cumulative percentage variance of biochemical environmental relation	99.5	99.9	100	0	
Sum of all eigenvalues					1
Sum of all canonical eigenvalues					0.373

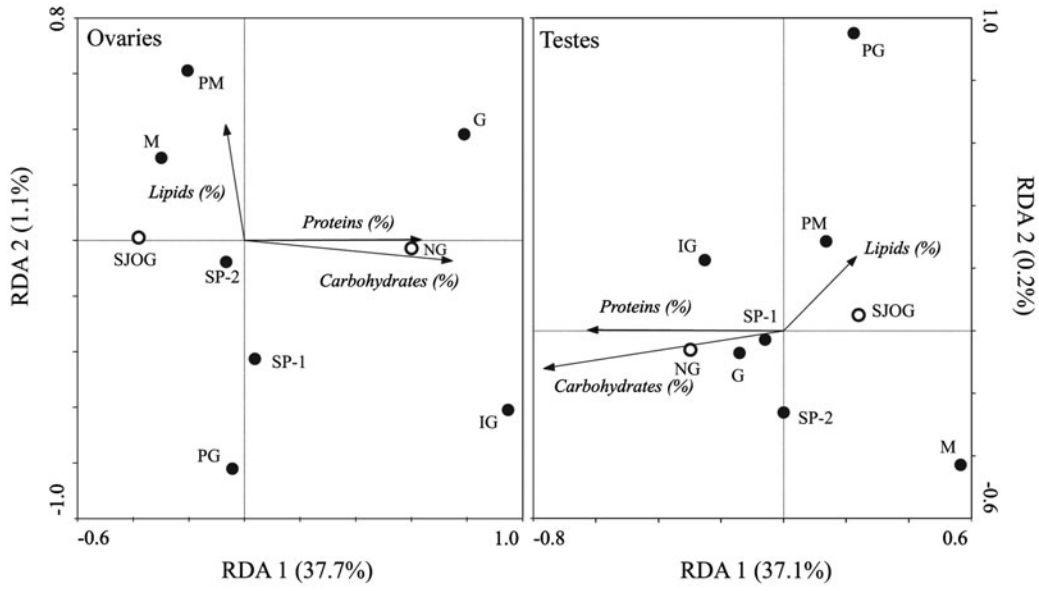

Figure 2. *A. dufresnii* Blainville, 1825, RDA ordination diagrams (biplot) of biochemical variables for testes and ovaries across study sites and reproductive stages. Filled circles represent reproductive stages: PG: pregametic; G: growth; PM: premature; M: mature; S1: spawning type-1, S2: spawning type -2; IG: intergametic. Open circles represent study sites: NG: Nuevo Gulf population; SJOG: San Jorge Gulf population.

between ovaries and testes while concentrations of lipids and carbohydrates differed significantly ($F_{(1.198)} = 47.80$; $F_{(1.182)} = 19.17$, $p < 0.0001$).

In ovaries, soluble protein concentration did not differ among stages ($F_{(6.64)} = 0.88$, $p = 0.51$). By contrast, lipid and TCA-soluble carbohydrate concentrations showed differences among stages ($F_{(6.79)} = 3.84$, $p < 0.005$; $F_{(6.80)} = 4.56$, $p < 0.001$). Lipid concentration was high at the mature stage and at spawning, when ova had high frequencies, while the TCA-soluble carbohydrate concentration showed the maximum value at the pregametic stage. Ash concentration remained nearly constant throughout the reproductive cycle, while the UOM concentration increased with gonadal development and decreased at the intergametic stage when ova frequencies diminished.

In testes, soluble protein concentration did not show significant differences among stages ($F_{(5.70)} = 0.45$, $p = 0.81$), although the intergametic stage had the lowest value. Lipid and TCA-soluble carbohydrate concentrations significantly varied with stages ($F_{(5.95)} = 2.42$, $p < 0.05$; $F_{(5.72)} = 2.55$, $p < 0.05$). Their concentrations decreased from the pregametic to the premature stage with the increase in the spermatozoan layer. Ash concentration remained nearly constant throughout the reproductive cycle, whereas UOM concentration increased with the amount of spermatogenic columns and spermatozoa.

San Jorge Gulf: The concentration values and the histological analysis are summarized in Figure 4. Soluble proteins were the main component of ovaries and testes while the concentration of TCA-soluble carbohydrate was nearly negligible. In this population, ovaries had significantly more soluble proteins and lipids than testes ($F_{(1.204)} = 8.76$; $F_{(1.213)} = 115.09$, $p < 0.0001$) and there were no significant differences for TCA-soluble carbohydrate concentration ($F_{(1.213)} = 0.13$, $p = 0.71$) between sexes. In ovaries, soluble protein and TCA-soluble carbohydrate concentrations remained nearly constant throughout the reproductive cycle ($F_{(4.107)} = 0.70$, $p = 0.59$; $F_{(4.104)} = 1.15$, $p = 0.33$). In contrast, lipid concentration differed among stages ($F_{(4.110)} = 14.93$, $p < 0.0001$), with higher values attained at premature and mature stages when the oocyte diameter frequency distribution was bimodal. Ash concentration was nearly constant throughout the reproductive cycle. UOM concentration was high throughout the reproductive cycle, with a maximum in mature stages. In testes, soluble protein and TCA-soluble carbohydrate concentrations were significantly different among stages ($F_{(5.88)} = 3.59$, $p < 0.01$; $F_{(5.82)} = 2.44$, $p < 0.05$). Minimum values occurred at the mature stage when spermatozoa were the most abundant cell type. Lipid concentration did not vary throughout the reproductive cycle ($F_{(5.94)} = 2.00$, $p = 0.08$). Ash concentration was nearly constant throughout the reproductive cycle. UOM was high at all gonadal stages. The mature stage had the highest value coincident with the widest spermatozoan layer.

Content

Nuevo Gulf: Content values are summarized in Table 4. Soluble protein content was significantly higher than

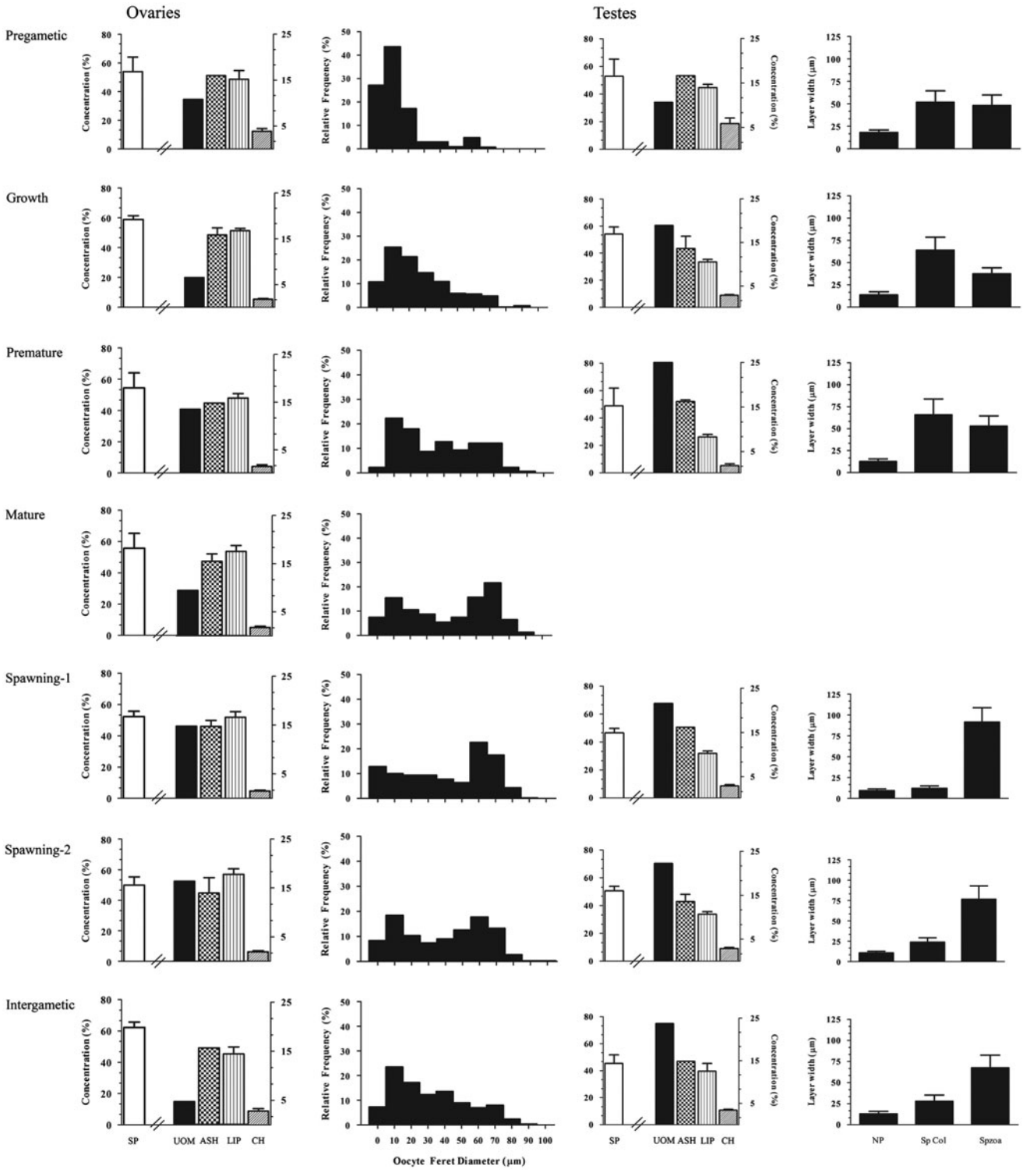

Figure 3. *A. dufresnii* Blainville, 1825, biochemical components concentration (% dry weight), oocyte maximum diameter frequency distribution in ovaries and spermatogenic, spermatozoa, and nutritive layers in testes of the Nuevo Gulf population. SP: Soluble proteins; LIP: Lipids; CH: Carbohydrates; UOM: unmeasured organic material.

lipid content while TCA-soluble carbohydrate content was very low in both sexes (♀: $F_{(2.242)} = 231.04$, $p < 0.0001$; ♂: $F_{(2.254)} = 331.74$, $p < 0.0001$). In ovaries, soluble protein and lipid content differed among stages. Both components increased from the pregametic stage to maturity. Values remained high at spawning and

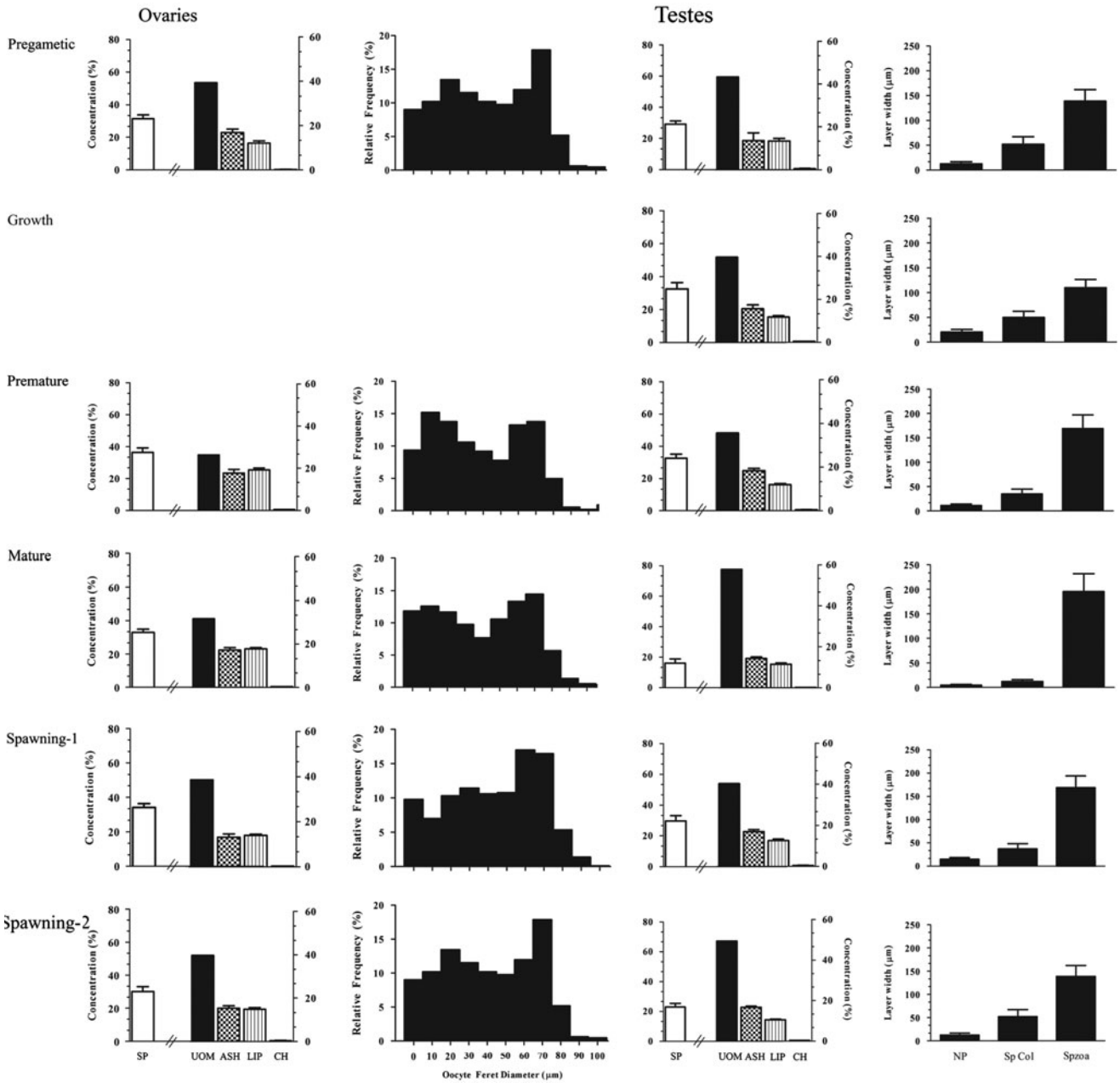

Figure 4. *A. dufresnii* Blainville, 1825, biochemical components concentration (% dry weight), oocyte maximum diameter frequency distribution and spermatogenic, spermatozoa, and nutritive layers in testes of San Jorge Gulf population. SP: Soluble proteins; LIP: Lipids; CH: Carbohydrates; UOM: unmeasured organic material.

decreased abruptly at the intergametic stage ($F_{(6.59)}$ = 5.41; $F_{(6.89)}$ = 16.42; $p < 0.0001$). TCA-soluble carbohydrate content was constant among stages ($F_{(6.79)}$ = 1.82, $p = 0.10$). In testes, all biochemical components showed significant differences among stages ($F_{(5.60)}$ = 2.87; $F_{(5.85)}$ = 3.46; $F_{(5.86)}$ = 17.47, $p < 0.05$). The content of soluble proteins, lipids, and TCA-soluble carbohydrate increased from the pregametic to the premature stages and then decreased to a minimum value at the intergametic stage.

San Jorge Gulf: Content values are summarized in Table 4. Soluble protein content was significantly higher than lipid and carbohydrate contents during the reproductive cycle (♀: $F_{(2.339)}$ = 206.53, $p < 0.0001$; ♂: $F_{(2.290)}$ = 139.03, $p < 0.0001$). In ovaries, the content of soluble proteins and lipids differed significantly among stages ($F_{(4.110)}$ = 5.21; $F_{(4.110)}$ = 8.35, $p < 0.01$). By contrast, TCA-soluble carbohydrate content remained constant among stages ($F_{(4.110)}$ = 2.33, $p = 0.06$). Lipids, soluble proteins, and TCA-soluble carbohydrates increased from

Table 4. Biochemical components contents (mg.individual^{-1}) of ovaries and testes at each gonadal stage in both populations (Mean ± SE). Different letters (a, b, c) denote significant differences between means ($p < 0.05$).

	Ovaries			Testes		
Gonadal Stage	Soluble proteins	Carbohydrates	Lipids	Soluble proteins	Carbohydrates	Lipids
Nuevo Gulf population						
Pregametic	11.75 ± 5.06^{a}	1.80 ± 1.09^{a}	3.65 ± 0.74^{a}	27.13 ± 11.49^{a}	1.85 ± 0.10^{a}	5.36 ± 1.21^{a}
Growth	46.21 ± 6.61^{b}	1.36 ± 0.19^{a}	11.55 ± 1.44^{b}	63.09 ± 11.20^{a}	2.36 ± 0.25^{a}	8.62 ± 0.76^{b}
Premature	47.40 ± 24.82^{b}	1.71 ± 0.07^{a}	15.08 ± 4.15^{b}	95.26 ± 23.48^{a}	2.63 ± 0.74^{a}	13.68 ± 1.23^{b}
Mature	80.09 ± 5.43^{d}	3.12 ± 0.94^{a}	29.87 ± 7.04^{c}	–	–	–
Spawning-1	80.56 ± 11.62^{d}	2.77 ± 0.62^{a}	31.21 ± 4.21^{c}	85.64 ± 8.87^{a}	4.06 ± 0.58^{a}	17.17 ± 1.95^{b}
Spawning-2	73.39 ± 11.04^{d}	3.11 ± 0.64^{a}	26.73 ± 3.89^{c}	75.27 ± 7.33^{a}	4.18 ± 0.48^{a}	14.56 ± 1.81^{b}
Intergametic	23.53 ± 6.10^{a}	1.20 ± 0.24^{a}	6.42 ± 1.09^{a}	54.69 ± 10.08^{a}	3.09 ± 0.85^{a}	10.04 ± 3.14^{a}
San Jorge Gulf population						
Pregametic	266.17 ± 92.10^{a}	30.30 ± 8.59^{a}	115.04 ± 37.47^{a}	66.10 ± 114.04^{a}	53.31 ± 18.28^{a}	194.00 ± 66.10^{a}
Growth	–	–	–	49.64 ± 145.53^{a}	76.06 ± 13.90^{a}	254.42 ± 49.64^{b}
Premature	691.70 ± 75.57^{b}	63.28 ± 7.63^{a}	381.48 ± 37.76^{d}	15.85 ± 75.90^{a}	74.48 ± 5.48^{a}	287.22 ± 15.85^{b}
Mature	766.48 ± 77.83^{b}	70.54 ± 6.13^{a}	423.81 ± 34.26^{d}	25.00 ± 96.21^{a}	115.30 ± 8.77^{a}	333.67 ± 25.00^{b}
Spawning-1	388.42 ± 75.45^{c}	61.48 ± 10.82^{a}	207.34 ± 40.42^{d}	17.65 ± 59.36^{a}	37.08 ± 6.68^{a}	109.78 ± 17.65^{a}
Spawning-2	434.33 ± 71.96^{c}	46.20 ± 6.71^{a}	219.98 ± 43.10^{a}	34.40 ± 90.69^{a}	51.22 ± 8.19^{a}	186.14 ± 34.40^{a}

the pregametic stage to the mature stage and decreased at spawning. In testes, these contents showed significant differences along the reproductive cycle ($F_{(5.94)} = 2.80$; $F_{(5.93)} = 8.01$; $F_{(5.90)} = 6.46$, $p < 0.05$). Soluble proteins accumulated from the pregametic stage to the premature stage and decreased at the mature stage. On the other hand, lipids and TCA-soluble carbohydrates were continuously stored until maturity. Lipid, soluble protein, and TCA-soluble carbohydrate contents decreased at spawning.

Energy

Nuevo Gulf: Energy values are summarized in Table 5. Soluble proteins were the main source of energy in ovaries (1.34 kJ ± 0.12), followed by lipids (0.68 kJ ± 0.07). There was a very low-energy input from of TCA-soluble carbohydrates (0.04 kJ ± 0.04). Energy provided by the three biochemical components increased from the pregametic to the mature stages, values remained high at spawning, and a decrease was evident at the intergametic stage.

The reproductive output for ovaries, expressed as the difference between the energy content of soluble proteins, lipids, and TCA-soluble carbohydrates at mature and intergametic stages was 0.76 kJ.

San Jorge Gulf: Energy values are summarized in Table 5. Soluble proteins and lipids were the main sources of energy in ovaries (14.67 kJ ± 1.00; 12.97 kJ ± 0.83). There was a low-energy input from TCA-soluble carbohydrates (1.05 kJ ± 0.07). There was an increase in energy provided by the three biochemical components from the pregametic stage to the mature stage and a decrease at spawning.

The reproductive output for ovaries, expressed as the difference between the energy content of soluble proteins, lipids, and TCA-soluble carbohydrates at mature and spawning type-1 stages was 17.66 kJ and between mature and spawning type-2 stages was 18.44 kJ.

Discussion

The biochemical composition of *A. dufresnii* gonads is affected by gonadal stage and location. Gonads in the

Table 5. Biochemical energy contents (kJ.individual^{-1}) of ovaries at each gonadal stage in both populations (Mean ± SE).

	Nuevo Gulf population			San Jorge Gulf population		
Gonadal Stage	Soluble proteins	Carbohydrates	Lipids	Soluble proteins	Carbohydrates	Lipids
Pregametic	0.28 ± 0.12	0.03 ± 0.02	0.14 ± 0.03	4.55 ± 1.48	6.30 ± 2.18	0.52 ± 0.15
Growth	0.98 ± 0.12	0.02 ± 0.00	0.46 ± 0.06	–	–	–
Premature	1.68 ± 0.30	0.03 ± 0.00	0.60 ± 0.16	15.09 ± 1.49	16.36 ± 1.79	1.09 ± 0.13
Mature	1.89 ± 0.13	0.05 ± 0.02	1.18 ± 0.28	16.77 ± 1.36	18.13 ± 1.84	1.21 ± 0.11
Spawning-1	1.89 ± 0.28	0.05 ± 0.01	1.18 ± 0.17	8.20 ± 1.60	9.19 ± 1.78	1.06 ± 0.19
Spawning-2	1.76 ± 0.26	0.05 ± 0.01	1.06 ± 0.15	8.70 ± 1.71	10.27 ± 1.70	0.79 ± 0.12
Intergametic	0.50 ± 0.06	0.02 ± 0.00	0.24 ± 0.04	–	–	–

growth stage (only found in the NG population) were characterized by a high soluble protein concentration, probably owing to the active germinal epithelium observed in ovaries and testes, or to the high number of nutritive phagocytes, rich in protein. Early gametogenesis is characterized by high-protein synthesis, mainly Mayor Yolk Protein (Unuma et al. 2003; Walker et al. 2013). Ova/spermatozoa were observed in the lumen of premature and mature gonads, in larger quantities in the latter stage. At these gonadal stages, ovaries accumulated lipids, probably in mature ova, to serve as energy source for larval development (Walker et al. 2013). The ratio % lipids ♀/♂ was 1.9 (premature stage) in the NG population and 1.5 (premature stage and mature stage) in the SJOG population. Magniez (1983) found a value of 2.8 for the brooding echinoid *Abatus cordatus*, while this ratio was about 1.0–1.3 in non-brooding echinoids and a high ovary/testis ratio of lipid concentration at maturity was also found for *Evechinus chloroticus* (Verachia et al. 2012). Two partial spawning stages (spawning types 1 and 2) were described for gonads of *A. dufresnii* (Epherra et al. Forthcoming). These were differentiated by the larger number of residual mature gametes in spawning type-1 and the more abundant primary oocytes and wider spermatogenic column layer in spawning type-2. However, the biochemical composition of ovaries did not reflect these differences. This may show that nutrients might be stored either in numerous non-spawned ova or in the arrested new generation of oocytes, since nutritive phagocytes were at their smallest size in both spawning stages. Histochemical studies may clarify this question. In testes, during the spawning period, the residual spermatozoa were the most abundant cell type. Thus, the relatively high UOM concentration in testes may reflect the DNA accumulation of residual spermatozoa as found in *Pseudocentrotus depressus*, (Unuma et al. 2003) and *Strongylocentrotus purpuratus* (Giese 1959). The intergametic stage was only observed in the NG population. Gonads in this stage were probably recycling nutrients, either by the NPs or by autophagia (Walker et al. 2013). Ovaries in this stage showed the maximum soluble protein concentration, attributable to lysis of residual ova. Similar results were found for *A. cordatus* (Magniez 1983).

Gonads of the NG population had higher soluble protein, total lipid, and TCA-soluble carbohydrate concentrations. In the SJOG population, on the other hand, ash and UOM together represented more than 50% of gonad composition. Since in both populations, ash did not exceed 20% of dry tissue and varied little across gonadal stages, the difference relies on UOM. Gonads of the SJOG population had a higher UOM concentration than those of NG (%UOM SJOG/NG ovaries = 3.5; %UOM SJOG/NG testes = 2.1). The adjusted gonad weight was about 10 times higher in SJOG. In this population, ova were larger and numerous and testes had about twice the amount of spermatozoa. These features were reflected in the higher reproductive output of ovaries from the SJOG population. In this population, ova were present throughout the reproductive cycle and primary and vitellogenic oocytes were present at higher frequencies at all stages of gonad development (Epherra et al. Forthcoming). Sewell et al. (2008) demonstrated that more than 70% of the protein found in ovaries of *E. chloroticus* containing ova is ribosomal proteins, proteins involved in turnover, chaperones, and proteins involved in cell function and metabolism. As the SJOG population had a higher frequency of oocytes and ova and larger ova than the NG population at all the stages, it may be inferred that these types of protein may be present throughout the cycle; thus, increasing the concentration of refractive protein (reflected in high UOM concentration) in the ovaries. However, a closer examination of protein components in different ovary stages would clarify this hypothesis. As for testes, the histological analysis indicated that the width of the spermatozoan layer was always wider in the SJOG population; thus, causing the high value of UOM by DNA accumulation in spermatozoa.

The content of a biochemical constituent gives information on the absolute quantity of a nutrient that has been consumed or accumulated in a specific tissue, being independent of the presence of the other constituents. Clear gonadal patterns of soluble protein, lipid, and TCA-soluble carbohydrate contents were observed in gonads of *A. dufresnii*, though the cycles differed between sexes and populations. However, values were significantly higher in ovaries than in testes in both populations, according to the role of energy source for larval development that these compounds play in the eggs. Maximum values were observed at mature or at spawning type-1 stages, especially in ovaries of both populations. Lipid accumulation is a way of storing energy in small volume (Nelson & Cox 2000). As for carbohydrate content, although the value was very low, a significant reduction in 25% of the content from the pregametic to the growth stages in the ovaries of the NG population was observed. As for the testes, a = 50% consumption was observed from the pregametic to the premature stages. This consumption may show that carbohydrates were used as energy fuel at the initial stages of the cycle. By contrast, the gonads from the SJOG population accumulated polysaccharides from the pregametic to the mature stages. It has been suggested that glycogen is one of the primary energy fuels utilized for gametogenesis in echinoderms (Unuma et al. 2003; Marsh et al. 2013). According to Walker et al. (2013), glycogen is released from the NPs early in oogenesis to be taken up by arrested oocytes as an energy source. On the other hand, the absolute quantity of TCA-soluble carbohydrates continuously increased in different gonadal stages in testes

from the NG population and in both sexes from the SJOG population. Thus, it appears that even if TCA-soluble carbohydrate concentration is comparatively reduced when gametogenesis progresses because of an increase in concentration of lipid or protein, glycogen storage may be taking place for other uses, either in NPs or in gametes or in both. As lipid and carbohydrate contents followed gonadal development, two non-exclusive hypotheses can be proposed for their continuous accumulation: either they were not used as an energy source for gametogenesis or they were continuously supplied by continuous food input. A slight decrease in content values was found at spawning stages. Fully spent gonads were not found in the NG and SJOG populations. As gonads of spawning types 1 and 2 stages were not empty, it is apparent that contents would not decrease as expected if spawning had been complete. Soluble protein, lipid, and TCA-soluble carbohydrate contents were significantly higher in SJOG population; thus, reflecting the higher frequency of oocytes and ova, larger ova size in ovaries, and wider spermatozoan layers in testes of this population (Epherra et al. Forthcoming). When the reproductive output is taken into account, there is a great difference in energy investment between both populations studied: females from the SJOG population invested nearly 25 times more energy than those of NG population. The different histological features found may explain this difference.

The different patterns in gonadal cycle in the two populations were reflected in the biochemical composition of gonads. The continuous presence of mature unreleased gametes throughout the reproductive cycle accounts for a larger proportion of refractive protein and DNA in gonads from the SJOG population. The higher content values and reproductive output in the SJOG population reflect the larger size of gonads and gametes produced in this population. According to Epherra et al. (Forthcoming), the differences found in the population parameters (body size and density) between both populations may influence gonadal production. Since gonadal production in sea urchins is highly sensitive to food quantity and quality (Byrne et al. 1998; Hernández et al. 2011), this immense difference in production of the gonads between population may indicate differences in food availability

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