

Temporal and spatial differences in the reproductive biology of the sea urchin *Arbacia dufresnii*

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Abstract. The reproductive cycle of *Arbacia dufresnii* was studied in two contrasting populations on the eastern coast of Patagonia (Nuevo Gulf and San Jorge Gulf) from January 2008 to August 2010. Sea urchins from San Jorge Gulf had larger test diameter and heavier gonads than did sea urchins from Nuevo Gulf. *A. dufresnii* showed an annual cycle in both populations, with gametogenesis occurring mainly in the autumn and the winter. An extended spawning period took place during the spring and the summer. Sea urchins from Nuevo Gulf had a strong seasonal pattern of reproduction, with presence of gonadal stages showing re-absortion and accumulation of reserves in nutritive cells at different seasons, whereas individuals from San Jorge Gulf had mature gametes during most of the year. Interannual variation of seawater temperature affected the gamete production and spawning duration in both populations. Higher temperatures during gametogenesis enhanced gonadal growth and gametes were released early. The major population differences found in gonadal cell composition highlight the plasticity in reproductive traits of *A. dufresnii*, and may be responsible for the wide distribution of *A. dufresnii* in different environments.

Additional keywords: Echinoidea, gametogenic cycle, nutritive phagocytes, Patagonia.

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Introduction

The reproductive cycle of sea urchins often has differences in timing and amount of gametes released from year to year and among different populations (Ernest and Blake 1981; Pearse and Cameron 1991). For example, studies on the genus *Diadema* showed that seasonality in the reproductive cycle presumably becomes more pronounced at higher latitudes (Pearse 1968, 1970). Spawning periods of the austral sea urchin *Loxechinus albus* occur at different times of the year and show latitudinal variation among populations (Zamora and Stotz 1992; Pérez *et al.* 2010; Schuhbauer *et al.* 2010).

Gonads of sea urchins contain somatic (nutritive phagocytes) and germinal cells, and during the reproductive cycle, a sequence of changes in these cells can be observed (Walker *et al.* 2007). Usually, the timing of the different stages of the reproductive cycle varies among species. A complex interaction of both endogenous and exogenous factors regulates the stages of gonads of a sea urchin population, in a way that there is a considerable variability within and among natural populations (Pearse and Cameron 1991). Periodicity in the reproductive cycle in echinoids is often correlated with factors such as

photoperiod, sea temperature, lunar periodicity, phytoplankton and food availability (Walker *et al.* 2007; Mercier and Hamel 2009). Interannual differences in the reproductive cycle are also likely to occur within the same population (Pearse and Cameron 1991; Byrne *et al.* 1998; Gil *et al.* 2009). In addition, reproductive success may be also affected by population density (Wahle and Peckham 1999). Sea urchins that release their gametes in dense populations would have more fertilisation success because eggs would be less likely to suffer from sperm limitation (Levitán 1995, 1998; Styan 1997; Yund 2000); however, reproductive success in sea urchins may be adversely affected by food limitation or low gonadal and somatic growth (Andrew 1986; Levitán 1989).

Arbacia dufresnii is a common sea urchin in the south-western Atlantic Ocean, and the only species of the genus *Arbacia* inhabiting both coasts of South America (Lessios *et al.* 2012). It is distributed from Río de la Plata, Argentina (~35°S), to Puerto Montt, Chile (~41°S), around Tierra del Fuego at a depth range between 0 and 315 m (Brogger *et al.* 2013). It is usually found in areas of coarse sediments and hard substrates. It is the most abundant sea urchin in northern

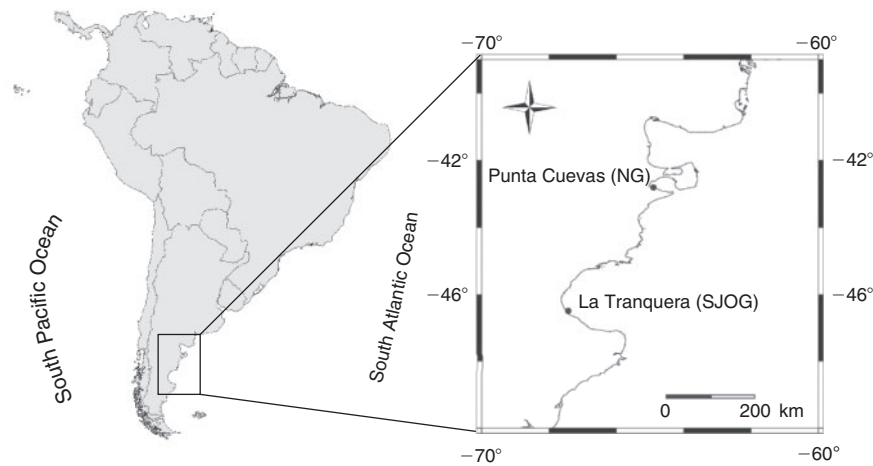


Fig. 1. Location of Punta Cuevas (Nuevo Gulf) and La Tranquera beach (San Jorge Gulf) in the coast of Patagonia, Argentina.

Patagonia, Argentina (Zaixso and Lizarralde 2000; Brogger *et al.* 2013). It has been described as carnivorous in circalittoral mussel beds (Penchaszadeh and Lawrence 1999). However, in shallow coastal areas of southern Chile, *A. dufresnii* plays an important ecological role through herbivory that affects benthic assemblages (Newcombe *et al.* 2012). Despite its wide distribution and ecological importance, the reproductive cycle of this species has been studied only in eastern coastal waters of Chiloé Island (Chile) in 1987–1988 (Kino 2010) and in Nuevo Gulf, Argentina, from 2001 to 2003 (Brogger *et al.* 2010). Reproductive studies of different populations are important so as to investigate the influence of biotic and abiotic factors and also to provide baseline data for future recruitment studies (Brewin *et al.* 2000). The wide geographic distribution of *A. dufresnii* provides an opportunity to examine latitudinal and interannual variability in reproductive traits and the influence of environmental conditions on them. We monitored two contrasting populations of *A. dufresnii* in natural conditions along the coast of Patagonia over a period of more than 2 years, so as to (1) describe and compare the reproductive cycle of *A. dufresnii*, (2) examine interannual variation in reproductive variables, and (3) assess the influence of environmental and some population parameters (e.g. body size and density) on reproductive traits at both populations.

Materials and methods

Study areas and field sampling

Sampling was conducted at two sites located on the coast of Patagonia, Argentina, namely, 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) (Fig. 1). Punta Cuevas is a wave-protected shallow rocky reef located in NG, which is a small semi-enclosed basin. Sea water temperature usually varies between 8 and 20°C, and salinity remains constant at ~34 psu (Rivas and Ripa 1989). The sea bottom has limestone platforms and sandy patches (Irigoyen *et al.* 2011). The benthic community is dominated by the algae *Codium* spp., *Dictyota* sp. and *Ulva* spp. (Piriz *et al.* 2003). However, since the invasion of the

alga *Undaria pinnatifida*, every late winter and spring, a dense forest of this invasive alga dominates the rocky reef (Casas *et al.* 2008). The population density of *A. dufresnii* ranges from 3 individuals m⁻² in winter to 10 individuals m⁻² in summer (L. Epherra, unpubl. data). However, it can reach densities of up to 30 individuals m⁻² (Irigoyen *et al.* 2011).

La Tranquera Beach is a wave-exposed rocky shore located in the central area of San Jorge Gulf (SJOG), which is a large gulf open to waves entering from the Atlantic Ocean (Isla *et al.* 2002). Sea water temperature usually varies between 7 and 16°C and salinity is ~33.5 psu (Vinueza *et al.* 2013). Extensive kelp forests of *Macrocystis pyrifera* occur in the sampling area, with a low incidence of the recently introduced *U. pinnatifida* (Zaixso *et al.*, in press). The complex biogenic structure of kelp forests and other biogenic habitats, as the ribbed mussel beds, provides important structural features, food and available space for settlement and recruitment to many species of invertebrates (Vinueza *et al.* 2013). *A. dufresnii* occurs at low densities, ranging from 0.3 to 0.6 individuals m⁻² (D. G. Gil, unpubl. data). They are usually found on hard bottoms, hidden in crevices, or in areas of high habitat complexity.

At each site, collections were made at 5–10-m depth by scuba diving. In NG, 30 individuals were collected each month between January 2008 and August 2010. In SJOG, because of low abundance of the population, ~20 individuals were collected each month from February 2008 to March 2010. Live specimens were placed in seawater and transported to a wet laboratory.

Laboratory analysis

Each individual was blotted dry and weighed to the nearest 0.01 g. Gonads were dissected out from each individual and weighed to the nearest 0.01 g. Sex was established by observing gonad coloration and histological analysis. One gonad from each individual was fixed in Davidson solution for 24 h and then preserved in ethanol 70%. Gonad tissues were dehydrated in increasing ethanol concentrations and embedded in paraffin wax, cut into sections with a microtome at 7 µm and stained with haematoxylin and eosin Y. Gonadal stages were categorised into

seven stages following the changes in the germinal cells and the nutritive phagocytes (NPs) as suggested by Walker *et al.* (2007).

Oocyte diameter frequency distribution (ODFD) was determined by image analysis of the ovary sections by using the software *ImageJ* (Abramoff *et al.* 2004; see also <http://rsb.info.nih.gov/ij/docs/faqs.html>, accessed 8 October 2014). A minimum of six females was randomly selected per month at each site and the maximum diameter of at least 200 oocytes was measured. Only oocytes sectioned through the nucleus were measured. Thickness of the spermatogenic cell layer (spermatogonia, spermatocytes, spermatids), spermatozoa, and nutritive layers were measured at the centre of the lumen by using transverse sections of at least six acini per male. The size of each acinus was also recorded by measuring the radius. Measurements were made of the gonads of all available males in the monthly sample. In both sexes, measurements were taken on different sections from each specimen as a precaution against potential heterogeneity of gonadal development within an individual.

Environmental parameters

Environmental data from Punta Cuevas (NG) and the central coast of SJOG were obtained from several sources at each monthly sample. Daylength data were provided by the Servicio de Hidrografía Naval (Argentina; <http://www.hidro.gov.ar/observatorio/sol.asp>, accessed 3 December 2010). Monthly mean seawater temperature and chlorophyll-*a* concentration were taken from Giovanni online data system, developed and maintained by the NASA GES DISC.

Data analysis

The sex ratio of *A. dufresnii* in each population was determined using a goodness of fit (*G*) test (Sokal and Rohlf 1995). Differences in body size (test diameter) between populations and sexes were tested by using a two-way ANOVA.

To evaluate the temporal pattern of the gonad cycle in each population, a GLM two-way ANCOVA, with test diameter as the covariate and sex and months as factors, was used to determine whether regressions of the average gonad wet weight (GWW) for each individual \times test diameter differed between months and sexes (Packard and Boardman 1999). In the NG population, a narrower subset of size classes was selected (NG: diameter >22 mm, $n = 22$ –30 per month). So as to examine reproductive synchrony in the gonadal cycle between sexes, populations and years, a series of cross-correlation analysis of the monthly adjusted gonad-weight means (AGW) were performed.

Monthly variations in oocyte diameter frequency distribution were assessed through a Kolmogorov–Smirnov two-sample test using Bonferroni correction procedures (Siegel and Castellan 1988). Heterogeneity of oocyte diameter development between females within each month was evaluated using the Kruskal–Wallis test. Monthly variations of spermatogenic, spermatozoa and nutritive layers were evaluated using separate one-way ANCOVA analysis, using the radius of the acini as a covariate. Correlation analyses between the layers were also performed on both populations. The assumptions of normality (Shapiro–Wilk test) and homogeneity of variances (Cochran's *C* test) were verified before all ANOVA analysis. Homogeneity of slopes

was also tested previous to the ANCOVAs analysis and showed no significant ($P > 0.05$) differences among slopes in all cases.

Multiple linear regressions and time-lag correlation analysis were used to examine relationships between environmental variables (temperature, daylength and chlorophyll-*a*) and gonadal development (AGW) for each sex and both populations. Regression models were estimated using forward-selection stepwise-regression procedures. The robustness of the reduced model was assessed by testing the residuals for normality (Shapiro–Wilk test). Standardised regression coefficients were used to understand the relative importance of predictor variables for explaining variations in the AGW. Collinearity among predictor variables was inspected using variance inflation factor (VIF) values, resulting in values below 1.4. All statistical analyses were performed with STATISTICA 7.0 (StatSoft, Inc., Tulsa, OK, USA). A significance level of 5% was assumed throughout the study.

Results

Sex ratio and body size

Monthly sex ratios did not differ significantly from 1 : 1 for both populations. In the NG population, the sex ratio of *A. dufresnii* for the pooled data was significantly different from the expected 1 : 1 ratio (females 1.17 : 1 males, $n = 907$, $G = 5.48$, $P < 0.05$), whereas it did not differ in the SJOG population ($n = 329$, $G = 0.003$, $P = 0.95$).

Mean test diameter differed significantly between populations. Sex differences were observed only in the SJOG population (ANOVA, sex \times population interaction, $F_{1,1175} = 6.8$, $P < 0.01$), where males were slightly larger than females (mean \pm s.e.; NG females: 28.9 ± 0.18 mm; NG males: 28.6 ± 0.19 mm; SJOG females: 51.7 ± 0.32 mm; SJOG males: 52.8 ± 0.32 mm). Test diameter distributions of the populations did not overlap, with larger body sizes in the SJOG population (see Fig. S1, available as Supplementary material for this paper).

Gonadal cycle

The adjusted gonad wet weight (AGW) showed a seasonal reproductive pattern in both populations. Sex, month and the sex \times month interaction were significant in the NG population, whereas only main effects were significant in the SJOG population (Table 1). Overall, testes weights were higher than ovary weights throughout the reproductive cycle of both populations. However, some differences in the pattern between sexes were observed.

The monthly gonadal pattern at NG population was synchronous between sexes ($r = 0.92 \pm 0.18$, $P < 0.001$), and the interaction sex \times month observed at this population (Table 1) indicated that males had a larger gonad most of the cycle, whereas the testes remained similar to ovaries after spawning periods (Fig. 2a). The reproductive pattern in the NG showed interannual differences. The mean AGW values increased from February 2008 (austral summer) to September–October 2008 (austral spring) when maximum values occurred. A prolonged spawning event in both sexes was indicated by the drop of AGW during late spring. Values slightly increased during the 2009 summer and decreased again in March. AGW recovered during the austral autumn and the winter and remained high until the

Table 1. Two-way ANCOVA test for differences in adjusted gonad wet weight of *Arbacia dufresnii* between sexes and months in the sites studied
The effect of test length was included as a covariate

	NG population					SJOG population				
	SS	d.f.	MS	F	P	SS	d.f.	MS	F	P
Intercept	97.54	1	97.54	278.46	<0.0001	4.75	1	4.75	19.96	<0.0001
In test length	40.42	1	40.42	115.39	<0.0001	13.76	1	13.76	57.86	<0.0001
Month	374.16	29	12.9	36.83	<0.0001	75.52	20	3.78	15.88	<0.0001
Sex	24.81	1	24.81	70.82	<0.0001	5.83	1	5.83	24.52	<0.0001
Month × Sex	15.37	29	0.53	1.51	<0.05	6.83	20	0.34	1.44	0.1
Error	266.93	762	0.35			68.01	286	0.24		

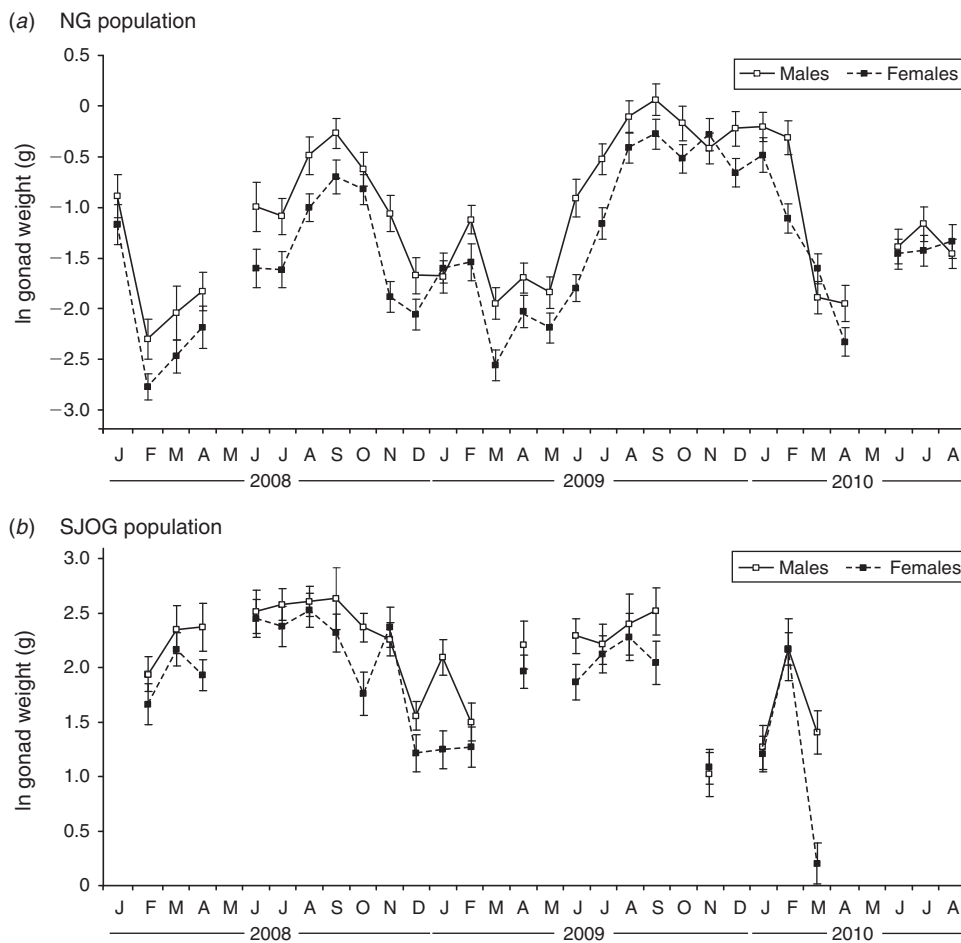


Fig. 2. In gonad weight mean plots for females and males of *Arbacia dufresnii* by month, adjusted for the effect of test length (log-transformed) in (a) Nuevo Gulf (NG) and (b) San Jorge Gulf (SJOG) populations. These are the adjusted means (± 0.95 confidence interval, $n = 25-30$ (NG), $n = 15-23$ (SJOG)) if test length was held constant at the overall mean of 28.60 mm for NG and 51.93 mm for SJOG.

next summer. Spawning in this year occurred during late summer. Interannual differences were also indicated by the presence of higher AGW during the second year of sampling. Time-lag correlation analysis of the gonad AGW cycle between years 2008 and 2009 showed maximum correlation at 1-month lag, indicating moderate interannual synchrony in the

reproductive cycle in both sexes (females: $r = 0.79 \pm 0.32$; males: $r = 0.75 \pm 0.32$; $P < 0.05$).

In the SJOG population, gonad weights were larger than those in the NG population and testes weight remained higher than ovary weight throughout the cycle (no interaction effect, Table 1), showing synchrony of male and female gonadal cycles

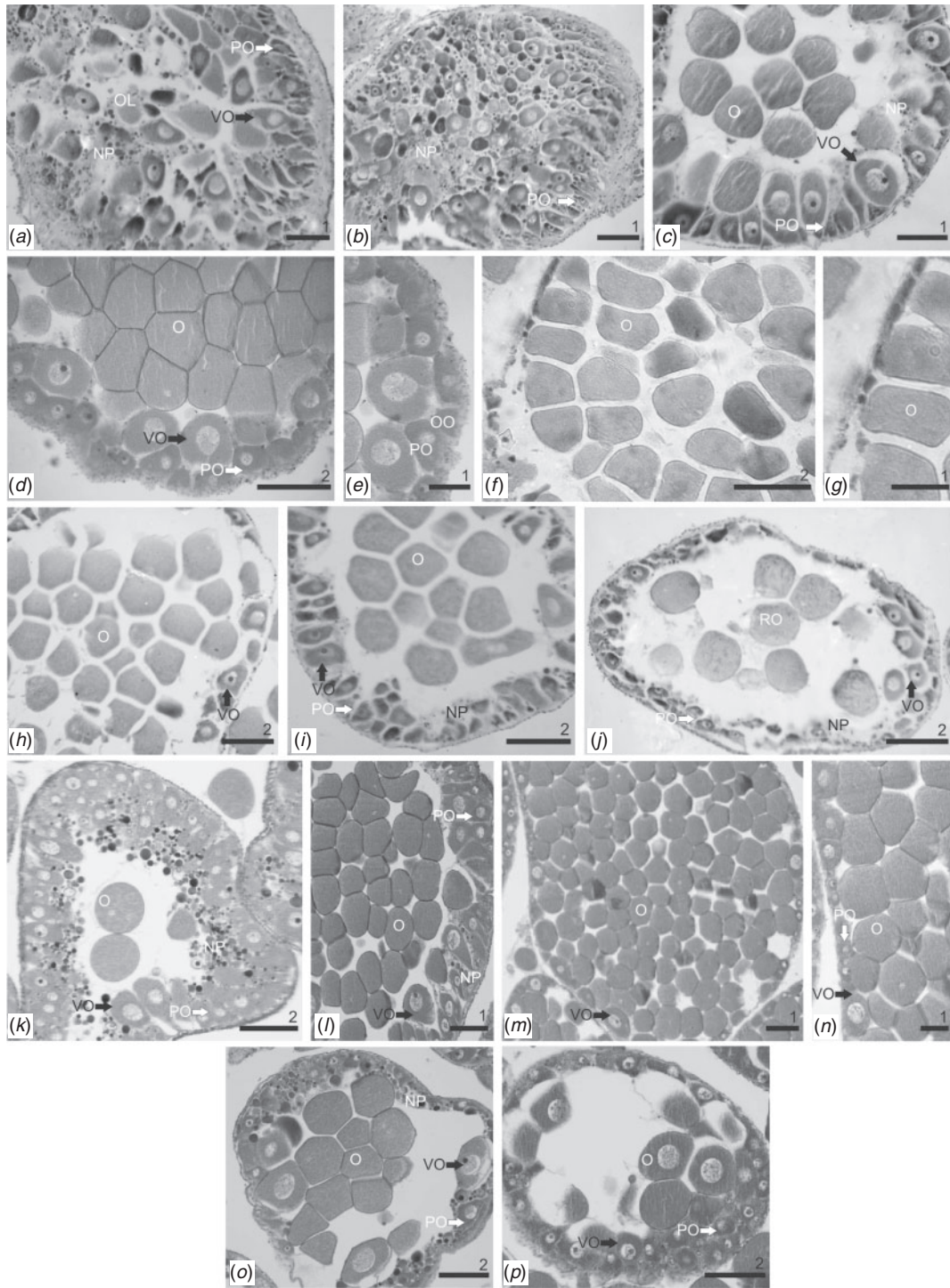


Fig. 3. Histology sections of ovaries of *Arbacia dufresnii*. (a–j) Ovaries from the Nuevo Gulf (NG) population. (k–p) Ovaries from the San Jorge Gulf (SJOG) population. (a) Pregametic stage. (b) Growth stage. (c) Premature stage. (d) Mature stage from the winter. (e) Detail of d. (f) Mature stage from the summer. (g) Detail from f. (h) Spawning Type-1. (i) Spawning Type-2. (j) Intergametic stage. (k) Pregametic stage. (l) Premature stage. (m) Mature stage. (n) Detail from m. (o) Spawning Type-1. (p) Spawning Type-2. NP, nutritive phagocytes; O, ova; OL, ova in lisis; OO, oogonia; PO, previtellogenic oocyte; RO, residual oocyte; and VO, vitellogenic oocyte. Scale bars 1 = 50 μm; 2 = 100 μm.

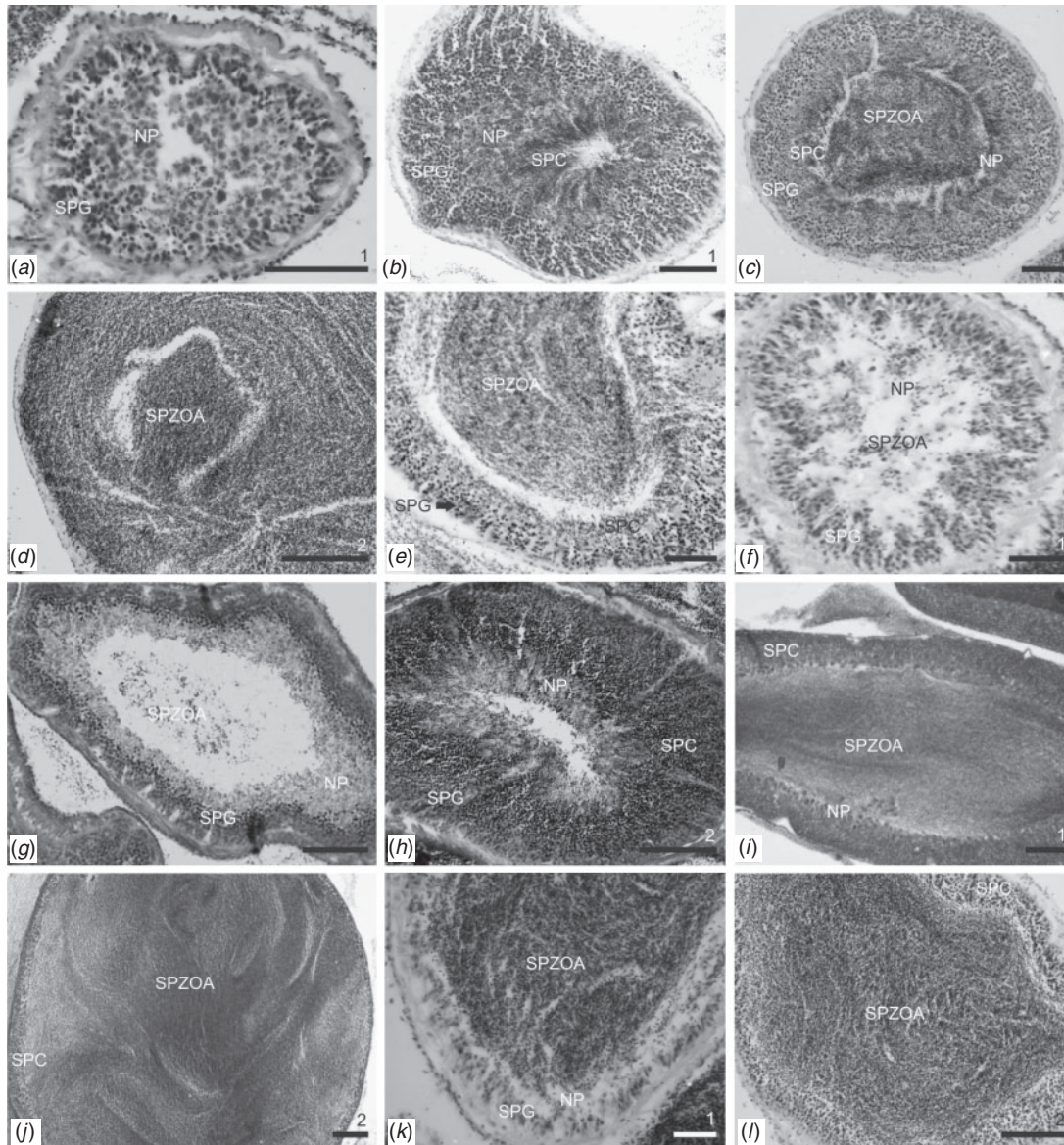


Fig. 4. Histology sections of testes of *Arbacia dufresnii*. (a–f) Testes from the NG population. (g–l) Testes from the SJOG population. (a) Pregametic stage. (b) Growth stage. (c) Premature stage. (d) Spawning Type-1. (e) Spawning Type-2. (f) Intergametic stage. (g) Pregametic stage. (h) Growth stage. (i) Premature stage. (j) Mature stage. (k) Spawning Type-1. (l) Spawning Type-2. NP, nutritive phagocytes; SPC, spermatogenic column; SPG, spermatogonia; and SPZOA, spermermatozoa. Scale bars 1 = 50 μm ; 2 = 100 μm .

($r = 0.08 \pm 0.21$, $P < 0.001$, Fig. 2b). The mean AGW values were high from summer to early spring 2008. A prolonged and intermittent spawning period was indicated by several drops of the AGW during spring 2008 and summer 2009. AGW recovered again and remained high from autumn to late winter 2009. A decrease in values of the average AGW during the spring indicated a spawning event. Values increased again in February 2010 and dropped sharply next month, suggesting another spawning event in the summer. Monthly patterns of AGW and spawning periods were similar between 2008 and 2009, particularly for males (females: $r = 0.45 \pm 0.38$, $P = 0.31$; males: $r = 0.77 \pm 0.38$, $P < 0.05$).

The gonadal cycle from the NG population showed a more seasonal pattern than that from the SJOG population. Cross-correlation analyses on monthly mean AGW values between populations indicated that the gonadal cycle did not show synchrony between populations (females: $r = 0.03 \pm 0.23$, $P = 0.899$; males: $r = 0.08 \pm 0.23$, $P = 0.74$).

Histological analysis

The NG and SJOG populations did not share exactly the same stages (Figs 3, 4). The features of each gonadal stage are described in Table 2.

Table 2. Gonadal stages of *Arbacia dufresnii*
NPs, nutritive phagocytes; NG, Nuevo Gulf; SJOG, San Jorge Gulf

Gonadal stage	Ovaries	Testes
Pregametic	Few or none residual ova in lysis Primary and vitellogenic oocytes from previous cycle Amitotic oogonias NPs start to fill the lumen	Few or none residual spermatozoa No activity in the germinal epithelium No spermatogenic columns NPs are abundant (larger at SJOG)
Growth	Activity in germinal epithelium New primary oocytes Vitellogenic oocytes from previous cycle NPs are filled with content and surrounding the vitellogenic oocytes Stage not present in the SJOG population	Activity in germinal epithelium Spermatogenic columns projecting into the lumen NPs are abundant and surrounding the columns
Premature	Activity in germinal epithelium Lumen filling with ova NPs surrounding the vitellogenic oocytes	Activity in germinal epithelium Lumen filling with spermatozoa NPs are abundant and surrounding the columns
Mature	Lumen filled with store mature ova NPs are small At NG: winter: new primary and vitellogenic oocytes; summer: no primary or vitellogenic oocytes In SJOG the germinal epithelium is active	Lumen filled with spermatozoa NPs still present Spermatogenic columns small or negligible In SJOG the germinal epithelium is active
Spawning		
Type-1	Large amount of residual ova Few primary and vitellogenic oocytes NPs in their smallest size	Large amount of residual spermatozoa No spermatogenic columns NPs in their smallest size
Type-2	Few residual ova Large amount of primary oocyte and vitellogenic oocytes NPs in their smallest size	Low amount of residual spermatozoa Spermatogenic columns present NPs in their smallest size
Intergametic	Phagocytosis of residual ova Arrested primary and vitellogenic oocytes from previous cycle NPs are few and depleted Amitotic oogonias Stage not present in the SJOG population	Residual spermatozoa No activity in germinal epithelium NPs are few and depleted Stage not present in the SJOG population

Synchrony of gonadal stages between sexes was more evident in gonads of the SJOG population. However, discrete reproductive cycles were more evident in the NG population. Generally, in both populations, gametogenesis began in the autumn until the end of the winter, when a long spawning period during the spring and the summer was observed. Spawning events involved individuals that had accumulated mature ova during the winter or had rapid maturation during the summer (Fig. 5). Two distinct reproductive cycles were detected in the NG population, the first of which began in February–March 2008 and ended by April 2009, and the second started in March–April 2009 and finished by April 2010. In contrast, the SJOG population did not show a clear cycle because of the lack of intergametic stage and dominance of premature stages most of the year. However, spawning occurred during a limited interval, resulting in a seasonal reproductive cycle.

Oocyte diameter frequency distributions (ODFDs) showed an annual cycle in both populations. In the NG population, a lack of synchrony of oocyte development between females at each month with a few exceptions was detected (K–W test, see Fig. S2, available as Supplementary material for this paper). However, frequency distributions showed an annual pattern, with significant monthly changes (K–S two-sample tests, $P < 0.001$ in all cases). Interannual variations in the monthly pattern were evident, although the overall ODFD remained

similar between 2008 and 2009 (K–S test, $P > 0.10$). Bimodal and unimodal distribution were observed in the NG population. Spawning events were recorded during summer and spring. In early summer (January) of each of the 3 years, there were two clear cohorts, one related to ova and a second to previtellogenic oocytes ($< 20 \mu\text{m}$). Following spawning, distributions were generally unimodal because ovaries were filled with previtellogenic oocytes. The oocyte size increased to $30\text{--}60 \mu\text{m}$ during the growth period (autumn–early winter), although previtellogenic oocytes were always present. Ova ($60\text{--}85 \mu\text{m}$) began to accumulate during the winter and formed a distinct cohort.

In the SJOG population, the oocyte distribution varied seasonally and synchrony among females in each month was absent (K–W test, see Fig. S3, available as Supplementary material for this paper). The ODFD was bimodal during most of the year. Interannual variations in the monthly pattern were also evident. The overall ODFD differed between 2008 and 2009 (K–S test, $P < 0.001$), with a major contribution of large oocytes in the year 2009. Spawning was evident during the spring in 2008 and the spring and the summer in 2009–2010. ODFD after spawning was generally bimodal with frequent previtellogenic and vitellogenic oocytes, suggesting a rapid or simultaneous recovery. Ova ($60\text{--}100 \mu\text{m}$) were nearly always present throughout the year, and most numerous during winter

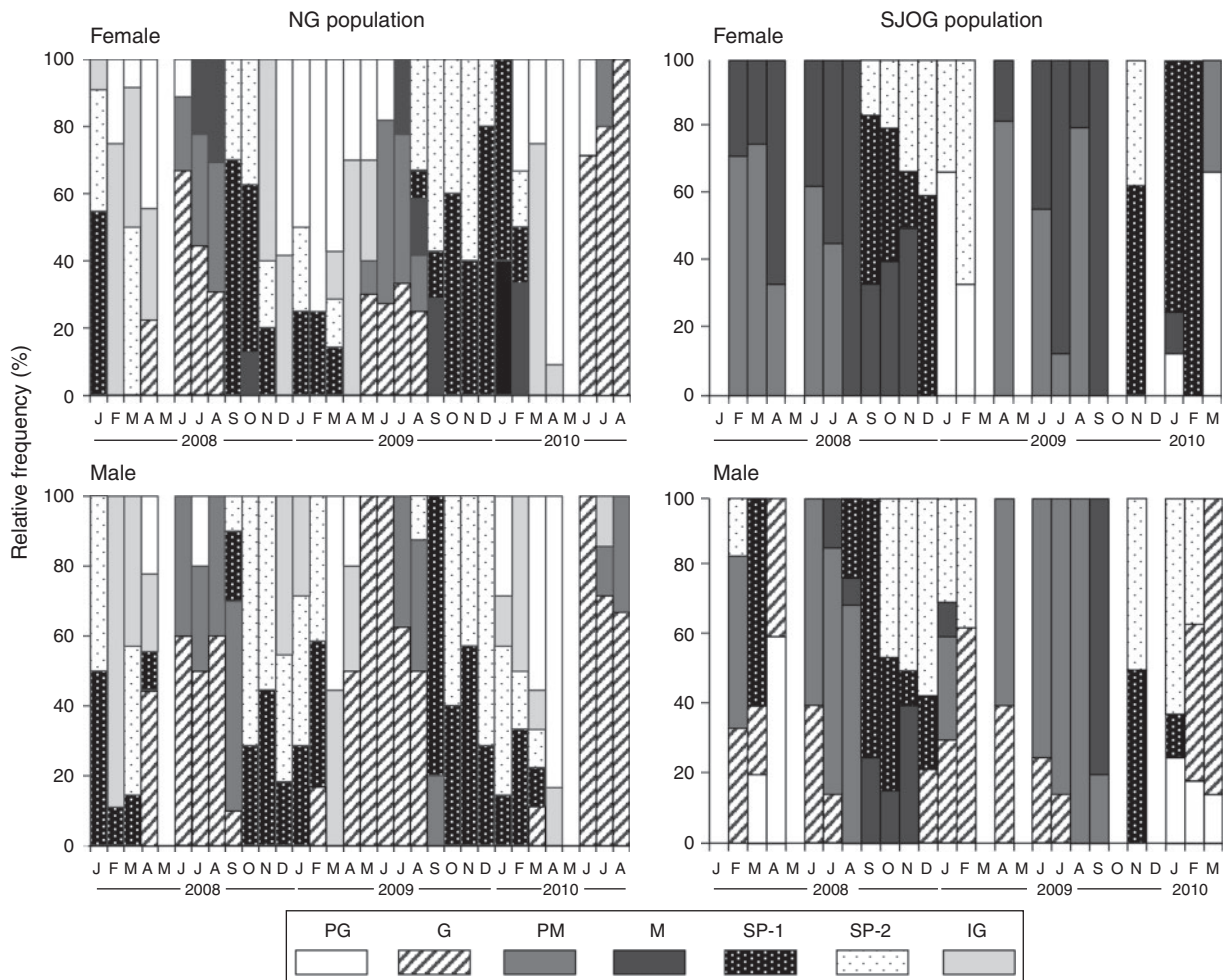


Fig. 5. Gametogenic cycle of females and males of *Arbacia dufresnii* from Nuevo Gulf (NG) and San Jorge Gulf (SJOG). Histograms show relative frequencies of gonad stages ($n = 25\text{--}30$ (NG), $n = 15\text{--}23$ (SJOG)). PG, pregametic; G, growth; PM, premature; M, mature; SP-1, spawning type-1, SP-2, spawning type-2; and IG, intergametic.

and early spring. After spawning, their abundance decreased significantly.

The pooled ODFDs among sites were dissimilar (K-S test, $P < 0.001$). Both populations showed a bimodal distribution, but the NG population had many more previtellogenic oocytes than mature ova, whereas in the SJOG population the pattern was inverted.

The ANCOVA analysis showed that the spermatogenic, spermatozoa and NPs layers varied monthly in each population (see Table S1 in the Supplementary material). The width of the spermatogenic and spermatozoa layers were negatively correlated in both populations (NG: $r = -0.92$, SJOG: $r = -0.93$; $P < 0.001$). The NP layer in the NG population showed a higher seasonal pattern than that in the SJOG population, owing to presence of intergametic stages in the testes of the NG population. The spermatozoa layer in sea urchins from the NG population was prominent during most of the cycle. However, when growth and premature stages occurred, the thickness of the spermatogenic column increased (Fig. 6a). This pattern was not observed in the testes of the SJOG population, and the

spermatogenic layer never exceeded the spermatozoa layer. The spermatogenic layer remained high during the spring and the summer (Fig. 6b). Spermatozoa layer was wider in the SJOG population than in the NG population during the entire reproductive cycle. In contrast, spermatogenic and NPs layers were less developed in the SJOG population than in the NG population.

Environmental parameters

Seawater temperature ranged from 10.2°C in the winter to 18.3°C in the summer in NG, showing interannual differences (Fig. 7). Cross-correlation with time-lag analysis between mean adjusted gonad weights (AGW) and seawater temperature indicated that maximum correlation occurred at a 1-month lag for both sexes (females: $r = -0.71 \pm 0.19$; males: $r = -0.69 \pm 0.19$, $P < 0.05$). In SJOG, seawater temperature ranged from 7.3°C in the winter to 17.1°C in the summer and was negatively correlated with AGW (no lag, $r = -0.42 \pm 0.22$, $P < 0.05$).

Chlorophyll-*a* concentration showed a more irregular pattern of variation, with peaks occurring mostly in spring and summer

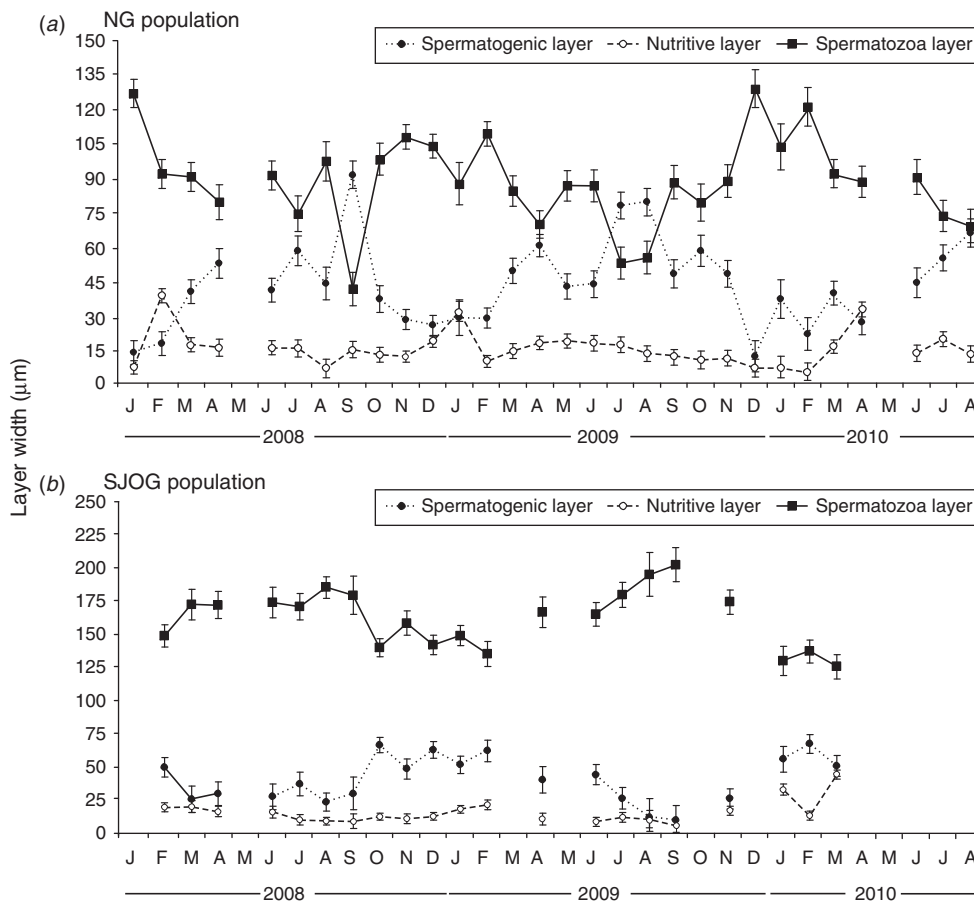


Fig. 6. Spermatozoa, spermatozoa and nutritive layers monthly variation in (a) Nuevo Gulf (NG) and (b) San Jorge Gulf (SJOG) populations. These are the adjusted means (± 0.95 confidence interval, $n = 8-15$ per month) if acini radius was held constant at the overall mean of $149 \mu\text{m}$ for NG and $209 \mu\text{m}$ for SJOG.

(Fig. 7). No association was found between AGW and mean monthly values. Cross-correlation analysis between AGW and daylength indicated that a positive correlation occurred at NG at a 2-month lag for both sexes (M: $r = 0.68 \pm 0.19$, H: $r = 0.69 \pm 0.19$; $P < 0.05$), whereas at SJOG, a negative correlation was found (no lag; females: $r = -0.54 \pm 0.21$; $P < 0.05$; males: $r = -0.64 \pm 0.22$).

In the NG population, the multiple regression analysis indicated that only seawater temperature and photoperiod explained more than 42% of the variation in AGW in both males and females (Table 3). In addition, a negative relationship was found for seawater temperature and a positive relationship for photoperiod. In SJOG population, forward selection found that only seawater temperature was a significant predictor of the reproductive cycle in females, with a negative relationship. In males, only photoperiod was a predictor of the reproductive cycle, with a negative relationship (Table 3).

Discussion

Annual reproductive cycles have been observed in many species of temperate sea urchins (Pearse and Cameron 1991). In Patagonia, Argentina, *Arbacia dufresnii* is a gonochoric species

with an annual reproductive cycle. In the NG population, *A. dufresnii* had a female-skewed sex ratio, whereas the SJOG population did not. Instead, Brogger *et al.* (2010) found a male-skewed sex ratio in the NG population for the period 2001–2003, indicating that these discrepancies may reflect either bias in random sampling or different environmental conditions affecting mortality or growth between sexes.

Both populations have contrasting differences regarding body size and density. *A. dufresnii* from the SJOG population had a lower density and a mean test diameter almost twice as large as sea urchins from NG. An inverse relationship between body size and population density has been observed in many species of sea urchins (Levitan 1988; Wahle and Peckham 1999). High densities may ensure high rates of fertilisation in broadcast spawners before the gametes are diluted in the sea (Levitan *et al.* 1992; Levitan and Young 1995). However, high densities may also negatively affect gonadal growth and gamete production because of competition and low food availability (Levitan 1989; Fernandez and Boudouresque 1997; Tomas *et al.* 2005; Siikavuopio *et al.* 2007). In addition, food availability may affect adult body size, because good nutritional conditions may enhance growth in sea urchins (Turon *et al.* 1995; Heflin *et al.* 2012). A positive relation between body size and gonad

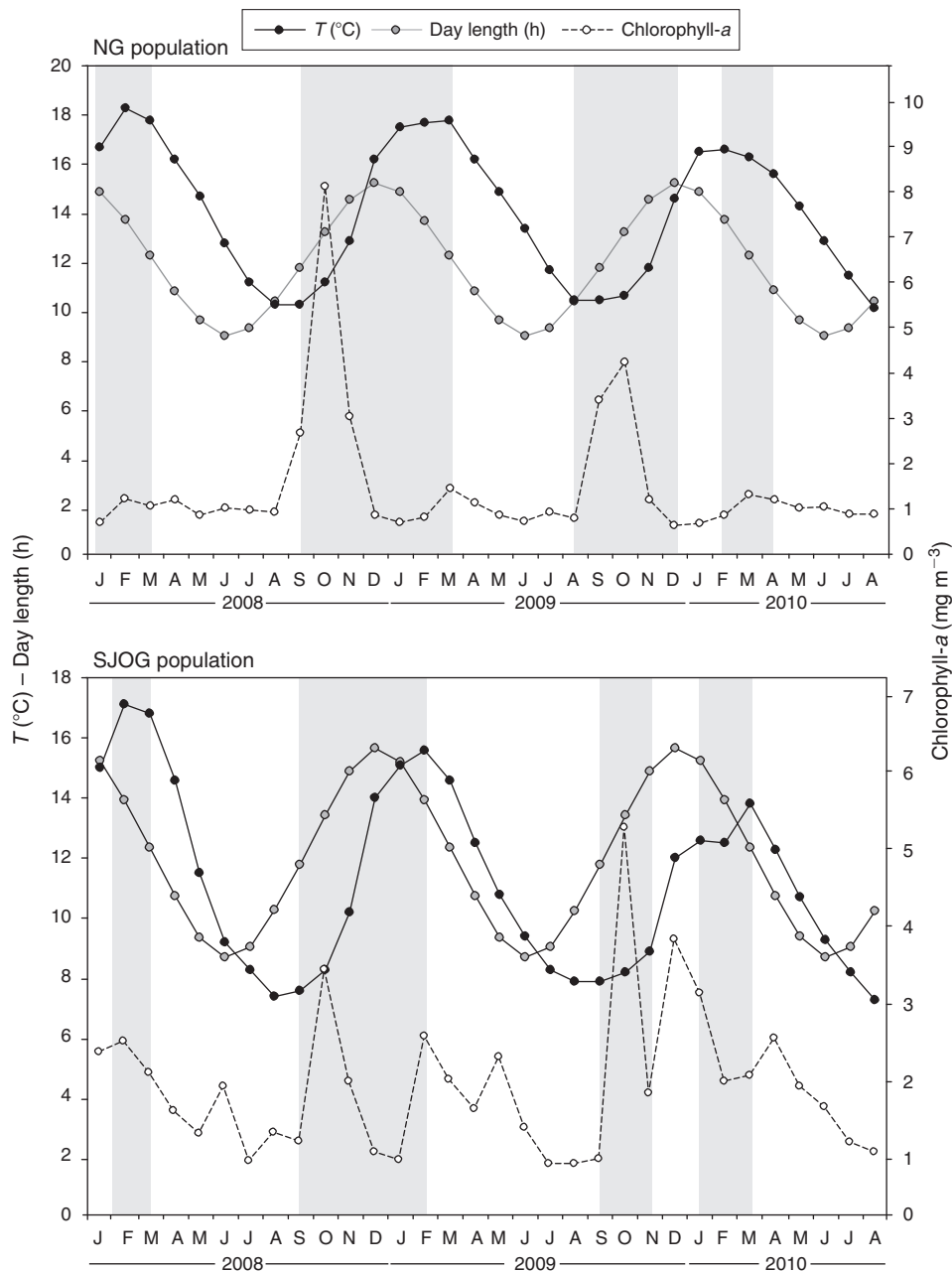


Fig. 7. Monthly variations of some environmental factors in both populations. Gray areas shows months with evidence of spawning.

weight is known to occur in sea urchins (Marsh and Watts 2007). In *A. dufresnii*, sea urchins from SJOG have gonads 20 times heavier than do sea urchins from NG. Although gonads from the NG population had a lower gamete production, the higher population density may compensate by increasing the reproductive success, as found in *Diadema antillarum* (Levitan 1991) and *Tetrapygus niger* (Zamora and Stotz 1993).

Although ovaries are usually larger than testes in sea urchins (Lawrence 1987), testes of *A. dufresnii* were larger than ovaries in both populations. A similar pattern was observed in brooding heart urchins *Abatus cavernosus* (Gil et al. 2009) or *A. cordatus*

(Magniez 1983). This could indicate that males produced more gametes because of the prolonged spawning period.

Reproductive cycles in both populations were synchronous between sexes. However, synchrony of gonadal stages was more evident in gonads of sea urchins from SJOG, which might be related to the low population density observed. The influence of population density on the synchrony of the reproductive cycle was important in the congeners of *Echinometra viridis* and *E. lucunter* (Lessios 1981).

The genus *Arbacia* has five valid species, four are distributed exclusively in neotropical regions; the only species able to live

Table 3. Summary of stepwise multiple regression analysis (forward selection) to examine the log adjusted mean of gonad weight of *Arbacia dufresnii* as a function of sea-water temperature (SST, °C), daylength (h) and chlorophyll-*a* (mg m⁻³) in Nuevo Gulf (NG) and San Jorge Gulf (SJOG) populations

Coefficient (*b*) and standardised regression coefficient (β) for each significant predictor are included. Variables entered and exited the model at $P = 0.15$, tolerance = 0.1

	Predictor	<i>b</i> (\pm s.e.)	<i>P</i>	β	Model <i>R</i> ²	Model <i>P</i>
NG – females	SST	-0.18 (0.04)	<0.001	-0.77	0.51	<0.001
	Daylength	0.16 (0.05)	<0.005	0.54		
	Constant	-0.70 (0.62)				
NG – males	SST	-0.17 (0.04)	0.001	-0.77	0.42	<0.005
	Daylength	0.12 (0.05)	0.028	0.54		
	Constant	-0.23 (0.69)				
SJOG – females	SST	-0.13 (0.04)	0.012	-0.59	0.35	<0.05
	Constant	3.19 (0.51)				
SJOG – males	Daylength	-0.12 (0.04)	<0.05	-0.62	0.38	<0.05
	Constant	-3.59 (0.51)				

in cold Subantarctic waters is *A. dufresnii* (Wangensteen 2013). Annual cycles are often found in temperate sea urchins (Pearse and Cameron 1991), and spawning periods in spring ensure the environmental conditions for the fertilisation of gametes and survival of larvae (Lessios 1981). Despite the observed inter-annual variations, the reproductive cycle of *A. dufresnii* displays an annual cycle at both sites. In Chiloé Island, Chile, mature individuals occur throughout the year and the spawning season begins in spring and extends through summer (Kino 2010). *A. lixula* from Brazil and Spain has mature individuals throughout most of the year and long spawning periods (Tavares 2004; Wangensteen *et al.* 2013). This pattern was also found in *A. dufresnii* from the SJOG population. This supports the hypothesis proposed by Wangensteen *et al.* (2013) that the presence of mature individuals throughout most of the year could be a conserved trait from the tropical past of the genus *Arbacia*.

In the NG population, mature stages were absent or poorly represented. Brogger *et al.* (2010) reported a partial spawning event in spring and a major one in summer in NG during 2000–2003. In the present study, the spawning period started in spring and lasted until the end of summer. However, no spent gonads were found. The major temporal differences found between these two studies may be due to interannual variation or the result of changes in the benthic community owing to the increase in biomass of the invasive macroalga *Undaria pinnatifida* (Irigoyen *et al.* 2011). The presence of *U. pinnatifida* is associated with a decrease in species richness and diversity of native algae in Nuevo Gulf (Casas *et al.* 2004). This probably affects the diet of *A. dufresnii*. Sea urchins often show plasticity regarding their feeding habits (Lawrence 1975; Lawrence and Lane 1982).

Maturation and spawning of gametes in marine invertebrates may also be influenced by environmental conditions (Lawrence and Lane 1982; Pearse and Cameron 1991). In *A. dufresnii*, there was a significant correlation between the photoperiod and AGW. In the SJOG population, the correlation was negative, whereas in NG population, it was positive. However, the temporal variability observed in the onset of the spawning period among years suggests that photoperiod may not be a

proximate cue for the release of gametes. In contrast, there was a significant negative correlation between seawater temperature and AGW for both populations. According to Byrne *et al.* (1998), seawater temperature may influence gamete storage and spawning duration. In NG, seawater temperature during gametogenesis in 2009 was higher than in the other years. Furthermore, gonadal mass was also higher and spawning started in August, 1 month earlier than in the Year 2008. During gametogenesis in 2010, seawater temperature was similar to that in 2008, and in August 2010 there were no individuals in the spawning stage. In addition, before spawning there were more ova and spermatozoa in 2009 than in the other years and mature individuals were also found during the summer. In *A. lixula* from the Mediterranean Sea, an increase of seawater temperature during gametogenesis resulted in larger gonads (Wangensteen *et al.* 2013). In the SJOG population, during 2008, spawning started in September, whereas in 2009, only mature gonads were found during the same month. The average seawater temperature in 2009 was lower than in the other years. A similar pattern was found for *P. lividus* from the Mediterranean waters (Lozano *et al.* 1995). It appears that seawater temperature may influence production of gametes and the onset of spawning in *A. dufresnii*. Although there was no correlation between chlorophyll-*a* and AGW, the spawning period in both populations coincided with phytoplankton blooms. Sea urchin spawning events have been observed close to phytoplankton blooms (Starr *et al.* 1991; Gaudette *et al.* 2006). The role of phytoplankton as inductor of spawning has been described by Reuter and Levitan (2010).

Seawater temperature may influence not only gonad production but also food availability; thus, the effects of these two factors in the gonadal cycle of *A. dufresnii* cannot be separated. Apparently, regardless of the environmental factors involved in triggering the gametogenesis process, the proximate cue to begin gonial cell mitosis is the availability of nutrients (Walker *et al.* 2007). Gonadal production in sea urchins is highly sensitive to food quantity and quality (Byrne *et al.* 1998; Hernández *et al.* 2011). Sea urchins with good nutritional state may invest more energy in gonad production, producing gametes during longer periods (Laegdsgaard *et al.* 1991; Tavares 2004; Walker

et al. 2007). The different types of spawning found in *A. dufresnii* may be related to the nutritional state of the individual. Sea urchins with a better nutritional state may produce and store new gametes while spawning. In the NG population, females not only had larger gonads in 2009, but also showed prevalence of spawning Type-2 stages, and the occurrence of mature gonads while spawning in the summer. This might indicate a higher input of nutrients during 2009, which allowed the production of more gametes. In contrast, in the SJOG population, females produced and stored new gametes while spawning in both years, since only spawning Type-2 was found. The pattern observed may also explain the absence of growth stage in the SJOG population. Males showed a higher synchrony in gonadal stages in NG, suggesting that nutrient availability may not affect the gonadal cycle of males as strongly as in females. In the NG population, males started spawning without reaching maturity and spermatogenic columns were well developed when spawning occurred. However, release of spermatozoa lasted several months. The proliferation of gametes while spawning may support this extended spawning period. In contrast, males and females at SJOG showed the same pattern and new gametes were produced during the spawning period. Spermatozoa were present in the gonads during the entire year. This same pattern was found in *A. lixula* in Brazilian and Spanish coasts (Tavares 2004; Wangensteen et al. 2013). According to Walker (1982), sperm differentiation is a fast process that would explain the rapid production of new spermatozoa in males. In summary, gamete production in the SJOG population was continuous during most of the year; suggesting that sea urchins from this population may have a higher availability of nutrients than those from the NG population.

Sea urchin gonads contain somatic cells (nutritive phagocytes, NPs) and germinal cells. NPs are responsible of accumulation of nutrients and lysis of residual gametes (Walker et al. 2007). The absence of intergametic and low frequency of pregametic stages in both sexes in the SJOG population may indicate that the NPs are not storing nutrients. In contrast, in NG population, the intergametic and pregametic stages had high frequencies of occurrence after and during spawning. The development of a NP layer observed at these stages indicates that recycling and storing nutrients is necessary in this population. Food availability influences the gonadal cellular composition in *Strongylocentrotus droebachiensis* (Garrido and Barber 2001).

This study has shown that *Arbacia dufresnii* displays spatial and temporal differences in its reproductive cycle. In NG, it showed a reproductive pattern different from those of other species of *Arbacia* that have been studied. These differences may be linked to diverse causes such as a population structure with prevalence of small body-size individuals, or be related to local hydrographic features. Besides, the steady increase in biomass of *Undaria pinnatifida* at NG is a major factor altering the benthic community (Casas et al. 2004). Therefore, the availability and predictability of food to *A. dufresnii* has probably changed, and a cascade effect on its reproduction is expected. The major population differences found in gonadal cell composition highlight the plasticity in reproductive traits of *A. dufresnii*. This high plasticity may be responsible for the wide distribution of *A. dufresnii* in different environments.

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