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Geographical implications of seasonal reproduction in the bat star *Asterina stellifera*

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Abstract

The reproductive cycle and environmental cues that regulate gonad production in *Asterina stellifera* were studied from April 2009 to April 2011 in a rocky subtidal habitat at the southernmost limit of its distribution (Mar del Plata, Argentina). The geographic variation in reproductive traits between latitudinal range limits was analyzed. The gonadal and pyloric caeca weight varied with sea star size and time in both sexes. Despite a previous study suggested absence of recruitment in a 4 years period, our data of the same period demonstrated that spawning happens from early spring to early summer. The gonad and pyloric caeca weight did not show an inverse relationship, this suggested that there is no dependence on energy transfer between the organs and that the bat star presented a good nutritional state. Seawater temperature appears to be the variable explaining gonad proliferation at the range limits of *A. stellifera* distribution. Furthermore, differences in sex ratio, oocyte production, oogenesis duration and capability of energy transformation into ova were found between range limits.

Key words: reproductive cycle; Asteroidea; range limits; environmental cues

1. **Introduction**

Seasonal patterns of gamete production followed by synchronized spawning periods have been documented for many asteroid species (Byrne et al., 1997; Chen and Chen, 1992; Chia and Walker, 1991). At high latitudes, sea stars show a marked seasonality in reproduction (Stanwell-Smith and Clarke, 1998) that fades in mid-latitudes (Rubilar et al., 2005; Ventura et al., 1997) and is not evident in tropical species (Guzmán and Guevara, 2002).

Many studies provide evidence about the relationship between reproductive cycles of sea stars and different environmental factors such as seawater temperature, day-length, pluvial precipitation and food supply (Benítez-Villalobos and Martínez-García, 2012; Benítez-Villalobos et al., 2007; Stanwell-Smith and Clarke, 1998; Tyler and Pain 1982a, 1982b; Tyler et al,. 1984). Understanding the fluctuation

of these factors is important to elucidate the coupling between reproductive patterns and the environment (Mercier and Hamel, 2009).

Asterina stellifera (Möbius, 1859) occurs in shallow waters from Cabo Frio, Brazil (23°S, 42°W) to Mar del Plata, Argentina (35°S, 56°W) in the southwest Atlantic (Clark and Downey, 1992). This bat star is one of the few asteroid species in the rocky coast of the South Atlantic Ocean and there is little published information on the life history of this species. *A. stellifera* is an omnivorous generalist predator, and modifies the abundance of other invertebrates and algae in subtidal marine communities (Farias et al., 2012).

A declining density has been reported for this species at the northern and central areas of its western latitudinal distribution (Calil et al., 2009) without an evident cause. For this reason, it was included in the Brazilian Red List of Endangered Species (MMA, 2004). The construction of coastal break waters along the coast of Argentina led to a decrease in abundance of intertidal populations in recent decades (Roux, 2004). In contrast, Farias et al. (2012) reported that the subtidal population of *A. stellifera* at Mar del Plata recently reached a high unexpected abundance despite the absence of recruitment during their four year study. Studies dealing with latitudinal inter-population differences in abundance and life history traits of marine benthic invertebrates are very scarce (Fenberg and Rivadeneira, 2011; Rivadeneira et al., 2010). Numerous studies have proposed several factors limiting geographic ranges of species, e.g., resource availabilities and physiological tolerances. Those tolerances may be forced in the limit of the geographic range; thus species may not persist in areas where environmental demands exceed these (Spicer and Gaston, 1999). Combinations of abiotic and biotic factors probably modify population traits such as abundance, reproduction cycles, fertility, larval survival and recruitment.

Understanding the population dynamics and the reproductive biology of the endangered bat star *A. stellifera* is important to elucidate potential causes of the scarce recruitment that was reported at range limits (Farias et al., 2012; MMA, 2004). In this study, we evaluate whether the Argentinian population of *A. stellifera* is sexually active and the geographic variation in reproductive traits at the distribution limits. For this purpose, we analyze the reproductive cycle and gametogenesis of *A. stellifera* at the southern limit of its distribution and we re-analyzed those data of reproduction previously published (Carvalho and Ventura, 2002) at the northern limit of its geographical distribution. Furthermore, we analyze the coupling of reproductive parameters with environmental factors at distribution range limits.

2. **Materials and methods**

2.1. Study area and environmental parameters

From April 2009 to April 2011, about 15 specimens of *Asterina stellifera* were randomly collected each month by SCUBA diving inside Mar del Plata port, Argentina (38º02’S, 57º31’30”W) (Fig. 1). The habitat is a rocky sublittoral consisting of walls of big orthoquartzite blocks limiting the harbor, and boulders of orthoquartzite rocks surrounding internal and external breakwaters. The breakwaters are surrounded by a fine-grained muddy bottom that limits individual´s dispersion. Depth in the sampling area ranged between 6 and 8 m. Environmental factors that potentially influence the gonad periodicity (seawater temperature, salinity, day-length and cumulative precipitation) were obtained from

official agencies. Seawater temperature and salinity from the sampling area were provided by the National Institute of Fisheries Research and Development (INIDEP). Average day-length was obtained from the web page of the Naval Hydrographic Service (http://www.hidro.gov.ar/observatorio/sol.asp), and precipitation was taken from the Mar del Plata Aerodrome database. Monthly means were calculated for all these parameters.

2.2. Reproductive cycle

Specimens were preserved in 10% formalin for at least 72 hours. The largest radius of each specimen (center of disc to arm tip) was measured to the nearest 1mm using a digital caliper. Water was blotted off by placing each individual on paper toweling for approximately 20 min and then weighted to the nearest 1mg (drained wet weight). Gonads and pyloric caeca of the five arms were dissected out, weighted to the nearest 0.001mg on a digital scale and stored in 70% ethanol.

Gonads were dehydrated in ascendant ethanol dilutions, cleared with xylol and embedded in paraffin wax. Tissues were sectioned at 7µm and stained with haematoxylin and eosin. The gonad cycle was divided into five stages based on the frequency of cell types and size and shape of acinus, based on an adaptation of the scale by Byrne (1992), Byrne et al. (1997) and Carvalho and Ventura (2002). Individual sex was determined by examining histological gonad sections of each animal.

In order to analyze gametic growth and proliferation, oocyte size frequency distribution was constructed by measuring the diameter of at least 100 oocytes per individual to the nearest 1µm. Only oocytes with visible nucleus were measured. The relative oocyte area (area of all oocytes present in an ocular field of optical microscope as a percentage of the field area) was estimated for each sample as: [mean number of oocytes per field/total area of a field] X 100.

2.3. Data analysis

Sex ratio was estimated considering the total sample over the study period. Statistically significant difference from the expected 1:1 sex ratio was tested using goodness of fit (G) test (Sokal and Rohlf, 1995).

A power function ($y=ax^b$) was fitted for organ wet weight (gonads and pyloric caeca) and sea star radius. To evaluate differences in gonad (GW) and pyloric caeca (PCW) wet weight between Brazilian and Argentinian populations, an ANCOVA analyses was performed after logarithmic transformation of both variables. Brazilian data was recorded by Carvalho and Ventura (2002) from Cabo Frio (Brazil) population.

Both *A. stellifera* GW and PCW are allometrically related (Fig. 2), i.e. the variables do not vary as a fixed proportion of the measure of body size (Packard and Boardman, 1999, 1988). Several studies had demonstrated that the use of a gonadosomatic index does not eliminate the relationship between individual's organ weights and body size and had criticized its use when these variables are not isometric (e.g., Ebert et al., 2011; García-Berthou, 2001; Jasienski and Bazzaz, 1999; Packard and Boardman, 1988, 1999; and bibliography there in). Therefore, we used organs wet weights data and included this size-dependent variation of GW and PCW into the analysis, as was performed in other echinoderm studies (Ebert et al., 2011; Gil and Zaixso 2007; Gil et al., 2009).

A two-way ANCOVA (with sea star radius as the covariate) was performed in order to evaluate if the relationship between organ wet weight (GW and PCW) and radius of each individual was different between months and sexes in the Argentinian population. The same analysis (two-way ANCOVA) was performed using data (GW and radius) of males and females from the Brazilian population (Carvalho and Ventura, 2002). This statistical procedure was performed as recommended by Packard and Boardman (1999) and Ebert et al. (2011). A Cochran's C test was performed to analyze the assumptions of homoscedasticity. The dependent variables (GW and PCW) and covariate (radius) were ln transformed to linearize and achieve homogeneity of variances.

Autocorrelation analysis was performed to detect temporal correlation within males and females of the monthly radius-adjusted GW throughout time. The synchrony between male and female GW cycle was investigated by using a cross-correlation analysis. The same analyses (cross-correlation) of the time series data from the Argentinian and Brazilian populations was performed to analyze the relationship between male and female organ wet weights (GW and PCW) with seawater temperature, salinity, monthly mean precipitation and day-length. Generalized Linear Models (GLM) were built in order to determine the environmental influence over the reproductive input. The explanatory variables were seawater temperature, salinity, day-length and monthly mean precipitation. A similar model was built to analyze the environment influence over the PCW. Two different time lags (t+1 and t+2) were added to the raw data matrix to analyze the response of organs wet weights and the environmental variables at different time scales. Cross-correlation and a GLM analysis were performed between GW and environmental variables (seawater temperature, day-length and salinity) data from Cabo Frio population taken from March 1999 to February 2000 (Carvalho and Ventura, 2002). For environmental analyses, monthly estimated radius-adjusted GW and PCW values were used to eliminate size effect. A radius of 45mm was selected as fixed size, which is a value within the range of *A. stellifera* individuals.

All GLM models analyzed in this study have a Gaussian distribution (Zuur et al., 2009). A model without any of the independent variables (i.e. null model) was fitted to test the hypothesis that none of the tested variables had an effect on the dependent variable. Model selection was performed with an Information Theory approach using Akaike's Information Criterion (IT-AIC) (Symonds and Moussalli, 2011). All statistical analyses were performed with the Open Access Software R (R Development Core Team, 2011).

3. **Results**

3.1. Sex ratio and organs characteristics

From a total of 243 bat stars analyzed from the Mar del Plata (Argentina) population, 143 were males and 100 were females. All individuals were adult. Sex ratio was significantly different from 1:1 ($G=9.225$, $p=0.002$).

Argentinian and Brazilian sea stars radius showed a large overlap (Table 1A, Figure 2). The gonad wet weight (GW) and the pyloric caeca wet weight (PCW) were not isometric with radius. The ANCOVA analysis showed significant differences in GW and PCW between populations. Sea stars from Argentina presented a higher GW and PCW than Brazilian specimens ($p<0.01$, Table 1, Fig. 2).

Macroscopically, fresh ripe testes presented a white to cream coloration, while ovaries generally presented an orange coloration. However, during early gametogenic stages, no macroscopic morphological differences were observed between gonads.

3.2. Gonad and pyloric caeca weight

The GW from the Argentinian population, adjusted using radius as a covariate, showed a seasonal reproductive pattern in both sexes (Fig. 3). The variables sex and month were included in the model, while the interaction term month x ln (radius) was excluded (Table 2). The significance of the term sex reflected differences in female and male GW values. Ripe females were found during winter (July 2010) and spring (October 2009). Values decreased until a minimum in late spring (December 2009) and summer (February 2011), this evidence a long spawning period. The radius-adjusted GW values started to rise in April, this indicates that oogenesis started in autumn (Fig. 3).

As in females, maximum male radius-adjusted GW values were reached during winter (August 2010) and early spring (October 2009). Then GW decreased gradually during spring (October-November 2010) and summer (December 2009-January 2010). Proliferation of gametes started in autumn (May) and lasted until late winter (Fig. 3).

The ANCOVA analysis performed on the PCW, with radius as the covariate, showed a temporal pattern (Fig. 3). The variables sex and month were included in the model, but the interaction term month x ln (radius) was excluded (Table 2). The significance of the term sex showed differences in female and male PCW. The male PCW values were always higher than GW during the two sampled years. There was no evident pattern in the male PCW progress throughout the years (Fig. 3). In 2009, PCW of males and females presented a similar pattern to GW during oogenesis. However, during 2010, PCW of females remained constant after spawning and started to increase during summer 2011 (Fig. 3).

Male and female GW presented no cross-correlation with male and female PCW ($p>0.05$). Therefore, reproduction was independent of PCW in both sexes. Autocorrelation analysis of male and female gonadal cycle showed no interannual variability within each sex ($p>0.05$). However, male GW presented a positive cross-correlation with GW of females (no lag, $r=0.87$, $p<0.01$). Therefore, both sexes appear to breed synchronously.

The ANCOVA analysis performed on GW data of individuals from the Brazilian population, with radius as the covariate, showed a temporal pattern in reproduction, as was found by Carvalho and Ventura (2002) (Fig. 4). The variables sex and month were included in the model (Table 2A). Ripe females were found during winter (July-August 1999). Radius-adjusted GW values decreased until a minimum in summer (January 2000), this indicates a long spawning period. Adjusted GW values rose during autumn (April to June), this indicates that oogenesis starts (Fig. 4).

3.3. Gonad development of females (Fig.5)

Growing phase. Primary oocytes ($\bar{x}$ =58.88µm, SE=13.91, n=800) attached to the ascinal wall and increased of oocyte diameter characterizes the ovaries in this stage. Some oocytes present a pear shape. Oocytes that increase in size are released into the lumen (Fig. 5A, B).

Mature phase. The ovary lumen is filled with large polyhedral oocytes ($\bar{x}$ =92.91µm, SE=15.23, n=1350). Few small previtellogenic oocytes ($\bar{x}$ =54.43µm, SE=12.81, n=250) are distinguished in the ovary wall (Fig. 5C).

Partly-spawned phase. Ovaries are similar in size to mature ones. However, there are spaces between oocytes, due to previous gamete release. Spawning characterizes by an increased frequency of individuals showingovaries in the partly spawned phase(Fig. 5D).

Spawned phase. Gonads become shrunken, and ovaries present few or no vitellogenic oocytes. Some of them contain few relict oocytes in degeneration and phagocytes (Fig. 5E).

Recovery phase. Ovaries are small and previtellogenic oocytes ($\bar{x}$ =45.37µm, SE=10.32, n=300) are found in the ovary wall. Some ovaries in recovery phase during November present small pear-shape oocytes in their walls, this indicates the beginning of the growing phase (Fig.5F).

3.4. Gonad development of males (Fig. 6)

Growing phase. A massive sperm production takes place. Spermatic columns are prominent. Later in this phase, sperm accumulates in the lumen of the ascinus. The ascinal wall of the testes is thick (Fig. 6A).

Mature phase. Spermatozoa fill the lumen, forming a dense mass. Spermatogenetic columns become narrow or absent (Fig. 6B).

Partly spawned phase. Testes are similar to those in mature phase. However, the ascinal lumen is not densely filled by sperm. Some empty spaces are present due to sperm release (Fig. 6C).

Spent phase. Testes lumens are almost empty, although some relict spermatozoa are found. Gonad walls become shrunken making evident a two sack structure. Also, a pale meshwork of phagocytes is present (Fig. 6D).

Recovery phase. Spermatic columns are evident along the thick ascinal testis wall and the germinal layer presents its characteristic folded shape (Fig. 6E). Relict sperm and phagocytes forming a meshwork are also present.

3.5. Seasonal analysis of gametogenesis

Ovaries in growing phase were present from early autumn (May) to spring (September). Mature ovaries with large oocytes ($\bar{x}$ =110.87μm) were found from winter (July-August) to summer (January) (Fig. 7, 8). Partly-spawned ovaries, with small oocyte diameter ($\bar{x}$ =80.59μm), were present from winter (August) to summer (January), this indicates a long spawning period. Spent ovaries were frequent in spring-summer (November-December) along with ovaries in recovery phase, evidenced by the decrease in the oocyte diameter ($\bar{x}$ =45.37μm) (Fig. 8). Mature individuals appeared in winter (July-August) until the beginning of summer (December-January), this was indicated by the presence of large vitellogenic oocytes ($\bar{x}$ =103.25μm) (Fig. 7, 8). Ovaries in recovery phase presented a new cohort of primary oocytes in their walls, this was evidenced by a decrease in the mean oocyte diameters ($\bar{x}$ =50.41μm).

Oocyte size frequency distribution was not unimodal. Instead, there was a bimodal distribution caused by the coexistence of oocytes in different stages of development. Ovaries in recovery phase produced new gametes for the long spawning period during the warm season (December-January) (Fig.

8). The relative oocyte area followed the same reproductive pattern described above. A small area was occupied by oocytes during autumn (~25.5%) and the maximum area was found in early spring–summer (~70%) (Fig. 8).

Spermatogenesis began in autumn (March-June) and lasted until spring (October). Ripe individuals were found from winter (July-August) to summer (January). Partly-spawned testes were present from winter (August) to summer (February), this indicates a long spawning period as was found in females. Individuals showing empty testes were present only in summer (December to March). Testes recovered during summer and autumn (Fig. 7).

3.5. Influence of environmental factors on gonad periodicity

From April 2009 to April 2011, the seawater temperature at Mar del Plata varied from 21°C in February to 9°C in August. Seawater salinity remained relatively constant throughout the studied period. Day-length varied from 8h 40minin winter (June) to 15h in summer (December), and the cumulative monthly precipitation ranged from 191.49mm to 13.72mm throughout the sampled period, being highly variable and with no clear annual pattern (Fig. 3).

In Cabo Frio, seawater temperature varied from 17.2°C in September to 24.5°C in April. The minimum value registered during September may be related to the upwelling existing in this area. Seawater salinity remained relatively constant throughout the year. Day-length ranged from 10h 45min in June to 13h 30min in December (Fig. 4).

Cross-correlation analyses showed that male and female radius-adjusted GW from the Argentinian population presented a maximum negative correlation with seawater temperature at a 1-month lag ($r=-0.95$ and $r=-0.68$, respectively, $p<0.01$) and a maximum negative correlation with day-length at a 2-month lag ($r=-0.89$, $r=-0.63$, respectively, $p<0.01$). Therefore, maximum development of testes occurred one month after the minimum value of seawater temperature and two month after the minimum value of day-length. However, radius-adjusted GW did not show a cross-correlation with seawater salinity and cumulative precipitation ($p>0.05$). In addition, radius-adjusted PCW of both sexes did not show a cross-correlation with the environmental factors ($p>0.05$).

Male and female radius-adjusted GW of sea stars from the Brazilian population presented a maximum negative cross-correlation with seawater temperature with 1-month lag ($r=-0.75$ and $r=-0.73$, respectively; $p<0.01$). No correlation was found between male and female radius-adjusted GW with day-length and seawater salinity ($p>0.05$).

The GLM analyses of radius-adjusted GW and environmental variables from the Argentinian population showed that models with day-length (with a 2-month lag) and seawater temperature (with a 1-month lag) presented the lowest AICc, highest *wi* and the consequent best fit. Therefore, these may be the variables triggering GW variation (Table 3). Thus, gametogenesis may be independent of cumulative precipitation and seawater salinity. Models showed that GW presented a negative relationship with seawater temperature, as was obtained by the cross-correlation analysis, and a positive association with day-length (Table 3, Fig. 3). The lowest seawater temperature values were found when individuals were in growing and mature phases (winter). In addition, spawning was coincident with an increase of seawater temperature (spring–early summer) (Fig. 3). No competent model was found to explain radius-adjusted

PCW relationship with environmental variables for both sexes (Table 3). The GLM analyses of radius-adjusted GW of males and females from the Brazilian population with environmental variables showed that models that include only seawater temperature (with a 1-month lag) as explanatory variable presented the best fit (Table 3). Thus, gametogenesis of sea stars from Brazil may be dependent on seawater temperature (Fig. 4).

4. **Discussion**

Most sea stars have an annual reproductive pattern, regulated by a complex interaction between endogenous and exogenous factors (Mercier and Hamel, 2009). In this study we found that *Asterina stellifera* presented a clearly defined annual and synchronous reproductive cycle at both limits of its distribution range. Seawater temperature and day-length are important environmental cues to increase chances of fertilization since they control gametogenesis in both populations.

Gonads of females from the Argentinian population were bigger than male ones, this differences is generally associated with differences in caloric investment between sexes (Scheibling, 1981). Carvalho and Ventura (2002) also found these differences between testes and ovaries production in the northernmost population of *A. stellifera*, at Cabo Frio. This indicates that there may be differences in caloric investment during gametogenesis between sexes in this species. Further studies on reproductive effort for this species at both populations are necessary to understand the meaning of these differences found in GW values of males and females.

Pyloric caeca are considered to be reserve organs in sea stars. An inverse relationship between gonads and pyloric caeca weights suggests nutrients transport between them (Byrne, 1992; Giese, 1966; Jangoux and van Impe, 1977; Lawrence, 1987; Lawrence, 1973; Rubilar et al., 2005; Ventura et al., 1997; Walker, 1982). If gametogenesis is supported by reserves from the pyloric caeca, a nutrients input to gonads have to occur and a decrease in pyloric caeca weight would be evident (Giese, 1966; Lawrence, 1973; Lawrence and Miller, 1999; Mercier and Hamel, 2009). If food is abundant throughout the year or enough to maintain gametogenesis, the gonads may not depend on the long-term nutrient storage of the pyloric caeca and this inverse relationship between GW and PCW may not be evident (Franz, 1986; Harrold and Pearse, 1980; Pastor de Ward et al., 2007; Xu and Barker, 1990b).

The Argentinian population of *A. stellifera* did not show a reciprocal relationship between PCW and GW for both sexes. Although storage of nutrients in pyloric caeca is a dynamic process, our results suggest that there is no clear transfer of nutrients between pyloric caeca and gonads. This species is able to feed over a wide range of preys (Farias et al., 2012). It seems that gamete proliferation in this population may not depend on the nutrients storage in the pyloric caeca because of a good nutritional level due to constant food supply. Similar results were found by Franz (1986) in *Asterias forbesi* and by Pastor de Ward et al. (2007) in *Cosmasterias lurida*.

Carvalho and Ventura (2002) studied two populations of *A. stellifera* in the northern limit of its distribution. According to the authors, although this pattern was not obvious, in one of those sites an inverse relationship was more evident than in the other site. The authors suggested nutrient transference from the pyloric caeca to the gonads under food shortage conditions. The Argentinian specimens presented a higher PCW than the Brazilian population, this indicates a higher storage capacity and

highlights the good nutritional level of the southern population of this species. Further studies on biochemical and energetic investment of *A. stellifera* are necessary to understand the allocation of energy during the reproductive cycle.

Reproductive success in benthic marine animals is influenced by a species capacity to quickly or slowly convert food into egg production. Nutrient allocation in response to food availability varies widely among species. Depending on the duration of gametogenesis, Eckelbarger (1994) characterized different reproductive strategies. Opportunistic species present a short vitellogenesis period, a high reproductive rate and fast egg production. This is generally associated with unpredictable food supply, where species allocate any excess energy (beyond maintenance) directly into reproduction. In contrast, *A. stellifera* from both populations have a long vitellogenesis period, and a long and slow spawning season, characteristic of predatory species. This is usually related to food supplies that are seasonal, continuous, or predictable; as well to a relatively stable environment. These are common features of subtidal shallow water populations at temperate seas.

Histological observations and gonad wet weight analyses supported these conclusions. Although, each approach properly describes gametogenesis and spawning stages, combining both methods enhances the determination of the reproductive cycle. Gametogenesis and spawning in *A. stellifera* is similar for males and females in both populations, despite of latitudinal differences. Cold water upwelling events found in Cabo Frio (Sumida et al., 2005) would explain the reproductive success of this temperate species at 22°S. *A. stellifera* seems to have a common reproductive pattern within its geographic distribution range.

The abundant-center hypothesis (Brown et al., 1995) proposes that species diminish their performance toward their distribution range limits, thus the abundance at the distribution limits should be low. Generally, it is assumed that low densities in the southern limit distribution of a species are caused by recruitment problems, low larval survival and/or juveniles and a decrease in adult reproductive output (Gilman, 2006a, 2006b). However, low densities in the northern limits are attributed to thermic limitations of adults which may be related to the species physiological and life history characteristics (Compton et al., 2007; Osovitz and Hofmann, 2007; Stillman, 2002). Farias et al. (2012) demonstrate that the Argentinian population is present in high densities. In contrast, the Brazilian population presents low densities (Meretta and Ventura, unpublished data). Thus, the abundant-center hypothesis is not supported by this species, since the abundance of A. *stellifera* declines from the northern to the southern limit distribution.

Furthermore, sex ratio differences were found between the populations under study. Brazilian population showed 1:1 sex ratio (Carvalho and Ventura, 2002) while in the Argentinian population, sex ratio was deviated towards males. The decreasing proportion of females found at the southern limit may be due to enhance survival of males, reduce survival of females, or a combination of both. Perhaps, the low temperatures found at the southern limit increase the cost of ova production, resulting in a low female survival compared to males. Similar results were found for five species of crustaceans (Rivadeneira et al., 2010).

The northern population of *A. stellifera* presented an extended spawning period and an unimodal oocyte diameter frequency (Carvalho and Ventura, 2002). In contrast, females in recovery phase from the

southern population produced new oocytes to support the continuous spawning along spring and early summer. Furthermore, the relative oocyte area did not decrease as it does in species with a punctual spawn (Pastor de Ward et al., 2007). In contrast, the relative oocyte area remained relatively constant during the extended spawning period. This fact may be related to differences in the fecundity of both populations. Although both populations present no differences in GW and a similar maximum oocyte diameter, the Brazilian population showed a higher mean frequency of oocytes diameter than the Argentinian population (40-50% and 30%, respectively). *A. stellifera* at the southern population may be balance this difference with the production of new oocyte cohorts during the reproductive season.

In the Argentinian population, oocyte growth of most females started during autumn (April-May). These sea stars, progressively accumulated ova until maturity was reached in winter (July-August), a month before gamete release. In the Brazilian population, oocyte growth also started in autumn (April). However, mature oocytes were found in May, and gamete release occurred in August (Carvalho and Ventura, 2002). This difference in duration of oogenesis between both populations suggests that sea stars from Argentina might transform energy from food directly into ova until ovaries are filled. This fact is consistent with the absence of a reciprocal relationship between PCW and gonad development. In contrast, sea stars from the northern population may present a more efficient energy transformation that promotes a large number of oocytes during vitellogenesis and therefore a higher mean frequency of oocytes diameter. Thus, the ability to transform food into yolk may be different in both populations. These differences in vitellogenesis strategies may be caused by different access to resources between limits distribution ranges (e.g. interspecific competition for food) that can be evidenced by reserves accumulated in pyloric caeca.

Reproduction is regulated by environmental factors such as seawater temperature, day-length and planktonic food supply, to maximize the fertilization success and offspring survival (Stanwell-Smith and Clarke, 1998; Mercier and Hamel, 2009). In Mar del Plata, chlorophyll concentration presents an annual cycle, characterized by two well-defined maxima: a main peak during spring and a secondary one during autumn (Carreto et al., 1995; Lutz et al., 2006). Although this pattern is in general agreement, there are regional differences regarding the time of the initiation of the blooms, their intensity and duration and their phytoplanktonic specific composition (Carreto et al., 1995). Similarly, in Cabo Frio the frequency of upwelling events (which increases phytoplankton abundance) becomes higher during spring and summer (Sumida et al., 2005). Although, there is little evidence that seasonal planktonic food supplies can directly trigger spawning in asteroids (Himmelman, 1975), the long spawning period of *A. stellifera* may correspond to the occurrence of distinct phytoplankton blooms in Mar del Plata and Cabo Frio.

The annual synchrony between sexes observed in *A. stellifera* suggests that external factors are controlling the annual reproductive cycle. In the Argentinian population, GW was negatively associated with seawater temperature and positively with day-length. In addition, these were the only two variables included in GLM analyses as explanatory factors of GW variation. Although, we have used an indirect approach to infer links between reproductive cycle and environmental variables, day-length and seawater temperature are considered the most probable factors to trigger reproduction in asteroids, at least for shallow-water species (Grange et al., 2007; Mercier and Hamel, 2009; Pastor-de-Ward et al., 2007; Pearse

and Bosch, 2002; Pearse and Eernisse, 1982; Pearse and Walker, 1986; Xu and Barker, 1990a). Furthermore, this relation between photoperiod and gametogenesis has been previously demonstrated by experimental surveys with several sea star species (Pearse and Beauchamp, 1986; Pearse and Eernisse, 1982; Pearse and Walker, 1986). In those studies, the long days during spring and summer synchronizes the initiation of gametogenesis in autumn. Gonad proliferation of *A. stellifera* occurs during short days (winter) and spawning when day-length starts to increase and persists during its maximum (late spring and summer).

In the Argentinian population, the gonad production period of *A. stellifera* occurred during low seawater temperature (winter). Moreover, this species showed a long spawning period when seawater temperature reached high values (spring–summer). In Cabo Frio, the photoperiod varied from 10h 45min to 13h 30min along the year, shorter and rather more constant throughout the year than in Mar del Plata. Furthermore, in the northern distribution, seawater temperature was the only variable explaining GW. While seawater temperature showed an annual variation from 17.2° to 24.5°C (amplitude of 7.3°C) in Cabo Frio; in Mar del Plata the value was larger (amplitude of 12°C). Thus, seawater temperature seems to be the environmental driving force for the gametogenic cycle on this species. Additional experimentation focused on endogenous and environmental factors are needed to elucidate the possible relationship between them and gonad development in this species.

At its southernmost distribution, *A. stellifera* is present in high densities, has a synchronous reproductive cycle and gametes are released to the ocean every year. Thus, the lack of production of gametes is not the cause of the recruitment failure found by Farias et al. (2012) during the same period as our sampling. Therefore, the absence of new settled individuals might be explained by pre- and/or post-settlement events. Pre-settlement processes might be larval dispersal due to hydrological port processes and larval mortality. Post-settlement processes that may influence recruitment rate might be predation on newly settled animals by benthic predators and/or auto-cannibalism of new recruits as suggested by Farias et al. (2012). Perhaps, recruits may be using a different habitat for settling, e.g. nursery areas (Rumrill, 1989), and then migrate into the adult population after the individuals have reached a certain size. Low densities reported at Brazilian population may be also be explained by these processes.

Factors that determine the species abundance and distribution range may be reflected in the geographic variation of life-history characteristics. Thus, further studies focusing on *A. stellifera* population dynamics, growth and age should be performed to understand the processes that shaped the populations at the limit distribution of this species. Furthermore, long-term studies in both geographical limits would be needed to reveal the causes of variation in the reproductive features associated to environmental cues of this endangered species and the possible effects over both population densities. Additionally, population genetics and larval features can help to understand similarities and differences found along the range of its distribution.

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Figure captions

Figure 1 Map showing *Asterina stellifera* western geographic range and sampling site in Mar del Plata (Argentina) and Cabo Frio (Brazil).

Figure 2 *Asterina stellifera*. Relationship of gonad wet weight (g) and pyloric caeca wet weight (g) with radius (mm) between populations at the distribution range limits, Argentina (Arg) and Brazil (Br). The lines correspond to adjusted power function. Sample sizes of each data set are given.

Figure 3 *Asterina stellifera*. Upper panels: Argentinian male (N=143) and female (N=100) monthly variation of radius-adjusted gonad [ln GW (g)] and pyloric caeca [ln PCW (g)] wet weights. Upper numbers on each graph represent the number of individuals collected each month. These are the radius-adjusted GW and PCW values (±0.95 confidence interval) if radius (ln transformed) was held constant at 45mm. Lower panels: Seasonality of seawater temperature, salinity, day-length and cumulative precipitation for the studied period.

Figure 4 *Asterina stellifera*. Upper panel: Brazilian male (N=73) and female (N=90) monthly variation of gonad wet weights [ln GW (g)], adjusted for the effect of sea star radius. The monthly means were adjusted (±0.95 confidence interval) if radius (ln transformed) was held constant at 45mm. Upper numbers represent the number of individuals collected each month. Lower panel: Seasonality of seawater temperature, salinity and day-length for the studied period.

Figure 5 *Asterina stellifera*. Histology sections of ovaries. **A** Growing phase. Denote the pear-shaped oocytes (*arrow*). **B** Detail of pear-shaped oocytes (arrow). **C** Mature phase. Denote the lumen filled by oocytes. **D** Partly spawned phase. Denote the spaces among cells. **E** Spawned phase. **F** Recovery phase. Denote the invaginations (*arrow*). *O* oocytes; *MO* mature oocytes; *UO* unspawned oocytes; *RO* relict oocytes; *P* phagocytes. *Scale bars*=100 µm.

Figure 6 *Asterina stellifera*. Histology sections of testes .**A** Growing phase. Denote the spermatic columns (*arrow*). **B** Mature phase. Denote the lumen filled of sperm. **C** Partly spawned phase. Denote spaces due to sperm release (*arrows*). **D** Spent phase. **E** Recovery phase. Denote invaginations of germinal epitelium (*arrow*). **F** Detail of germinal epithelium (*arrow*) and relict spermatozoa. *SZ* spermatozoa; *SC* spermatic columns; *RS* relict spermatozoa. *Scale bars*=50 µm.

Figure 7 *Asterina stellifera*. Argentinian male and female gametogenic cycle. Histograms show relative frequencies of gonad stages in histological sections.

Figure 8 *Asterina stellifera*. Argentinian monthly oocyte diameter distribution throughout the reproductive cycle (black bars) and relative oocyte area (empty circles).

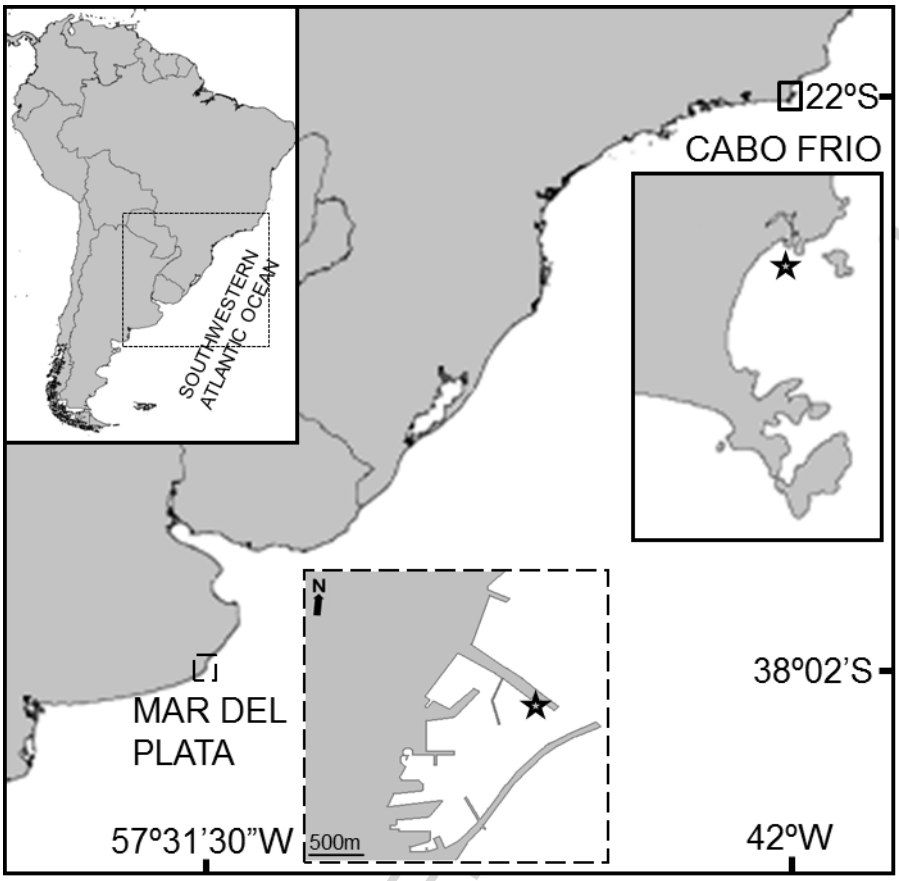

Figure 1

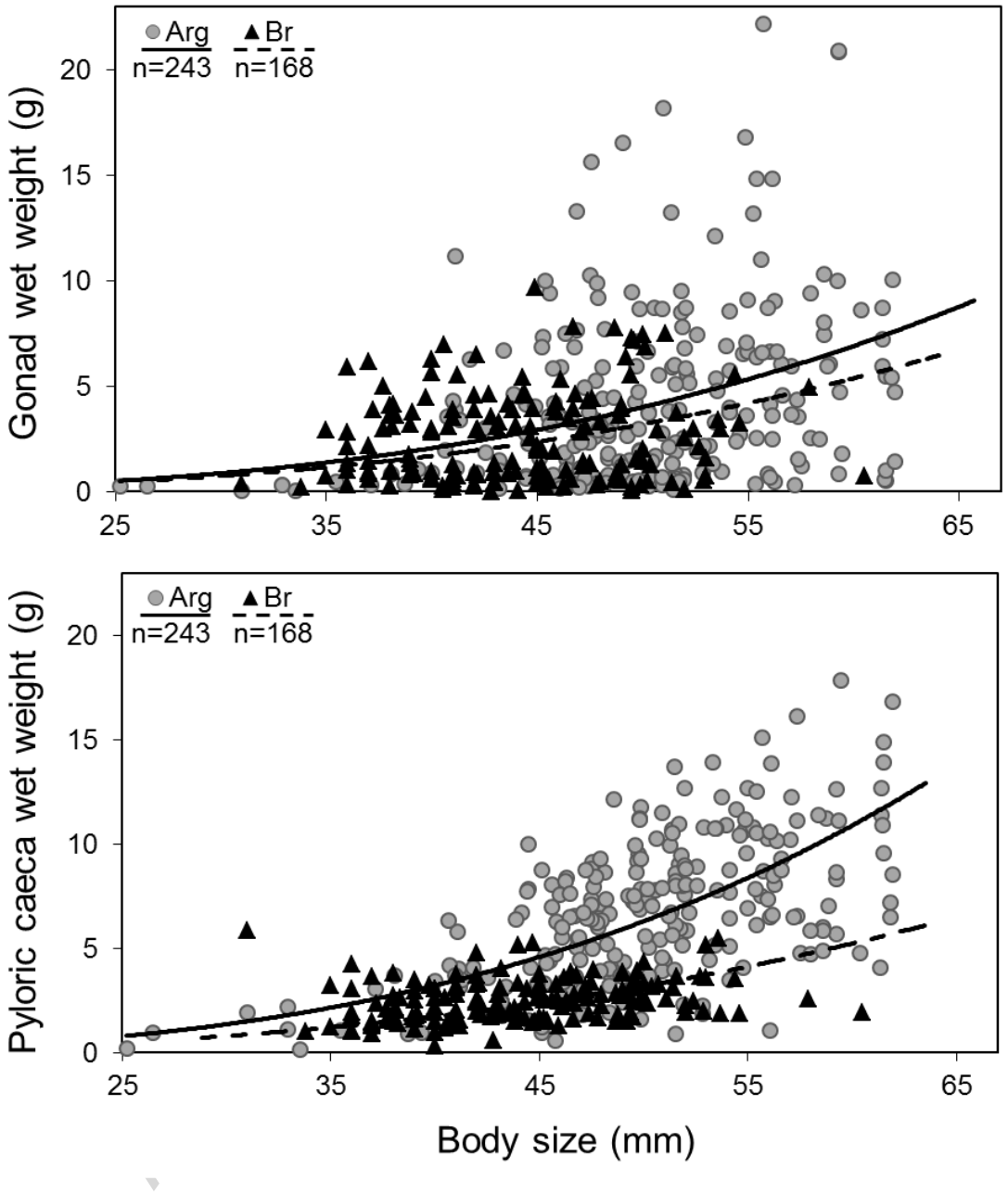

Figure 2

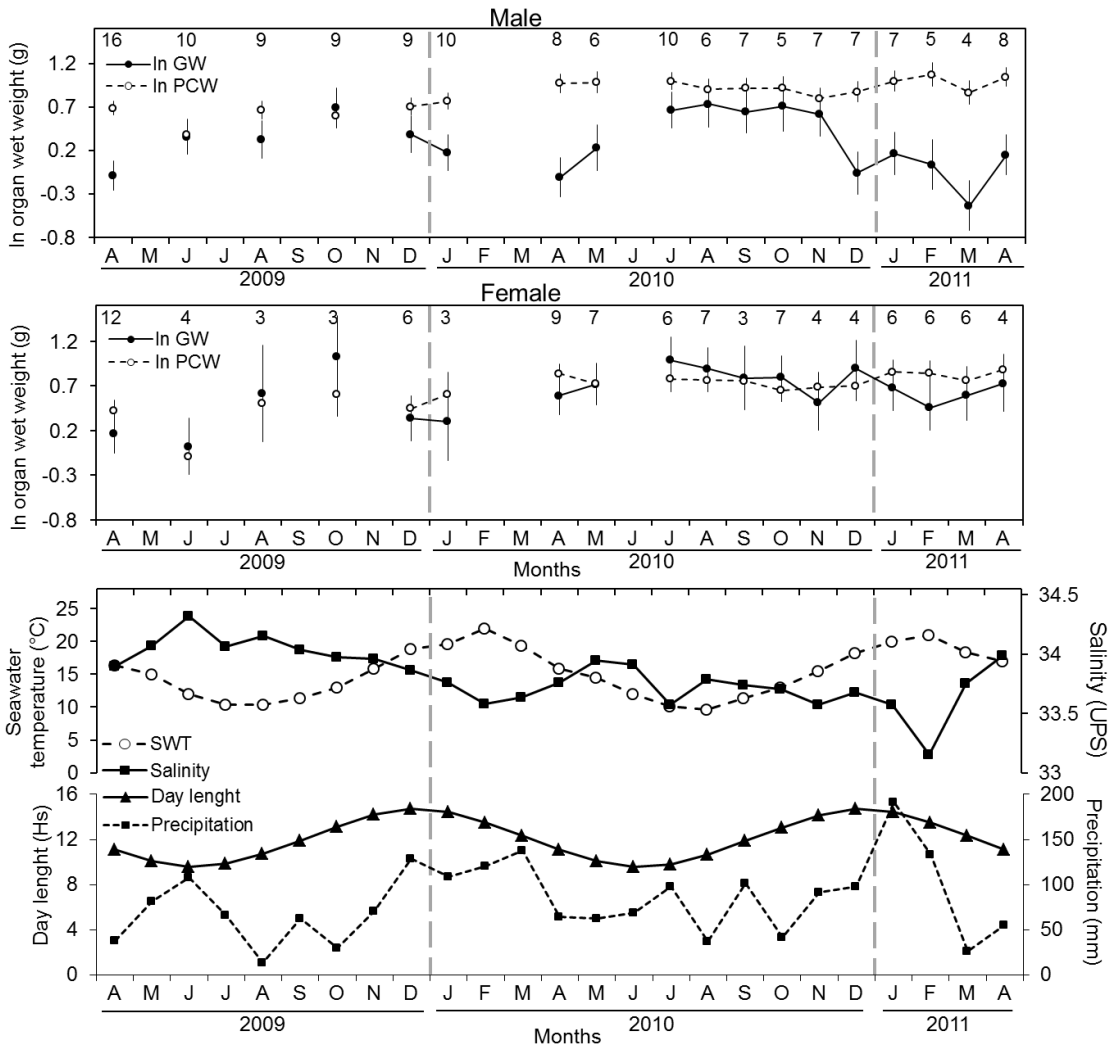

Figure 3

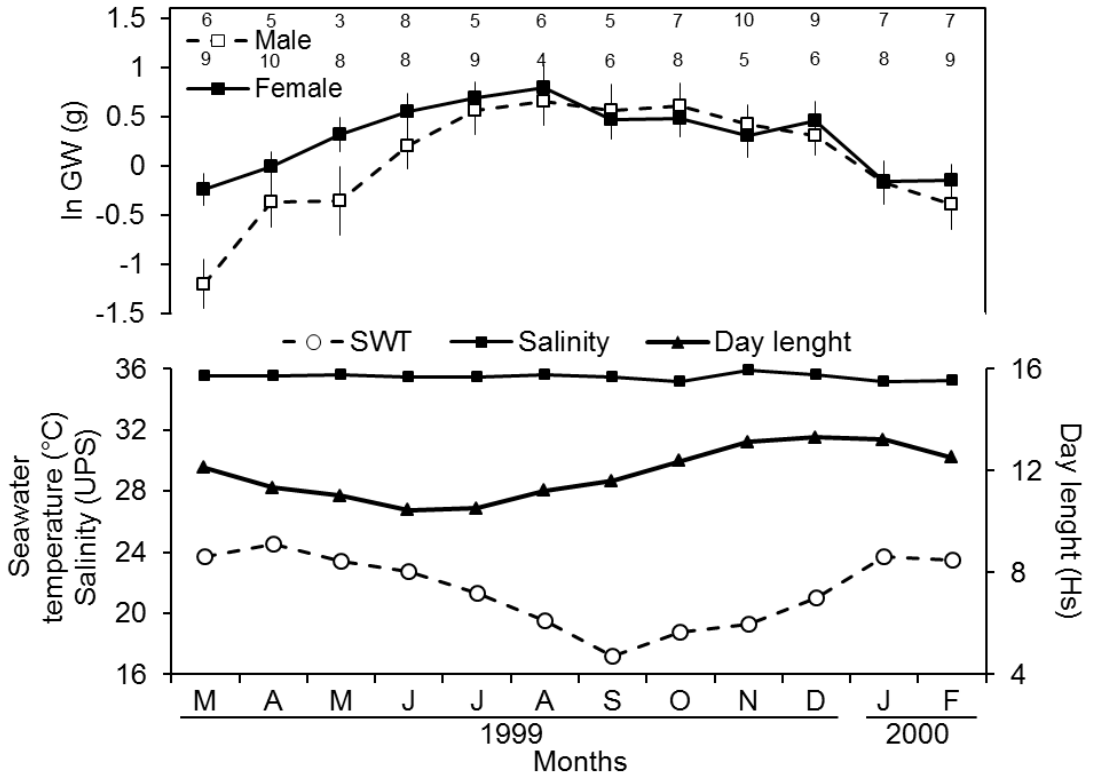

Figure 4

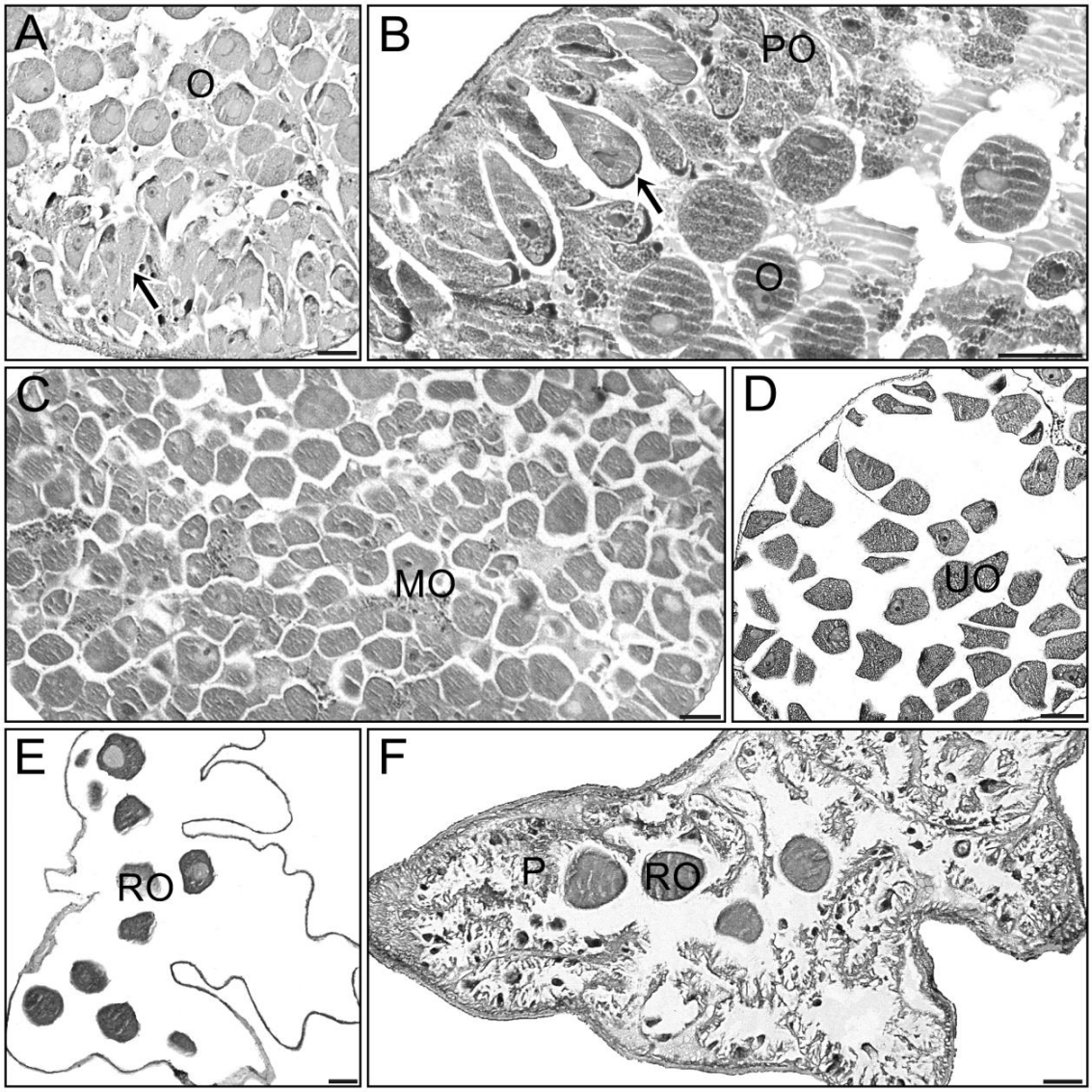

Figure 5

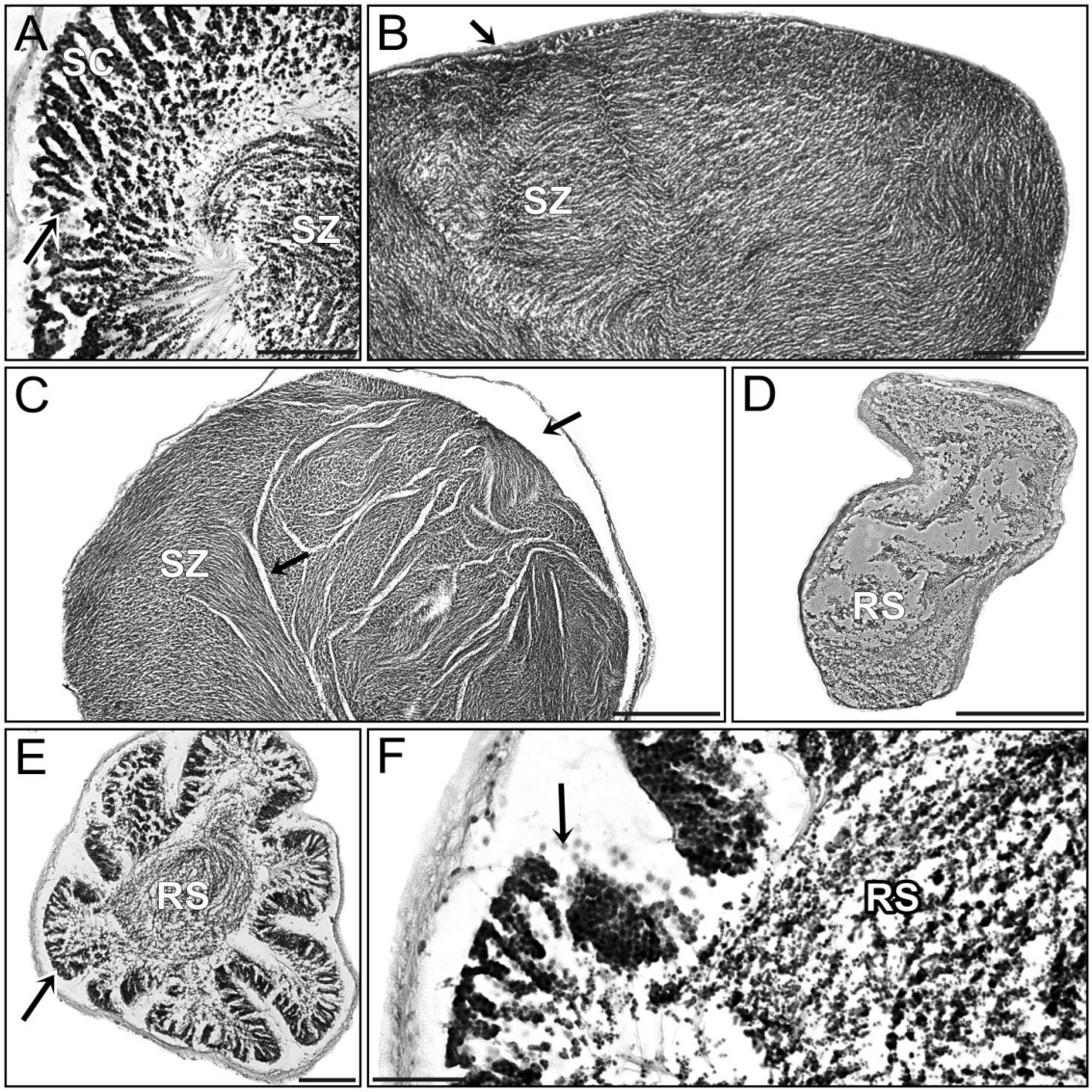

Figure 6

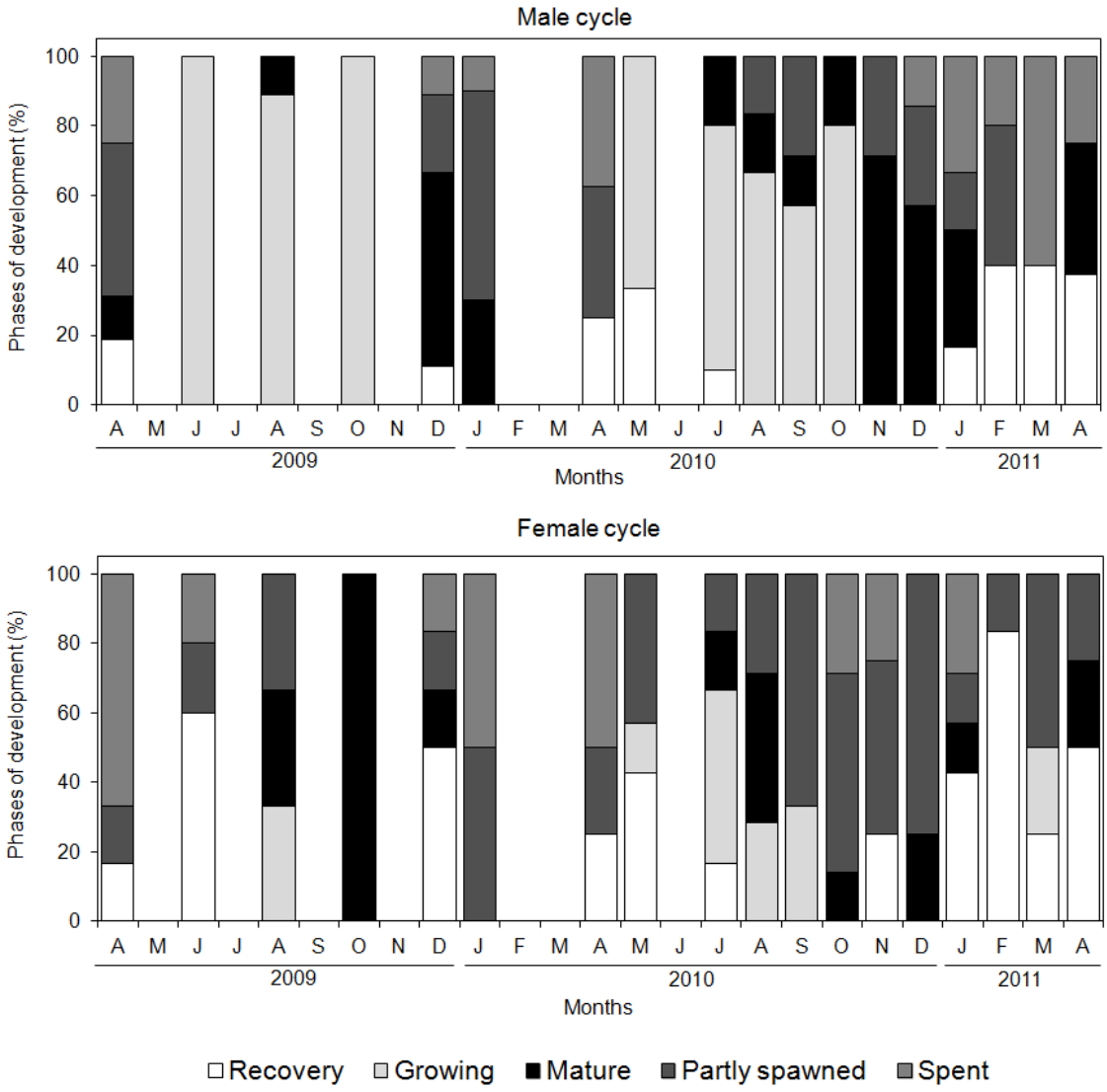

Figure 7

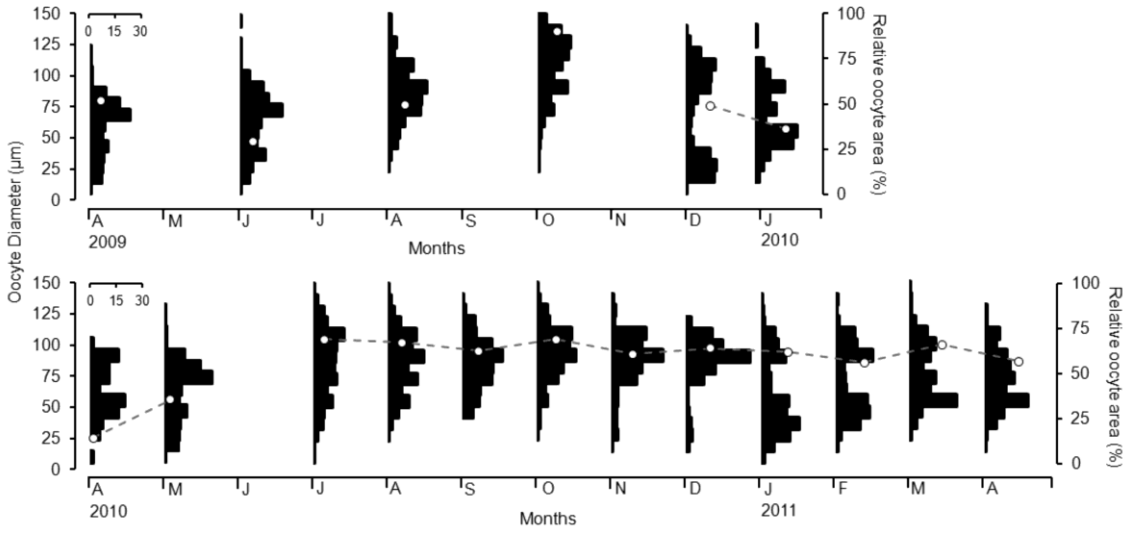

Figure 8

Table 1 *Asterina stellifera*. Comparison of gonad wet weight and pyloric caeca wet weight between populations from Argentina (Arg) and Brazil (Br): using summary statistics (A) and ANCOVA with raw data (B). Mean of *X* is the body size (mm) and mean of *Y* is the organ wet weight (g). GW, gonad wet weight; PCW, pyloric caeca wet weight.

Response variables	Site	Mean *X* (SE)	Mean *Y* (SE)	Equations	
A. *Summary statistics*					
GW	Arg	50.25 (6.35)	4.35 (4.12)	$y=2.976X^{0.000035}$	
GW	Br	44.10 (5.22)	2.51 (2.09)	$y=2.839X^{0.000048}$	
PCW	Arg	50.25 (6.35)	6.85 (3.48)	$y=3.002X^{0.000050}$	
PCW	Br	44.10 (5.22)	2.51 (5.22)	$y=2.715X^{0.000078}$	
Response variables	SS	*df*	MS	*F*-ratio	*p*
B. *ANCOVA with raw data*					
GW: Arg – Br					
ln (radius)	69.33	1	69.33	63.83	<0.0001
Site	57.55	2	28.74	26.46	<0.0001
Site x ln (radius)	24.82	2	12.42	11.44	<0.0001
Error	436.64	402	1.09		
PCW: Arg – Br					
ln (radius)	99.06	1	99.06	403.32	<0.0001
Site	27.26	2	13.63	55.49	<0.0001
Site x ln (radius)	8.41	2	4.21	17.13	<0.0001
Error	98.74	402	0.25		

Table 2. *Asterina stellifera*. Two-way ANCOVA test with raw data for differences in ln gonad wet weight and ln pyloric caeca wet weight between sexes and months in the Argentinian (Arg) and Brazilian (Br) populations. The effect of radius was included as a covariate.

Response variable	SS	*df*	MS	*F*-ratio	*p*
Gonad wet weight – Arg					
ln (radius)	56.97	1	56.97	93.24	<0.0001
Month	85.27	17	5.02	8.21	<0.0001
Sex	56.64	1	56.64	92.69	<0.0001
Month x ln (radius)	14.88	17	0.88	1.34	0.124
Error	124.04	203	0.61		
Pyloric caeca wet weight – Arg					
ln (radius)	51.08	1	51.08	400.65	<0.0001
Month	39.25	17	2.31	18.11	<0.0001
Sex	2.66	1	2.66	20.83	<0.0001
Month x ln (radius)	12.24	17	0.72	1.67	0.115
Error	25.88	203	0.13		
Gonad wet weight – Br					
ln (radius)	1.57	1	1.57	4.15	0.04
Month	143.38	11	13.03	29.09	<0.0001
Sex	9.57	1	9.57	21.36	<0.0001
Month x ln (radius)	7.45	11	0.68	1.51	0.133
Error	64.08	143	0.45		

Table 3. Generalized linear set of best-ranked models examining the variation of the gonad and pyloric caeca wet weight due to environmental variables between distribution limits. Parameter estimate (SE) for the supported models is given. Organs wet weights correspond to estimated radius-monthly adjusted values, as if radius was held constant at 45mm. The null models and models with a support of *wi*>0 are provided. Models are listed in decreasing order of importance. The model with the most support is shown in bold. AICc, Akaike information criterion for small samples; ∆i, AICc differences; *wi*, normalized weights of AICc; DL, day-length; Pp, precipitation, SWT, seawater temperature, Sal, seawater salinity. -1 and -2 indicate that the independent variable corresponds to 1 and 2 month lag, respectively.

Dependent variable	N	k	AICc	∆i	*wi*	Parameters					
						Intercept	DL	DL–2	Pp	Sal	SWT	SWT–1
Argentinian Gonad wet weight	34	**2**	**69.11**	**0.000**	**0.704**	**2.485 (1.071)**		**0.138 (0.104)**				**-0.275 (0.061)**
		1	72.7	3.590	0.117							X
		1	73.91	4.800	0.064			X				
		3	75.47	6.360	0.029			X	X			X
		2	75.6	6.490	0.027		X				X	
		4	75.89	6.780	0.024			X	X	X	X	
		2	76.12	7.010	0.021		X			X	X	
		4	77.45	8.340	0.011		X		X	X	X	
		Null	87.811	18.701	0							
Argentinian Pyloric caeca wet weight	34	2	29.93	0.000	0.255	0.082 (0.724)		0.254 (0.119)				-0.093 (0.055)
		Null	30.04	0.110	0.241							
		1	30.5	0.570	0.192							X
		3	30.84	0.910	0.162			X	X			X
		1	31	1.070	0.149			X				
		4	44.54	14.610	<0.001			X	X	X		X
Brazilian Gonad wet weight	24	**1**	**47.88**	**0.000**	**0.670**	**9.771 (2.131)**						**-0.386 (0.089)**
		2	50.54	2.660	0.177			X				X
		3	51.48	3.600	0.111			X		X		X
		1	54.72	6.840	0.022						X	

1	56.63	8.750	0.008			X		
2	56.69	8.810	0.008		X			X
3	58.58	10.700	0.003		X		X	X
1	67.35	19.470	<0.001		X			
Null	71.48	23.600	0					

Highlights

1. We study the geographic variation of seasonal reproduction of *Asterina stellifera*
2. An extended spawning period and no dependence on energy transfer is detected
3. Seawater temperature appears to influence gonad proliferation at both range limits
4. Gamete release failure is not the cause of the recruitment scarcity detected
5. Reproductive traits differences were found in populations at both range limits