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# Reproductive cycle of the rock oyster, *Striostrea prismatica* (Gray, 1825) from two locations on the southern coast of Ecuador

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## Abstract

The reproductive cycle of the rock oyster *Striostrea prismatica* was determined at two fishing areas, General Villamil and Ayangue (located on the southern coast of Ecuador), between May 2012 and April 2013. Monthly sampling campaigns were performed at both locations. The tissues were histologically examined to determine gonadal index (GI), oocyte development, follicular area coverage and sex ratio. Surface seawater temperature, salinity and chlorophyll *a* concentration were measured during samplings. Our results show a similar annual reproductive pattern at both locations. The GI reached maximum values during the summer. Oysters reached highest ripeness in January and February, while spawning occurred in February–March. Gametogenesis was linked to a consistently increasing follicular area (from 1.3 ± 0.7% to 85.8 ± 7.8%) and associated to surface seawater temperature. Spawning coincided with warm water temperature fluctuations and a seawater salinity decrease. No correlation was found with Chlorophyll *a* concentration. The sex ratio of sampled populations was 1:1, suggesting that oysters sizing more than 10 cm in shell length present a stable sex proportion in the population. The diameter of mature oocytes was significantly reduced (32.7%) during histologically preparations in comparison to fresh oocytes. Our study provides useful information of environmental factors that may control the observed gametogenesis and spawning activity of *S. prismatica*.

**Keywords:** *Striostrea prismatica*, gametogenesis, condition index, sex determination, *Crassostrea iridescens*

## Introduction

The rock oyster, *Striostrea prismatica* (Gray, 1825) (Mollusca, Bivalvia, Otreidae), is a commercially valuable species along the Pacific Ocean coast of America, from La Paz, Baja California (Mexico), to Mancora, Tumbes (Peru) (Poutiers 1995). It is found on rocky intertidal and shallow subtidal substrates down to 7 m of depth (Fournier 1992; Coan & Valentich-Scott 2012). The meat is highly appreciated as food plate and the valves are used by craftsmen, thus significantly contributing to the economy of coastal communities of Ecuador. Nevertheless, in coastal fishing villages such as Ayangue (Santa Elena), a shortage of individuals and the extraction of smaller oyster are reported (Artisanal Fishermen 2012, personal communication), suggesting a possible over-exploitation of the resource. To mitigate human and environmental impacts on *S. prismatica* populations, laboratory studies focusing on broodstock maturation, spawning and larval development have been recently conducted (Loor 2012; Argüello-Guevara, Loor & Sonnenholzner 2013). However, studies to determine the reproductive cycle of this oyster in Ecuadorean waters have not been undertaken to our knowledge, which are essential for proper management policies of the resource and efforts for its restoration.

The reproductive cycle of marine bivalves vary among species and locations (Chávez-Villalba, Barret, Mingant, Cochard & Le Pennec 2002; Serdar & Lök 2009), and is regulated by exogenous and endogenous factors (Mackie 1984; Barber & Blake 2006). Water temperature and food availability are the main factors that control

gonadal development (Giese & Pearse 1974; Frías-Espericueta, Páez-Osuna & Osuna-López 1997; Chávez-Villalba, Cochard, Le Pennec, Barret, Enríquez-Díaz & Cáceres-Martínez 2003; Fabioux, Huvet, Souchu, Le Pennec & Pouvreau 2005), and influence fertility and gamete quality (Gabbott 1983; Utting & Millican 1997; Park, Lee, Choy, Choi & Kang 2011). Microscopy and histology provide accurate analysis of gametogenic stages, while oocyte size is determined by image analysis (Lango-Reynoso, Chávez-Villalba, Cochard & Le Pennec 2000; Meneghetti, Moschino & Da Ros 2004; Enríquez-Díaz, Pouvreau, Chávez-Villalba & Le Pennec 2009).

The annual reproductive cycle for *S. prismatica* formerly referred to as *Crassostrea iridescens* and/or *Ostrea iridescens*, has been investigated in other latitudes (Cuevas-Guevara & Martínez-Guerrero 1979; Fournier 1992; Frías-Espericueta *et al.* 1997). Histological analyses carried out by Cuevas-Guevara and Martínez-Guerrero (1979) and Fournier (1992) on *S. prismatica* oysters collected year round in the Northwest coast of Mexico and Pacific coast of Costa Rica, respectively, evidenced a clear seasonal reproductive cycle, where temperature and salinity appear to modulate the gametogenesis and trigger their spawning. Warm tropical waters of the Panama Bay and cold subtropical waters of the Humboldt Current from the south, seasonally influence the oceanography of the Ecuadorean coast. These water masses display marked thermohaline variability (Cucalón 1989), showing an appealing opportunity for studying the effect of environmental parameters on the reproductive cycle of marine invertebrates, which can be applied to aquaculture techniques.

Determinations of physical and chemical parameters that regulate broodstock conditioning are essential for aquaculture projects of *S. prismatica*. A one-year baseline study was performed in wild populations of *S. prismatica* at two contrasting environmental locations of the southern coast of Ecuador; Ayangue, characterized by coastal marine waters; and General Villamil, characterized by a mixture of coastal marine and estuarine waters of the Gulf of Guayaquil. Our study aimed two objectives: (i) to determine the reproductive cycle of wild S. *prismatica* by determining mean monthly Gonadal index (GI), follicular area, oocyte development and population sex ratio; and (ii) to correlate reproductive patterns with changes in environmental factors.

## Materials and methods

### Study areas and biometric data samples

The samples of *S. prismatica* were obtained at General Villamil (2°38′34″ S; 80°26′09″ W) and Ayangue (1°58′05″ S; 80°45′21″ W) between May 2012 and April 2013. Both sites are located on the southern coast of Ecuador (Fig. 1). We sampled fifteen oysters off the coast at water depths between 3 and 6 m every month. Only oysters having shell length of 10 cm or larger were considered in the study. Oysters were transported in plastic coolers to the Centro Nacional de Acuicultura e Investigaciones Marinas (CENAIM) at San Pedro de Manglaralto (Santa Elena – Ecuador). Fouling organisms on oyster shells were removed by brushing and scrubbing. Oyster were measured with a calipre to the closest 1 mm to determine shell dimensions (length, height and width) and soft body weight was determine in an electronic balance with precision 0.1 g. Dimensional terms were applied according to Coan and Valentich-Scott (2012).

### Environmental parameters

Water samples were collected at both locations during oyster sampling for temperature, salinity and chlorophyll *a* determination. Temperature and salinity were measured on site with a mercury thermometer and refractometer respectively. Chlorophyll *a* was analysed in the laboratory following the spectrophotometric method (Greenberg, Clesceri & Eaton 1992).

### Histology

Transverse 5-mm-thick cross-sections of the posterior visceral mass (between the pericardial cavity and digestive gland) from each oyster were sliced, placed in histology cassettes and fixed in Davidson's solution for 48 h (Howard & Smith 1983), dehydrated in a graded series of ethanol solutions, cleared in xylene and embedded in paraffin. Paraffin blocks were then sliced into 4 μm on a rotary microtome, mounted on slides and stained with Haematoxylin-eosin for analysis. Pictures were taken with a Olympus CH-2 microscope coupled to a Nikon E995 camera.

Oyster gonads were analysed microscopically for sex differentiation. Gonadal development was

  

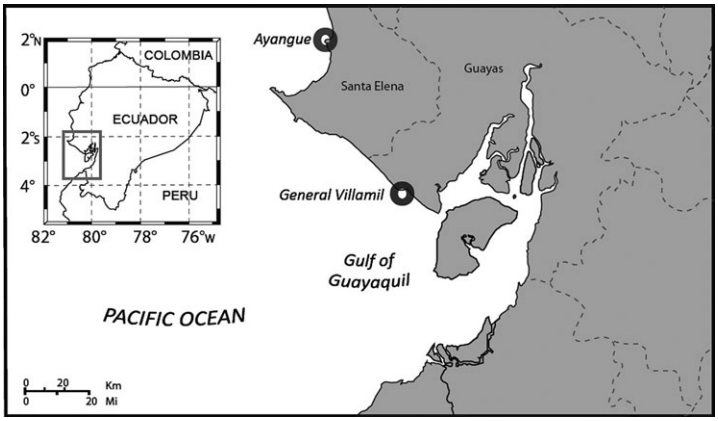


**Figure 1** Map of the southern Ecuadorian coast showing sampling sites (grey circles).

classified into six stages according to the classification system described by Cuevas-Guevara and Martínez-Guerrero (1979) and Fournier (1992) for *Striostrea prismatica*: Resting (S0), Early development (S1), Late development (S2), Ripe (S3), Spawning (S4) and Spent-Reabsorbing (S5).

### Mean gonadal index

Monthly mean Gonadal Index (GI) was determined for each sampling site. The GI ranged between 0 (if all oysters in the monthly sample had a resting stage S0) and 1 (if all oyster were in the ripe stage S3). GI was calculated following the formula described by Machensen, Brey and Sonnenholzner (2011):

$$\mathrm{GI} = (N_{S0} \times 0 + N_{S1} \times 0.33 + N_{S2} \times 0.66 + N_{S3} \times 1 + N_{S4} \times 0.66 + N_{S5} \times 0.33)/(N_{S0+S1+S2+S3+S4+S5})$$

where $N_x$ was the number of oysters found in x-gonadal stage and $N_{S0+S1+S2+S3+S4+S5}$ the total number of oysters evaluated in the monthly sample.

### Oocyte measurements

Oocyte sizes in accordance with gonadal development stages were determined by examining digitalized images (400 X) of histological slides. Because oocytes within follicles may present irregular shapes, digital images were analysed with Adobe Illustrator 10® software using the Path Area plug-in filter. Thus, the outline of the oocyte area ($a$) was digitally marked and the theoretical diameter (TD) determined with the formula proposed by Lango-Reynoso *et al.* (2000) and Rodríguez-Jaramillo, Hurtado, Romero-Vivas, Ramírez, Manzano and Palacios (2008):

$$\mathrm{TD} = \sqrt{4a/\pi}$$

A total of 45 oocytes located at the centre of the follicle with visible nucleus were measured at 10 randomly selected regions per slide. A digital calibration was previously carried out to scale measurements in μm units.

Fresh ripe oocytes were also measured and compared to histologically processed oocytes. Fresh oocytes were mounted on glass slides, photographed and measured with the same digital procedure described above. In addition, the size of hydrated fresh oocytes was also determined for comparison purposes. Slices 3-mm thick were removed from the gonad, stripped and placed into a 20-ml test tube containing seawater for 30'. Oocytes were then filtered through a 30-μm mesh filter and measured ($n$ = 45 per female) as previously described.

### Follicular area

The proportion of the follicles occupied by the gonad was calculated as a percentage of the total follicular area. Histological slides of the one-year study at both sites were grouped into gonadal stages. Ten slides of each gonadal stage were randomly selected to determine the follicular area. Five points within the total area of the gonad were randomly selected in each slide using an Olympus CH-2 microscope (100 X) and photographed with a Nikon E995 camera. Adobe Illustrator 10® software was used to analyse each image (image area = 1396.1 × 1047.1 $\mu m^2$).

### Statistical analyses

All data were tested for homogeneity of variance and normality using Levene and Kolmogorov–Smirnov tests respectively. Non-parametric procedures were used when data failed to pass these tests. Follicular area and population weight were, respectively, transformed to $x^2$ and ln(x), to comply with homogeneity of variance. The one-way ANOVA followed by *post hoc* Scheffé test was applied to statistically determine differences in follicular area among gonadal stages. The non-parametric Kruskal–Wallis test and the Mann–Whitney test for pairwise independent comparisons were employed to determine statistical differences in oocyte diameter at different stages. A *t*-test was used to compare means between weights at each site and between Processed and non-treated groups, while the Mann–Whitney test was used to detect differences between non-treated and hydrated groups. The Pearson Chi–Square test compared sex ratio between sampling sites. The Pearson correlation coefficient helped to determine the presence of linear relationships between GI and exogenous factors. For all cases, statistical significant differences were considered at $P < 0.05$. All Statistical analyses were run on SPSS software (version 11.0).

## Results

### Environmental parameters

Seawater temperature followed a cyclic pattern at both sites (Fig. 2 a and b). Lowest water temperatures were recorded in August; 23.4°C at Ayangue and 24.1°C at General Villamil, whereas highest water temperature were evidenced during January; 29.6°C at General Villamil and 28.5°C at Ayangue. Salinity was influenced by the rainy season that normally occurs during summer (January–April). The large salinity fluctuation registered at General Villamil (27 g $L^{-1}$ in April and 34 g $L^{-1}$ in October) was most likely caused by variations in freshwater flow from the Guayas River estuary of the Gulf of Guayaquil. Salinity fluctuation at Ayangue was smaller, ranging between 30 g $L^{-1}$ in March to 33 g $L^{-1}$ and 34 g $L^{-1}$ during May–October.

Chlorophyll *a* at General Villamil reached maximum levels in May (2.4 μg $L^{-1}$) and the minimum in December (0.5 μg $L^{-1}$); while for Ayangue, the chlorophyll *a* fluctuated from

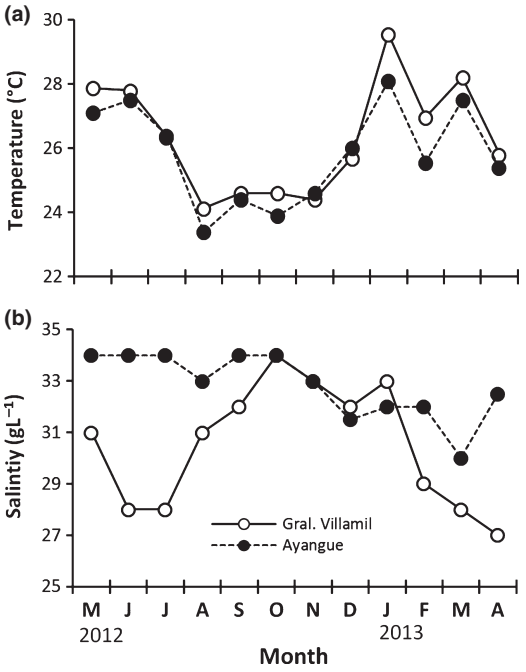


**Figure 2** Monthly variation in seawater temperature (a) and salinity (b) at General Villamil and Ayangue.

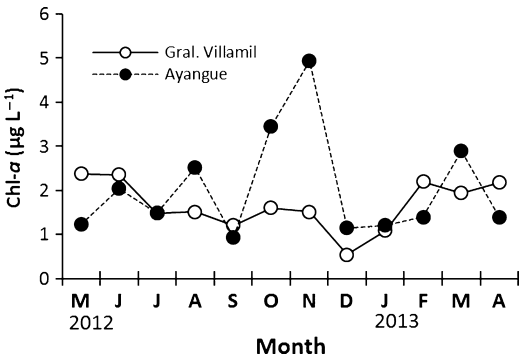


**Figure 3** Monthly variation in chlorophyll *a* concentration in the water column at General Villamil and Ayangue.

0.9 μg $L^{-1}$ to 4.9 μg $L^{-1}$ in September and November respectively (Fig. 3). The annual average of chlorophyll *a* was 1.7 ± 0.6 μg $L^{-1}$ for General Villamil and 2.1 ± 1.2 μg $L^{-1}$ for Ayangue.

### Biometric data

A total of 360 oysters were collected from both sites to determine the gametogenic cycle. The

  

average total weight of oysters from Ayangue was 308.5 ± 136.3 g, with 23.3 ± 11.0 g in average soft body weight. Average shell dimensions from this location were 12.1 ± 0.7 in length, 9.3 ± 0.4 cm in height and 3.6 ± 0.7 cm in width. Oysters collected at General Villamil were significantly larger ($t$ = 11.013, df = 360, $P$ = 0.000) in average total weigh (494.8 ± 189.9 g); with average soft body weight = 59.5 ± 32.5 g, and shell dimensions: 14.5 ± 0.9 cm in length, 11.5 ± 1.1 cm in height and 4.4 ± 0.5 cm in width.

### Reproductive cycle and GI

Histological sections of gonad showing male and female gonadal developmental stages are presented in Fig. 4. The gametogenic cycles of *S. prismatica* at General Villamil and Ayangue are illustrated in Fig. 5a and b. The reproductive patterns were similar in oysters of the two studied sites. According to histological analysis, the onset of gametogenesis (S1) was detected in September and October at General Villamil and Ayangue, with 13.3% and 46.7% of oysters in early gonadal development respectively. Ripe oysters (S3) were first observed in early summer for both populations. About 25% of the oysters were ripe at Ayangue in December and 93.3% at General Villamil in January 25%. Subsequently, the release of gametes (S4) began in January (12.5%) at Ayangue followed by General Villamil in February (46.7%). The spawning period persisted throughout the next 3 months at each location concomitantly with variations of environmental conditions. From April to September, all oysters sampled at Ayangue were either spawned and reabsorbing their gametes (S5) or in resting stage (S0), while at General Villamil, S5 and S0 oysters prevailed between June and September.

The GI of *S. prismatica* presented a clear annual cycle (Fig. 6). The lowest GI value was recorded in winter at both sampling sites (0.02 in August at General Villamil; 0.00 in August–September at Ayangue), and peaked in summer (0.96 and 0.89 in January for General Villamil and Ayangue respectively). A progressive GI increase throughout the winter–summer transition (October 2012–January 2013) followed by a decline during the summer months (January 2013–April 2013) was observed.

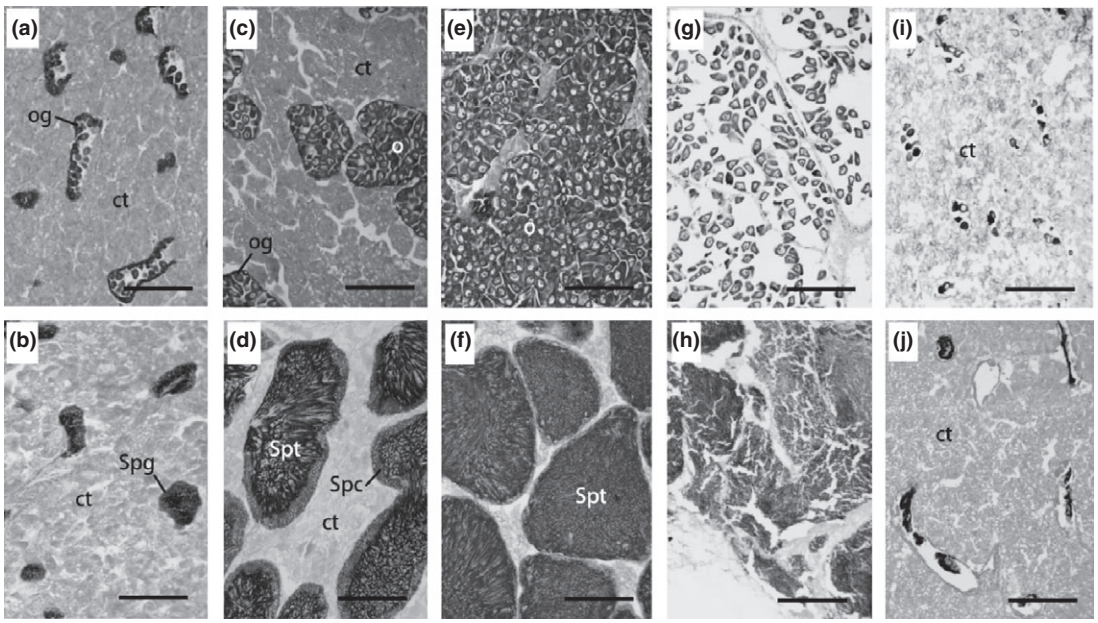


**Figure 4** Histological sections of gonadal stages of females (top row) and males (bottom row): S1, early development (a, b); S2, late development (c, d); S3, ripe (e, f); S4, spawning (g, h); S5, spent-reabsorbing (i, j). ct: connective tissue, og: oogonia, o: oocytes, Spc: spermatocytes, Spg: spermatogonia, Spt: mature spermatids. Bars: 200 μm.

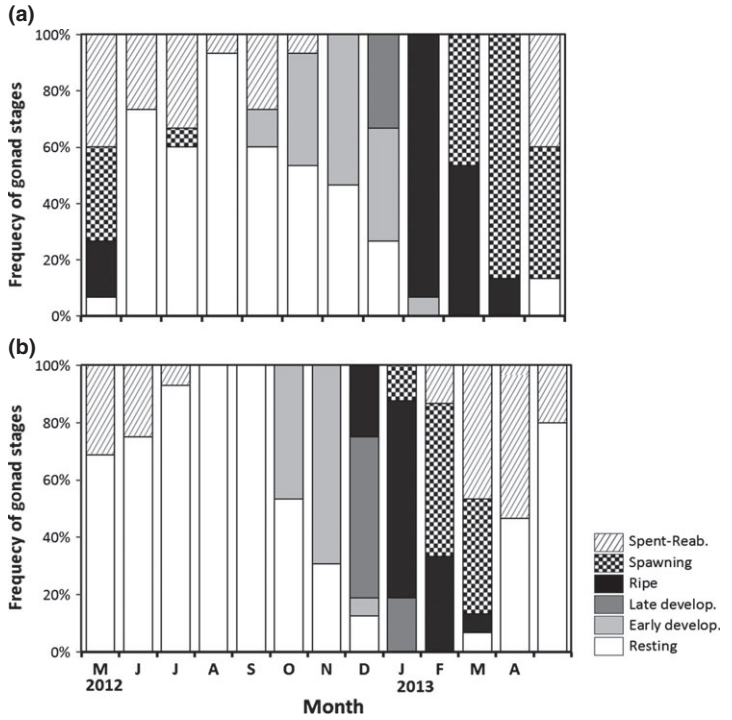


**Figure 5** Monthly frequency distribution of gonadal stages (%) of *Striostrea prismatica* at General Villamil (a) and Ayangue (b) between May 2012 and April 2013.

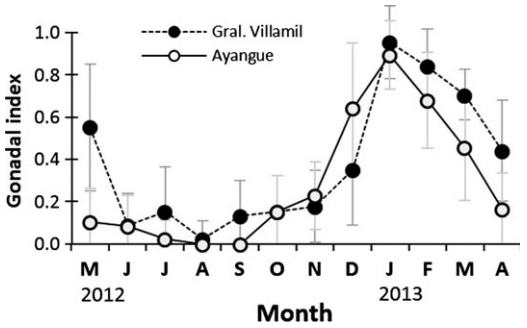


**Figure 6** Monthly gonadal index (mean ± SD; $n = 15$) of *S. prismatica* registered between May 2012 and April 2013 at General Villamil and Ayangue.

At General Villamil, the GI was positively correlated with temperature ($r = 0.748$, $P = 0.005$, $n = 12$), but not with salinity. In contrast, a negative correlation between GI and salinity was found at Ayangue ($r = -0.705$, $P = 0.010$, $n = 12$), but not with temperature. GI values were strongly correlated with temperature during gonadal development (October 2012–January 2013) at General Villamil ($r = 0.998$, $P = 0.002$, $n = 4$) and Ayangue ($r = 0.982$, $P = 0.018$, $n = 4$). Finally, no correlations were evidenced between GI and chlorophyll *a* at both locations.

### Sex ratio

About 34.4% of sampled oysters at General Villamil were female while 28.9% were male (male:female ratio of 1:1.19), the remaining individuals (36.7%) were sexually indeterminate (Resting, S0). At Ayangue, proportion of male predominated with 28.3% compared to 22.8% of female oysters (male: female, 1.24:1.00); 48.9% were indeterminate (S0). Nevertheless, Pearson Chi Square tests showed no significant differences between proportions of males and females at either location ($X^2 = 2.831$, $P = 0.092$). The general sex ratio of all sexually determined oysters from both populations was 1:1 (28.6% = male; 28.6% = female, and 42.8% = indeterminate). Hermaphrodites were not found at either site.

### Follicular area and oocyte sizes

The estimation of the follicular area occupied by the gonad of *S. prismatica* oysters (males and females from both sampling sites) along the reproductive cycle ranged from 0.4 ± 0.6% (S0) to 96.8 ± 2.4% (S3). Significant differences were observed in follicular area between gonadal stages ($F = 78.402$, gl = 4, $P = 0.000$) (Fig. 7). On average, oysters in stage S3 had significantly higher

  

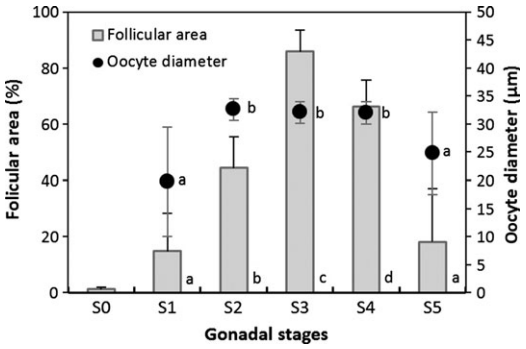


**Figure 7** Diameter (µm) of the oocyte (mean ± sd; $n$ = 9–23) and follicular area (mean ± SD; $n$ = 10) determined among different gonadal stages. Different letters indicate significant differences at $P < 0.05$.

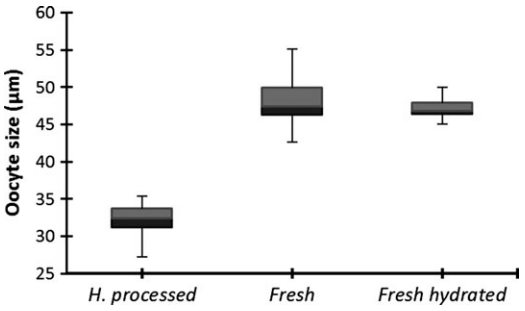


**Figure 8** Differences in oocyte size (mean ± sd; $n$ = 27) of selected *S. prismatica* female oysters with late development (S2) and ripe (S3) gonadal stages. *Histologically processed*; *Fresh* and *Fresh hydrated* oocytes.

follicular area (85.8 ± 7.8%) when compared to all other stages. S4 oysters presented statistically higher follicular area (66.3 ± 9.2%) compared to S2 oysters (44.3 ± 11.0%), S1 oysters (14.8 ± 13.4%) and S5 oysters (18.2 ± 24.8%). S2 oysters were also statistically different to S1 and S5 oysters, while S1 and S5 did not differ in follicular area. A dissimilar size pattern was observed in oocytes diameter between oysters in the same stage (Fig. 7). Among them, significant differences were found between S5 and S2, S3 and S4 ($X^2$ = 43.912, gl = 4, $P$ = 0.000).

### Fresh, histologically processed and hydrated oocytes size analysis

No statistically significant difference was found between fresh oocytes (48.0 ± 2.8 µm) and fresh hydrated oocytes (47.0 ± 1.2 µm) (Mann–Whitney $U$ = 685.500, $P$ = 0.705) (Fig. 8). In contrast, there was a significant 16.7 µm decrease in diameter (32.7%) of histologically processed oocytes compared to fresh oocytes ($t$ = 22.214, gl = 74, $P$ = 0.000), which measured 32.3 ± 1.9 µm on average. This analysis was carried out with oocytes in stages S2 and S3.

## Discussion

In both oyster populations, the reproductive cycle evidenced a similar unimodal pattern over the one-year study, suggesting that the cues for the reproductive modulation can be similar in both studied populations. Exogenous factors such as temperature and food availability play a significant role on growth and timing of gonad maturation in oysters (Gabbott 1983; Mackie 1984; Chávez-Villalba *et al.* 2003; Fabioux *et al.* 2005). The seawater temperature reported in this study exhibited a distinctive seasonal cycle as described by Cucalón (1989), which can be summarized in two differentiated periods: summer (January–April) and winter (July–October). These thermal periods are associated to the onset of gametogenesis (oogenesis and spermatogenesis) that began after a 4 month resting period (S0) during the winter (September at General Villamil and October at Ayangue).

The relatively colder water registered during winter time at both locations is usually rich in plant nutrients due to coastal upwelling, giving raise to higher primary productivity levels (Cucalón 1989). In fact, high Chlorophyll *a* concentration measured at General Villamil and Ayangue appear not to have affected the energy demands required for the reproductive conditioning. In addition, it is remarkable that oysters in early development stage (S1) at Ayangue coincided with the highest chlorophyll *a* values of the studied period (3.4 µg $L^{-1}$ and 4.9 µg $L^{-1}$ in October and November, respectively), suggesting that high phytoplankton biomass associated to lower temperatures may leads to the onset of gametogenesis (S1). On the other hand, many authors have suggested that chlorophyll *a* levels may not provide a precise estimation of appropriate food, since energy inputs necessary for reproductive activities may also depend on other factors like phytoplankton concentration, quality (nutritional composition of the diet) or on non-algal food supply, such as microbes or dissolved organic matter (Nell, Skeel & Dunkley 1983; Utting & Millican 1997; Baines,

Fisher & Cole 2007; Palais, Mouneyrac, Dedourge-Geffard, Giambérini, Biagianti-Risbourg & Geffard 2011).

Gamete development advanced rapidly during the winter-summer transition, and most oysters attained maximal sexual maturity (S3) in January 2013 at both sites, when the water reached the highest annual temperature (29.6°C at General Villamil; 28.1°C at Ayangue). These results suggest that the rising of temperature may play an important role in regulating the gonadal development of *S. prismatica*, as mentioned for several species of the Crassostreinae subfamily, such as *Crassostrea gigas* (Chávez-Villalba *et al.* 2002; Fabioux *et al.* 2005), *C. plicatula* (Li, Liu, Shirasu, Chen & Jiang 2006) and *C. corteziensis* (Rodríguez-Jaramillo *et al.* 2008). For *S. prismatica*, a similar reproductive pattern in relation to temperature has been previously reported on the West coast of Mexico (Stuardo & Martínez 1975; Cuevas-Guevara & Martínez-Guerrero 1979; Frías-Espericueta *et al.* 1997), where gametogenesis and ripeness of this species occurred seasonally when the water temperature increased from about 24 to 27°C (February–August). Moreover, on the Pacific coast of Costa Rica, higher annual water temperatures fluctuating between 29 and 32°C were required to maintain the reproductive activity all over the year for *S. prismatica* populations (Fournier 1992). Our results are consistent with laboratory data of Argüello-Guevara *et al.* (2013), who reported the achievement of Late development stage (S2) starting from Resting stage (S0) in *S. prismatica* oysters (collected in September 2011 at Ayangue), by increasing the seawater temperature in lab conditions from 23°C to 28°C in 3 days and then keeping the warm temperature for seven consecutive weeks. The Pearson correlation between gonadal index (GI) and water temperature at both sampling sites during the 12-month study was weak for the post-spawning period (low GI) that occurred during the months of May to July 2012. However, a strong correlation was found for the October 2012–January 2013 period, coinciding with an increase in both water temperature and gonadal index.

The observed synchronized gonadal development of males and females may contribute to the reproductive success of the species, due to simultaneous release of gametes and thus achieving a higher probability of fertilization. Oyster spawning was evidenced in summer at the two populations. A vast majority of bivalve species tend to spawn during the summer (Mackie 1984), when environmental conditions are propitious for larval development (Chávez-Villalba *et al.* 2002). Surface salinity also varied notably during warmer periods due to freshwater intrusion caused by runoff water and river flows, which increase particularly during the rainy season (December–April). These cyclic varying environmental conditions in temperature and salinity appear to influence on *S. prismatica* physiology triggering its spawning. In studies conducted with S. *prismatica* on the Pacific coast of Mexico and Costa Rica, spawning coincided with a pronounced decrease in salinity from 32 g $L^{-1}$ to 0 g $L^{-1}$ at Mexico and from 32 g $L^{-1}$ to 29 g $L^{-1}$ at Costa Rica (Stuardo & Martínez 1975; Cuevas-Guevara & Martínez-Guerrero 1979; Fournier 1992). Moreover, Frías-Espericueta *et al.* (1997) found that populations of *S. prismatica* begin to spawn when water temperature reaches 30°C in the Northwest coast of Mexico. Several environmental factors that induce spawning were recently tested under controlled conditions for *S. prismatica*. Those involving changes in water temperature (from 28°C to 33°C) and salinity reduction (from 32 g $L^{-1}$ to 15 g $L^{-1}$) succeeded in the release of gametes (Argüello-Guevara *et al.* 2013). These results are in agreement with previous studies carried out with tropical oysters (Angell 1986; Quayle & Newkirk 1989).

Spawning was usually incomplete, particularly in oysters from General Villamil. In those cases, the reabsorption of gametes (S5) was observed (May–October 2012). It has been generally accepted that reabsorption occurs after partial spawns to clean the gonad and prepare it for a new cycle (Fabioux *et al.* 2005). Unspawn or partially spawned oysters can reabsorb gametes and reconvert them into glycogen (Quayle 1988).

Although statistically differences in sizes and soft body weights were found in oysters collected between study sites, their similarities in sex ratio suggest that adult *S. prismatica* measuring between 9.8 and 19.0 cm in shell length (13.3 ± 2.3 cm) present even sex ratios, which in our study was 1:1 at both locations. In contrast, a dissimilar sex ratio of 3:1 (male to female) and hermaphroditism (2%) has been reported in other populations of the same species (Fournier 1992). However, the author reported smaller individuals in shell length (7.9 ± 2.6 cm in males and 9.0 ± 2.6 cm in females) as compared to the ones collected in this

  

study. Information on sex determination mechanisms for *Striostrea* genus in natural populations is limited. However, based on the present findings, it can be speculated that *S. prismatica* may present features of hermaphroditism and protandric dioecy, showing a higher proportion of males to females at an early stage but decreasing with age or size due to sex reversal, such as described for *C. gigas* and *C. virginica* (Coe 1932, 1943; Guo, Hedgecock, Hershberger, Cooper & Allen 1998).

Follicular area occupied by oyster gonads varied considerably in *S. prismatica*, between 1.3 ± 0.7% (S0) and 85.8 ± 7.8% (S3), increasing accordingly with the advancement of gonadal developmental stages. Similar trends have been reported for *C. gigas* (Enríquez-Díaz *et al.* 2009) ranging from 1% to 69%, and *C. corteziensis* (Rodríguez-Jaramillo *et al.* 2008) ranging from 1% to 29%. However, dissimilarities in gonadal area coverage (follicular area) among these studies could be related to differences in the measurement methods or selection of histological sections. The increment in follicular area during S1 and S2 was correspondent with oocyte development. Ripe oocytes diameter in this study (32.0 ± 1.9 μm) were similar to those reported for *C. gigas* and *C. corteziensis*, with 36.1 ± 4.4 and 31.6 ± 0.8 μm, respectively (Lango-Reynoso *et al.* 2000; Rodríguez-Jaramillo *et al.* 2008). Mean oocyte diameter, determined histologically, reflects the reproductive cycle, increasing in size as they develop (Barber & Blake 2006). The absence of statistical differences in oocyte diameter found between S2, S3 and S4 gonadal stages might indicate the possibility of finding ripe oocytes in S2 stage at least in the centre of the follicles (where measurements were carried out), and thus spawning might be possible even at this early stage. Although this reproductive condition has not been validated in the environment, spawning of *S. prismatica* in S2 stage has been recently observed in the laboratory (Argüello-Guevara *et al.* 2013).

Oocytes diameter, as determined through image analysis, is a useful tool to describe monthly variations of oyster oocyte development throughout its annual reproductive cycle (Lango-Reynoso *et al.* 2000; Meneghetti *et al.* 2004). However, it is important to highlight if measurements were performed on fresh or histologically treated tissues in studies involving oocyte analysis. Our results indicate than histologically treated oocytes tend to be smaller in size compared to fresh or histologically untreated oocytes. The diameter of histologically processed mature S2 and S3 oocytes were on average 32.7% smaller when compared to histologically untreated oocytes. Fortunately, non-invasive techniques such as magnetic resonance imaging (MRI) have shown to be appropriate for quantifying the growth of somatic and gonadic tissues in live oysters (Davenel *et al.*, 2010; Smith & Reddy 2012). Nevertheless, further studies are necessary to enhance preciseness of oocytes measurements. On the other hand, the diameter of ripe hydrated oocytes measured in this study (47.0 ± 1.2 μm) was similar to that reported byArgüello-Guevara *et al.* (2013, 47.0 ± 1.5 μm) and Li *et al.* (2006, 49.6 μm) for *S. prismatica* and *C. plicatula* respectively.

In conclusion, this study shows evidence that the reproductive cycle of two wild population of *S. prismática* is linked to seasonal surface seawater temperatures patterns characteristic of the southern coast of Ecuador. This information about levels and variability in environmental parameters such as temperature and salinity required for gonad development can be used for broodstock conditioning in aquaculture projects. As an alternative, mature organisms could be collected from wild populations during January and February for artificial spawning under laboratory settings. Our findings of February and March being the highest spawning periods of wild *S. prismatica* populations may help biologists and administrators to establish conservation management strategies and policies for this resource. Despite this contribution in describing the natural reproductive cycle of *S. prismatica* and other recent studies of larval development in hatchery settings (Loor 2012), it is considered that further research is still required to understand larval dynamics of *S. prismatica* in spawning sites such as larvae distribution and development cycles in the nature, settlement characteristics such as timing and substrate preference, just to mention a few.

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