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OPTIMIZING BROODSTOCK CONDITIONING FOR THE TROPICAL CLAM *ANOMALOCARDIA BRASILIANA*

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ABSTRACT The tropical clam *Anomalocardia brasiliana* is a commercially important bivalve living along the south Atlantic coast of Latin America. Harvest of natural stocks is the principal method for supplying market demand—the market average price ranging from 5 to 10 US$ per kilogram of meat. As a first step in the development of hatchery techniques for *A. brasiliana* seed production, this study examined the effects of temperature and salinity on conditioning broodstock for spawning. Two treatments tested conditioning at 25°C at salinities of either 30 or 35. A third treatment examined the effects of an initial 10-day conditioning at 16°C followed by a gradual temperature increase to 25°C at a salinity of 35. As a control, clams were sampled from the wild over the same experimental period. Tissue samples were taken at the initiation of the experiment and at 15, 30, 45, and 55 days of conditioning and examined histologically to determine changes in the sexual development of the clams. Four reproductive stages were identified during the experimental period: gametogenesis, mature, spawned, and absorption. The treatment with the initial 10-day conditioning period at 16°C demonstrated that it is possible to synchronize gamete development in both males and females to result in the maximum proportion of mature broodstock at 55 days of conditioning.

KEY WORDS: Berbigão, clam, sexual stage, broodstock, reproductive synchrony, *Anomalocardia brasiliana*

INTRODUCTION

Over recent decades, global aquaculture has continued to grow more rapidly than any other food production sector (FAO 2018). According to FAO (2016), global aquaculture production in 2014 equaled 47.03 million tons and clam production equaled 5.2 million tons. Clam aquaculture has increased at a rapid rate relative to that of other bivalve commodity groups; for example, clam production was only half of total global oyster production in 1990, but had reached 4.7 million tons in 2010, exceeding oyster production (Pulvenis 2012). The most commonly cultured clam species is *Ruditapes philippinarum* (Adams & Reeve, 1850), with a production of 3.9 million tons in 2014 (FAO 2016); however, other clam species, such as the Veneridae *Anomalocardia brasiliana* (Gmelin, 1769) (syn. *Anomalocardia flexuosa*) have considerable potential in tropical and subtropical regions.

According to Rios (2009), *Anomalocardia brasiliana* occurs in many estuaries of the Atlantic coast of South America, ranging from the West Indies and Suriname in the north to Uruguay in the south. It is a eurythermal and euryhaline species, having a high resistance to low oxygen concentrations (Schaeffer-Novelli 1976). This species is native to Brazil and is commonly known as the Berbigão or Vôngole clam and is mainly harvested from natural stocks. In addition, this clam species is of social and economic importance to native communities in the south and northeast of Brazil (Silva-Cavalcanti & Costa 2011). The species is susceptible to overexploitation, water pollution, and environmental factors such as flooding; therefore, hatchery production of seed for either aquaculture or restoration of wild stocks, may help ensure long-term sustainable production.

Studies of the natural reproductive biology of *Anomalocardia brasiliana* have been reported by Grotta and Lunetta (1982), Araújo (2001), Rocha-Barreira and Araújo (2005), Boehs et al. (2008), Lavander et al. (2011) and Luz and Boehs (2011); however, little is known about the reproductive cycle of *A. brasiliana* under experimentally controlled conditions. Control of the reproductive cycle by conditioning adults to provide fertile broodstock outside of the natural reproductive season is an important step in seed production. Endogenous and exogenous factors control the maturation and spawning of marine bivalves, and temperature is commonly reported to be important in determining the timing and rate of gamete development (Sastry 1968, Bayne et al. 1976, Mann 1979, Newell et al. 1982, Muranaka & Lannan 1984, Barber & Blake 2006).

Asynchronous reproductive development of wild-collected broodstock often makes it difficult to condition them in commercial hatcheries to obtain synchronous spawning. Another challenge is unwanted, premature spawning due to broodstock differing in their reproductive stage at the start of the conditioning period. In this study, the effect of temperature and salinity on the reproductive cycle of *Anomalocardia brasiliana* was studied under three different laboratory conditioning regimes.

MATERIALS AND METHODS

Broodstock Collection

The broodstock conditioning experiment was conducted during the spring, from September to November 2011, at the Laboratory of Marine Molluscs, Federal University of Santa Catarina (LMM), Florianópolis, Brazil. Specimens of *Anomalocardia brasiliana* (1,200 animals) with an average shell height

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of 24.65 ± 1.36 mm, measured according to Caill-Milly et al. (2012), were collected at 0.5 m depth in the sediment at low tide at 18°C, from Praia da Daniela (27° 27′ 25.40″ S; 48° 32′ 31.51″ W), Florianópolis. After collection, the animals were transported in coolers, at ambient temperature, without addition of seawater to LMM.

Acclimation to Experimental Conditions

On arrival at LMM (September 27, 2011), the clams were cleaned by first rinsing with clean freshwater, immersion in a solution of sodium hypochlorite (NaClO 2%) for 10 min, and then rinsing with clean freshwater.

The animals were spawned before the beginning of the experiment to obtain a uniform postspawn stage across all broodstock and to reduce premature spawning during the conditioning period. Gamete release was stimulated by an initial 2-h exposure to air followed by placement in 100-L tanks filled with seawater (filtered to 1 μm and treated with UV) at 18°C and a salinity of 35. After 10 min, the temperature of the seawater in the tanks was increased to 24°C, and the animals were kept overnight at this temperature. The next day, after gamete release, the animals were rinsed with freshwater and transferred to experimental units (EU).

Once in the EU, temperature and salinity were adjusted gradually over 24 h, until the initial values for each treatment were attained. After reaching the desired initial value for each treatment, animals were held for another 24 h before the experiment (D0) was initiated, to discard any mortality due to cleaning and handling.

Reproductive Conditioning of Broodstock Clams

The reproductive conditioning experiment was carried out at LMM and lasted for 55 days (from September 30 to November 23, 2011). Three treatments were tested simultaneously. Each treatment was set up with eight EU, totaling 24 EU for the experiment that were randomly distributed in the system. Two treatments started conditioning at a temperature of 21°C, gradually increasing to 25°C at day 10 (0.4°C day^{-1}) and maintaining this temperature until the end of the experiment. One of these 21°C–25°C treatments was maintained at a salinity of 30 (T21–25/S30) and the second treatment at a salinity of 35 (T21–25/S35). Broodstock in the third conditioning treatment was initially exposed to a temperature of 16°C for 10 days at a salinity of 35, then the temperature was gradually increased to 25°C from day 11 at a rate of 0.26°C day^{-1} to 25°C (day 45) and maintained at this temperature until the end of the experiment on day 55 (T16–25/S35). The initial conditioning temperature of 16°C was approximately 3.5°C lower than ambient seawater temperature in spring, whereas the initial conditioning temperature of 21°C was approximately 1.5°C warmer than ambient temperature (Fig. 1).

Semi-recirculation aquaculture systems were constructed for each treatment. Each semi-recirculation aquaculture systems was made up of eight 10-L conditioning tanks supplied with 1 μm–filtered seawater from a common 100-L sump tank and supplied with algae from a 200-L feed tank. A plastic grid was installed on the bottom of each EU to prevent clams from contacting their feces and pseudofeces. A water chiller (Gelaqua; 1/2 HP) and a heater (titanium electric heater; 500 W), both coupled to a digital thermostat, were installed to maintain treatment temperatures. Fifty clams (400 clams per treatment) were added to each EU. The EU were aerated and 200% of the seawater was replaced per hour (330 mL min^{-1}) with new filtered seawater and microalgal diet. Every 24 h, the EU sump and algal tanks were cleaned and the seawater replaced at the experimental salinity and temperature. Each day, temperatures and salinities were recorded and dead clams removed. Every 15 days, the whole experimental system was cleaned with freshwater.

The diet was composed of *Isochrysis* aff. *galbana* (Parke, 1949) and *Chaetoceros muelleri* (Lemmermann, 1898) at a cell ratio of 1:1. This diet was selected because of its optimal fatty acid profile (Sühnel et al. 2012). Microalgae were grown with additions of f2 nutrients and silicate (Guillard 1975), and harvested in the exponential phase of growth. Microalgae was continuously supplied daily, based on clam dry meat weights and daily algal consumption. The algal ration was initially set at

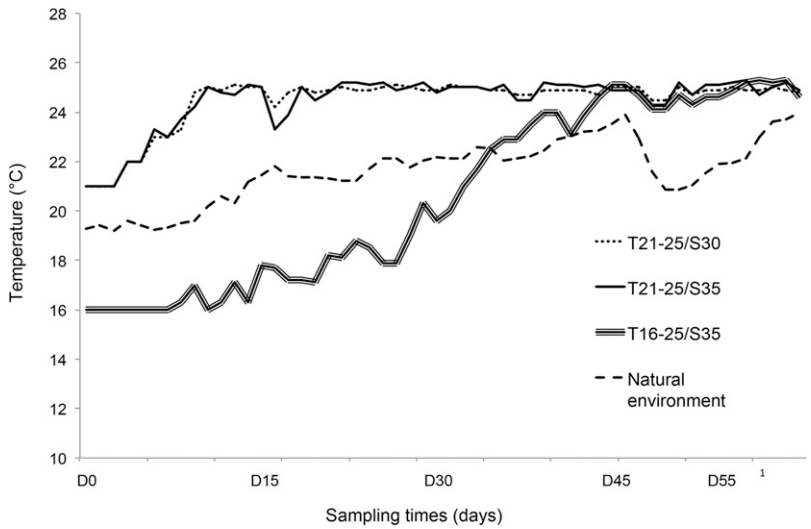

Figure 1. Daily seawater temperatures recorded during the 55-day (September 30 to November 23, 2011) conditioning period of treatments T21–25/S30, T21–25/S35, and T16–25/S35, as well as the natural environment (Praia da Daniela, Florianópolis, Brazil).

3%–4% of clam dry meat weight (Helm et al. 2004) and was increased daily when concentrations of residual uneaten microalgae decreased to less than 20,000 cell mL^{-1}. Treatments T21–25/S30 and T21–25/S35 received a ration of 4% dry meat weight from day 1 to 9 and a ration of 3% from day 10 to 55. Treatment T16–25/S35, received a ration of 2% from day 1 to 9, when the temperature was initially low, 1.5% from day 10 to 22, and 4% ration from day 23 to 55.

Samples of three clams per EU ($n = 24$ from each treatment) were sampled for histological analysis of reproductive development at the beginning of the experiment (D0) and then every 15 days (D15, D30, and D45), with the last sampling on the 55th day (D55) of conditioning.

Reproductive Development of Sampled Wild Clams

Samples of wild clams ($n = 24$; 24.62 ± 1.25 mm average shell height) were collected from Praia da Daniela from September 30 to November 23 of 2011 (time points D0, D15, D30, D45, and D55) to compare reproductive development with that of conditioned clams. Clams were collected at 0.5 m depth in the sediment at low tide. Seawater temperatures were recorded at the time of collection.

Histological Examinations

Cross-sections (2 mm) were taken of gonadal tissues of sampled clams from both the conditioning experiment and the wild (Howard & Smith 1983). Sections were subjected to standard histological procedures (Sühnel et al. 2010), using Davidson fixative and Harris hematoxylin and eosin. The sex and the reproductive stage of each sample were divided into four categories, based on descriptions by Sühnel et al. (2010) and Luz and Boehs (2011), namely: gametogenesis, mature, spawning, and absorption (see Table 1).

Statistical Analysis

Feeding data were analyzed by analysis of variance and Tukey's test (SAS 2003). Reproductive stages of wild and conditioned clams, within and among treatments, were compared using a *t*-test and permutations of a nonparametric proc multitest (Westfall et al. 1999; SAS).

RESULTS

Temperature and Clam Mortalities

The seawater temperatures during the first 10 days of the experiment for the treatments T21–25/S30 and T21–25/S35 were 22.61 ± 1.48°C and 22.62 ± 1.43°C, respectively, rising to 24.80 ± 0.57°C and 24.81 ± 0.64°C, respectively, during the 45 remaining days. For treatment T16–25/S35, the temperature during the first 10 days was 16.13 ± 0.31°C, gradually rising (approximately 0.5°C per day) to 25°C during the remaining 45 days (Fig. 1). The seawater temperature at Daniela beach (the collection site of wild clams) during the experimental period ranged from 19.30°C at D0–23.97°C at day 55 (D55) (Fig. 1).

Clam mortalities were low, and by the end of the 55-day conditioning period, mortalities were 1.5%, 0.75%, and 0.25% in treatments T21–25/S35, T21–25/S30, and T16–25/S35, respectively.

Algal Consumption

Consumption of microalgal rations in treatments T21–25/S30 and T21–25/S35 was constant throughout the experimental period, with a daily rate of 3.68% ± 0.47% and 3.68% ± 0.48% dry meat weight, respectively. Lower microalgae consumption was observed during the first 22 days by broodstock clams in treatment T16–25/S35, with a daily feeding rate of 1.59% ± 0.43% dry

TABLE 1.

Categorization of the reproductive stages of the clam *Anomalocardia brasiliana*.

Sexual stage name	Abbreviation	Females	Males
Gametogenesis	GA	Presence of germ cells (oogonia); few oocytes of different sizes	Different stages of germ cells (spermatogonia and spermatocytes); few spermatids and spermatozoa in the follicles
		Presence of interfollicular connective tissue; thick follicle walls with intrafollicular spaces	Presence of interfollicular connective tissue; thick follicle walls with intrafollicular spaces
Mature	MA	Oocytes present in lumens of follicles; rounded oocytes present; possibly some germ cells in early stages of development	Presence of intrafollicular spermatozoids with or without eosinophils; some germ cells in early stages of development
		Many follicles filled with gametes; gonoducts empty; little or no intrafollicular and interfollicular connective tissue or spaces	Many follicles filled with gametes; gonoducts empty; little or no intrafollicular and interfollicular connective tissue or spaces
Spawning	SP	Some oocytes in lumens of follicles; possible presence of gametes in the genital ducts. Interfollicular and intrafollicular spaces present; follicles with irregular shapes due to recent spawning; partially empty follicles, with remaining oocytes may be evident. Phagocytosis of oocytes may be evident	Few spermatozoids in lumens of follicles; possible presence of gametes in the genital ducts. Interfollicular and intrafollicular spaces present; follicles with irregular shapes due to recent spawning; partially empty follicles with remaining spermatozoids. Phagocytosis of spermatozoids may be evident
Absorption	AB	No or very few remaining oocytes. No or few remaining follicles with small diameters; intrafollicular and interfollicular connective tissue evident	No or very few remaining sperm. No or few remaining follicles with small diameters; intrafollicular and interfollicular connective tissue evident

meat weight. This initial feeding rate was significantly different ($P < 0.05$) compared with consumption during the remaining 33 days (3.95% ± 0.22%) when the temperature increased higher than 18°C.

Histological Analysis

Histological analysis of samples of *Anomalocardia brasiliana* showed four reproductive stages, gametogenesis, mature, spawned, and absorption (Table 1; males Fig. 2A–D and females Fig. 2E–H). Among the 472 animals initially examined, the sex ratio of males and females was 1.48:1 (M:F), with 55.51% males, 37.50% females, and 6.99% of indeterminate sex. Before the experiment started and after induction of spawning, 48.61% of the clams ($n = 72$) were classified as spawned, 43.06% in the absorption stage, 5.56% in the mature stage and 2.78% in gametogenesis. It was not possible to determine the sex of some animals because only connective tissue and limited areas of germinal tissue, without the presence of germ cells, were observed in the gonad region. These clams were classified as being in the absorption stage.

Reproductive Stages within Treatments

Female Reproductive Stages

At the beginning of the experiment (D0), females of treatment T21–25/S30 were either in the spawned (50%) or in the absorption (50%) stages (Fig. 3). Mature clams were observed

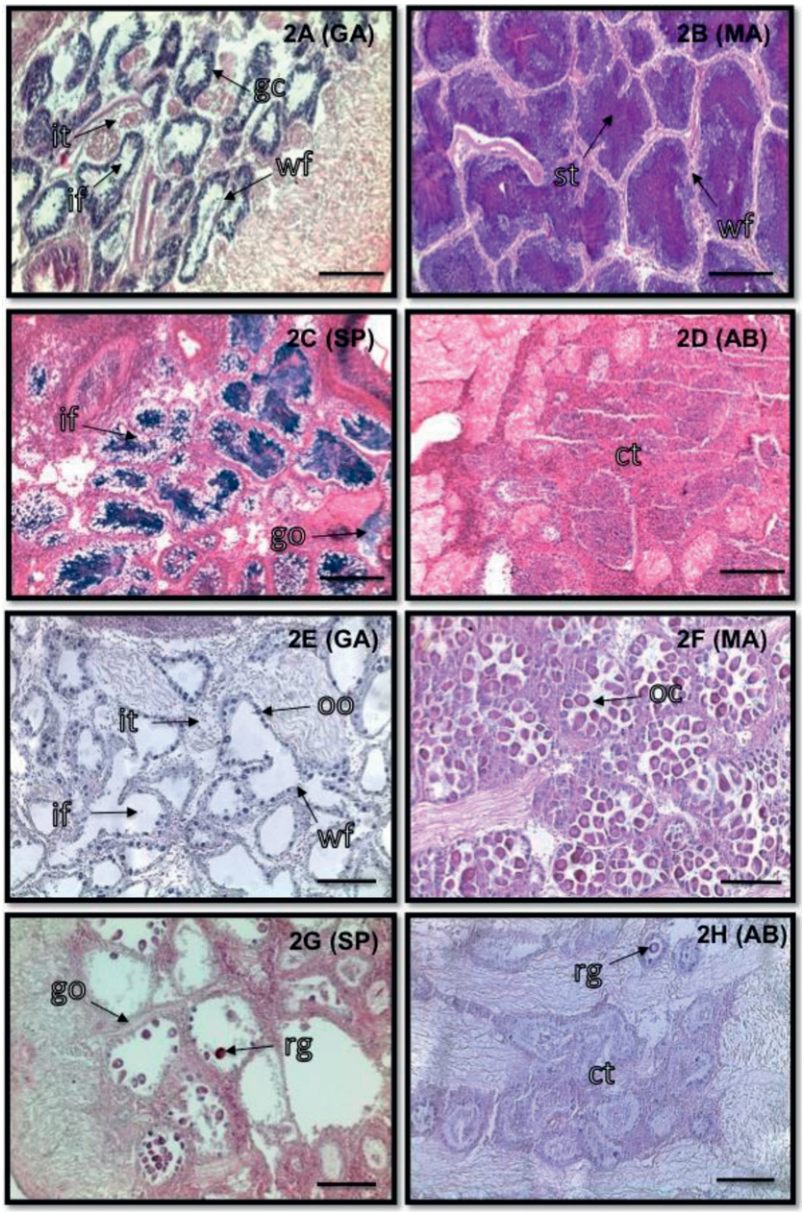

Figure 2. Reproductive stages: gametogenesis (GA), mature (MA), spawning (SP), and absorption (AB) for females (Fig. 2A, B) and males (Fig. 2E–H) of the clam *Anomalocardia brasiliana*, where: ct = connective tissue; gc = germ cells; go = gonoduct; if = intrafollicular space; it = interforllicular space; oc = oocytes; oo = oogonia; rg = remaining gametes; st = spermatozoids; wf = follicle wall. Bar = 200 microns; objective: 100× of magnification.

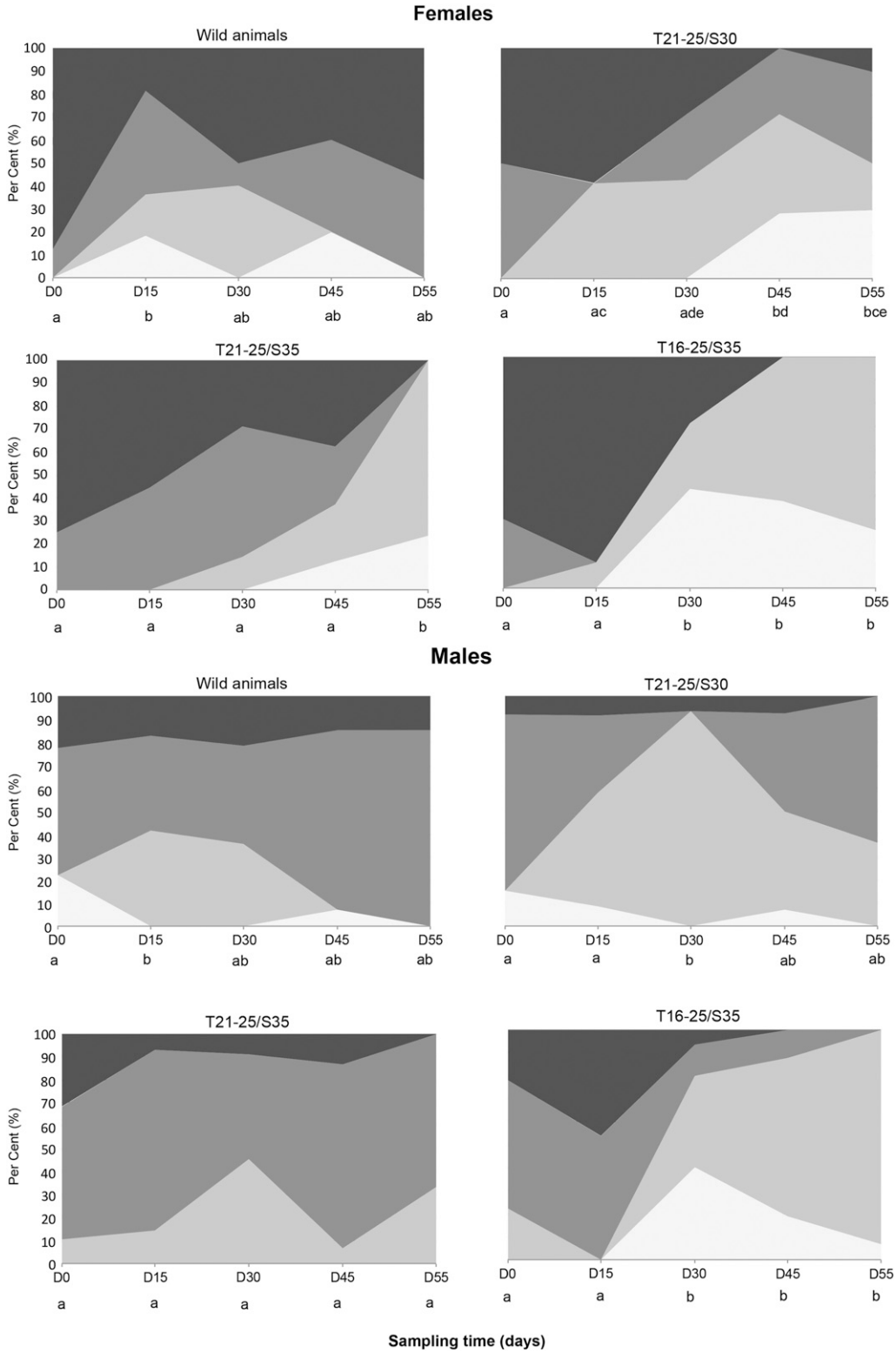

Figure 3. Reproductive stages of females and males (gametogenesis, mature, spawning, and absorption) of the clam *Anomalocardia brasiliana*. Clams were conditioned (from September 30 to November 23, 2011) at temperature increasing from 21°C to 25°C at salinities of 30 (T21–25/S30) or 35 (T21–25/S35), and from 16°C to 25°C at a salinity of 35 (T16–25/S35). Data for clams sampled from the wild (Praia da Daniela) are also shown. Data shown for the first day of the experiment (D0) and after 15, 30, 45, and 55 days (D0, D15, D30, D45, and D55). Small letters represent significant differences ($P < 0.05$) using *t*-test with permutation. ☐ Gametogenesis; ▒ Pre-spawning; ▓ Spawning; ■ Rest.

after 15, 30, and 45 days of conditioning (42%, 43%, and 43%, respectively), but this proportion decreased at D55 to 20% (Fig. 4). This decrease may have been because of spawning after 30 days of conditioning (28%) that continued until D55 (40%). Gametogenesis was observed in samples taken at D45 (29%) and D55 (30%).

The reproductive stages of clams in treatment T21–25/S35 showed significant differences only on D55, compared with D0, D15, D30, and D45. At the beginning of the experiment (D0) females showed signs of either spawning (25%) or absorption (75%) (Fig. 3). The proportion of mature clams increased after 30, 45, and 55 days (14%, 25%, and 76%, respectively; Fig. 4). Spawned clams were observed at all sampling times (D0, D15, D30, and D45) except on D55, with a high proportion of spawned clams (57%) occurring on D30.

In treatment T16–25/S35, the reproductive stages at D0 and D15 did not differ from each other but were significantly different ($P < 0.05$) from those of later samples at D30, D45, and D55. At the beginning of the experiment (D0), most of the clams were either in the absorption (70%) or in the spawning stages (30%; Fig. 3). A high proportion of clams (89%) remained in the absorption stage until D15 but this proportion decreased to 29% after D30. The proportion of mature clams gradually increased from 11% on D15 to 75% on D55 as conditioning temperatures increased (Fig. 4). The proportion of clams showing signs of gametogenesis increased from 0% on D15 to 43% on D30, decreasing to 25% on D55.

Wild females showed significant differences ($P < 0.05$) in reproductive condition between D0 and D15, with 88% of females in the absorption stage at D0, but most females were in the spawning stage (45%) by D15 (Fig. 3). At D30, 50% of the females were in the absorption stage and 40% in the mature stage (Fig. 4). At D45 and D55, 40% and 43%, respectively, of females were in the spawning stage and 40% and 57%, respectively, in the absorption stage.

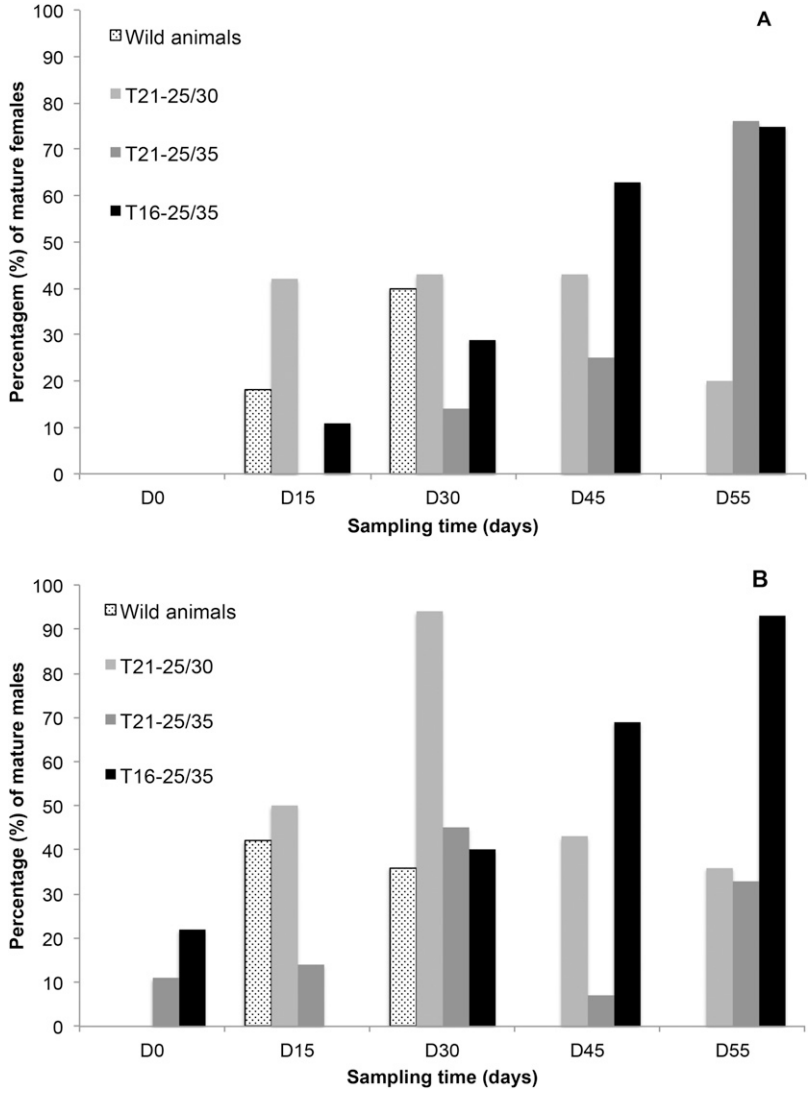

Figure 4. Percentage (%) of mature females (A) and males (B) of the clam *Anomalocardia brasiliana*. Treated clams were conditioned (from September 30 to November 23, 2011) at temperatures increasing from 21°C to 25°C at salinity of 30 (T21–25/S30) and salinity of 35 (T21–25/S35), and from 16°C to 25°C at salinity of 35 (T16–25/S35) and wild animals (Praia da Daniela). Samples were taken on the first day of the experiment (D0) and after 15, 30, 45, and 55 days of conditioning (D0, D15, D30, D45, and D55).

The proportions of mature stage clams among treatments and wild controls showed no differences at D0, D15, and D30; however at D45 and D55, significant differences ($P < 0.05$) were observed (Fig. 4). Females from treatment T16–25/S35 at D45 showed a high proportion of mature-stage animals compared with wild females. At D55, a high proportion of mature females were observed in treatments T16–25/S35 and T21–25/S35 compared with clams from the wild and treatment T21–25/S30.

Males Reproductive Stages

The reproductive condition of male clams in treatment T21–25/S30 showed significant differences ($P < 0.05$) between D0 and D30. At the beginning of the conditioning period, 77% of the males were in the spawning stage declining to 33%, 43%, and 64% on D15, D45, and D55, respectively (Fig. 3). A high proportion of mature males was observed on D30 (94%) and lower proportions on D15, D45, and D55 (50%, 43%, and 36%, respectively; Fig. 4). Gametogenesis decreased from D0 (15%) to D45 (7%), with no males found in this stage on D30 and D55.

In treatment T21–25/S35, no significant differences were observed among clam reproductive stages during conditioning. Males were mostly in the spawning stage during the experiment, with 58% at D0, 79% at D15, 45% at D30, 80% at D45, and 67% at D55 (Fig. 3). Mature clams were observed in high proportions on D30 (45%) and D55 (33%; Fig. 4). No male clams in the gametogenesis stage were observed in this treatment.

In treatment T16–25/S35, clams sampled during the initial 15 days of conditioning showed significant differences compared with those sampled at D30, D45, and D55. At the beginning of conditioning, most of the males were in the spawning stage (56%) and fewer in the mature and absorption stages (both 22%; Fig. 3). Gametogenesis was high on D30 (40%) and decreased to only 7% on D55. Mature stage clams (40%) started to appear at D30 and increased to 95% at D55 (Fig. 4).

No significant differences were observed in the proportions of wild clams in different sexual stages during the experimental period. At D0, 56% of the males were in the spawning stage, increasing to 79% and 86% at D45 and 55, respectively.

Reproductive Stages of Wild versus Conditioned Clams

Comparing the sexual stages of wild and conditioned females, two spawning peaks of wild clams were evident at D15 and D45 (45% and 40%, respectively). By contrast, in T16–25/S35 most of the conditioned female clams (89%) were in the absorption stage on D15 or in the mature stage (45%) on D45. Also, 57% of female wild clams were in the absorption stage on D55, a proportion that was significantly different ($P < 0.05$) from those in all treatments.

Wild males showed a significant difference ($P < 0.05$) at D15 compared with clams in treatment T16–25/S35, where spawned and absorption stages were high for conditioned clams (54% and 46%, respectively) compared with wild clams (42% and 17%, respectively). At D15, mature clams were only observed in wild males. On D30, 43% of wild males were in the spawning stage, a significant difference ($P < 0.05$) compared with clams in treatments T21–25/S30 and T16–25/S35 where most were in the mature stage (94% and 40%, respectively). On D45 and D55, 79% and 86%, respectively, of wild males clams were in the spawning stage, whereas, by contrast, a significantly ($P < 0.05$) higher proportion of clams in treatment T16–25/S35 (69% on D45 and 93% on D55) were in the mature stage.

Effects of Salinity and Temperature on Reproductive Stages

No significant differences in the proportions of different reproductive stages were observed for either male or female clams, between the two different salinities (30 and 35) under the T21–25 conditioning temperature regime. By contrast, significant differences ($P < 0.05$) were observed based on temperature regime at D45 when most of the females and males were in the mature stage (63% and 69%, respectively) in the T16–25/S35 treatment, whereas 38% of females were in the absorption stage and 80% of males were in the spawning stage in the T21–25/S35 treatment. At D55, the reproductive stage of female clams showed no differences between treatments T21–25/S35 and T16–25/S35.

DISCUSSION

The present study showed that it is possible to obtain high proportions of reproductively mature *Anomalocardia brasiliana* under controlled salinity and temperature conditions. After 55 days of conditioning, the treatment that included an initial cooler period of 16°C and a salinity of 35 (T16–25/S35), resulted in a higher proportion of mature clams than in other treatments. In addition, greater reproductive synchronization of females and males was observed with this treatment than in the other two treatments, where the initial conditioning temperature was higher (21°C). Temperature and food availability are known to be the main factors affecting bivalve mollusc reproduction (Bayne & Newell 1983, Barber & Blake 1991, 2006). Araújo (2001) observed an increase in gametogenesis of *A. brasiliana*, with increasing temperature in southern Brazil (latitude - 27°), where water temperatures range from 16°C to 30°C throughout the year (Suplicy et al. 2003).

Lower microalgal consumption of clams was observed in treatment T16–25/S35 than in the other treatments and this may be related to the effects of lower initial temperature on filtration rates. According to Kittner and Riisgard (2005), temperature (within the range of tolerance) and filtration rates in bivalves are positively correlated. This was observed in treatment T16–25/S35, where consumption increased with conditioning temperature. Gonadal maturation also improved, with 75% of the females and 93% of the males mature after 55 days of conditioning.

Another difficult problem in conditioning bivalves from tropical zones is spontaneous spawning. In this study, gradually increasing the conditioning temperature from 16°C to 25°C (T16–25/S35) made it possible to limit female spawning during conditioning. Control of male reproductive development was also improved by this treatment. Higher proportions of both males and females showed signs of spawning when conditioning started at a higher temperature of 21°C (T21–25/30 and T21–25/35), resulting in a loss of gametes and fewer reproductively mature broodstock clams at the end of the conditioning period.

Different salinities tested in the present study (30 and 35), under the same 21°C–25°C temperature regime, did not significantly affect the reproductive cycles of females and males of *Anomalocardia brasiliana*. Mature female and male clams were observed after 55 days of conditioning at both salinities, but a high proportion of clams spawned during the conditioning period, resulting in a lower proportion of mature broodstock at

D55. The tested salinities are within the range tolerated by *A. brasiliana* (Leonel et al. 1983).

No mature animals (females and males) were evident in samples of the wild population at D45 and D55 and a high proportion at these sample times had either spawned or were in the reabsorption stage of reproductive development. These observations agree with the findings of Araújo (2001), who observed reproductive peaks in both the spring and autumn in *Anomalocardia brasiliana* populations from southern Brazil (Florianópolis/SC). Similarly, Grotta and Lunetta (1982) observed that *A. brasiliana* sampled from wild populations in São Paulo (latitude - 23°) showed two reproductive peaks in contrast to a low level of spawning throughout the year at lower latitudes (Paraíba; latitude - 7°). Giese and Pearse (1974) report that continuous spawning and maturation processes in molluscs occur where variation in environmental factors are low, such as in the tropics. In temperate climates, bivalves are subject to greater variations in temperature and food availability throughout the year and, consequently, the maturation process is seasonal, with one to two reproductive peaks per year, usually occurring between the spring and fall (Helm et al. 2004).

In conclusion, reproductive conditioning of *Anomalocardia brasiliana*, with temperature conditions increasing from 16°C to 25°C and a salinity of 35 (T16–25/S35), proved to be effective in conditioning both male and female clams for spawning after a 55-day period. In addition, this treatment did not result in spawning during the conditioning period. These results should enhance the effectiveness of broodstock conditioning and hatchery production of *A. brasiliana* for both aquaculture and restoration of wild populations.

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