

REPRODUCTIVE BIOLOGY OF THE NONNATIVE OYSTER, *CRASSOSTREA GIGAS* (THUNBERG, 1793), AS A KEY FACTOR FOR ITS SUCCESSFUL SPREAD ALONG THE ROCKY SHORES OF NORTHERN PATAGONIA, ARGENTINA

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ABSTRACT *Crassostrea gigas* was introduced in Anegada Bay (North Patagonia, Argentina), in 1981 for aquaculture purposes. The species has, since established in the field, covered all available hard substrata in the bay and gradually expanded south along the coast of the neighboring province of Río Negro, 90 km away from its original introduction site. Our work focused on the reproductive dynamics of the species at the introduction site, with emphasis on the thermal thresholds needed for each stage of gonad development. During early spring, the oysters in Anegada Bay go through active gonad proliferation. Maturity extends mainly from October to January. The first spawning occurs in November, when seawater temperature exceeds 17°C, and peaks from December to February, when seawater temperature lies in the range of 19–21.5°C. The timing of gonad development is size dependent. Larger (≥ 70 mm) and medium-size oysters begin spawning first, whereas small oysters (<40 mm) represent the late spawners of the season. The *C. gigas* population in Anegada Bay is a clear and well-documented case of an introduction site where optimal environmental conditions are met, both for complete gonad maturation and for successful larval survival and settlement. We compiled a series of *C. gigas* cases from different parts of the world in which reproduction is related to seawater temperature. We concluded that the thermal threshold for full female gonad ripening in this species is strict (temperature > 17°C), and spawning only occurs in those sites where this threshold is reached.

KEY WORDS: oysters, nonnative, *Crassostrea gigas*, reproduction

INTRODUCTION

The Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), has been extensively introduced around the world for aquaculture purposes, and in many locations it has been established in the field (Mann et al. 1991, Carlton 1992, Shatkin et al. 1997, Mckindsey et al. 2007). In South America, *C. gigas* was introduced in the Pacific Ocean in Chile and Peru (Winter et al. 1984), and in the Atlantic in Brazil and Argentina (Pascual & Orensanz 1996, Pascual & Castaños 2000, Orensanz et al. 2002). In 1981, a stock of commercial size *C. gigas* (approximately 500 individuals) was brought from Coquimbo (Chile) and was introduced to the southern part of Anegada Bay (39°50'S–40°40'S and 61°59'W–62°28'W, North Patagonia, Fig. 1) with the purpose of implementing an aquaculture project. After a year of experimentation, the project was interrupted and the remaining stock abandoned, but the species became established in the bay (Pascual & Castaños 2000, Orensanz et al. 2002, Escapa et al. 2004, Borges 2005).

Despite its significant representation in world oyster production (98%), and the fact that it is one of the most widely introduced species, the Pacific oyster *C. gigas* has not become adapted to many of the natural environments where it has been introduced. It seems paradoxical that a species that tolerates such a wide range of environmental conditions for grow-out (Shatkin et al. 1997) could be so strict regarding the conditions needed to accomplish successful larval settlement. In Chile, for example, female *C. gigas* do not spawn naturally in any of the sites where it is cultivated along the shore, from Chiloé to Coquimbo, because of low water temperatures. Spawning has been detected only in one shallow coastal lagoon located in Cahuil (region VI), where summer seawater temperature exceeds 20°C (Pereira, pers. comm.).

In Brazil, where *C. gigas* was introduced in the 1970s in Santa Catarina, high seawater temperatures (>30°C) similarly precluded massive reproduction. A recent contribution, however, presents the finding through molecular identification of some *C. gigas* individuals mixed in natural oyster grounds with *Crassostrea brasiliensis* and *C. rizophorae* in protected sites in the south of Florianópolis (Melo et al., 2009).

In both countries, oyster production relies on hatchery seed, and broodstock conditioning at 20–22°C guarantees normal gonad ripening and gamete release (Hickey 1997). The lack of widespread natural recruitment has impeded a greater expansion of the Pacific oyster industry in both countries.

Escapa et al. (2004) carried out from 2001 to 2003 a thorough study of *C. gigas* oyster grounds of Anegada Bay with the main purpose of evaluating the number, distribution, and extension of *C. gigas* beds, and to evaluate, through sampling and field experiments, its effects on the benthic community and shorebird habitat use. This research provided, among other information, knowledge regarding the distribution of the oyster beds in the bay. According to them, in 2003, oyster reefs covered a total of 36.45 ha, which is less than 0.05% of the bay's intertidal area. Besides the areas colonized by oysters, there are no free superficial hard substrata along the intertidal zone. Spat was found attached to limestone outcrops, empty shells, shells of the native small mussel *Brachidontes rodriguezii* and to the basal portion of cordgrass (*Spartina alterniflora*). Oyster densities were higher in the middle and middle-low intertidal zones compared with other intertidal zones, and oysters were not found in the low intertidal or subtidal zones.

Although this study demonstrated that the expansion of this exotic species is limited by the availability of hard substrata, spatfall has occurred regularly, and the oyster is well established in this area. During the past 5 years, this species expanded south (90 km from Anegada Bay) and reached the estuary of the Negro River (pers. obs.; Fig. 1B).

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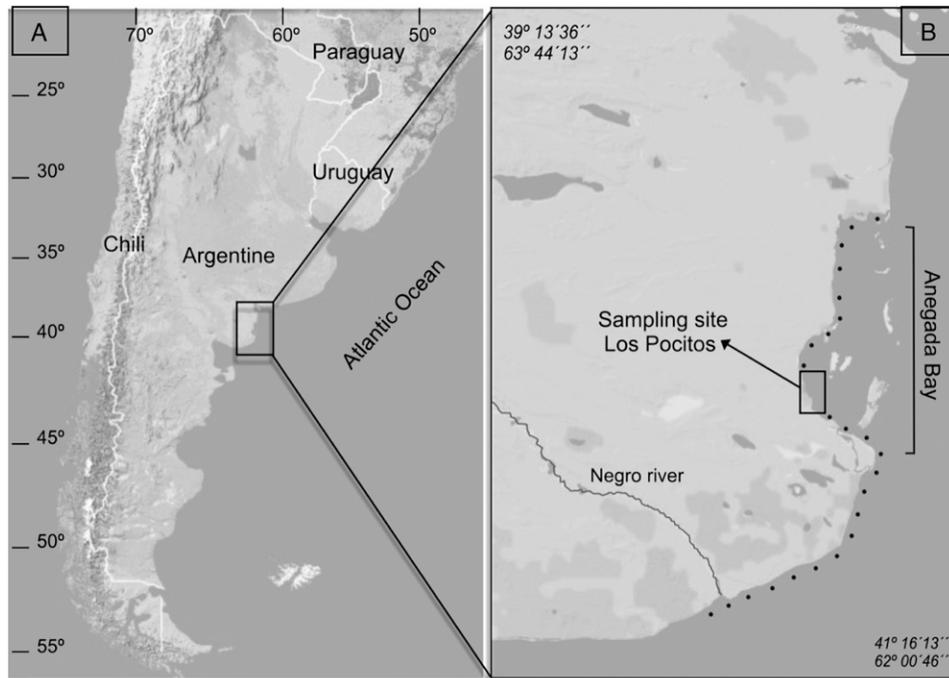


Figure 1. (A) Map showing the study area in North Patagonia, Argentina. (B) Sampling site (Los Pocitos) on Anegada Bay. Black circles indicate the current distribution of *C. gigas* along the coast from Anegada Bay to the Negro River.

Small lots of hatchery seed were also experimentally introduced in 1998 in the North of Buenos Aires Province (Las Toninas; Lat., 36°27'S; Long., 56°41'W) and in the southern province of Santa Cruz (San Julián Bay; Lat, 49°11'S; Long, 67°39'W) for aquaculture purposes. The species grew normally both in suspended and off-bottom culture, but no reports of establishment exist. No further documented introductions were performed in these sites.

In Anegada Bay, the presence of *C. gigas* and the development of an oyster industry based on natural spatfall and off-bottom culture has produced controversial points of view among growers and conservationists.

We report the results of a study intended to describe the annual cycle of gonadal condition of *C. gigas* in Anegada Bay as a means to understand one of the main processes underlying its successful establishment and spread in northern Patagonia. In addition, the effect of temperature on gonad maturation and spawning is discussed in relation to previous studies and observations around the world.

MATERIALS AND METHODS

Study Area

The study was carried out on the intertidal oyster bed located at Los Pocitos, Anegada Bay, Northern Patagonia (39°50'S–40°40'S and 61°59'W–62°28'W, Fig. 1). Samples were collected from September 1998 to August 1999.

Temperature, Salinity, and pH

Temperature, salinity, and pH were recorded monthly during the sampling period. The measurements were made

with a mercury thermometer, a digital pH meter, and a refractometer.

Sample Collection and Processing

Monthly samples comprising 100 oysters of sizes between 10 and 100 mm total height were manually collected. The oysters in the sample were distributed in size classes as follows: 30 individuals < 40 mm, 40 individuals within the size range 40–69 mm, and 30 individuals \geq 70 mm. Oysters were transported in coolers to the laboratory for histological analyses.

Condition Index

At monthly intervals throughout the year, 40 oysters larger than 60 mm were sampled for condition index (CI). The oysters were sacrificed and total weight (TW) and fresh meat weight (FMW) were recorded. The estimated index was

$$CI = FMW/TW \times 100.$$

Histological Treatment

The sex and stages of gonadal maturity were histologically determined on each oyster. The visceral mass was separated from the shells and a section across the gonadal tissue (approximately 1 cm³), from the dorsoventral area of the body, was excised from each individual. Each tissue section was fixed in Davidson's solution, stored in 70% alcohol, and dehydrated in consecutive baths of ethanol and a 1:1 mix of 100% ethanol and xylene. Tissue samples were embedded in paraffin (56–58°C). Histological sections of 5–7 μ m were made with a rotary microtome and stained with hematoxylin and eosin as described

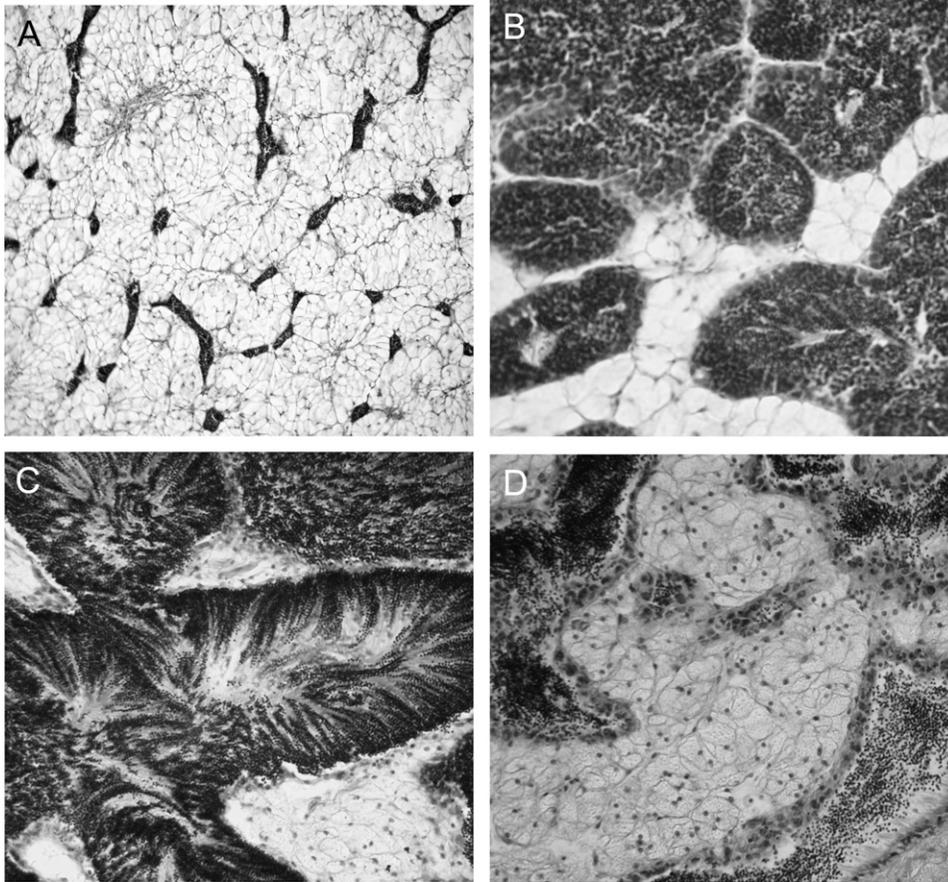


Figure 2. Histological section showing the male gonad stages. (A) Proliferation ($\times 100X$). (B) Early maturation ($\times 400X$). (C) Maturation ($\times 400$). (D) Totally spawned ($\times 400$).

by Humanson (1967). Slides were examined and photographed under a light microscope.

Analysis of the Reproductive Cycle

Gonadal tissues were classified on a scale of maturity modified from the one used by Sugiwaru (in Imai 1982) for the same species. The gametogenic cycle was then divided into the following categories for males (Fig. 2) and females (Fig. 3), respectively:

Stage 0: Sexually undeveloped. Follicles do not exist or are small and elongated in shape. Walls are covered by a germinal undifferentiated epithelium. Connective tissue is widely developed.

Male Developmental Stages

Stage I: Proliferation. The spermatogonia and some spermatocytes are visible along the germinal epithelium. Connective tissue is abundant. As proliferation increases, spermatocytes and spermatids form a narrow band along the follicular wall. No free spermatozoa are observed in the lumen.

Stage IIa: Early maturation. Cell components of the spermatogenic series are oriented toward the follicle lumen. Connective tissue decreases as maturation advances. Free spermatozoa may be visualized in the lumen.

Stage IIb: Total maturation. The interfollicular tissue and germinal epithelium are inconspicuous. The follicles are

completely full of spermatozoa densely packed, with their tails pointing toward the lumen. Sometimes, a few spermatocytes may be seen around the follicular wall.

Stage IIIa: Partially spawned. Follicles partially emptied, with a large number of spermatozoa loosely arranged. A small band of spermatocytes and spermatids is observed along the follicular wall.

Stage IIIb: Totally spawned. Follicles have drastically reduced their size and few spermatozoa still remain in the lumen. The follicle wall contains few scattered germinal cells.

Stage IV: Postspawning. Connective tissue rapidly develops among the follicles, which are collapsed. Residual spermatozoa and phagocytes can be observed in the small lumen of the follicles.

Female Developmental Stages

Stage I: Proliferation. Tiny follicles isolated in the gonad begin to develop secondary sexual cells that cover the follicle wall. In a more advanced stage, follicles grow at the expense of the connective tissue and oocytes can be observed.

Stage IIa: Early maturation. Few oogamies and previtellogenic oocytes are seen on the follicle walls. Vitellogenic pedunculated oocytes are visible in the lumen. Connective tissue is scarce.

Stage IIb: Total maturation. Boundaries between follicles are not distinguishable. Lumen are completely filled with

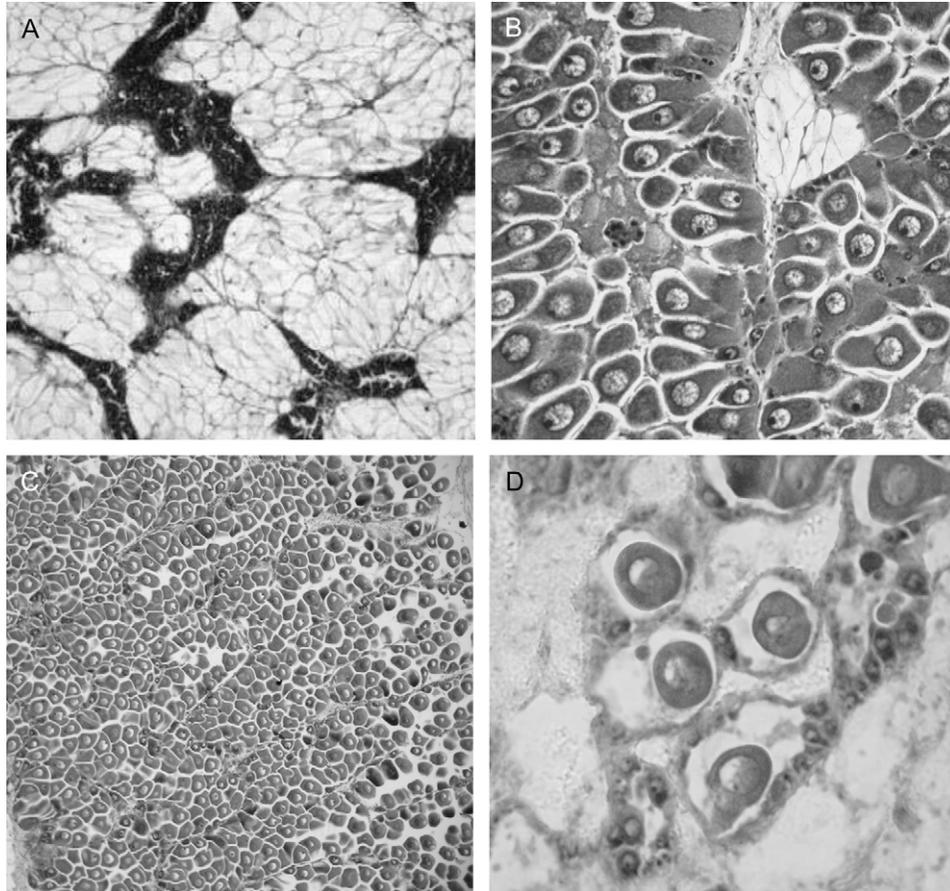


Figure 3. Histological section showing the female gonad stages. (A) Proliferation ($\times 400$). (B) Maturation ($\times 400$). (C) Partially spawned ($\times 100$). (D) Totally spawned ($\times 400$).

vitellogenic polyhedral oocytes. Macroscopically, the gonad appears white and creamy.

Stage IIIa: Partially spawned. A large number of free oocytes is loosely arranged and free space can be observed in the lumen.

Oocytes attached to the follicle wall may still be present.

Stage IIIb: Total spawning. Collapsed follicles are noted. Few nonreleased oocytes remain free in the lumen.

Stage IV: Postspawning. Follicles are collapsed and small. Large numbers of phagocytes appear and the residual oocytes are being "cytolized." Connective tissue begins to develop.

Relative frequencies of gonad developmental stages were obtained throughout the annual cycle. Sex ratio of the population was tested by chi-square analysis.

Frequency Distribution and Mean Size of Oocytes

Oocyte size was determined from histological sections using an eyepiece graticule calibrated with a stage micrometer ($\times 400$). Five to 8 females were randomly selected from the lot sampled during each collection date, and at least 100 oocytes (among those with clearly visible nucleoli) per female were measured through the longest axis, transverse to the nucleus. Individuals with scarce oocytes and extensive phagocytosis (spent and undifferentiated individuals) were not ana-

lyzed, following the criteria recommended by Grant and Tyler (1983).

RESULTS

Temperature, Salinity, and pH

Seawater temperature ranged from a minimum of 7.6°C in July to a maximum of 21.5°C in January; salinity ranged from 33.2–36.1‰ (Fig. 4). pH values remained slightly alkaline, fluctuating between 8.6 and 8.75 through the year.

Condition Index

CI oscillated inversely to seawater temperature. In September it showed its maximum value (CI = 15.4), decreasing steadily through spring and summer until reaching its lowest value in March (CI = 8.5). In April, an abrupt decrease in seawater temperature occurred ($\Delta 5^\circ\text{C}$) and condition began to recover (Fig. 4).

Sex Ratio

Sexual differentiation of reproductive cells occurs during fall and winter. As the cold season progresses, the number of individuals sexually differentiated gradually increases. Sex ratio

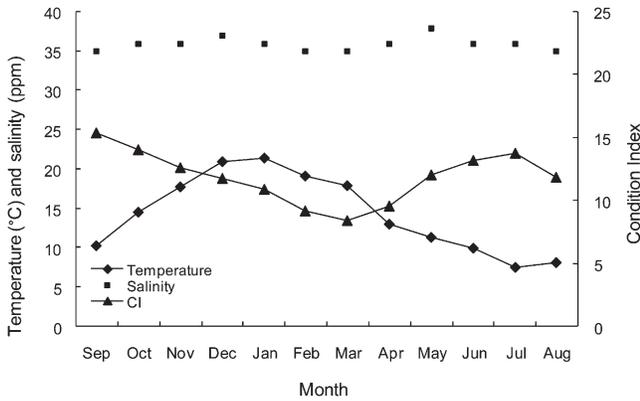


Figure 4. Monthly values of seawater temperature (measured in degrees Celsius), salinity (measured in parts per million), and the condition index (CI).

of the whole population (all months considered) was statistically even, with a trend toward relatively more males (46% vs.54%; chi-square = 0.93; n = 684 oysters).

Young oysters mature only as males, and only oysters larger than 27 mm may develop as females. Sex ratio approaches 1:1 in oysters larger than 40 mm, whereas older oysters (>80 mm) show a slight female predominance.

No true hermaphrodites were found. Only two male individuals were observed at the end of the spawning season bearing one or two previtellogenic oocytes per follicle.

Gametogenic Cycle

In September, the entire population entered the proliferation stage, which progressed during early spring (Fig. 5). Seawater temperature from September to October ranged from 10.3–14.6°C. By December, no oysters were found in this stage. Maturation began in October and peaked in November, at 70% of the population. The first spawners were detected in early November (27% of the population) when seawater temperature surpassed the 17°C barrier (November temperature, 17.8°C). Mass spawning occurred in January (66%) and February

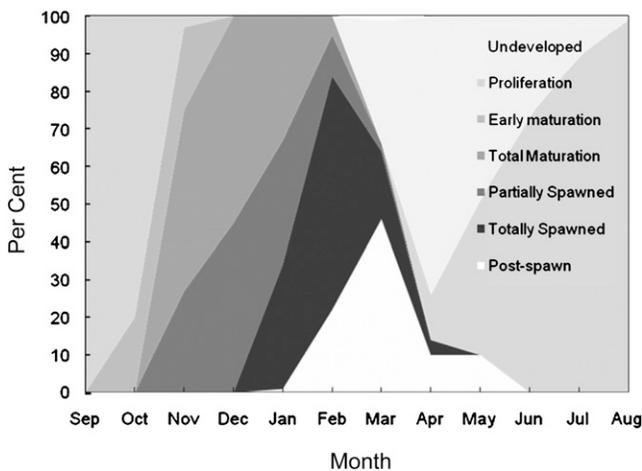


Figure 5. Annual gametogenic cycle of *C. gigas* in Anegada Bay.

(73%), during which time the seawater temperature was 19–21.5°C. Spawning continued with lower intensity through March until April, when the last spawners were detected and seawater temperature fell abruptly to 13°C.

In January, 1% of the population was found in the postspawning stage, with these individuals corresponding to the early November spawners. By February, the number of oysters that had already spawned increased to 22%, and in March and April 79–84% of the population was represented by individuals that already spawned (stage V) or was inactive (stage I). The remainder in April consisted of oysters in the proliferation phase (12%) and late spawners (4%). From May to August, the number of oysters in the proliferation phase gradually increased, whereas seawater temperature decreased to its minimum July value (7.6°C). The oysters with sexually undeveloped gonads decreased steadily until August, when they only represented 1% of the population.

The spawning season is more extended for males than for females; both began in November, but males ended spawning later (April vs. March). At the start (November), there were more male than female spawners (13% vs. 1%). Spawning peaked 1 month earlier in females than in males (females, 26% in January; males, 37% in February).

Oocyte Growth

Oocyte size increased slowly through the winter and early spring, showing an abrupt increase in November coincident with an increase in seawater temperature (from 14.6–17.8°C; Fig. 4). Ripe oocytes reached a maximum mean size of 41 μm (SD, 6 μm) at full maturity. These oocytes were found in the samples of oysters collected between November and March (Fig. 6).

When oocyte mean diameter is related to oyster size, the following pattern is found: Medium (40–69 mm) and large (≥70 mm) oysters may reach oocyte ripeness in November, whereas small oysters (<40 mm) present immature oocytes from September to November (oocyte mean diameter, 14–17 μm) that do not reach ripeness until December. Large and medium oysters present ripe oocytes until March, whereas in April, only small oysters have gonads with ripe oocytes.

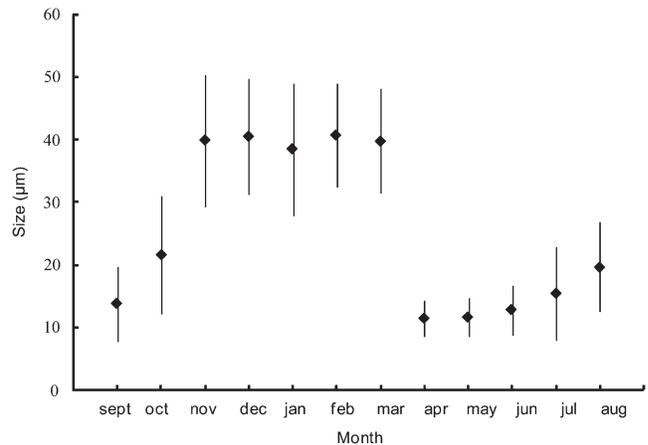


Figure 6. Seasonal variation in mean oocyte size. Vertical bars represent standard deviations.

TABLE 1.
Size and sex-specific spawning behavior of *C. gigas* during the reproductive season.

Gonad Stages		Proliferation		Maturation		Spawning	
Month	Size	F	M	F	M	F	M
September	Small	33	67				
	Medium	44	56				
	Large	72	28				
October	Small	29	57	0	14		
	Medium	59	16	0	24		
	Large	63	17	0	20		
November	Small	33	0	0	67		
	Medium	0	0	29	51	2	0
	Large	0	4	25	32	18	17
December	Small			17	33	17	21
	Medium			37	18	18	33
	Large			29	26	18	26
January	Small			0	33	0	67
	Medium			16	18	34	32
	Large			9	22	44	22
February	Small			0	0	0	57
	Medium			2	5	36	36
	Large			0	3	10	62
March	Small					0	26
	Medium					7	17
	Large					0	0
April	Small	0	0			0	11
	Medium	21	0			0	2
	Large	8	8			0	0

Size ranges are expressed as: small (<40 mm), medium (40–69 mm), and large (≥ 70 mm). Frequencies are expressed as percentages of the total number of oysters sampled in each month. F, female; M, male.

Size and Sex-Specific Spawning Behavior

When spawning behavior is analyzed in relation to both sex and size of oysters, a different pattern appears (Table 1). Large and medium oysters began spawning in November, whereas small oysters began spawning one month later. Small oysters spawned from December to April, but only in December included females, which probably represent the slow growers of the year class. Large oysters ended spawning in February, earlier than the rest, and the peak of gamete emission occurred in January (48%). Medium oysters spawning also peaked in January (35%). Small and medium oysters spawned until April but were only represented by males.

DISCUSSION

A small number of adult oysters, *C. gigas*, were introduced for aquaculture purposes in 1981 in Anegada Bay and shortly abandoned thereafter, when the enterprise in charge of the project shut down the facility for unknown reasons (Pascual & Castaños 2000, Orensanz et al. 2002). No reports on this stock were available until 1990, when some dead oysters were sent to our laboratory by local fishermen. Both the huge size of the oysters and the existence of overspawning on their shells suggested that the 1981 oysters had survived and might have been naturally spawning in the bay (Pascual, unpublished). In 1994, cupped oysters were found scattered in the intertidal zone of Anegada Bay, fixed to limestone outcrops and to clumps of the small mussel *Brachydontes rodriguezii* (Pascual, pers. obs.).

Since 1998, recruitment has increased, and hard substrata were gradually colonized. As a result, an artisanal aquaculture industry started in the bay based both on wild and hatchery seed (Pascual & Castaños 2000, Borges 2005). Fishery activity also began in 2001, but the artisanal hand gathering of oysters on some spots was low mainly as a result of the weak demand of the domestic market. Oyster fishing did not seem capable of controlling the spreading of the wild population.

This introduction has been, and still is, a matter of an intense controversy. Concerns arose, for instance, that the invader could become a threat to migratory shorebirds by decreasing the biodiversity of the benthic community upon which they fed. Escapa et al. (2004) addressed this particular topic and evaluated the oyster beds and their ecological impact, finding no negative impacts on benthic biodiversity. They also reported that, in contrast to most cases of successful introductions of *C. gigas* elsewhere, the success (in terms of the total area occupied to that date) of this species in Anegada Bay was low. They suggested that expansion of the oyster reefs was mainly constrained by the availability of hard substrata.

Despite the fact that no further thorough and complete surveys of these populations had been performed since 2003, the observed expansion of the species is obviously strong, both within and outside the bay. The species, 28 y after its introduction in Anegada Bay, is currently colonizing the coast of the neighboring province of Río Negro (Fig. 1), where natural spatting was found on hard substrata 90 km south from its primary introduction site (pers. obs., unpublished).

TABLE 2.
Summary of information on the relation between gametogenic stages of *C. gigas* and seawater temperature in different geographic locations.

Country and Regions	Locality	Proliferation (°C)	Maturation (°C)	Spawning (°C)	Total Evacuation Females (°C)	Annual Seawater Temperature Range (°C)	Sources
Alaska	Prince William Sound			No, <17			Prince William Sound Regional Citizens' Advisory Council (2004)
Argentina	Anegada Bay	13.1	14.6	17.8	21.5	7.6–21.5	Current study
Australia	Port Stephens, NS			18–26		13–27	Mason and Nell (1995)
Brazil	Cabo Frio			Only males	No	18.2–28.9	Costa Muñoz et al. (1986)
	Santa Catarina			No	No	16–30	Bastos Maccacchero et al. (2007)
Canada	Cananea, Sao Paulo			23	26.3	17.4–29.8	Akaboshi et al. (1983)
Chile	Guaratuba Bay, Parana			No, <17	No	20–27	Christo (2006)
	British Columbia			>20			Waldichuk et al. (2008)
	Cahuil			Yes			Perreira (pers. comm.)
Denmark	Isefjord	11	>17	18–21		0–20	Wang et al. (2007)
France	Bay of Brest		18–19	20–22.5	19.6	7.5–25	Lango-Reynoso et al. (1999)
	Arcachon					1.5–24.5*	His and Robert (1985), Robert et al. (1987), Maurer et al. (2003)
	Normandy, Baie des Veys	10	19	>19	19.4	5–19.2 (mean)	Chávez-Villalba et al. (2003), Enriquez-Díaz (2004)
	Arcachon/Marennes-Oleron	10–10.3	>15	>18		6–19.4	Chávez-Villalba et al. (2003), Goulletquer and Héral (2003) Enriquez-Díaz (2004)
Germany	Gironde			No, < 18			
	Brittany	10	10–14	No, 22–30			
	Mediterranean			Yes		22–30	Diederich et al. (2005)
	List Basin and Hornum Basin			Yes		2.3–18.2 (>20 inches)	Riese (1988), Kochmann (2008)
	Stylt			Yes		3.5–19.5	Spencer et al. (1994)
Great Britain	Devon/Gwynne			>18			Steele and Mulcahy (1999)
Ireland	Cork Harbor			No		2.7–18.6	
	Dungarvan			17.5	18.4	2.7–18.45	
Israel	Gulf of Eilat	12–23		17–23	22–26	14–28	Shpigel (1989)
Japan	Hiroshima Bay and Matsushima Bay	16–22	19–25	>19	Yes		Ventilla (1984)
Korea	Hansan-Koje and Jaran Bay	10.5–15		18–20		6.5–26.5	Kang et al. (2000)
Mexico	Mina Vieja	16.8–17	17–20	19–23	19.8	15.8–23	Paniagua-Chávez & Acosta-Ruiz (1995)
	La Boca	12.8	15–17	17–18.5	15–17	12.8–19	
	Bahia Falsa (Baja California)	20	20–21	20–24	21–25	19–25	Cáceres-Martínez et al. (2004)
Netherlands	Oosterschelde			Yes		0–5/18–22	Smaal et al. (2009), Troost et al. (2009)

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TABLE 2.
continued

Country and Regions	Locality	Proliferation (°C)	Maturation (°C)	Spawning (°C)	Total Evacuation Females (°C)	Annual Seawater Temperature Range (°C)	Sources
New Zealand	Northland, Mahurangi Harbor	14–16	16	18–20		14–20	Dinamani 1987
Portugal	Ria Formosa, Algarve	>19	20–24	19–25	20–22	19–25	Massapina et al. (1999)
South Africa	South and east coast			Yes		10–24	Robinson et al. (2005)
Spain	O Grove, Galicia	13	13–15	16–19.5		17.6 (mean)	Ruiz et al. (1992)
Taiwan	West and south coast			19–25	Yes	4.8–24.8 9.6–24.9	Bardach et al. (1972), Kyu Lim (2006)
Tunisia	Bizert Lagoon	14		20	23–27		Dridi et al. (2006)
United States	Chesapeake Bay	8.5	>17	18–23	>24	4–27	Barber (1996)
	Willapa Bay, WA		15	19		5–15 (means)	Magoon and Vining (1981), Pauley et al. (1988)
	Oakland Bay, WA	12	12–15	18.7 (mean) 20–22 (after ebb tide)		9–18.7	Perdue and Erickson (1984)
Laboratory experiments	Yaquina Bay, OR	12–15	13–15 15–18	No 18–20			Robinson (1992) Mann (1979)

Our results show that *C. gigas* develops an active and normal reproductive behavior in this area, suggesting that this behavior accounts for its successful spread in northern Patagonia. Many introductions of *C. gigas* have occurred worldwide, but not all of them have resulted in established populations (Ruesink et al. 2005). One main reason may be the temperature thresholds required for complete female gonad maturation.

During early spring, the oysters in Anegada Bay go through active gonad proliferation. Maturity mainly extends from October to January. The first spawning is reported in November, when seawater temperature exceeds 17°C, and peaks from December to February, when seawater temperature ranges from 19–21.5°C. At the end of the summer and into fall, spawning is weak and mainly male, with the majority of the oysters undifferentiated.

In general, the entire female population spawns from November to February. An analysis of gonad stages in relation to oyster size shows that the timing of gonad development is size dependent. Larger (≥ 70 mm) and medium oysters begin spawning first, whereas small oysters (<40 mm) represent the late spawners in the population. Because of the high growth rate of the species in this area (Pascual & Castaños 2000), it is feasible that these small autumn spawners could be the first settlers of the season.

The *C. gigas* population established in Anegada Bay offers the case of an introduction where optimal environmental conditions are met, both for complete gonad maturation and for successful larval survival and settlement. An analysis of the Argentinean coast enables us to predict that *C. gigas* may theoretically continue its expansion southward along the Patagonian coast through a chain of particularly protected sites, at least up to the locality of Comodoro Rivadavia (Chubut province: 47°59'S, 67°29'W), where summer seawater temperature can reach an average maximum of 17°C (DeCarli & Corbacho 2000). This conclusion should be taken cautiously because we are analyzing only one variable in the complex array of biological and physical factors that influence natural establishment of this species in nature.

Currently there are complete reviews on the status of establishment of *C. gigas* around the world (Mann 1983, Chew 1990, Shatkin et al. 1997, FAO, Fisheries Global Information System 2000–2009, Ruesink et al. 2005, McKindsey et al. 2007). There is great concern about the ecological consequences of the introduction of the species. Even when the need of assessing precisely the environmental conditions for establishment in the field has been emphasized (Ruesink et al. 2005, McKindsey et al. 2007), there are very few studies that document in detail thermal thresholds for successful reproduction, and larval survival and settlement.

Because scarce experimental work has been published on this matter (but see Mann 1979, Enriquez-Díaz 2004, Fabioux et al. 2005), we compiled cases relating reproduction to seawater temperature, with emphasis on those where spawning has been confirmed (Table 2).

Several authors have concluded that gametogenesis in *C. gigas* is directly related to seawater temperature (Mann 1979, Neudecker 1981, Deslous-Paoli et al. 1982, Bourne 1984, Lango-Reynoso et al. 1999, Diederich et al. 2005), indicating that if temperature lies within the range of 17–25°C, spawning will occur in this species. However, the amount of spawning activity will depend on temperature; these values will determine whether

the oyster will fully or partially spawn in nature. Paniagua-Chávez and Acosta-Ruiz (1995) showed that, in *C. gigas* established in Baja California, Mexico, when food requirements are fully met, temperature is the limiting factor for successful reproduction. Low mean temperatures enlarge the maturation stage and impede the arrival to massive spawning, despite food availability. The permanence through time of high condition indexes is an additional proof for the absence of total spawning.

The relation between temperature and food is also discussed by Ruiz et al. (1992) in a study of the reproductive behavior of *C. gigas* in one of the most highly productive marine ecosystems (El Grove, Galicia, Spain). At this site, oysters show 2 spawning peaks: the first occurring when temperature surpasses 17°C and a second, weaker peak occurring later in the summer when temperature decreases to 15–16°C and coincides with a major algal bloom. They suggest that the high productivity of the *ría* may explain this behavior, perhaps confirming that in the best of food scenarios, the temperature threshold may only decrease 1–2°C with respect to the repeatedly mentioned 17°C barrier.

Mann (1979) found, through laboratory experiments, that gametogenesis in this species progresses when seawater temperature ranges from 15–18°C, and spawning occurs only when temperature reaches 18–20°C. At temperatures less than 10.5°C, gametogenesis ceases. Mann (1979) also suggested that not only absolute temperature matters, but also the period of exposure to those temperatures, meaning the requirements of day-degrees accumulated (Fabioux et al. [2005] show similar results).

It has been suggested that there could be “strains” of *C. gigas* able to spawn in cold waters (Imai 1980, Ruiz et al. 1992). Even when specific research is necessary to prove this, our survey leads us to conclude that temperature thresholds for complete female gonad ripening stand in all the documented cases, in conditions of normal food availability. The cases of “unexpected” establishment and expansion of this species correspond to abnormal temperature increase above average values. These rises can be the result of special local conditions found in environments such as coastal embayment, fjords, or lagoons (permanent or seasonal) located within a larger region characterized by lower seawater temperatures. Spawning events can also occur in warmer summers, during which seawater temperature increases from its average, sometimes for few days. Female gonads in this species can reach advanced ripeness if temperatures reach approximately 15–16°C (in fact, proliferation initiates at 8–11°C [Mann 1979, Fabioux et al. 2005]) and may remain in that condition without changes for some time. If temperature suddenly increases to 17–18°C, full ripeness is achieved very quickly and spawning occurs. However, the quality of gametes and the intensity of the spawning event will also depend on food availability and perhaps photoperiod (Fabioux et al. 2005).

To show a case of “unexpected” establishment of this species, we chose the case of the slow and successful expansion of *C. gigas* along cold waters of the Wadden Sea. The case is by

now very well documented (Riese 1998, Diederich et al. 2005, Nehls & Büttger 2007, Wang et al. 2007, Smaal et al. 2009, Troost et al. 2009). It is interesting to note that, since the introduction of *C. gigas* on Texel (The Netherlands) in 1983 (Nerhing 2006) and on the Isle of Sylt (Germany) in 1986 (Riese 1998), the species has established local populations along the coasts of Germany (Diederich et al. 2005), the Netherlands (Drinkwaard 1999, Smaal et al. 2009, Troost et al. 2009), and Denmark (Diederich et al. 2005, Wang et al. 2007). The thermal thresholds that operate to control full maturation and spawning apparently have been met, because the species has spawned naturally in particular coastal sites where seawater temperature increases above the average regional values (Nerhing 2003, Diederich et al. 2005). This fact, combined with the occurrence of warmer summers, explains the successful reproductive spots from which the populations further expanded (e.g., summers of 1975–1976, 1982, 1986, 1989 [Drinkwaard 1999]; and 1991, 1994, 1997, 2001–2003 [Diederich et al. 2005]). Van Aken (2008) documents in detail these temperature oscillations that correspond, according to his conclusions, to the last “warm” 25-y cycle, during which seawater temperature shifted 1.5°C in the North Atlantic.

Chile, on the other hand, is the case of a cold seawater region where *C. gigas* has not established. The Pacific oyster was introduced to a hatchery in Coquimbo (29°54'S, 71°20'W) in 1978, and since then it was transferred repeatedly to many locations along the roughly broken Chilean coast, sustaining commercial farms from Coquimbo to the island of Chiloé. However, there are no records in the literature of the establishment of this species in any of the transplantation sites. We could collect only one oral communication from Dr. Pereira of UCN-Coquimbo who reports that natural spawning occurs in Cahuil (34°30'S, 72°1'W; region VI) during summer when a sand barrier encloses a coastal lagoon in which temperature exceeds 20°C. Even when adults have survived, only a few dead spats were observed on submerged rocks. No further reports of natural reproduction exist for Chile.

The only documented case of establishment of *C. gigas* in warmer waters is presented in the recent paper by Melo et al. (2009). The authors demonstrated, through the analysis of DNA sequences, the presence of adult individuals in the rocky intertidal zone of the southern coast of Florianópolis (Brazil), on oyster grounds dominated by the tropical native species *Crassostrea braziliiana* and *C. rizophorae*. The areas where the specimens were found have an annual temperature range of 13–30°C.

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