Broodstock Conditioning, Spawning Induction, and Early Larval Development of the Tropical Rock Oyster *Striostrea prismatica* (Gray 1825)

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BROODSTOCK CONDITIONING, SPAWNING INDUCTION, AND EARLY LARVAL DEVELOPMENT OF THE TROPICAL ROCK OYSTER *STRIOSTREA PRISMATICA* (GRAY 1825)

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ABSTRACT The tropical rock oyster *Striostrea prismatica* is a commercially valuable bivalve mollusc found along the Pacific tropical coast. A laboratory study was conducted to investigate broodstock acclimation at two temperatures (28.3 ± 0.9°C and 22.2 ± 0.9°C) fed a combined microalgae diet (*Chaetoceros gracilis* and *Isochrysis galbana* at 150 cells/µL/day and 100 cells/µL/day, respectively) for 7 wk, and to investigate seven treatments to stimulate spawning of individuals acclimated at both temperatures. Treatments to induce spawning consisted of a 10°C temperature decrease for 60 min (HS1), a temperature increase to 30°C for 30 min (HS2), a 5°C temperature increase for 60 min followed by 30 min of desiccation (HSD), a decrease in salinity to 15 psu for 30 min (SS1), an increase in salinity to 96 psu for 30 min (SS2), overfeeding with *C. gracilis* microalgae (OF), and the addition of oyster sperm (SPM). All treatments were treated with oyster sperm after 1 h of the last stimulus. Spawning success was evaluated by measuring egg production, fertilization percentage, and response time of spawning. Only broodstock held at 28°C spawned (51.8%). All organisms in the HSD treatment spawned after 1.40 ± 1.01 h. Oocytes released per individual and fertilization rate averaged $34.88 \pm 23.81 \times 10^6$ and 89.82 ± 5.90%, respectively. Addition of sperm enhanced spawning success in the HS1, HS2, and SS1 treatments.

KEY WORDS: rock oyster, *Striostrea prismatica*, gonad and embryonic development, broodstock acclimation, spawning stimulation

INTRODUCTION

The rock oyster, *Striostrea prismatica* (Bernard, 1983) formerly named *Crassostrea iridescens* (Hanley, 1854), is a bivalve mollusc distributed along the eastern Pacific coast from southern Baja California to northern Peru (Mora 1990). In Santa Elena, Ecuador, this mollusc is extracted and commercialized by local fishermen, thus contributing to the economy of their fishing communities. The fishery of this resource is not managed and landings are not documented; however, smaller size individuals, and greater efforts reported by fishermen to obtain them, suggest that current extraction volumes exceed the resilience of this resource in the natural environment. The development of reproduction techniques to produce oyster seeds under laboratory conditions may help to recover overexploited fishing grounds if seed is restocked successfully at these sites. Mollusc breeding programs require successful broodstock maturation, which is usually achieved by mimicking environmental conditions that occur under controlled laboratory settings, mainly temperature handling (Barber & Blake 2006), and various feeding regimes that promote viability of gametes for subsequent embryonic and larval development (Wilson et al. 1996, Farías & Uriarte 2001, Helm et al. 2004). Environmental conditions required by *S. prismatica* for gonadal development and reproduction is scarce. Gonadal maturity of *S. prismatica* has been reported to occur in the Pacific coastal waters of Mexico at water temperatures between 28°C and 32°C, and at constant salinities (Stuardo & Martínez 1974, Cuevas & Martínez 1979, Fournier 1992). Biological parameters such as natural productivity or microalgae type, unfortunately, were not documented in these studies.

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Environmental conditions that trigger spawning are usually not unknown, and ripe broodstock is induced to spawn in the laboratory with external stimulation. Although there are no previous records of spawning inductions for *Striostrea prismatica,* common methods in other bivalve species involve external stimulation, such as changes in water temperature (De la Roche et al. 2002, Velasco et al. 2007, Pronker et al. 2008, Matias et al. 2009, Nava & García de Severeyn 2010, Soria et al. 2010), changes in salinity (Southgate & Lee 1998, Nava & García de Severeyn 2010), overfeeding (Breese & Robinson 1981), and the addition of mature gametes (Ellis 1998). The injection of chemicals directly into the gonad tissue or muscle of bivalve species with visible organs has also been reported (Vélez et al. 1990, Velasco et al. 2007). Reproductive organs are not exposed readily in *S. prismatica*; thus, internal chemical stimulation would probably cause tissue damage and stress. The effectiveness of these aforementioned methods varies depending on species, maturity stages, and environmental conditions. The aim of this study was to evaluate two culture temperatures for gametogenesis in *S. prismatica* broodstock obtained from the wild and, second, to test different laboratory treatments to induce spawning in *S. prismatica*. Last, we describe early embryonic developmental stages.

MATERIALS AND METHODS

Collection and Laboratory Conditioning of Broodstock

This work was carried out at the Centro Nacional de Acuicultura e Investigaciones Marinas "Edgar Arellano M." (CENAIM-ESPOL), San Pedro de Manglaralto (Santa Elena Province, Ecuador). A total of 152 individuals of *Striostrea prismatica* (weight, 325.1 ± 128.3 g; height, 11.37 ± 1.55 cm; length, 8.37 ± 1.51 cm) were collected on September 9, 2011, at

two locations near the village Ayangue, Santa Elena, Ecuador (01°57'54" S, 80°44'30" W and 01°58'27" S, 80°45'53" W). Water temperature and salinity in the collection area was 23°C and 32.3, respectively. All oysters were placed in plastic containers with seawater and transported to the laboratory. Epibionts, algae, and other shell encrustations were scraped from the oysters. Oysters were placed in a 1-m^3 tank and provided with continuous seawater circulation and aeration. Organisms were deprived of food for 3 days. Oysters were split into 2 groups. One group was acclimated to 28°C; the second group was held at ambient water temperature. Water temperature in the 28°C treatment was maintained by a thermostat coupled thermal plate. Oysters were later transferred to larger tanks containing 6 m^3 filtered seawater (1 μm) and constant aeration. Both groups were fed with a 1:1 (by cell count) mixture of *Chaetoceros gracilis* and *Isochrysis galbana* at a concentration of 150 cell/μL/day and 100 cell/μL/day, respectively. Salinity varied slightly during the trial, registering 32.2 ± 0.5. Feces and settled algae were removed from culture tanks daily. About 60% of the water in each tank was renewed daily. Epibionts and other debris were removed weekly from oyster shells. Water temperature and dissolved oxygen were recorded twice daily with a YSI 55D oxygen meter.

Gametogenesis Stage Determination

Gametogenesis in oysters was determined histologically. Ten organisms of each treatment were sacrificed at the start and end (week 7) of the acclimation experiment.

Gonads were fixed in Davidson solution for 24 h, followed by dehydration, clarification, inclusion, and cross-section (4 μm), and, last, were stained with hematoxylin-eosin stain (Bell & Lightner 1998). Gonadal stages were determined based on morphological characteristics, presence or absence of gametes, and degree of development considering 5 stages: indeterminate, early development, late development, mature, and spawning (Fournier 1992). Samples were examined with an Olympus BH-2 microscope and were photographed using a Nikon E995 camera.

Induction of Spawning

Eight individuals were selected randomly from each conditioning tank (28°C and 22°C) and placed into 2 15-L buckets (4 oysters each) and allocated to 7 spawning stimulation treatments. A total of 28 buckets were prepared for this trial. The experiment had 2 replicates (buckets) per spawning treatment, as follows:

Heat shock 1 (*HS1*): Oysters were subjected to a 10°C decrease in water temperature for 1 h, then were brought back to the initial conditioning temperature during the next hour, and then subjected to another 10°C decrease for another hour. Microalgae *Isochrysis galbana* (200 cells/μL) were added during the process.

Heat shock 2 (*HS2*): The oyster holding water temperature was increased to 30°C for a half hour and then was reduced to 22°C for 30 min. The procedure was repeated twice, reaching a final temperature of 23°C.

Heat shock and desiccation (*HSD*): The oyster water temperature was increased 5°C for 1 h, after which the oysters were removed from the water and exposed to air for 30 min. Oysters were then returned to +5°C warmer water for 1 h. Air temperature was 23°C.

Salinity shock 1 (*SS1*): Oysters were transferred from a salinity of 32 to containers with 15 psu and were kept there for 30 min, then returned back to 32-psu water containers for the next half hour. The procedure was repeated twice. Water temperature was kept constant at their corresponding conditioning water temperature.

Salinity shock 2 (*SS2*): Oysters were transferred from 32 psu to containers with 96 psu and were kept there for 30 min, then returned back to 32-psu water containers for the next half hour. The procedure was also repeated twice. Water temperature was kept constant at their corresponding conditioning water temperature.

Overfeeding (*OF*): Both groups of oysters received an inoculum of *Chaetoceros gracilis* at a concentration of 2,800 cell/μL. Because the microalgae water temperature was 23°C, oyster belonging to conditioning treatment 28°C, also experienced a decrease in temperature to near 23°C.

Sperm addition (*SPM*): Gametes extracted from ripe *Striostrea prismatica* males were placed in a 1-L beaker (10 million spermatozoa/mL). About 100 mL spermatic material was added to the buckets every 30 min for 2 h. Water temperature of the buckets was kept constant at their corresponding conditioning water temperature.

All treatments were inoculated with sperm after 1 h of the last corresponding spawning induction procedure described.

Data and Statistical Analysis

Induced spawning success was evaluated through visual observation of the presence or absence of gametes in each container. The response time to first spawning was measured as the elapsed time from the start of each treatment. If spawning occurred, water samples were collected to determine the number of eggs and the amount of fertilized eggs. Counts were submitted to the Levene test to verify homogeneity of variance; normality was verified graphically. Response time were transformed to $1/x$ to comply with normality and equal-variance assumptions required for parametric analysis of variance tests. Treatment HS1 was not included in the statistical analysis because of the absence of data. All variables were subjected to 1-way analysis of variance (ANOVA) at a 95% level of confidence ($\alpha = 0.05$). When ANOVAs were significant, the post hoc Scheffe multiple comparison test was used to determine which treatments differed. All statistical tests were analyzed using Statistica software version 5.5.

RESULTS

Gametogenesis

Ninety percent of the oysters collected from the wild presented undefined gonadic development stage; 10% presented an early developmental stage. Highest maturity was achieved after conditioning in treatment 28°C. All organisms in this treatment presented the late gametogenesis developmental stage, of which 60% were female. Only 20% of oysters conditioned at ambient water temperature (22°C) presented an early developmental stage, and the rest were undefined (Fig. 1).

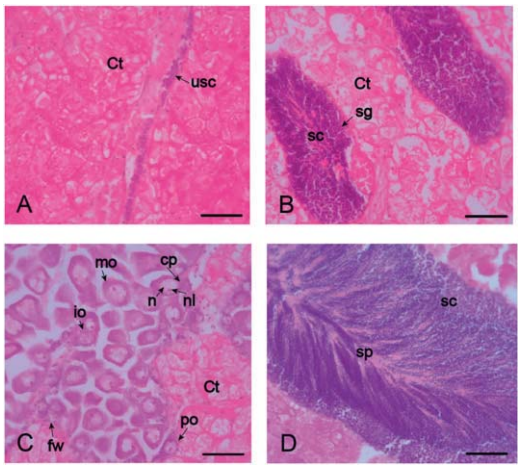

Figure 1. Gonadal development in *Striostrea prismatica* (×400 magnification). (A) Indeterminate. (B) Early development, male. (C) Late development, female. (D) Late development, male. cp, cytoplasm; Ct, connective tissue; fw, follicular wall; io, immature oocyte; mo, mature oocyte; n, nucleus; nl, nucleolus; po, primary oocyte; sc, spermatocytes; sg, spermatogonia; sp, sperm; usc, undifferentiated sex cells. Bar = 50 μm.

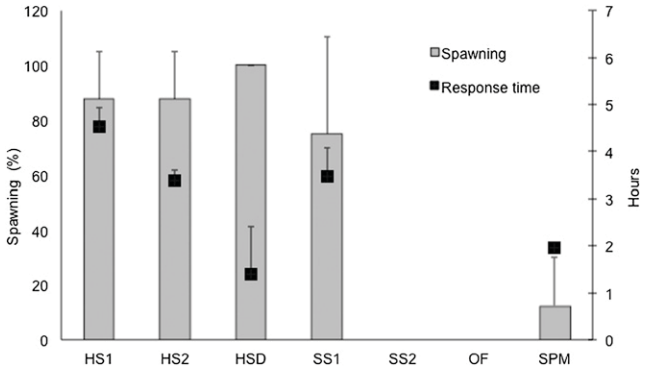

Figure 2. Spawning percentage and average time required to spawn after initiation of different induction treatments in oyster *Striostrea prismatica* previously conditioned to mature at 28°C for 7 wk. Induction treatments included decreasing 10°C of magnitude by 1 hour departing from the rearing condition, twice (HS1); changes in water temperature between 30°C and 22°C by 30 minutes, twice (HS2); heat shock in 5°C of magnitude and desiccation (HSD); salinity shocks from the rearing condition to 15 psu (SS1) and 96 psu (SS2); overfeeding with *Chaetoceros gracilis* (OF); and addition of oyster sperm (SPM).

Induced Spawning

Significant spawning differences were observed between oysters conditioned at 22°C and 28°C for 7 wk. Independent of the spawning induction method, 51.8% and 5.4% of oysters conditioned at 28°C and 22°C, respectively, spawned. Because of such a low spawning percentage obtained at 22°C, efficacy of different spawning methods tested was analyzed only with oysters conditioned at 28°C. The greatest spawning was observed in treatments HS1, HS2, HSD, and SS1 (Fig. 2). There were no statistical differences ($P > 0.05$) among these 4 treatments. No spawnings were recorded in treatments SS2 and OF, and only a few spawned in treatment SPM. About half the oyster population in treatments HS2 and HSD were female; 4 spawnings in both treatments corresponded to oocytes.

The addition of sperm at the end of treatment procedure HSD did not influence spawning because they occurred before, on average, 1.4 h after starting the induction treatment. However, sperm addition appears to have had a significant effect in treatments HS1, HS2, and SS1, because about 75% of spawning occurred after sperm inoculation (Fig. 2). The average spawning time after the start of treatments HS1, HS2, and SS1 was 4.5 h, 3.4 h, and 3.5 h, respectively. These 3 response times were not statistically different, but were significantly different ($P < 0.05$) when compared with treatment HSD.

Egg Production and Fertilization

The greatest amount of spawned eggs was observed in treatment HSD, with an average number of $47.2 \pm 32.1 \times 10^6$, followed by treatments HS2 and SS1 with $24.5 \pm 16.4 \times 10^6$ and $20.8 \pm 6.2 \times 10^6$, respectively, with no significant differences ($P > 0.05$) among them. The average size of eggs was 46.95 ± 1.53 μm (Fig. 3A). Fertilization percentage ranged between 82% and 98%. Treatment HSD had the greatest fertilization percentage (95.9 ± 1.4%), which was significantly greater ($P < 0.05$) compared with all other treatments.

Embryonic Development

Fertilization occurred about 30 min after gametes came into contact, the time at which the formation of the first polar body was observed (Fig. 3B), and subsequent cell segmentations into 2 unequal cells started (Fig. 3C). The second cell division occurred after 1 h, and blastomeres divided in 4 cells (Fig. 3D). Subsequent divisions resulted in the formation of morula and blastula after 6–8 h (Fig. 3E, F). The gastrula stage, followed by the formation of active trochophore larvae, occurred between 10 h and 12 h (Fig. 3G). After 22–24 h, straight-hinge D-larvae were observed. Height and shell length at this starting larval developmental stage was 58.0 ± 0.4 μm and 67.9 ± 2.3 μm, respectively (Fig. 3H).

DISCUSSION

Temperature is the main environmental factor that regulates gametogenesis in marine bivalves (Barber & Blake 2006). In natural environments, the onset of gametogenesis in *Striostrea prismatica* has been related to an increase in water temperature (Stuardo & Martínez 1974, Cuevas & Martínez 1979, Fournier 1992). The oysters in our study were obtained from the wild in early September at water temperatures of 23°C. At this time, about 90% of sampled oysters showed undetermined stage by histology. After 7 wk of conditioning at 28°C, all developed mature gonads. On the contrary, only 20% of oysters subjected to ambient water temperatures (22–23°C) matured during the same time period. Working with the same species, Marín (2011) observed advanced gonadal stages when oysters were cultured at 27.0 ± 2.0°C in a shrimp farm reservoir for 30 days. Cuevas and Martínez (1979) described the gametogenic cycle of *S. prismatica* in San Blas, Nayarit, Mexico, during 1972 and 1973, recording a gonadal resting phase from October to April, gonadal maturity stage from June to August, and spawning during August and September. Another study in the same location and same time period also reported maturation and spawning of *S. prismatica* at warm water temperatures (30–32°C) between June and October, and a gonadal resting phase

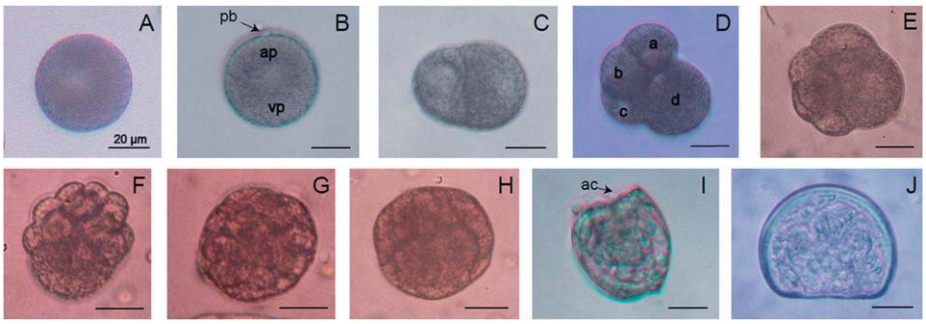

Figure 3. Embryonic development of *Striostrea prismatica* within 24 hours. (A) Egg spawned (46.95 ± 1.53 µm). (B) Zygote. (C) First cleavage. (D) Second cleavage. (E) Third cleavage. (F) Fourth cleavage. (G) Blastula. (H) Gastrula. (I) Trochophore larvae. (J) Straight-hinge D-larva. ac, apical cilium; ap, animal pole; pb, polar body; vp, vegetal pole. In view D, a, b, c, and d are blastomeres. Bar = 20 µm.

at colder water temperatures (23–26°C) between November and April (Stuardo & Martínez 1974). Several species of *Crassostrea* have shown similar gametogenic cycles linked to varying seasonal coastal water temperatures. Paniagua-Chávez & Acosta-Ruíz (1995), who worked with *Crassostrea gigas* in Mina Vieja, Mexico, and Brousseau (1995), who worked with *Crassostrea virginica* in Bridgeport, CT observed gonadal maturity of wild populations at warmer water temperatures, 20–23°C and 20–30°C, respectively. Gonadal resting periods are, however, not always observed. Fournier (1992), while working with *S. prismatica* along the Pacific Coast of Costa Rica, observed continuous reproductive activity between 1986 and 1987, with an absence of extended resting periods. Years 1986 and 1987, however, were ENSO years, characterized by the presence of abnormal warm-water temperatures along the eastern Pacific coast (Fiedler & Philbrick 2002). Chávez-Villalba et al. (2002a), in their experiments with *C. gigas*, provided evidence of new gonadal development after short periods of conditioning (a resting phase), through water temperature and photoperiod manipulation, even in the presence of residual eggs observed during the start of the experiment, suggesting that this species does not require a prolonged dormancy period. We cannot infer from our study whether a resting phase is required by *S. prismatica* to mature successfully when conditioned at warm temperatures as those tested in our study. It is clear that more studies addressing the annual reproductive cycle of *S. prismatica* throughout its geographical range of distribution are required to understand fully the role of environmental factors on their reproductive physiology. Nevertheless, most studies reviewed suggest that temperature constitutes a key environmental factor that regulates gametogenesis in *S. prismatica*, triggering gonadal development at temperatures warmer than 27°C.

Gametogenesis in bivalve molluscs is also strongly influenced by diet value (Wilson et al. 1996, Chávez-Villalba et al. 2002b). Lipid content in bivalve eggs for effective embryonic and larval development depends on food availability during gametogenesis (Wilson et al. 1996, Farías & Uriarte 2001). The microalgae *Chaetoceros gracilis* and *Isochrysis galbana* used in our study are reported to have high levels of unsaturated fatty acids (Volkman et al. 1989), and therefore may have contributed to the nutritional profile required for gonadal development, which is reflected in egg viability and vigor of the embryo (Utting & Spencer 1991, Helm et al. 2004). A combination of microalgae species has been used successfully in *Nodipecten nodosus* (Linné, 1758) and *Ostrea chilensis* (Philippi, 1845) (Wilson et al. 1996, De la Roche et al. 2002). However, some studies have shown that mature oysters can be obtained in laboratories without food supply during conditioning, because colder conditions have proved to favor the accumulation of nutritional reserves and energy for gametogenesis (Chávez-Villalba et al. 2002b). Other studies, on the contrary, have shown that food supplement is essential for gonad development (Heasman et al. 1996, Martínez et al. 2000, Madrones-Ladja et al. 2002). In fact, accumulated reserves in the digestive gland prior to gametogenesis may not be sufficient to support this process (Sastry 1966). In this study, oyster conditioning at low water temperatures (22°C) was not as effective as treatment with a warmer temperature (28°C) for gametogenesis.

Of all the stimuli tested, those involving changes in temperature and salinity reduction induced spawning. Although spawning of *Striostrea prismatica* under laboratory conditions has not been reported, our results coincide with wild spawns observed during warmer seasonal temperatures (Stuardo & Martínez 1974, Cuevas & Martínez 1979, Fournier 1992). Increasing temperature followed by oyster desiccation was found to be the most successful external stimulation treatment to induce *S. prismatica* to spawn in a relatively short period of time (1.40 ± 1.01 h). Increasing water temperature to promote spawning has also been reported for other bivalves (De la Roche et al. 2002, Pronker et al. 2008, Matias et al. 2009). Salinity changes have been reported to induce spawning in tropical oysters (Rao 1951, Southgate & Lee 1998). It has been suggested that salinity-induced spawning may affect larval survival (Southgate & Lee 1998). Increasing the salinity did not induce spawning in our study, but has been reported to work in bivalve mollusc species such as *Rangia cuneata* (Sowerby, 1831), *Crassostrea virginica* (Gmelin, 1791), *Geukensia demissa* (Dillwyn, 1817) (Nava & García de Severeyn 2010), and *Crassostrea madrasensis* (Preston) (Rao 1951). Sudden salinity changes have been reported to affect the contraction of the adductor muscle of *Crassostrea gigas* (Thunberg, 1793), and prolonged exposure to high salinities can cause its mortality (Hopkins 1936). It is likely that *S. prismatica* fully closed their valves to avoid saltier test water, preventing any physiological effect.

Although the presence of gametes has been reported to stimulate spawning in clams (Ellis 1998), and overfeeding in *Siliqua patula* (Breese & Robinson 1981), treatments in the current study involving the addition of sperm (SPM) and a high concentration of microalgae (OF) were not effective in inducing

spawning. However, most spawning in treatments HS1, HS2, and SS1 occurred after sperm addition. In some studies, the temperature stimulation only was not sufficient, and the addition of sperm or eggs from mature gonads was required to induce spawning (Galtsoff 1938). Our observations in *Striostrea prismatica* may corroborate the use of mature gonads in addition to other exogenous stimuli to induce spawning. Mature gonads in molluscs may contain pheromones that trigger spawning (Heslinga et al. 1990).

There were no significant differences in the number of spawned eggs among treatments that resulted in effective spawning. This would suggest that the release of gametes was independent of the exogenous stimulation that promoted spawning. The number of eggs released in bivalves may also depend on other factors such as maturity and size of broodstock (Helm et al. 2004, Soria et al. 2010). Egg fertilization in this study was relatively high, varying between 82% and 98%. This fertilization rate is greater than those reported for other species such as *Argopecten nucleus* (Born, 1778) (45%–76%) and *Mytilus edulis* (51%) (Pronker et al. 2008). Our findings may suggest that *Striostrea prismatica* has a high fecundity once gonadal maturity is reached, and a high percentage of viable gametes can be obtained through exogenous spawning stimulus. Size, shape, and characteristics of different embryonic stages of *S. prismatica* registered in this study were similar to those previously described for other bivalve species (Utting & Spencer 1991, Wallace 2001, Helm et al. 2004).

In conclusion, water temperature is probably one of the main environmental factors influencing gonadal development of the rock oyster *Striostrea Prismatica*, with 28°C being optimal to reach gonadal maturity in this study, with continuous good-quality feeding. Spawning can occur under laboratory conditions with specific external stimulation, such as changes in temperature followed by oyster desiccation, as long as oysters have reached advanced stages of gonadal maturity. Sperm addition enhanced spawning of oysters previously subjected to some external stimulus.

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LITERATURE CITED

Barber, B. J. & N. J. Blake. 2006. Reproductive physiology. In: S. E. Shumway & G. J. Parsons, editors. Scallops: biology, ecology and aquaculture. Amsterdam: Elsevier. pp. 357–416.

Bell, T. & D. Lightner. 1998. A handbook of normal shrimp histology. Baton Rouge, LA: World Aquaculture Society. 198 pp.

Breese, W. P. & A. Robinson. 1981. Razor clams, *Siliqua patula* (Dixon): gonadal development induced spawning and larval rearing. *Aquaculture* 22:27–33.

Brousseau, D. 1995. Gametogenesis and spawning in intertidal oysters (*Crassostrea virginica*) from western Long Island Sound. *J. Shellfish Res.* 14:483–487.

Chávez-Villalba, J., J. Barret, C. Mingant, J. C. Cochard & M. Le Pennec. 2002a. Autumn conditioning of the oyster *Crassostrea gigas*: a new approach. *Aquaculture* 210:171–186.

Chávez-Villalba, J., J. Pommier, J. Andriamiseza, S. Pouvreau, J. Barret, J. C. Cochard & M. Le Pennec. 2002b. Broodstock conditioning of the oyster *Crassostrea gigas*: origin and temperature effect. *Aquaculture* 214:115–130.

Cuevas, C. A. & A. Martínez. 1979. Estudio gonádico de *Crassostrea corteziensis* Hertlein, *C. palmula* Carpenter y *C. iridescens* Hanley de San Blas, Nayarit, México (Bivalvia: Ostreidae). *An. Inst. Cienc. Mar Limnol.* 6:81–98.

De la Roche, J. P., B. Marín, L. Freites & A. Vélez. 2002. Embryonic development and larval and post-larval growth of the tropical scallop *Nodipecten* (=*Lyropecten*) *nodosus* (L. 1758) (Mollusca: Pectinidae). *Aquat. Res.* 33:819–827.

Ellis, S. 1998. Spawning and early larval rearing of giant clams (Bivalvia: Tridacnidae). *Center for Tropical and Subtropical Aquaculture Publication* 130:1–55.

Farías, A. & I. Uriarte. 2001. Effect of microalgae protein on the gonad development and physiological parameters for the scallop *Argopecten purpuratus* (Lamarck, 1819). *J. Shellfish Res.* 20:97–105.

Fiedler, P. C. & V. A. Philbrick. 2002. Environmental change in the eastern tropical Pacific Ocean: observations in 1986–1990 and 1998–2000. Administrative report LJ-02-15. National Marine Fisheries Service, NOAA La Jolla. California. 16 pp.

Fournier, M. L. 1992. The reproductive biology of the tropical rocky oyster *Ostrea iridescens* (Bivalvia: Ostreidae) on the Pacific coast of Costa Rica. *Aquaculture* 101:371–378.

Galtsoff, P. S. 1938. Physiology of reproduction of *Ostrea virginica*: I. Spawning reactions of the female and male. *Biol. Bull.* 74:461–486.

Heasman, M. P., W. A. O'Connor & A. W. Frazer. 1996. Temperature and nutrition as factors in conditioning broodstock of the commercial scallop *Pecten fumatus* Reeve. *Aquaculture* 143:75–90.

Helm, M. M., N. Bourne & A. Lovatelli. 2004. Hatchery culture of bivalves. FAO fisheries technical paper no. 471. Rome: FAO. 177 pp.

Heslinga, G., T. Watson & T. Isamu. 1990. Giant clam farming. Honolulu, HI: Pacific Fisheries Development Foundation (NMFS/NOAA). 179 pp.

Hopkins, A. E. 1936. Adaptation of the feeding mechanism of the oyster (*Ostrea gigas*) to changes in salinity. *Bull. Bureau Fish.* 21:345–364.

Madrones-Ladja, J. A., M. de la Peña & N. P. Parami. 2002. The effect of micro algal diet and rearing condition on gonad maturity, fecundity and embryonic development of the window-pane shell, *Placuna placenta* Linnaeus. *Aquaculture* 206:313–321.

Marín, H. 2011. Evaluación de diferentes regímenes de alimentación para el acondicionamiento reproductivo de la ostra nativa *Crassostrea iridescens* (Hanley, 1854). B.Sc. thesis, Facultad de Ingeniería Marítima y Ciencias del Mar, ESPOL. 94 pp.

Martínez, G., C. Aguilera & L. Mettifogo. 2000. Interactive effects of diet and temperature on reproductive conditioning of *Argopecten purpuratus* broodstock. *Aquaculture* 183:149–159.

Matias, D., S. Joaquim, A. Leitão & C. Massapina. 2009. Effect of geographic origin, temperature and timing of broodstock collection on conditioning, spawning success and larval viability of *Ruditapes decussates* (Linné, 1758). *Aquacult. Int.* 17:257–271.

Mora, E. 1990. Catálogo de bivalvos marinos del Ecuador. Boletín Científico y Técnico. Guayaquil: Instituto Nacional de Pesca 10. 51 pp.

Nava, D. & Y. García de Severeyn. 2010. Desove inducido en moluscos bivalvos del sistema de Maracaibo. *Ciencia* 18:153–164.

Paniagua-Cháves, C. & M. Acosta-Ruiz. 1995. Desarrollo gonadal de *Crassostrea gigas* en Bahía San Quintín, Baja California, México. *Cienc. Mar*. 20:225–242.

Pronker, A. E., N. M. Nevejan, F. Peene, P. Geijsen & P. Sorgeloos. 2008. Hatchery broodstock conditioning of the blue mussel *Mytilus edulis* (Linnaeus 1758): part I. Impact of different micro-algae mixtures on broodstock performance. *Aquacult. Int*. 16:297–307.

Rao, K. V. 1951. Observations on the probable effects of salinity on the spawning, development and setting of the Indian backwater oyster, *Ostrea madrasensis* Perston. *Proc. Indiana Acad. Sci*. 33B:231–256.

Sastry, A. N. 1966. Temperature effects in reproduction of the bay scallop, *Aequipecten irradians* Lamarck. *Biol. Bull*. 130:118–134.

Soria, G., J. Tordecillas-Guillen, R. Cudney-Bueno & W. Shaw. 2010. Spawning induction, fecundity estimation, and larval culture of *Spondylus calcifer* (Carpenter, 1857) (Bivalvia: Spondylidae). *J. Shellfish Res*. 29:143–149.

Southgate, P. C. & P. S. Lee. 1998. Hatchery rearing of the tropical blacklip oyster *Saccostrea echinata* (Quoy and Gaimard). *Aquaculture* 169:275–281.

Stuardo, J. & A. Martínez. 1974. Relaciones entre algunos factores ecológicos y la biología, de población de *Crassostrea corteziensis* Hertlein, 1951, de San Blas, Nayarit, México. An. Inst. Cienc. Mar Limnol. 45 pp.

Utting, S. D. & D. E. Spencer. 1991. The hatchery culture of bivalve mollusk larvae and juveniles. Lab. Leafl., MAFF Fish Res., Lowestoft 68. 31 pp.

Velasco, L. A., J. Barros & E. Acosta. 2007. Spawning induction and early development of the Caribbean scallops *Argopecten nucleus* and *Nodipecten nodosus*. *Aquaculture* 266:153–165.

Vélez, A., E. Alifa & O. Azuaje. 1990. Induction of spawning by temperature and serotonin in the hermaphroditic tropical scallop, *Pecten ziczac*. *Aquaculture* 84:307–313.

Volkman, J. K., S. W. Jeffrey, P. D. Nichols, G. I. Rodgers & C. D. Garland. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol*. 128:219–240.

Wallace, R. K. 2001. Cultivating the eastern oyster, *Crassostrea virginica*. Southern Regional Aquaculture Center (SRAC) Publication 432. 4 pp.

Wilson, J., O. Chaparro & R. Thompson. 1996. The importance of broodstock nutrition on the viability of larvae and spat in the Chilean oyster *Crassostrea chilensis*. *Aquaculture* 139:63–75.