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Effect of temperature on gonad development of the Pacific geoduck clam (*Panopea generosa* Gould, 1850)

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ABSTRACT

Temperature is well known to be a significant factor influencing bivalve gonad development and has major implications for the success of broodstock conditioning in a hatchery setting. Little is known, however, about how temperature affects the reproductive development of the Pacific geoduck clam, *Panopea generosa*, despite it being a major commercial species. To determine the appropriate broodstock conditioning temperatures for this species, adults were held in the laboratory for 155 days at fixed temperatures of 7, 11, 15, and 19 °C. Clams held at the two lower temperatures (7 and 11 °C) displayed a more advanced state of reproductive development than those at the two higher temperatures (15 and 19 °C). Significantly higher percentages of individuals with mature gonads were noted at 7 and 11 °C than at 19 °C and significantly more oocytes follicle^{-1} were evident at 7 and 11 °C than at 15 and 19 °C. Clams in the 7 °C treatment had a significantly higher gonadosomatic index than those in the 11 °C one (which were not significantly different from clams held at 15 or 19 °C), but that was likely because there was a significantly lower incidence of spawning activity at 7 than at 11 °C. The 11 °C treatment had a significantly higher overall percentage of individuals spawning between weeks 15 and 17 of the experiment than any of the three other temperature treatments. Gonads of individuals in the 19 °C treatment degenerated, with 0 oocytes follicle^{-1} and 90% of the gonad occupied by connective tissue after 113 days. In a hatchery setting, *P. generosa* should be held at a temperature of 11 °C if reproductive output is to be maximized. Lower temperatures (*e.g.* 7 °C), however, could be used to hold ripe broodstock for extended periods.

1. Introduction

Pacific geoduck clams (*Panopea generosa* Gould, 1850) are the world's largest burrowing clam and a commercially important species in north-western North America, British Columbia (BC), Canada and Washington (WA), U.S.A. being the largest producers (Beattie, 1992; Beattie and Blake, 1999; Bureau et al., 2003). This species, however, typically has low recruitment rates (Breen and Shields, 1983; Campbell et al., 2004; Harbo et al., 1983; Zhang and Campbell, 2004) and fishery landings from wild stocks have remained stable since the 1990s at approximately 2 million kg annually in BC (James, 2008). Limitations of market supply, combined with a high value [over US $16 kg^{-1} (James, 2008)], have led to significant efforts toward aquaculture production over the last 20 years, especially in WA (Gordon, 2007).

Despite the efforts of the aquaculture industry to increase supply – 397,000 kg were produced in 2007 in WA alone (Gordon, 2007) – surprisingly little is known about the various biological (*e.g.* food quantity/quality, adult density, predation) and physical (*e.g.* temperature, salinity, oxygen concentration, current, substratum type) factors that may affect reproductive development, gamete production, and spawning of either wild or cultured clams. Reproductive development is of particular importance to broodstock conditioning in a hatchery, but no published studies on the subject are available for *P. generosa* [but see notes on spawning methods in early larval-rearing studies by Goodwin (1973) and Goodwin et al. (1979)]. Increased knowledge could improve broodstock conditioning and therefore hatchery production.

Temperature is a factor known to influence the reproductive biology of bivalves (Bayne, 1976; Loosanoff, 1937; Loosanoff and Davis, 1952, 1963; Ropes and Stickney, 1965). Manipulation of temperature under controlled conditions (*i.e.* laboratory or hatchery) can be a

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powerful tool for increasing the rate of gametogenesis — as seen in *Tapes philippinarum* (Mann, 1979b), *Crassostrea gigas* (Chávez-Villalba et al., 2002; Fabioux et al., 2005; Robinson, 1992), and *Ostrea edulis* (Mann, 1979a) for example. It can also increase larval production [*e.g. O. lurida* (Santos et al., 1993)] and subsequent larval survival and spat fall [*e.g. C. gigas* (Robinson, 1992)]. Specific studies on the effects of temperature on *P. generosa* gonad development, however, have not been conducted. Seasonal reproductive cycles of this species do show a strong association between reproductive state and season (Andersen, 1971; Campbell and Ming, 2003; Goodwin, 1976; Sloan and Robinson, 1984) suggesting a likely correlation with temperature. Similar reproductive-cycle studies on other species of geoduck [*P. zelandica* (Gribben et al., 2004), *P. abbreviata* (van der Molen et al., 2007), and *P. globosa* (Aragón-Noriega et al., 2007)] have suggested that temperature is a major factor in reproductive development.

The objective of the present study was to investigate the reproductive responses of *P. generosa* to being held at fixed temperatures in a controlled environment for prolonged periods. The response variables investigated were: timing of spawn events, condition index, gonadosomatic index, gametogenic (development) stage, oocyte maturity, oocytes per follicle, oocytes per unit area, and connective tissue occupation index. The ultimate goal of the study was to identify temperatures that are suitable for the maintenance and conditioning of *P. generosa* broodstock in a hatchery setting.

2. Materials and methods

2.1. Algal culture

Live algal cultures of *Isochrysis* sp. (TISO clone, CCMP 1324) and *Chaetoceros muelleri* (CCMP 1316) were used as feed. Specific levels of fatty acids required for successful gametogenesis in *P. generosa* are unknown, but these two phytoplankton species were selected to provide a balanced complement of polyunsaturated fats known to be typically required for successful bivalve broodstock conditioning; *C. muelleri* has high levels of eicosapentaenoic acid (20:5n-3) (Helm et al., 2004) and moderate levels of arachidonic acid (20:4n-6) (Soudant et al., 1996b) while TISO has high levels of docosahexaenoic acid (22:6n-3) (Soudant et al., 1996a). The algae were grown semi-continuously in 300-L fibreglass columns and 500-L polyethylene bags at a temperature of 18.7 ± 0.1 °C (mean ± SD, $n = 588$) under full-spectrum fluorescent bulbs (Philips DayLight Deluxe®, Philips Electronics Ltd., Markham, Canada). Seawater for algal culturing was filtered to 0.2 μm, sterilized with sodium hypochlorite, neutralized with sodium thiosulfate, and fertilized with a modified Harrison's formula (Harrison et al., 1980). That modification was the partial substitution of organic phosphates by inorganic phosphates.

2.2. Broodstock collection and initial maintenance

Broodstock were collected from a natural geoduck bed on December 2, 4, and 7, 2006 in Comox, BC (49° 39.07′ N latitude, 124° 52.93′ W longitude). The clams were taken from a single genetic population (VanKoeveringe, 1998) as close to the same date and location as possible to minimize the influences of genetic variability (Barber et al., 1991), season, and location (Chávez-Villalba et al., 2002, 2003a) on reproductive condition. Clams were collected by SCUBA diving at a depth of 9–12 m using a hydraulic harvester (consisting of a water jet supplied by a surface pump). Water temperature at depth during collection ranged from 7.3 to 8.9 °C and salinity was 28. The substratum was a cobble and mud mixture. After being gathered, the clams were placed in totes, covered with wet burlap sacks, and delivered within 7 h to the Pacific Biological Station in Nanaimo, BC. Clams were subsequently held in seawater tables at 11.1 ± 0.8 °C (mean ± SD, $n = 14{,}400$) with sand-filtered seawater until the start of the acclimation phase.

Shell length (SL) (measured to the nearest 1.0 mm on the anterior-posterior axis of the right valve using vernier callipers) of the clams averaged 151 ± 18 mm (mean ± SD, $n = 112$). Live weight [to the nearest 0.1 g using a Mettler PM4800 Delta Range balance (Mettler Toledo, Mississauga, Canada)] averaged $1{,}412 \pm 443$ g (mean ± SD, $n = 112$). The minimum size of clam used in the experiment was 110 mm SL to ensure an even sex ratio and sexual maturity, as reported by Andersen (1971), Sloan and Robinson (1984), Campbell and Ming (2003), and Gribben and Creese (2005). Reproductive senility has not been reported in *P. generosa* (Sloan and Robinson, 1984), but larger (over 2000 g) and presumably older individuals were avoided as reproductive success can diminish with age in bivalves (Sukhotin and Flyachinskaya, 2009).

2.3. Broodstock conditioning: experimental design

On December 12, 2006 the clams were set vertically – to provide a more natural orientation – inside PVC tubes (H: 55 cm, Diameter: 12.5 cm) with one individual per tube (Fig. 1). A U-shaped, PVC-coated, wire-mesh "girdle" (2.5 × 2.5 cm mesh size, 3 mm gauge wire, cut into 8 × 34-cm strips) was moulded around the shell of each clam. Without external pressure on a geoduck (normally provided by the natural substratum) the shell can gape, causing tears in the periostracum (which covers the shell, siphon, and muscular mantle) making the clam susceptible to infection. Necrotic tissue typically develops in the immediate area where the shell separates from the periostracum. The tubes had an outflow 2.5 cm from the top, giving a functional volume of 6.4 L. Tubes were placed vertically in Plexiglas water-bath tanks (L × W × H: 58 × 58 × 30 cm, Volume: 100.9 L) (Fig. 1). Eight tubes were placed in each tank (total of 16 tanks) and seven clams were randomly assigned to each tank with one tube per tank kept clear as a control for testing water quality parameters. Water was fed to each tube from a plastic header tank (L × W × H: 30 × 25 × 64 cm, Volume: 47.3 L) and entered the geoduck tubes approximately 10 cm from the bottom (Fig. 1). The average seawater flow rate in the tubes was 25.4 ± 6.8 L h^{-1} (mean ± SD, $n = 336$). Flow rate to each tube was set to exceed the average clearance rate of the clams which was determined to be 13.2 L h^{-1} $clam^{-1}$ at 11 to 19 °C. Average clearance rate was determined through a preliminary study using the method of Quayle, as described by Coughlan (1969). Clearance rates remained steady or declined with increasing temperature, indicating that food availability was not a limiting factor. Water flowed to waste except during feeding when it was

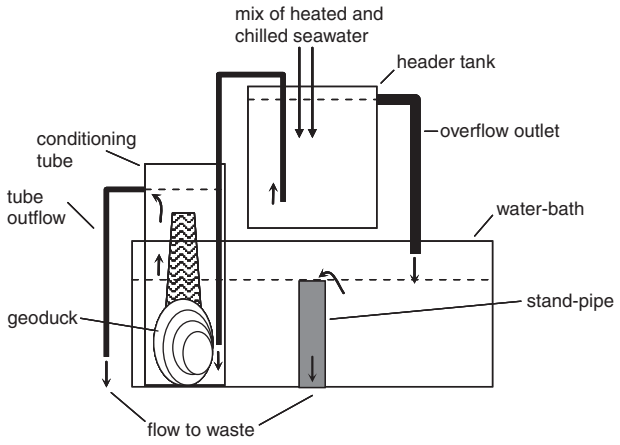

Fig. 1. Schematic showing the *Panopea generosa* conditioning tube system. Dashed lines depict water levels in the respective containers. Arrows depict water flow. Actual configuration was eight tubes per water-bath tank with seven containing one clam each and one remaining empty for water quality testing. There were four water-bath tanks for each temperature treatment. Not to scale. See text for dimensions.