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TEMPERATURE AND FOOD-RATION OPTIMIZATION IN THE HATCHERY CULTURE OF JUVENILES OF THE PACIFIC GEODUCK *PANOPEA GENEROSA*

BIANCA ARNEY,[1,2] WENSHAN LIU,[3] IAN FORSTER,[4] R. SCOTT MCKINLEY[1,2] AND CHRISTOPHER M. PEARCE[3]*

[1]*Faculty of Land and Food Systems, The University of British Columbia, 248-2357 Main Mall, Vancouver, British Columbia V6T 1Z4, Canada;* [2]*Centre for Aquaculture and Environmental Research, The University of British Columbia/Fisheries and Oceans Canada, 4160 Marine Drive, West Vancouver, British Columbia V7V 1N6, Canada;* [3]*Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, British Columbia V9T 6N7, Canada;* [4]*Fisheries and Oceans Canada, West Vancouver Laboratories, 4160 Marine Drive, West Vancouver, British Columbia V7V 1N6, Canada*

ABSTRACT This research examined the individual effects of temperature and ration on growth and survival of juveniles of the Pacific geoduck *Panopea generosa* Gould, 1850, in two separate experiments. Growth parameters measured included shell length, daily shell increment, individual total body wet weight, specific growth rate, individual total body dry weight, and total body ash-free dry weight (AFDW). Larvae in all treatments were fed a binary microalgal diet of *Chaetoceros muelleri* and *Tisochrysis lutea* mixed at a 1:1 ratio by AFDW. The temperature experiment examined the effect of four temperatures (7°C, 11°C, 15°C, and 19°C) using two geoduck cohorts. One cohort was comprised of larger juveniles (mean initial shell length ± SE, 3.22 ± 0.05 mm) and the other included smaller individuals (0.54 ± 0.01 mm) which were cultured for 28 days and 21 days, respectively. Using a separate cohort, the food-ration experiment examined the effect of nine rations (0.0, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, and 128.0×10^6 equivalent *T. lutea* cells per individual per day) on four size classes of geoduck juveniles obtained from the same spawning batch (mean initial shell length ± SE, size class 1, 2.34 ± 0.04 mm; size class 2, 3.32 ± 0.04 mm; size class 3, 4.13 ± 0.04 mm; and size class 4, 4.98 ± 0.04 mm) for 7 days. On the final sampling day, there were significant differences among all four temperatures for all growth parameters except AFDW, with each parameter increasing significantly for each increase in temperature. For AFDW, there were no significant differences between 7°C and 19°C, nor between 11°C and 15°C, with the first set of two temperatures producing juveniles with significantly less AFDW than the latter set. In general, optimal ration levels increased with increasing geoduck size. To optimize growth in shell length, individual total body wet weight, and/or individual total body dry weight, the following rations are recommended for size classes 1, 2, 3, and 4, respectively: 4.0 or 8.0, 8.0, 16.0 or 32.0, and 32.0 or 64.0×10^6 equivalent *T. lutea* cells per individual per day. Refinement of an understanding of optimum juvenile geoduck culture conditions contributes to the general knowledge of the species' physiology and helps maximize the commercial hatchery production of geoduck seed.

KEY WORDS: aquaculture, food ration, juveniles, Pacific geoduck, *Panopea generosa*, temperature

INTRODUCTION

The Pacific geoduck *Panopea generosa* Gould, 1850, supports the most valuable commercial clam fishery in the northeast Pacific Ocean. Landed values for commercially captured geoducks increased dramatically between 2008 and 2011 in both Washington (USD26.4–49.0 million) and British Columbia, Canada (CAD 25.8–41.3 million) (Ministry of Agriculture, British Columbia 2011, Washington Department of Fish and Wildlife unpubl. data), with the majority of product demand driven by the Chinese market. Because of its lucrative value, the Pacific geoduck has become an attractive species for aquaculture in British Columbia, garnering much interest from First Nations and commercial entities in recent years. The adoption of geoduck aquaculture is further encouraged by the unknown resilience of wild populations to prolonged fishery pressure (Orensanz et al. 2004) and the occurrence of illicit harvesting activity (Campbell et al. 1998, Orensanz et al. 2004). Currently, geoduck aquaculture in British Columbia aims to outplant hatchery-raised juveniles either into designated tenured plots for later commercial harvest (Hand & Marcus 2004) or into the common marine domain to enhance wild stocks (James 2008). Juvenile geoducks are fed live microalgae and cultured in a hatchery until they reach a shell length of 3–6 mm (Pinfold & IEC International 2001). At that time, juveniles may be transferred to nursery culture for further growth before field outplanting; transfer typically occurs at a shell length of 12–20 mm (Pinfold & IEC International 2001). Final harvest is then estimated to occur between 6 y and 9 y after outplanting (Heath 2005).

Published research concerning Pacific geoduck hatchery rearing technologies is surprisingly sparse, given the level of interest in the culture of the species, and is limited predominantly to that on larvae and broodstock. Seminal hatchery work by Goodwin refined the temperature and salinity requirements of geoduck embryos and larvae (Goodwin 1973), and examined the various stages of larval development (Goodwin et al. 1979), whereas more recent work by Marshall et al. (2014b) examined the combined effects of food ration and stocking density on larval growth and survival. Further studies by Marshall et al. have investigated the effects of temperature (Marshall et al. 2012) and food ration (Marshall et al. 2014a) on Pacific geoduck broodstock conditioning. In addition, recent work by García-Esquivel et al. (2013), on the closely related subtropical species *Panopea globosa* (Dall, 1898), has examined broodstock conditioning and maturation under varied temperature regimes and with live microalgae supplementation. In the only known peer-reviewed publication examining the culture of early postset juveniles of Pacific geoducks, Ren et al. (2015) studied the effect of

*Corresponding author. E-mail: Chris.Pearce@dfo-mpo.gc.ca
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binary-species phytoplankton diets on juvenile growth and survival and reported that *Chaetoceros muelleri* and *Isochrysis* sp. (Tahitian strain), mixed at a 1:1 ratio by ash-free dry weight (AFDW), was the best of 11 diets examined. This paucity of basic biological information concerning early postsettlement juveniles is a major obstacle to reliable geoduck hatchery production. Indeed, British Columbia hatchery protocols are ill defined (Hand & Marcus 2004) and production is limited (Heath 2005). This absence of data necessitates prioritization in pilot investigations, isolating the culture parameters most influential to geoduck growth optimization. Out of a multitude of culture conditions—including temperature, salinity, food type, food ration, and stocking density—research has indicated that temperature and ration may be the most important factors dictating growth rate in bivalve juveniles (Walne & Spencer 1974, Broom & Mason 1978, Beiras et al. 1993).

Typically, temperature elevation accelerates poikilothermic growth until a species-specific thermal maximum is attained, beyond which growth and/or survival is depressed (Kinne 1970, Hochachka & Somero 2002). This growth trend has been shown for juveniles of a variety of temperate and subtropical bivalve species (e.g., Almada-Villela et al. 1982, Laing et al. 1987, Kleinman et al. 1996, Sicard et al. 1999, Rico-Villa et al. 2009). In aquaculture, the rearing temperature can therefore be manipulated to the culturist's advantage if the optimal thermal range of the target culture species is known. The thermal optimum remains undefined for juvenile Pacific geoducks, with thermal investigations on this species limited to embryos (Goodwin 1973) and broodstock (Marshall et al. 2012).

Food ration is the quantity of food available for consumption by the animals in culture. Energy obtained from the selected ration cannot be used for shell or tissue growth until maintenance (i.e., respiration, excretion) requirements are exceeded (Thompson & MacDonald 2006). Beyond this threshold, elevated food availability has been demonstrated to enhance food consumption and growth in multiple bivalve species (e.g., Walne & Spencer 1974, Langton et al. 1977, Beiras et al. 1993, Coutteau et al. 1994a, Coutteau et al. 1994b, Lu & Blake 1996, García-Esquivel et al. 2000). Indiscriminate elevation of the offered ration can, however, elicit an insignificant or even reduced growth response in the cultured animals (Coutteau et al. 1994a, Coutteau et al. 1994b, García-Esquivel et al. 2000). This may be the result of detrimental organismal responses associated with excessive food concentrations, including reduced food handling rates resulting from overloaded ctenidia (Kahlil 1996, Riisgård et al. 2011), elevated pseudofeces production (Winter 1978, Wilson 1979, Riisgård et al. 2011), and reduced efficiencies in absorption (Winter 1974, Widdows et al. 1979) and/or digestion (reviewed by Griffiths and Griffiths [1987]). The deterioration of water quality resulting from overfeeding may also incur negative feeding and growth responses. Application of an incorrect or excessive food ration is costly, especially during the juvenile growth stage, as juveniles often represent the largest biomass in hatchery culture and are the greatest consumers of microalgae (Claus 1981, Manzi & Castagna 1989, Helm 1990). Optimal temperature and food-ration requirements may be controlled ontogenetically (Kinne 1970, Helm et al. 2004), thus requiring an examination of thermal and ration requirements of various organismal size classes. Therefore, the specific objective of the present research was to determine the optimal temperatures and food rations for growth and survival of various size classes of juveniles of Pacific geoducks. The results of the research are important for refining hatchery technology and maximizing juvenile production in commercial culture facilities.

MATERIALS AND METHODS

Common Protocols for All Experiments

Experimental Animal Source and Husbandry

Broodstock were collected in the Strait of Georgia, British Columbia, Canada (between 48°50.9′ N, 123°23.5′ W and 49°18.2′ N, 124°11.1′ W) in October 2011. The mean (±SD) shell length and live weight of the broodstock were 149.6 ± 14.7 mm and 1.4 ± 0.3 kg ($n = 50$), respectively. Animals were reared at the Pacific Biological Station (Nanaimo, British Columbia, Canada) in indoor holding tanks (L × W × H, 1.2 × 0.9 × 0.3 m) stocked with 20–30 individuals per tank. The tanks received seawater (8–12°C, sand filtered and UV treated) at 3–4 L/min. A single-algal diet of *Chaetoceros muelleri* or *Tisochrysis lutea* (formerly *Isochrysis* sp., Tahitian strain [Bendif et al. 2013]) was drip-fed into the tanks at a rate of $4–6 \times 10^9$ cells per individual per day.

Spawning was induced by excessive provision of *Tisochrysis lutea* to ripe broodstock. Fertilized eggs were collected and hatched in holding tanks (L × W × H, 1.2 × 0.9 × 0.3 m) at a density of less than 30 eggs/mL at a temperature of 12–15°C. After 48–60 h, the newly developed D-larvae were transferred to a 300-L cylindrical–conical tank and reared at 3–8 individuals/mL. After 18–20 days of culture, pediveligers ready for settlement were collected from the water column, transferred to a floating tray fitted with a mesh bottom (diameter, 36 cm; base area, 1,018 cm^2; mesh size, 200–240 μm), and reintroduced to the cylindrical–conical tank in the floating tray. Geoducks were reared on the mesh tray at a mean (±SE) temperature of 15.5 ± 0.4°C until the desired experimental size was achieved.

During the larval stage, animals were fed a mixed algal diet of *Chaetoceros calcitrans* and *Tisochrysis lutea* (in equal AFDW proportions) at a ration level of 10,000–20,000 AFDW equivalent *T. lutea* cells/mL/day. During the postsettlement stage, animals were fed a mixed algal diet of *Chaetoceros muelleri* and *T. lutea* (in equal AFDW proportions) at a ration level of 20,000–100,000 equivalent *T. lutea* cells/mL/day. The mean AFDW of *C. calcitrans*, *T. lutea*, and *C. muelleri* was 8.33, 15.99, and 16.79 pg/cell, respectively (Arney 2013, W. Liu unpubl. data). A photoperiod of 16 h/8 h light/dark was maintained for the full culture period.

Live Algal Culture

Microalgal species were sourced from the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA, formerly the CCMP, East Boothbay Harbor, ME). The following strains were used: *Chaetoceros muelleri* (CCMP 1316), *Tisochrysis lutea* (CCMP 1324; formerly *Isochrysis* sp., Tahitian strain), and *Chaetoceros calcitrans* (CCMP 1315; *C. calcitrans* was not used in the juvenile experiments, only in larval culture). All experimental treatments received a mixed diet of *C. muelleri* and *T. lutea* (50%/50% by AFDW). This binary-species diet supports the best growth throughout the geoduck juvenile stage compared with a variety of alternative monospecies and mixed-species diets (Ren et al. 2015; W. Liu unpubl. data). Strains of *C. muelleri* and *T. lutea* were grown in batch cultures in 20-L carboys, and *C. calcitrans* was batch-cultured in 4-L flasks. Cultures were maintained at 18°C

and grown under continuous cool-white fluorescent light. All species were harvested during the late logarithmic growth phase. Cultures were grown in sand-filtered, 1-μm cartridge-filtered, and UV-treated seawater enriched with an artificial growth medium of Harrison's formula (Harrison et al. 1980), modified by the partial substitution of organic phosphates by inorganic phosphates. Algae were used between 4 days and 8 days after carboy or flask inoculation. Daily algae culture density was determined before feeding, with cell counts obtained using a hematocytometer.

System Monitoring

All experiments were conducted indoors at the Pacific Biological Station. Rearing water temperature was monitored daily with a glass thermometer and with four temperature loggers (HOBO Tidbit v2; Onset Computer Corporation, Bourne, MA), recording at 5-min intervals. Water pH was measured three times throughout each trial or subtrial. Water samples were collected from each rearing container in 10-mL glass vials and were allowed to equilibrate to room temperature before measurement with a pH probe (Orion AquaPro Professional pH/ATC triode; Thermo Fisher Scientific Inc., Waltham, MA). Salinity of the incoming seawater was measured on each water change day with a refractometer (VEE GEE Stx-3; VEE GEE Scientific Inc., Kirkland, WA). A photoperiod of 16 h/8 h light/dark was maintained for all experiments.

Growth Parameters

The following clam growth parameters were determined during each experiment: shell length (measured from the anterior to posterior axis in millimeters), daily shell increment (DSI; measured in micrometers per day), individual total body wet weight (measured in milligrams per individual), specific growth rate (SGR), individual total body dry weight (measured in milligrams per individual), and total body AFDW (measured as percent dry weight). Smaller geoduck juveniles in the temperature experiment were too small to weigh accurately; therefore, only shell length and DSI were considered in that experiment. Percent survival was calculated for all experiments. The metrics DSI, SGR, AFDW, and percent survival were calculated using the following equations:

$$\text{DSI}\ (\mu\text{m/day}) = \frac{\text{Final shell length} - \text{Initial shell length}}{T}$$

$$\text{SGR} = \frac{\ln(W_2) - \ln(W_1)}{T}$$

$$\text{AFDW}\ (\%\ \text{dry wt.}) = \frac{\text{Organic weight (mg)}}{\text{Dry weight (mg)}} \times 100\ \text{(Laing 2000)}$$

$$\text{Percent survival}\ (\%) = \frac{\text{No. of final individuals}}{\text{No. of initial individuals}} \times 100 \text{ of each culture period}$$

(cumulative survival in the temperature trial was calculated from the product of each sampling period)

where W_2 is the final mean individual total body wet weight (measured in milligrams per individual) of the geoduck seed, W_1 is the initial mean individual total body wet weight (measured in milligrams per individual), and T represents the duration of the experiment (in days).

Temperature Experiment: Larger Juveniles

Rearing Conditions

A summary of culture protocols is given in Table 1. Four temperature treatments averaging 7.5 ± 0.2°C, 11.2 ± 0.3°C, 14.8 ± 0.3°C, and 18.6 ± 1.0°C (±SD, $n = 8{,}026$) were tested in triplicate for 28 days. These treatments are abbreviated as 7°C, 11°C, 15°C, and 19°C from this point forward. Each treatment replicate was represented by 100 randomly chosen geoduck juveniles collected from a common rearing batch (age, 58 days postfertilization). Shell length and individual total body wet weight (mean ± SE) of the initial sample were 3.22 ± 0.05 mm ($n = 50$) and 7.04 ± 0.25 mg/individual ($n = 3$ groups of 100 individuals), respectively. Geoducks for each replicate were maintained in a PVC container (diameter × H, 10 × 25 cm) fitted with a 300-μm Nitex mesh bottom. The initial stocking density was 8.96 mg total body wet weight/cm^2. The PVC containers were suspended individually in 19-L plastic buckets filled with 18 L sand-filtered, 1-μm cartridge-filtered, and UV-treated seawater of the appropriate treatment temperature. Buckets were covered with lids and gentle aeration was provided by an air stone placed beneath the suspended PVC container, which facilitated water exchange across the mesh bottom (culture water in the buckets was static). Buckets were held in water bath tanks receiving a constant flow of temperature-controlled water to maintain treatment temperatures. To reduce temperature shock at initial stocking, all experimental geoducks were placed in buckets filled with 15°C seawater (culture temperature of the initial population) before transfer in the appropriate water bath. Bucket culture water attained the treatment temperatures gradually within less than 24 h.

After trial initiation, full water exchanges occurred every second day up to day 10. Commencing at day 11, water exchange occurred daily because of increased geoduck biomass. Buckets and lids were cleaned with a 10% bleach solution and were rinsed with freshwater during each water exchange. Air stones were also rinsed with freshwater at this time. As a result of seasonal acidity (pH, <7.8) in the seawater source, 3 mL 0.5 M NaOH was added to each bucket during water exchanges to adjust system pH to normalized levels (>7.8).

Algal Feeding

Batch food delivery occurred daily after the water exchange, with the exception of the 7°C treatment. This treatment received food every second day between day 0 and day 10, because of the reduced feeding activity of the clams at this temperature. All treatments received an initial ration of 100,000 equivalent *Tisochrysis lutea* cells/mL. Ration quantity was *ad libitum* in all treatments to ensure food availability did not become limiting as geoduck size increased. Ration was adjusted for each temperature treatment through the observation of residual algal densities in the rearing water. The delivered ration was increased to 150,000 equivalent *T. lutea* cells/mL to the 15°C and 19°C treatments at day 10, and to the 11°C treatment at day 14. After these adjustments, ration levels remained unchanged for the duration of the experiment.

Sampling

With the exception of the 7°C treatment (which displayed little growth over time), geoduck biomass in each replicate container was standardized at 14 days and 21 days to account

TABLE 1.

Culture protocols followed for each experiment in the study (two temperature experiments and four food-ration experiments).

Culture parameter	Temperature experiments		Food-ration experiments			
Geoduck size class	Large	Small	1	2	3	4
Initial shell length (mm)	3.22 ± 0.05	0.54 ± 0.01	2.34 ± 0.04	3.32 ± 0.04	4.13 ± 0.04	4.98 ± 0.04
Initial total body wet weight (mg/ind)	7.04 ± 0.25	—	2.96 ± 0.15	6.73 ± 0.27	12.31 ± 0.16	21.23 ± 0.36
Spawning batch	1	2	3	3	3	3
Age (days postfertilization)	58	29	53	64	72	83
Treatments (°C or $\times 10^6$ equivalent *Tisochrysis lutea* cells/ind/day)	7, 11, 15, 19	7, 11, 15, 19	0.0–64.0*	0.0–64.0*	0.0–128.0†	0.0–128.0†
Replicates	3	3	3	3	3	3
Container size (L)	19	4	19	19	19	19
Initial juveniles per replicate (*n*)	100	100	100	50	25	25
Stocking density (mg/cm^2)	8.96	—	3.77	4.28	3.92	6.75
Initial ration (*T. lutea* cells/mL)	100,000	100,000	—	—	—	—
Final ration (*T. lutea* cells/mL)	7°C: 100,000 11°C: 150,000 15°C: 150,000 19°C: 150,000	100,000	—	—	—	—
Ration modification days	7°C: — 11°C: 14 15°C: 10 19°C: 10	—	—	—	—	—
Biomass standardization days	14, 21	9	—	—	—	—
Subsampling days	7, 14, 21	7, 14	—	—	—	—
Subsampled juveniles (*n*)	15	15	—	—	—	—
Trial duration (day)	28	21	7	7	7	7
Final sampled juveniles (*n*)	7°C: 50 11°C: 30 15°C: 19 19°C: 19	7°C: 70 11°C: 38 15°C: 29 19°C: 23	100	50	25	25
Juveniles used for SL and DSI (*n*)	15	15	20	20	20	20
Juveniles used for AFDW (*n*)	3	—	3	3	3	3
Growth parameters (*n*)	6‡	2§	6‡	6‡	6‡	6‡

—, Not applicable; AFDW, ash-free dry weight; DSI, daily shell increment; ind, individual; SL, shell length. * Rations tested: 0.0, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, and 64.0×10^6 equivalent *Tisochrysis lutea* cells/individual/day. † Rations tested: 0.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, and 128.0×10^6 equivalent *T. lutea* cells/individual/day. ‡Measured growth parameters: SL (measured in millimeters), DSI (measured in micrometers per day), individual total body wet weight (measured in milligrams per individual), specific growth rate, individual total body dry weight (measured in milligrams per individual), AFDW (measured as percent dry weight). §Measured growth parameters: SL (measured in millimeters) and DSI (measured in micrometers per day). Initial shell length and individual total body wet weight are given as mean ± SE.

for the large size differences among temperature treatments (11°C, 15°C, and 19°C). Combined with routine weekly samplings (discussed next), randomly chosen geoducks were removed from each replicate container to reduce the total live biomass to 1,200 mg/bucket at day 14 and 1,000 mg/bucket at day 21.

Subsampling occurred at day 7, day 14, and day 21. At each sampling time, empty shells were extracted and counted in each replicate to determine percent survival. Fifteen randomly selected geoducks were removed and used to calculate shell length, DSI, individual total body wet weight, SGR, individual total body dry weight, and total body AFDW. At final sampling (28 days), all remaining geoducks were counted and removed (final geoduck number was treatment dependent based on the biomass standardization that occurred on days 14 and 21). Fifteen random individuals from each replicate were used to determine final shell length and DSI. All individuals from each replicate were measured to determine final individual total body wet weight, SGR, and individual total body dry weight. Three geoducks from each replicate were used to determine final AFDW. Percent survival was multiplied across sampling periods to determine cumulative survival across the entire trial. Growth parameters and percent survival were averaged within each replicate and then treatment for each sampling period.

At sampling, all replicates were rinsed three times with 10 mL 0.5-M ammonium formate for salt removal and were transferred to a Petri dish. Geoducks were photographed with a digital SLR camera (EOS Digital Rebel XSi; Canon, Melville, NY) mounted on a dissecting microscope. Shell length was measured using the imaging software Motic Images Advanced 3.2 (Motic, Xiamen, China). To determine total body wet weight, geoducks were blotted on a paper towel and weighed as a group. Mean individual total body geoduck wet weight was calculated by dividing the total group weight by the number of individuals weighed for each replicate. Sampled geoducks were placed in 50-mL centrifuge tubes and stored at –80°C before lyophilization.

To determine total body dry weight, frozen geoducks were lyophilized for 48 h in a freeze dryer (FreeZone; Labconco, Kansas City, MO) and then reweighed. Mean individual total body dry weight was calculated by dividing the total group weight by the number of individuals weighed for each replicate.

To determine ash at the initial (3 individuals per replicate), final (3 individuals per replicate), and subsampled intervals (15 individuals per replicate), lyophilized geoducks were weighed, combusted in a muffle furnace for 4 h at 500°C, and then reweighed; AFDW was calculated for each replicate by subtracting the ash weight from the dry weight of the sample.

Temperature Experiment: Smaller Juveniles

Rearing Conditions

This experiment tested the same treatment temperatures (7°C, 11°C, 15°C, and 19°C) as the previous temperature experiment, but examined a smaller initial size class (obtained from a separate cohort age 29 days postfertilization) of juveniles for 21 days (see Table 1 for a summary of culture protocols). Each of the three treatment replicates was represented by 100 randomly selected geoducks (mean initial shell length ± SE, 0.54 ± 0.01 mm; $n = 50$) collected from a common rearing batch. Geoducks for each replicate were placed directly on the bottom of a 4-L bucket fitted with a lid. These containers were filled with 2 L sand-filtered, 1-μm cartridge-filtered, and UV-treated seawater of the appropriate treatment temperature. Culture water was agitated for 20 sec twice a day with a glass rod mixer in lieu of an air stone. Complete water exchanges occurred every second day. Other experimental conditions were maintained as in the previous temperature experiment with larger juveniles.

Algal Feeding

Treatments were fed every second day immediately after water exchange. All temperature treatments received a fixed ration of 100,000 equivalent *Tisochrysis lutea* cells/mL for the trial duration. Qualitative observation of residual algae in the water column at the end of the feeding periods indicated that this ration fulfilled satiation requirements of all treatment replicates at each feeding.

Sampling

At day 9, geoduck culture density was reduced to 55 individuals per replicate in treatments 11°C, 15°C, and 19°C to account for the size variation between these treatments and the 7°C treatment. Subsampling occurred at 7 days and 14 days, with 15 randomly selected geoducks sampled destructively from each replicate to calculate shell length and DSI. Empty shells were extracted and counted for each replicate to determine percent survival. At final sampling (21 days) all remaining geoducks (number being treatment dependent) were removed and counted in each replicate. Samples were preserved in 4% formalin for later shell length determination, according to the same protocol as the previous temperature experiment. Percent survival was multiplied across sampling periods to determine cumulative survival across the entire trial. Growth parameters and percent survival were averaged within each replicate and then treatment for each sampling period.

Food-Ration Experiment

Rearing Conditions

A summary of culture protocols is given in Table 1. The food-ration experiment was subdivided into four size classes of juveniles, separated by 810–980-μm shell length increments. Each size class was tested for 7 days. These animals were obtained from a new spawning cohort, different from the temperature trials, with size classes 1, 2, 3, and 4 age 53 days, 64 days, 72 days, and 83 days postfertilization, respectively. Size class 1 had a mean (±SE) initial shell length of 2.34 ± 0.04 mm; size 2, 3.32 ± 0.04 mm; size 3, 4.13 ± 0.04 mm; and size 4, 4.98 ± 0.04 mm ($n = 50$). The respective mean individual total body wet weights were 2.96 ± 0.15 mg/individual, 6.73 ± 0.27 mg/individual, 12.31 ± 0.16 mg/individual, and 21.23 ± 0.36 mg/individual ($n = 3$ groups of 200, week 1; 3 groups of 50, weeks 2–4). Initial stocking densities for size classes 1, 2, 3, and 4 were 3.77, 4.28, 3.92, and 6.75 mg total body wet weight/cm^2, respectively. Eight food rations (0.0, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, and 64.0×10^6 equivalent *Tisochrysis lutea* cells per individual per day) were tested in triplicate in size classes 1 and 2. To reflect geoduck growth, food rations were shifted upward to 0.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, and 128.0×10^6 equivalent *T. lutea* cells per individual per day for size classes 3 and 4.

Geoducks were taken from a common rearing batch at the weekly stocking intervals of the experiment. These nonexperimental animals were maintained in a cylindrical–conical tank for the duration of the trial and were fed to satiation to ensure mean population size correlated with the growth of experimental animals. For size class 1, each treatment replicate was represented by 100 geoduck juveniles collected randomly from the common rearing batch. Because of increased size, each treatment replicate was represented by 50 randomly selected geoduck juveniles for size class 2 and by 25 individuals for size classes 3 and 4.

Geoducks in each replicate were maintained at 15°C in the same water bath tanks, bucket setup, and water source as the larger juvenile temperature experiment (pH, however, did not require modification because of a seasonal increase; pH, >7.8). Treatment replicates were distributed randomly among these water bath tanks. Cleaning protocols also followed the same procedure as the previous temperature experiments. Complete water exchanges, followed by food delivery, occurred daily.

Sampling

Sampling occurred at the conclusion of each 1-week subtrial to determine mean shell length, DSI, individual total body wet weight, SGR, individual total body dry weight, total body AFDW, and percent survival using the same protocols as the temperature experiments, with the exception of the geoduck number used to determine shell length (20 individuals per replicate). The optimal ration level was determined for each size class growth parameter. For practical hatchery application, this optimal level was designated as the minimal food amount that elicited the greatest growth beyond which an additional ration increase did not result in significant growth improvement (see Liu et al. [2011]). To permit literature comparisons, weight-specific ration – grams (algae organic weight) per gram (initial geoduck wet weight) per week (as in Laing and Psimopoulous [1998] and Laing [2000]) was calculated for each optimal ration amount. Algal organic weight (AFDW of *Tisochrysis lutea*, 15.99 pg/cell) was determined as in Arney (2013).

Statistics

Treatment means were calculated for each replicate for each parameter. The effects of treatment (temperature or food ration) on intermediate (temperature) and final (temperature or food ration) geoduck shell length, DSI, individual total body

wet weight, SGR, individual total body dry weight, total body AFDW, and percent survival ($n = 3$ for each tested parameter) were examined with a one-way analysis of variance followed by Tukey's multiple comparisons test. An exception was the temperature experiment with smaller juveniles, which examined shell length, DSI, and percent survival only. Normality and equal variance of the data were tested using Kolmogorov–Smirnov and Levene's tests, respectively. In the temperature experiment with larger juveniles, SGR at 14 days was inverse-transformed, and dry weight at 28 days was natural–log-transformed to ensure normality. In the food-ration experiment, size 2 dry weight data required reciprocal transformation to ensure normality. Statistical analyses were performed with the statistical software SigmaPlot 12.3 (Systat Software Inc., San Jose, CA). A P value of 0.05 was used to indicate significance in all statistical analyses.

RESULTS

Culture Parameters

In the larger juvenile temperature trial, the mean ± SD pH and seawater salinity measured among all temperature treatments were 8.06 ± 0.08 ($n = 36$) and 28.2 ± 0.5 ($n = 88$), respectively. In the smaller juvenile temperature trial, the mean pH and seawater salinity measured among all temperature treatments were 8.02 ± 0.06 ($n = 36$) and 28.2 ± 0.5 ($n = 40$), respectively. In the ration trial, the mean temperature and pH maintained across all ration treatments for the entire trial period (weeks 1–4) were 15.2 ± 0.3°C ($n = 31{,}562$) and 8.30 ± 0.18 ($n = 264$), respectively. Mean ambient pH and salinity of the seawater source were 7.98 ± 0.04 ($n = 5$) and 28.0 ± 0.5 ($n = 27$), respectively.

Temperature Experiment: Larger Juveniles

Temperature affected all tested growth and survival variables significantly in the larger juveniles. At the final sampling day (day 28), there were significant differences among all four temperatures for all growth parameters except AFDW, with growth parameters increasing significantly for each increase in temperature (Fig. 1). For AFDW, there were no significant differences between 7°C and 19°C, and between 11°C and 15°C, with the former set of two temperatures resulting in juveniles with significantly less AFDW than the latter set (Fig. 1F). Final percent survival was high for all temperatures (96.1%, 97.0%, 98.5%, and 100.0% for 7°C, 11°C, 15°C, and 19°C, respectively) and did not vary significantly ($P = 0.159$) among treatments.

Temperature Experiment: Smaller Juveniles

Temperature also had a significant effect on all tested growth and survival variables in the smaller juveniles. There were significant differences among all temperature treatments for all sampling days for both shell length and DSI, with growth parameters increasing significantly for each increase in temperature (Fig. 2A–B). Percent survival at 28 days was 92.2%, 93.9%, 97.2%, and 96.4% at 7°C, 11°C, 15°C, and 19°C, respectively. Survival at 7°C was significantly less compared with the 15°C and 19°C treatments. Survival did not vary among 11°C, 15°C, and 19°C, nor between 7°C and 11°C (survival results not shown).

Food-Ration Experiment

Size Class 1

For size class 1, mean shell length, DSI, individual total body wet weight, and SGR increased significantly with ration up to 4.0×10^6 equivalent *Tisochrysis lutea* cells per individual per day (Fig. 3A–D). Beyond this ration level, increased ration did not improve growth significantly in these parameters. This optimal ration converted to a weight-specific ration of 0.15 g algal organic weight/g initial geoduck wet weight/week (Table 2). In all measured parameters, growth typically displayed a declining trend at rations greater than 8.0×10^6 equivalent *T. lutea* cells per individual per day, except for total body AFDW, which displayed an asymptotic trend (Fig. 3A–F). The optimal ration for individual total body dry weight occurred at 8.0×10^6 equivalent *T. lutea* cells per individual per day (Fig. 3E), with the converted weight-specific ration equaling 0.30 g/g/wk (Table 2); the optimal ration for AFDW was 16.0×10^6 equivalent *T. lutea* cells per individual per day (Fig. 3 F). Percent survival was high in all treatments and ranged from 93.7% to 100.0% without significant variation ($P = 0.423$) among treatments.

Size Class 2

For size class 2, the optimal ration shifted upward to 8.0×10^6 equivalent *Tisochrysis lutea* cells per individual per day for mean shell length, DSI, individual total body wet weight, and SGR (Fig. 4A–D). The optimal food level identified for individual total body dry weight remained at 8.0×10^6 equivalent *T. lutea* cells per individual per day (Fig. 4E). For this ration, the converted weight-specific ration equaled 0.11 g/g/wk (Table 2). The optimal ration for AFDW also shifted upward to 32.0×10^6 equivalent *T. lutea* cells per individual per day (Fig. 4F). Percent survival was 100.0% in all treatments.

Size Class 3

For size class 3, shell length, DSI, and individual total body dry weight increased significantly with elevated ration up to 32.0×10^6 equivalent *Tisochrysis lutea* cells per individual per day (Fig. 5A, B, E), which converted to a weight-specific ration of 0.29 g/g/wk (Table 2). Optimal individual total body wet weight and SGR both occurred at 16.0×10^6 equivalent *T. lutea* cells per individual per day (converted weight-specific ration, 0.15 g/g/wk; Fig. 5C, D and Table 2). Once again, the optimal ration for AFDW shifted upward to 64.0×10^6 equivalent *T. lutea* cells per individual per day (Fig. 5F). Percent survival was 100.0% in all treatments.

Size Class 4

The optimal ration for both shell length and DSI for size class 4 remained unchanged from size class 3 at 32.0×10^6 equivalent *Tisochrysis lutea* cells per individual per day (Figs. 5A, B and 6A, B). The optimal ration for individual total body wet weight was elevated to 32.0×10^6 equivalent *T. lutea* cells per individual per day from the previous size class (Fig. 6C). The converted weight-specific ration for shell length, DSI, and individual total body wet weight equaled 0.17 g/g/wk (Table 2). The optimal ration for SGR and individual total body dry weight was 64.0×10^6 equivalent *T. lutea* cells per individual per day (converted weight-specific ration, 0.34 g/g/wk; Fig. 6D, E and Table 2), whereas that for AFDW shifted upward again to

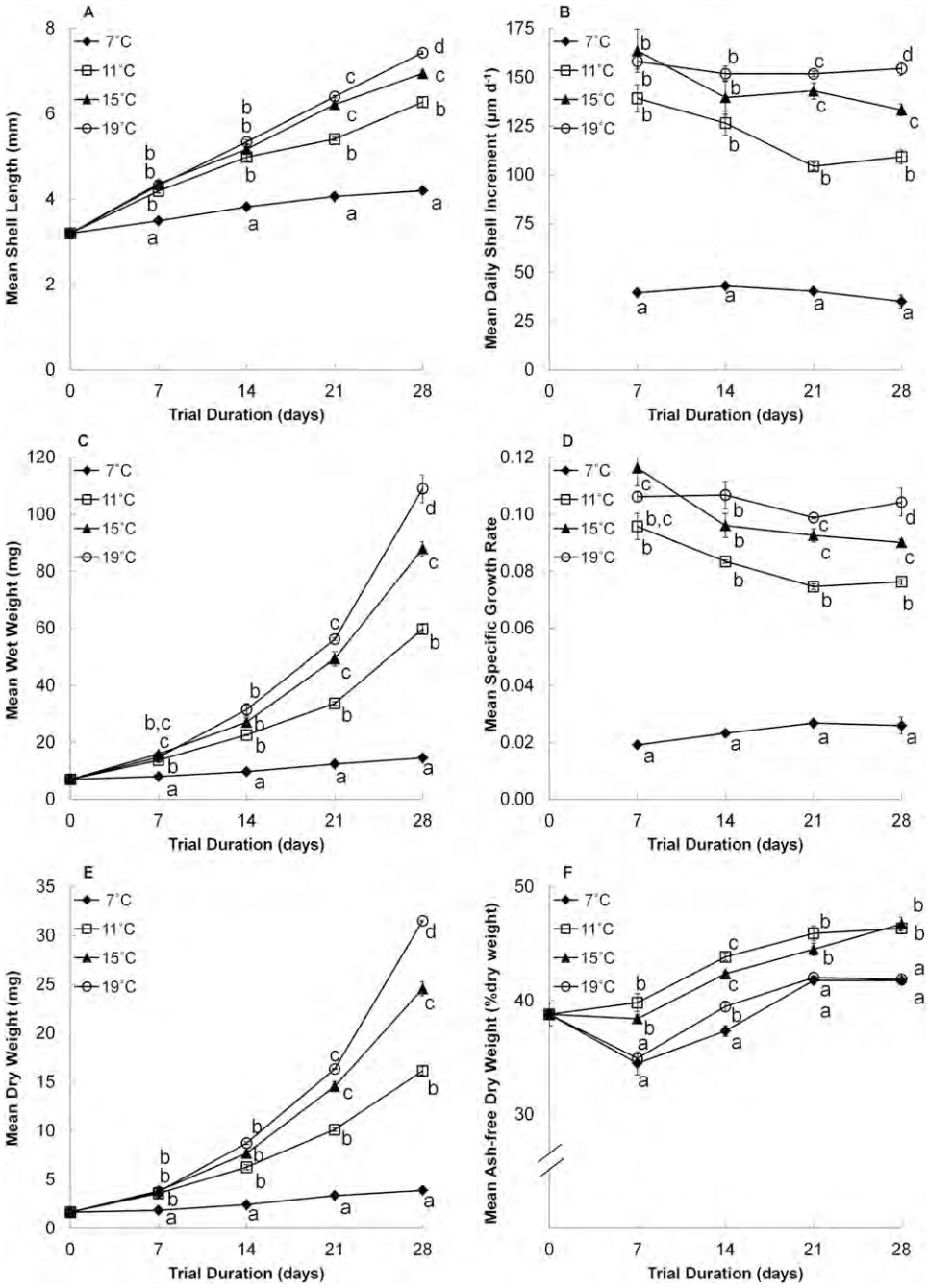

Figure 1. (A–F) Mean shell length (A), daily shell increment (B), individual total body wet weight (C), specific growth rate (D), individual total body dry weight (E), and total body ash-free dry weight (F) of larger juvenile geoducks (*Panopea generosa*) reared at different temperatures (7°C, 11°C, 15°C, and 19°C). Different letters indicate significant differences (Tukey's test, $P < 0.05$) among temperatures within sample days. Error bars represent SE ($n = 3$, except day 0 shell length when $n = 50$).

128.0×10^6 equivalent *T. lutea* cells per individual per day. Percent survival was 100.0% in all treatments.

DISCUSSION

Temperature

In both large and small juveniles, growth rate (except AFDW) was significantly greater at 19°C than in all other temperature treatments. Survival was suppressed significantly at the lowest culture temperature (7°C) in the smaller juveniles, but the highest temperature (19°C) did not inhibit survival in either geoduck size class. The successful growth and survival at 19°C contrasts with the results of previous research conducted with embryonic Pacific geoducks. Goodwin (1973) cultured geoduck embryos at 6°C, 10°C, 14°C, and 18°C, and found that 10°C and 14°C produced a greater percentage of normal straight-hinge larvae, with a second experiment in the same study establishing 16°C as the upper thermal limit for the embryos. Shifts in thermal tolerance during ontogenetic

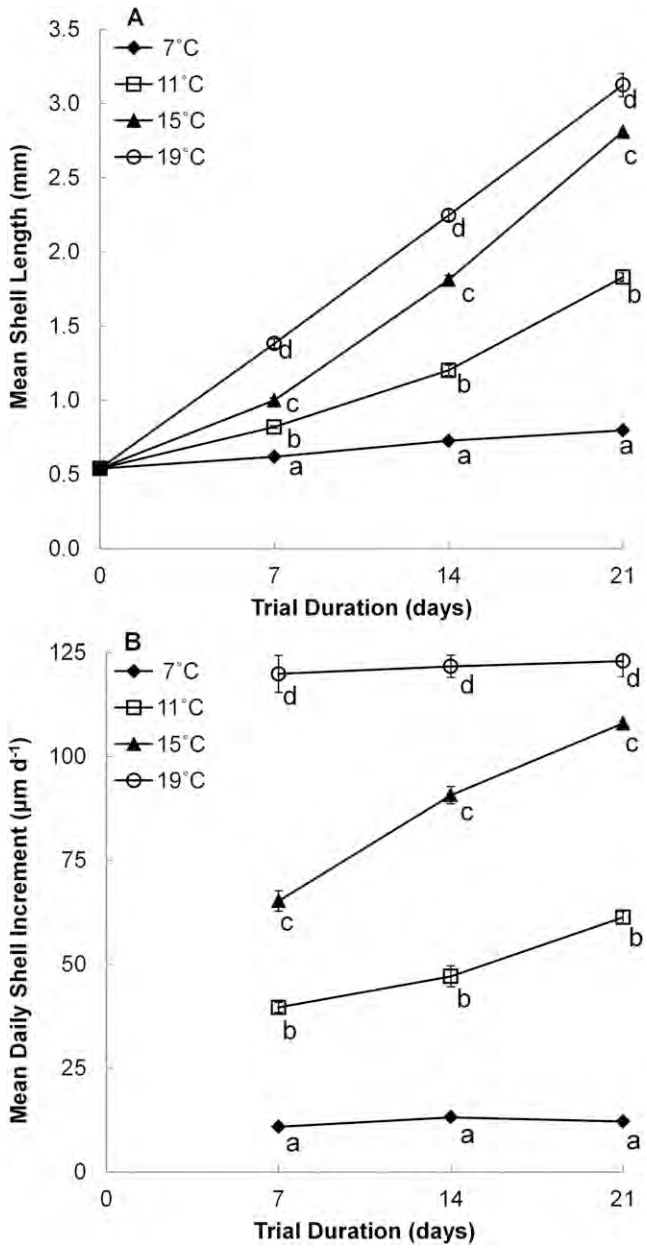

Figure 2. (A, B) Mean shell length (A) and daily shell increment (B) of smaller juvenile geoducks (*Panopea generosa*) reared at different temperatures (7°C, 11°C, 15°C, and 19°C). Different letters indicate significant differences (Tukey's test, $P < 0.05$) among temperatures within sample days. Error bars represent SE ($n = 3$, except day 0 shell length when $n = 50$).

development are typical in marine invertebrates, with the tolerance range of an environmental parameter generally widening as the animal grows (Kinne 1970). Anecdotal hatchery observations by Goodwin and Pease (1989) corroborated this generalized trend (and the results of the present study), indicating that high larval survival occurred in Pacific geoducks at 17°C, with juvenile growth continuing to increase up to 18°C. A similar ontogenetic shift was reported by Tettelbach and Rhodes (1981) for the northern bay scallop (*Argopecten irradians irradians* Lamarck, 1819). Normal embryonic development to the straight-hinge veliger stage occurred in this species within a narrow thermal range (20–25°C), with optimal development (100%) at 20°C, whereas the larvae displayed diminished thermal sensitivity with high survival rates at 20°C (100%), 25°C (91%), and 30°C (93%). In contrast, only 39% of embryos completed normal development at 30°C.

The temperature range (7–19°C) tested in the present study corresponds closely with thermal variation in the Pacific geoduck's natural environment. Examination of Fisheries and Oceans Canada sea surface temperature time series data since 2000 (locations: Chrome Island and Departure Bay, the closest sampling points to the region of broodstock collection) indicated that the mean minimum and maximum sea surface temperatures were 7.2 ± 0.8°C and 18.0 ± 0.8°C ($n = 12$), respectively (Fisheries and Oceans Canada 2013). The high growth performance exhibited by juvenile geoducks held at 19°C suggests that the thermal tolerance maximum of the source population may exceed the typical average maximum of its natural range. Anecdotal observations from Puget Sound (Washington state) further indicate the elevated thermal tolerance of wild Pacific geoducks; adults in intertidal and shallow subtidal habitats experience periodic temperature elevations up to 21–22°C in July and August without apparent mortality (Goodwin & Pease 1989). It is unknown whether similar thermal elevations are tolerated by Pacific geoducks in British Columbia. Future investigations are required to establish the upper thermal limit of British Columbia geoduck juveniles and adults.

Despite accelerating geoduck growth in shell deposition and soft tissues with increasing temperature, the 19°C treatment suppressed organic weight (AFDW) accumulation significantly in larger juveniles when compared with geoducks held at 11°C and 15°C. A similar thermal trend was described by Laing et al. (1987) in which the organic weight (AFDW) of juvenile Manila (*Ruditapes philippinarum* A. Adams & Reeve, 1850) and hard (*Mercenaria mercenaria* Linnaeus, 1758) clams increased with temperature between 10°C and 25°C, but the highest tested culture temperature (28°C) inhibited organic accumulation significantly. In the culture of European flat oyster (*Ostrea edulis* Linnaeus, 1758) spat, Walne and Spencer (1974) demonstrated that shell growth surpassed tissue growth at warmer temperatures. In addition, Laing (2000) reported that elevated temperatures (20°C and 23°C) accelerated shell growth in king scallops (*Pecten maximus* Linnaeus, 1758), but the condition index (dry-meat to dry-shell ratio) was reduced compared with that in animals reared at lower temperatures; culturing at 17°C maximizing this parameter. Laing (2000) indicated that ration requirements of king scallop spat increased with rearing temperature, with scallops grown at the highest culture temperatures (20.0°C and 23.0°C) displaying the greatest weight-specific ration requirement and food consumption compared with all other treatments (5.0°C, 6.5°C, 8.0°C, 10.0°C, 15.0°C, 17.0°C). Laing (2000) also indicated that 20% (20.0°C) and 65% (23.0°C) food increases were required to reduce the king scallop culture period by 2–4 days compared with 17–18°C. Despite the food increase, the scallop condition index declined at the warmer temperatures, possibly indicating that thermal elevation can inhibit bivalve organic weight gain despite adequate ration provision.

Ash-free dry weight could not be measured in the smaller geoducks because of size constraints; therefore, it is unknown whether similar organic tissue suppression occurred at the warmest culture temperature with this size class. The 19°C temperature, however, accelerated geoduck shell growth of the

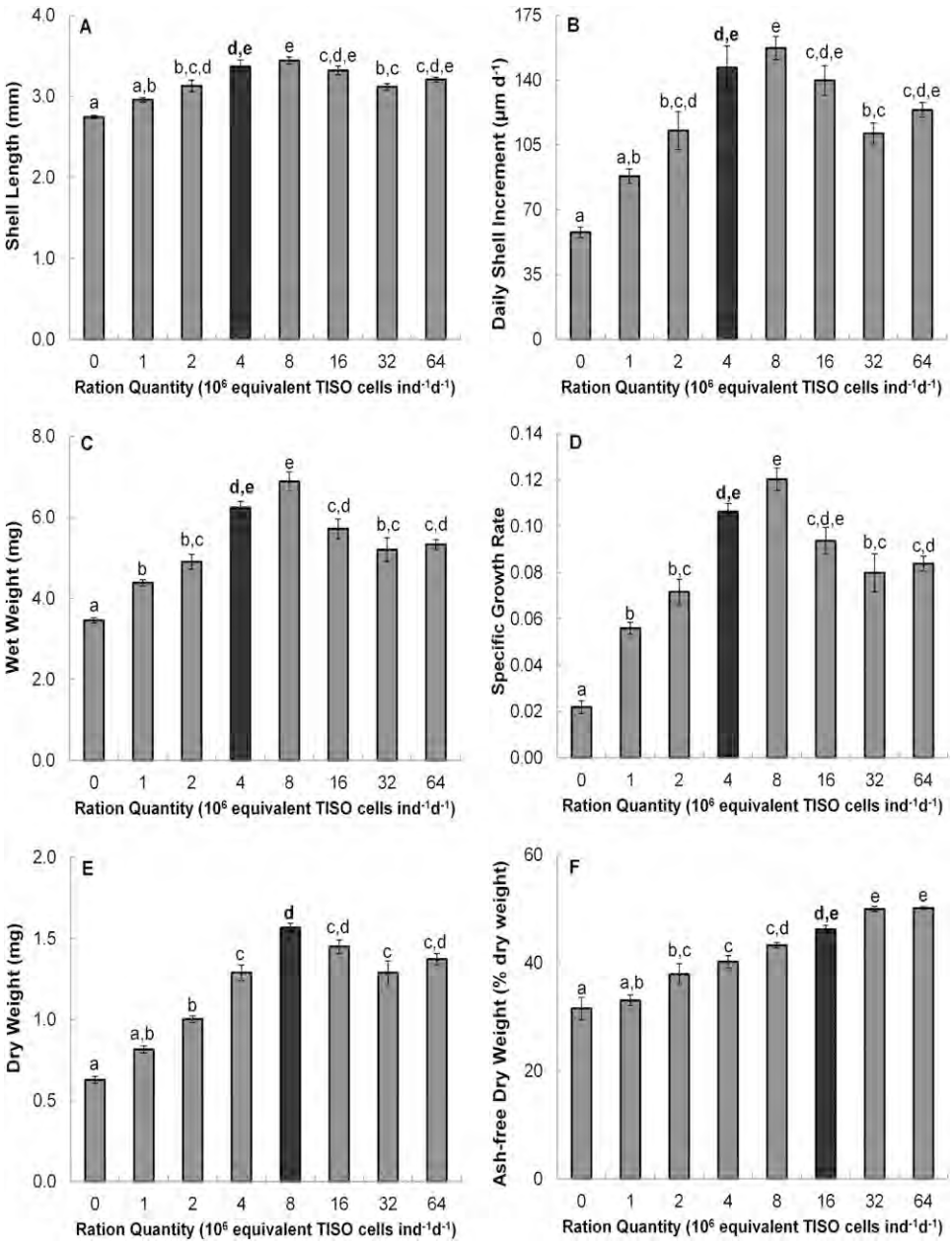

Figure 3. (A–F) Mean shell length (A), daily shell increment (B), individual total body wet weight (C), specific growth rate (D), individual total body dry weight (E), and total body ash-free dry weight (F) of juvenile geoducks (*Panopea generosa*) fed different rations for the week 1 size class. Week 1 initial mean (±SE) shell length, 2.34 ± 0.04 mm ($n = 50$); initial mean (±SE) wet weight, 2.96 ± 0.15 mg/individual ($n = 3$). Different letters indicate significant differences (Tukey's test, $P < 0.05$) among rations. Error bars represent SE ($n = 3$). Black bars indicate the optimal ration level determined for that growth parameter. TISO, *Tisochrysis lutea*.

smaller individuals significantly at all sampling periods. At final sampling (21 days), the mean shell length of geoducks held at 19°C exceeded that of those held at 15°C by 315.1 µm. The mean DSI (107.98 µm/day) exhibited by geoducks reared at 15°C at 21 days indicated that 2.9 days are required to recover the growth deficit between the 15°C and 19°C treatments. This may translate to an immediate economic benefit of rearing at 19°C if reduced expenses associated with a shortened production period can exceed seawater heating (if required) costs. The economics of production acceleration and seawater heating/cooling, however, are beyond the scope of this study.

Although culturing at 19°C may accelerate shell growth, it may come at the expense of organic weight gain, as demonstrated in the larger geoduck size class in the present study. Laing and Millican (1986) indicated that lipid accumulation in juvenile *Ostrea edulis* correlated with growth and survival performance in the natural environment, which suggests the longevity of culture influence after animals are outplanted. Condition index (improved by increased AFDW) is also used as a general quality indicator of physiological status and product value in aquaculture (Lucas & Beninger 1985, Marin et al. 2003). The applied culture temperature recommended for

TABLE 2.

Weight-specific optimal rations (grams of algal organic weight per gram initial geoduck wet weight per week) determined for tested growth parameters of different size classes of juvenile Pacific geoducks (*Panopea generosa*).

Size class	Shell length	Daily shell increment	Wet weight	Specific growth rate	Dry weight
1	0.15	0.15	0.15	0.15	0.30
2	0.11	0.11	0.11	0.11	0.11
3	0.29	0.29	0.15	0.15	0.29
4	0.17	0.17	0.17	0.17	0.34

Optimal ration is designated as the minimal food amount that elicited the greatest growth beyond which a further ration increase did not result in significant growth improvement. Mean (±SE) initial individual total body wet weights of size classes 1, 2, 3, and 4 were 2.96 ± 0.15 mg/individual, 6.73 ± 0.27 mg/individual, 12.31 ± 0.16 mg/individual, 21.23 ± 0.36 mg/individual, respectively. Mean *Tisochrysis lutea* ash-free dry weight, 15.99 pg/cell ($n = 6$).

hatchery production may therefore be dependent on commercial objectives. To accelerate the hatchery period, 19°C is recommended, but to maximize AFDW (and hence seed quality) before outplanting, a somewhat lower temperature (≥15°C, but <19°C) may be required. Additional research is required to examine outplant success of various sizes of seed with differing organic levels to determine the importance of AFDW in field production.

Food Ration

The optimal food ration identified for each growth parameter (i.e., shell length, DSI, individual total body wet weight, SGR, individual total body dry weight, and total body AFDW) increased in general with increasing size class. The upward shift in the optimum ration highlights the size dependence of geoduck food requirements and emphasizes the necessity of frequent ration modification in late-stage hatchery culture. The optimal ration in the present study was designated as the minimal food level beyond which an additional ration elevation did not accelerate growth significantly. Because ingestion rate was not measured, growth was used as the sole indicator of food and satiation requirements (e.g., Liu et al. 2011). The high geoduck growth at an intermediate ration level suggested the occurrence of satiation feeding, as demonstrated previously in juvenile *Ostrea edulis* (Beiras et al. 1993) and the grooved carpet shell clam (*Ruditapes decussatus* Linnaeus, 1758) (Albentosa et al. 1996).

Various growth parameters measured demonstrated reduction (sometimes significant) beyond the optimal identified ration level. The growth inhibition exhibited at these excessive rations may have resulted from elevated ingestion rates incurred as a result of increased food availability (Epifanio 1979, Beiras et al. 1993, Lu & Blake 1996, Rico-Villa et al. 2009). Ingestion rate increases as a function of available food ration until a saturation level is achieved (Beiras et al. 1993, Rico-Villa et al. 2009). At saturation and beyond, the ingested food may exceed stomach capacity; partial digestion may occur before the food is diverted to the midgut and egested as feces, leading to reduced absorption efficiency (e.g., Winter 1974, Widdows et al. 1979). The excess energy required for partial digestion and egestion of unused food as feces may contribute to reduced growth displayed at high food concentrations. Furthermore, feeding studies with the blue mussel (*Mytilus edulis* Linnaeus, 1758) indicate that high algal concentrations can severely overload the ctenidia and reduce filtration rates (Riisgård et al. 2011). Because of pseudofeces production and the presence of undigested algal cells in the feces of mussels receiving high food rations, Riisgård et al. (2011) suggested that this reduced filtration is the result of digestive and ctenidia overloading, rather than a physiological regulation. This implicates the negative effects of high ration on feeding activity and growth. Clams may also demonstrate inherent feeding constraints at high food levels. Tenore and Dunstan (1973) studied feeding and biodeposition rates in *M. edulis*, the eastern oyster (*Crassostrea virginica* Gmelin, 1791), and *Mercenaria mercenaria* under varying food concentrations and indicated that the last species exhibited the poorest feeding efficiency at the highest ration level (reduced feeding rate, with increased pseudofecal production). The authors (Tenore & Dunstan 1973) suggested that the hard clam is less adapted to high food concentrations compared with *M. edulis* and *C. virginica*, both of which may be found in areas of high productivity.

Ration influenced geoduck growth significantly within each size class. With the exclusion of size class 3, the ration required for optimal shell and individual total body wet weight growth matched within each size class, indicating similarity in the food requirements for these parameters. The food intake required to optimize individual total body dry weight was greater than that required to optimize shell length for size classes 1 and 4, exceeding that for optimized shell length twofold. Ash-free dry weight was optimized with additional food increases, exceeding the optimal shell length ration fourfold (except size class 3, which was twofold). The elevated ration for optimal AFDW growth emphasizes the high food requirement for organic tissue accumulation. It is speculated that a greater ration is required to support continued siphon and mantle growth through the juvenile culture stage. Geoducks are characterized by their large, fleshy siphon and mantle, which cannot be retracted fully into the shell at the adult stage (Goodwin & Pease 1989). In the present study, external siphon protrusion became evident in sampled geoducks in size classes 3 and 4. It is possible that the high energy requirement of this tissue growth (e.g., Bayne & Hawkins 1997) resulted in increased ration optima for AFDW beyond the levels identified for shell deposition.

Weight-specific ration requirements remained relatively stable among size classes, especially classes 1 and 4. For optimal shell length, DSI, individual total body wet weight, and SGR, the weight-specific ration was 0.15 g/g/wk for size class 1 and

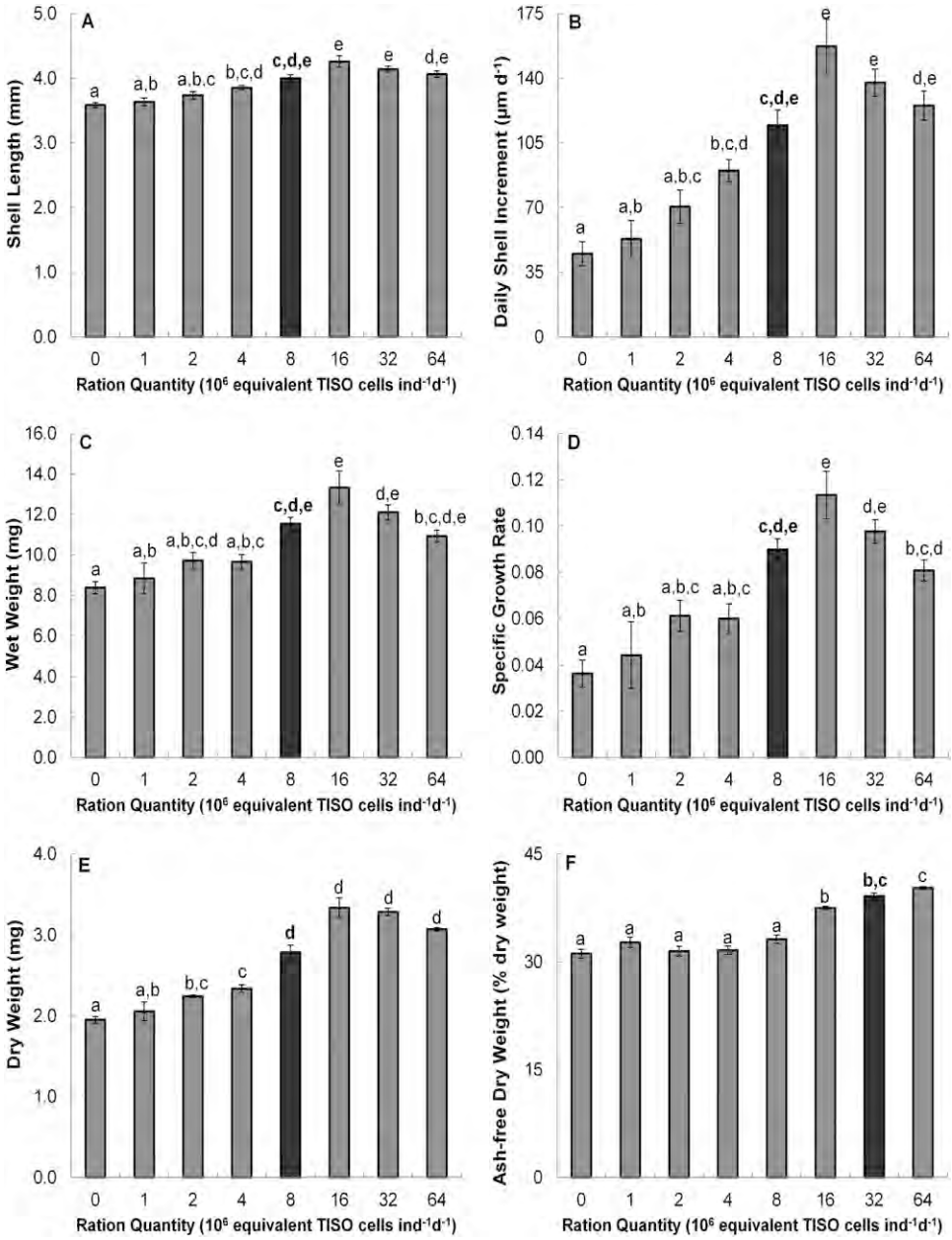

Figure 4. (A–F) Mean shell length (A), daily shell increment (B), individual total body wet weight (C), specific growth rate (D), individual total body dry weight (E), and total body ash-free dry weight (F) of juvenile geoducks (*Panopea generosa*) fed different rations for the week 2 size class. Week 2 initial mean (±SE) shell length, 3.32 ± 0.04 mm ($n = 50$); initial mean (±SE) wet weight, 6.73 ± 0.27 mg/individual ($n = 3$). Different letters indicate significant differences (Tukey's test, $P < 0.05$) among rations. Error bars represent SE ($n = 3$). Black bars indicate the optimal ration level determined for that growth parameter. TISO, *Tisochrysis lutea*.

0.17 g/g/wk for size class 4, whereas optimal dry weight occurred at 0.30 g/g/wk and 0.34 g/g/wk, respectively. In bivalve culture, weight-specific food requirements typically decline with increased animal size (Urban et al. 1983, Coutteau et al. 1994b, Liu et al. 2011). In the 3-wk culture of large juvenile *Crassostrea virginica*, the weight-specific food ration decreased with each successive week (Urban et al. 1983), whereas the feeding activity of small (final shell length, 1–3 mm) juvenile *Mercenaria mercenaria* fed "on demand" demonstrated that larger animals in the second week of culture achieved satiation at a reduced ration compared with the initial week (Coutteau et al. 1994b). Liu et al. (2011) cultured five size classes (initial mean shell length, 0.74–3.00 mm) of juvenile basket cockle (*Clinocardium nuttallii* Conrad, 1837) and demonstrated that the optimal weight-specific ration generally declined with each successive week, before stabilizing in the larger size classes (weeks 1–5: 7.1, 1.7, 1.1, 1.0, 1.0 g/g/wk, respectively). The selection of larger geoducks in the present study (initial mean shell length, 2.34–4.98 mm) possibly restricted the weight-specific ration variation displayed in previous studies, resulting in the maintenance of food requirements among size classes. Individuals tested in Liu et al. (2011) and the present

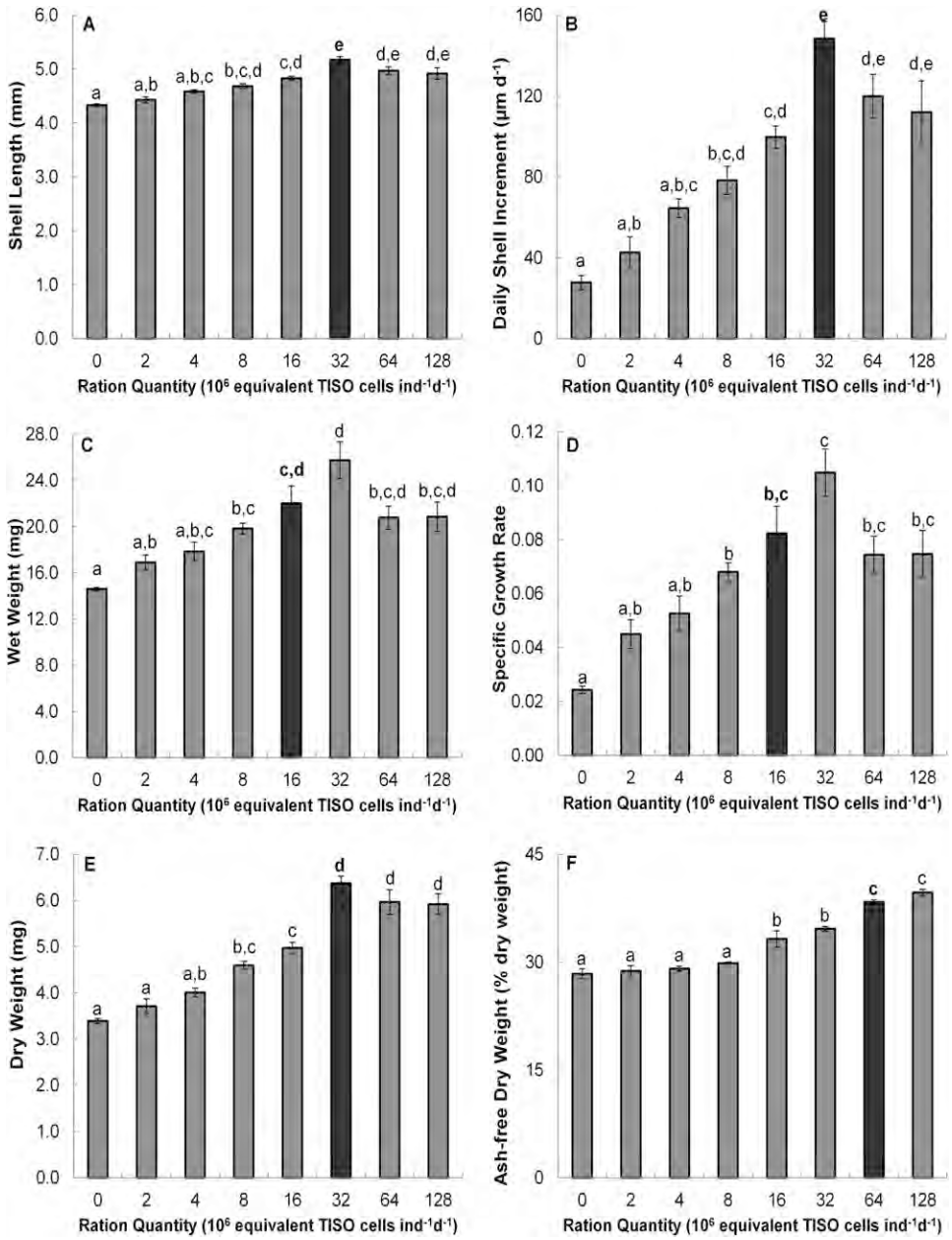

Figure 5. (A–F) Mean shell length (A), daily shell increment (B), individual total body wet weight (C), specific growth rate (D), individual total body dry weight (E), and total body ash-free dry weight (F) of juvenile geoducks (*Panopea generosa*) fed different rations for the week 3 size class. Week 3 initial mean (±SE) shell length, 4.13 ± 0.04 mm ($n = 50$); initial mean (±SE) wet weight, 12.31 ± 0.16 mg/individual ($n = 3$). Different letters indicate significant differences (Tukey's test, $P < 0.05$) among rations. Error bars represent SE ($n = 3$). Black bars indicate the optimal ration level determined for that growth parameter. TISO, *Tisochrysis lutea*.

study, however, represented a smaller size class than the oysters (wet weight, 20–50 mg/individual) examined by Urban et al. (1983). The variation among studies likely emphasizes species specificity in feeding requirements and further emphasizes the need to customize feeding regimens to both bivalve species and size.

For Pacific geoduck juveniles, it is recommended that weekly rations follow the optima established in the present study for shell length, individual total body wet weight, and individual total body dry weight. In contrast, the optimal rations identified for growth in organic weight (AFDW) extended beyond the levels required for satiation feeding. Accretion of organic matter can continue with increased food delivery despite possible reduction in absolute growth (i.e., shell length, wet and dry weight), yielding inefficiencies in both food and animal production. Adhering to this recommendation, the following ration levels should be applied for juvenile culture: size class 1 (mean initial shell length, 2.34 mm), 4.0×10^6 or 8.0×10^6 equivalent *Tisochrysis lutea* cells per individual per day; size class 2 (3.32 mm), 8.0×10^6 cells per individual per day; size class 3 (4.13 mm), 16×10^6 or 32.0×10^6 cells per individual per day; and size class 4 (4.98 mm), 32.0×10^6 or 64.0×10^6 cells per

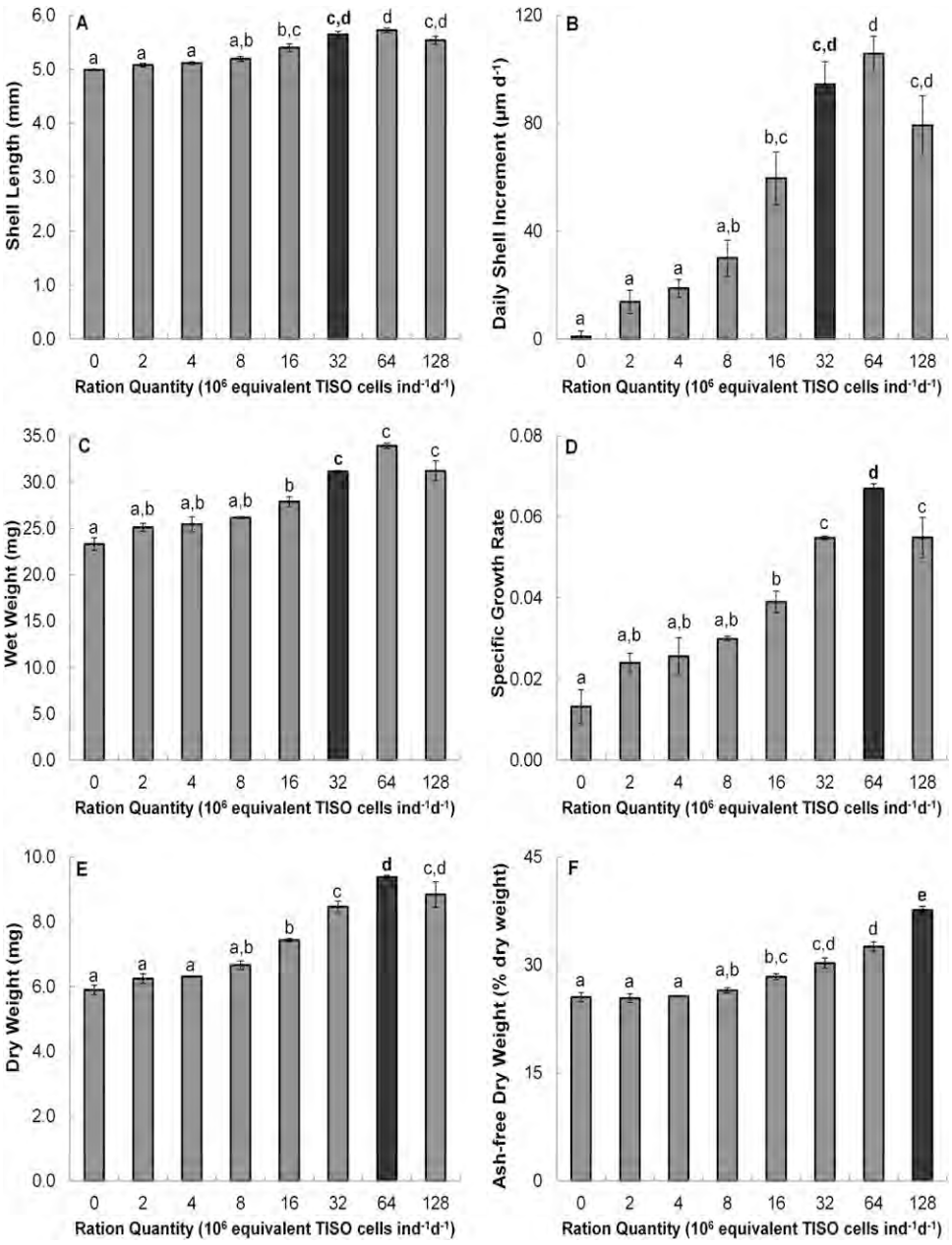

Figure 6. (A–F) Mean shell length (A), daily shell increment (B), individual total body wet weight (C), specific growth rate (D), individual total body dry weight (E), and total body ash-free dry weight (F) of juvenile geoducks (*Panopea generosa*) fed different rations for the week 4 size class. Week 4 initial mean (±SE) shell length, 4.98 ± 0.04 mm ($n = 50$); initial mean (±SE) wet weight, 21.23 ± 0.36 mg/individual ($n = 3$). Different letters indicate significant differences (Tukey's test, $P < 0.05$) among rations. Error bars represent SE ($n = 3$). Black bars indicate the optimal ration level determined for that growth parameter. TISO, *Tisochrysis lutea*.

individual per day, depending on which growth parameter is to be maximized.

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