THERMAL AND DIETARY OPTIMIZATION IN THE HATCHERY CULTURE OF
JUVENILE PACIFIC GEODUCK CLAMS (PANOPEA GENEROSA, GOULD 1850)

by

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Abstract

This research is the first to examine thermal and dietary optimization in the hatchery culture of juvenile Pacific geoduck (*Panopea generosa*). Chapter 2 investigated temperature and feed ration optimization; chapter 3 examined live algae substitution with the spray-dried species, *Schizochytrium* spp. or *Spirulina* (*Arthrospira platensis*). Geoduck growth and survival were measured to quantify treatment success. The temperature trial tested four temperatures (7, 11, 15, 19 °C) in juvenile and post-larval culture. Temperature promoted a significant growth effect in both sizes. The 19 °C culture elicited a delayed growth benefit in juveniles, and suppressed ash-free dry weight (AFDW), which recommends utilization of 15 °C. In contrast, geoduck post-larvae displayed immediate (post 7 d) shell growth acceleration at 19 °C. The 19 °C temperature shortened the rearing period by 2.9 d, suggesting its application in post-larval culture.

The ration experiment examined the feed ration requirements of four geoduck juvenile size classes. Ration quantities between 0.0 - 128.0x10^6 equivalent *Isochrysis* cells individual^{-1} day^{-1} were tested. All treatments received *Chaetoceros muelleri* and *Isochrysis* sp. mixed by AFDW. Following shell length/wet weight optimization, the following rations (10^6 equivalent *Isochrysis* cells individual^{-1} day^{-1}) should be applied between week 1 and 4 of the tested geoduck culture: 4.0 (1); 8.0 (2); 16.0 or 32.0 (shell length or wet weight optimum, respectively; 3); and 32.0 (4).

The algae substitution trial replaced the above bi-algae diet with variant levels (0, 25, 50, 75, 100%) of *Schizochytrium* or *Spirulina* in two size classes. Between spray-dried diets, *Spirulina* treatments enhanced geoduck growth. However, overall growth displayed a general decline with elevated spray-dried inclusion and maximal growth occurred between 0 – 25% substitution. The superior bi-algae diet contained a polyunsaturated fatty acid Σn – 3/n – 6 ratio.
of 2.9, a value conserved in the bivalve literature. This may indicate its importance in future bivalve studies. Tissue analysis suggested potential limited digestion of spray-dried derived protein or carbohydrate, but indicated general lipid/fatty acid sequestration. These findings do not recommend dietary incorporation of *Schizochytrium* or *Spirulina*, but culture refinement may enhance juvenile geoduck hatchery production.
Preface

This thesis was completed under the Aquaculture Collaborative Research and Development Program, project number: P-10-01-007, under the supervision of Dr. R. Scott McKinley. Research design was formulated with the assistance of Dr. McKinley, Dr. Christopher M. Pearce, and Dr. Wenshan Liu, the project’s postdoctoral fellow and primary investigator.

Live animal experimentation occurred at the Pacific Biological Station (PBS) under the supervision of Dr. Pearce, with the approval of the DFO Pacific Region Animal Care Committee (PRACC) and the guidelines of the Canadian Council on Animal Care (CCAC). The DFO PRACC Animal Use Protocol approval number was: 10-019. Dr. Liu maintained geoduck broodstock and larvae at PBS, and produced the geoduck post-larvae and juveniles used in experimentation. I performed the experimental husbandry and data collection, with assistance from Dr. Liu.

Protein, lipid, carbohydrate, and fatty acid analyses were conducted at the Center for Aquaculture and Environmental Research, under the supervision of Dr. McKinley, and followed the recommendations of Dr. Ian Forster and Dr. Liu. I performed the analyses and data collection with assistance from Dr. Liu.

Statistical analyses, data interpretation, and preparation of this thesis manuscript was completed by myself, with critical input provided by my graduate committee (Dr. McKinley, Dr. Pearce, Dr. Forster, Dr. Marina von Keyserlingk) and Dr. Liu.

Chapters 2 and 3 of this thesis will be submitted for potential publication [Chapter 2: Arney, B., Liu, W., Forster, I., McKinley, R.S., Pearce, C.M. 2013. Temperature and feed ration optimization in the hatchery culture of juvenile Pacific geoduck clams (Panopea generosa Gould, 1850). Chapter 3: Arney, B., Liu, W., Forster, I., McKinley, R.S., Pearce, C.M. 2013.
Feasibility of live algae dietary substitution with spray-dried *Schizochytrium* sp. or *Spirulina (Arthrospira platensis)* in the hatchery culture of juvenile Pacific geoduck clams (*Panopea generosa* Gould, 1850)).

Funding was provided by the Aquaculture Collaborative Research and Development Program.
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(P<0.05) were used to determine the effect of algae type (A), substitution level (S), and their interaction (AxS) on final tissue composition. Detection of a significant interaction effect between dietary algae species and substitution level (n = 3) is denoted by *. Different letters within rows indicate significant differences between substitution levels (two-way ANOVA, Tukey’s test, P<0.05, n = 3). Substitution levels across algae species were pooled (n = 6) if the effect of algial type and/or interaction was not significant (P>0.05). These results are summarized in the statistical analysis column. Letters (A, S, AxS) in bold indicate that parameter induced a significant treatment effect.

Table 3.4. Trial 1 initial (day 0) and final (day 14) proximate and fatty acid composition of juvenile geoduck tissue (mean ± SE). The 0% substitution level is represented by a full live algae diet of Chaetoceros muelleri and Isochrysis sp. (Tahitian strain) mixed at equal AFDW; Spirulina (Sp.) replaced this live bi-algal diet at variant inclusion levels (25 – 100%). Total fatty acid (% dry weight) is represented by total identified content. See Table 3.3 for explanation of statistical analyses and superscript notation.

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Figure 2.6. Mean (A) shell length (mm), (B) daily shell increment (μm d\(^{-1}\)), (C) wet weight (mg ind\(^{-1}\)), (D) specific growth rate, (E) dry weight (mg ind\(^{-1}\)), and (F) ash-free dry weight (% initial)
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**Figure 3.1.** Mean (A) individual shell length (mm), (B) daily shell increment (μm d\(^{-1}\)), (C) wet weight (mg ind\(^{-1}\)), (D) specific growth rate, (E) dry weight (mg ind\(^{-1}\)), and (F) ash-free dry weight (% initial) of juvenile geoduck at final sampling (14 d; trial 1). Geoduck were reared on a live algae diet (CM+TISO delivered in equal AFDW proportion) substituted with variant levels of the dried algae, *Schizochytrium* sp. or *Spirulina* (*Arthrospira platensis*). No significant interaction effects were detected between dietary algae species and substitution level (two-way ANOVA, P>0.05, n = 3). Algal type was a significant source of variation for: shell length, DSI, SGR, and dry weight (two-way ANOVA, P<0.05, n = 12). Different letters indicate significant differences between substitution levels (two-way ANOVA, Tukey’s test, P<0.05, n = 3). Substitution levels across algae species were pooled (n = 6) if the effect of algal type was not significant (P>0.05; n = 12). Error bars denote standard error.

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**Figure 3.3.** Trial 1 mean dietary composition of (A) protein (mg g\(^{-1}\) AFDW), (B) carbohydrate (mg g\(^{-1}\) AFDW), (C) total fatty acid (mg g\(^{-1}\) AFDW), (D) EPA (%TFA), (E) DHA (%TFA), (F) AA (%TFA), (G) n – 6 DPA (%TFA), (H) ∑SAT (%TFA), (I) ∑MUFA (%TFA), (J) ∑n – 3 PUFA (%TFA), (K) ∑n – 6 PUFA (%TFA), and (L) ∑n – 3/∑n – 6 PUFA (%TFA) (X; calculated from mean values in Table 3.2) correlated against the same parameters measured in geoduck tissue (%DW for proximate components, %TFA for fatty acids) after 14 d of experimental dietary exposure (Y; trial 1). Error bars represent standard error.

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List of Abbreviations and Symbols

Abbreviations in text

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<tr>
<td>°C</td>
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SE     standard error
SGR    standard growth rate
SR     survival rate
t     ton, tonne (1,000 kg)
TAC    total allowable catch
TISO   *Isochrysis* sp. (Tahitian strain)
UV     ultraviolet
W      width
WW     wet weight

**Symbols in equations**

\[ W_1 \] initial geoduck wet weight
\[ W_2 \] final geoduck wet weight
\[ T \] trial duration in days
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Fresh saline river,

My girl is broken anew.

I will heal her now.
To my beloved Nonna. Con tanto amore per sempre. ♥
1 Introduction

The Pacific geoduck clam (Panopea generosa, Gould 1850) supports the most valuable commercial clam fishery in the Pacific Northwest. Landed values for commercially captured geoduck increased between 2008 and 2010 for both Washington and British Columbia (BC) fisheries, with the majority of product demand driven by the Asian market. Due to its lucrative market value, the geoduck presents an attractive species for aquaculture. The unknown resilience of wild populations to fishery pressure further encourages the adaptation of aquaculture techniques to supplement the commercial fishery supply and enhance wild stocks. Current aquaculture knowledge on the Pacific geoduck is limited in the published literature and has emphasized the larval culture stage, with little or no information available for post-settled juveniles. This absence of information inhibits the success of geoduck aquaculture and provided the impetus for my thesis research. I examined thermal and dietary optimization in the hatchery culture of juvenile geoduck to refine and economize the hatchery protocols required for successful geoduck cultivation.

1.1 The Pacific geoduck

The Pacific geoduck (incorrectly described in recent literature as Panopea abrupta, Conrad 1849 (Vadopalas, 2010)) is a large, long-lived, hiattellid clam (Anderson, 1971) that is distributed throughout the Pacific Northwest from Baja California to Alaska (23–58°N) (Bernard, 1983). Geoduck can exceed 100 years in age, with the oldest geoduck recorded at 168 years (Bureau et al. 2002). Goodwin and Pease (1991) determined the mean adult shell size and wet weight of geoduck extracted from the Puget Sound (Washington, USA) measured 135.2 mm
and 872.2 g, respectively, with the largest individual recorded at 212 mm (shell length) and 3.25 kg (wet weight).

The geoduck is a dioecious broadcast spawner; eggs and sperm are released into the water where external fertilization occurs (Goodwin and Pease, 1989). Following fertilization, the geoduck life history is comprised of seven main development stages: the fertilized egg (diameter: 80 µm), the trochophore (80–100 µm), two larval stages (shell length: 110–400 µm), one post-larval stage (400–1,500 µm), one juvenile stage (1.5–7.5 mm), and the final adult stage (75–200+ mm) (Goodwin and Pease, 1989). As an adult, the geoduck is found buried up to 1 m in sand, mud, gravel, and silt substrate from the lower intertidal to depths of 110 m (Jamison et al., 1984). When the geoduck is a small juvenile, it uses a well-developed ciliated foot to burrow in the substratum. As the juvenile develops, its foot becomes disproportionally smaller to total body size and larger geoduck lose the ability to dig (Goodwin and Pease, 1989). Burrowing as a juvenile provides the adult geoduck safety from most benthic predators and natural mortality is low at the adult stage (Goodwin and Pease, 1989). As a filter feeder, a long fused siphon is extended to the seabed surface to intake food particles, which can be retracted below the surface if feeding conditions are not favorable (Goodwin and Pease, 1989).

1.2 Commercial fisheries

The geoduck is a species of commercial interest in the Pacific Northwest, supporting the most lucrative commercial clam fishery in the region (British Columbia Seafood Industry in Review, 2011; Washington Department of Fish and Wildlife, unpublished data). Concentrated in the Puget Sound and coastal BC (Westely et al., 1990), wild geoduck populations comprise a significant portion of the region’s benthic infaunal biomass (Feldman et al., 2004; Goodwin,
1978; Goodwin and Pease, 1987). The majority of the global geoduck supply is sourced from this population center, through the commercial harvest activity of the Washington and BC fisheries (Orensanz et al., 2004). Separately managed, the Washington geoduck fishery harvested 2,369 t of geoduck in 2011, generating a landed value of $48.9 million (USD) (Washington Department of Fish and Wildlife, unpublished data), while the BC fishery captured 1,600 t of geoduck in 2011 for a landed value of $41.3 million (CAD) (BC Seafood Industry in Review, 2011). In both fisheries, harvest is required by hand. Commercial divers dislodge individual geoduck from the substrate with a high-volume water hose and nozzle, termed a stinger, which liquefies the surrounding sediment prior to extraction (Westely et al., 1990). Harvested geoduck are primarily exported live to Asia; 99% of commercially captured BC geoduck were directed to the Asian market in 2010 (BC Seafood Industry in Review, 2011). China receives the dominant portion of this export (90% of the 2008 BC export), where the geoduck siphon is considered a delicacy and is valued for its sweet flavour and crunchy texture (James, 2008). The landed value of harvested geoduck increased between 2008 and 2011 for both Washington ($26.4 to 48.9 million) and BC fisheries ($25.8 to $41.3 million), perhaps indicating an upward trend in product demand and value (BC Seafood Industry in Review, 2011; Washington Department of Fish and Wildlife, unpublished data).

To sustain population levels, both Washington and BC annual harvest regimes exploit a small percentage of the estimated available biomass, quantified as the annual total allowable catch (TAC) (Bradbury et al., 2000; Zhang and Hand, 2007). For designated harvest beds in Washington, Bradbury et al. (2000) recommended a 2.7% annual TAC, while in BC the annual TAC is set between 1.2% (Vancouver Island west coast) and 1.8% (the remainder of the coast) (Zhang and Hand, 2007). However, the geoduck’s longevity prohibits the immediate assessment
of long-term harvest effects. This lag may result in erroneous projections of prolonged population resilience and stability under current fishery management regimens (Orensanz et al., 2004). Additionally, Valero et al. (2004) warned that accurate estimation of geoduck abundance must consider long-term data in the context of large-scale environmental variability.

Environmental variability can influence geoduck recruitment success, inhibiting or promoting a population’s ability to buffer against harvest events (Valero et al., 2004). Back calculations of age-frequency distributions collected from large sampling events in Washington and BC (Bureau et al., 2002; Breen and Shields, 1983; Goodwin and Shaul, 1984) revealed a correlation between large-scale coastal abiotic shifts and recruitment ability (Valero et al., 2004). These authors suggested increased sea surface temperature enhanced recruitment in both Washington and BC populations, while elevated freshwater discharge depressed recruitment in the BC population. Geoduck reproduction and larval development may be repressed by prolonged periods of cold water; most hatcheries incite spawning via temperature elevation (Feldman et al., 2004; Goodwin and Pease, 1989). Intense freshwater runoff may not inhibit spawning, but instead may displace larvae from suitable coastal habitats (Valero et al., 2004). During periods of suppressed recruitment (i.e. low water temperature, high freshwater input), it is possible the selected TAC levels may not permit adequate population growth for sustained commercial harvest. Delayed recognition of inhibited recruitment may not be visualized until future sampling efforts, permitting unsustainable stock exploitation under the harvest regimen.

Population studies further indicate that commercial harvesting may target older cohorts that have accumulated in the geoduck population over time (Bureau et al., 2002, 2003); this age-biased removal may further hamper long-term recruitment success. Geoduck appear to maintain
fecundity with age; all old BC geoduck (50+ years) collected by Sloan and Robinson (1984) displayed gonad ripeness and reproductive capability. Population samples indicate that the time required for reproductive maturity is variable and may require 2–7 years (Campbell and Ming, 2003; Sloan and Robinson, 1984). Largely depleted of fecund adults, a geoduck population dominated by young juveniles may be incapable of adequate re-seeding and the cohort imbalance may create localized stock collapses.

Illicit quality grading may also occur during the underwater harvest (Campbell et al., 1998; Orensanz et al., 2004), further exacerbating exploitation vulnerability. White siphon colour identifies high quality individuals, while dark brown meat is considered the poorest grade (Campbell et al., 1998; Goodwin and Pease, 1991). Determination of geoduck quality requires complete extraction of the animal from the substrate; if rejected, a discarded geoduck will die on the seafloor, incapable of reburial (Campbell et al., 1998). The number of geoduck lost in this cryptic manner is difficult to detect and quantify. This illegal harvest activity may therefore inflate harvest quotas through overfishing and depress mortality estimates (Campbell et al., 1998), inhibiting the accuracy of biomass estimations.

1.3 Aquaculture

Due to the geoduck’s high market value and unknown resilience to prolonged fishery pressure, aquaculture production may present an attractive alternative to wild stock collection. Geoduck aquaculture is not a novel endeavour. In the early 1990s, hatchery and grow-out techniques developed at the Point Whitney research laboratory initiated aquaculture production in Washington state (Feldman et al. 2004; Hand and Marcus, 2004) with BC aquaculture following in 1996 (Hand and Marcus, 2004). At that time, Fisheries and Oceans Canada
instituted a pilot-scale BC aquaculture project in collaboration with the existing commercial geoduck fishery, Underwater Harvesters Association (UHA), and the aquaculture constituent, Fan Seafoods Ltd. (Hand and Marcus, 2004).

To initiate geoduck production, both Washington and BC hatcheries utilize thermal manipulation (i.e. an elevation in temperature) to trigger broodstock spawning (Feldman et al., 2004; Goodwin and Pease, 1989). Cultured geoduck are fed with live microalgae and reared in the hatchery until the juveniles reach a shell length of 3–6 mm (Pinfold, 2001). Due to indications that increased shell size may support enhanced survival in the natural environment (Beattie, 1992; Pinfold, 2001), hatchery reared juveniles may be transferred into nursery culture for further growth prior to field out-planting (Pinfold, 2001). A nursery may consist of a land-based (i.e. pond or raceway) or protected marine culture system. In Washington, geoduck are transferred into the marine substrate between a shell length of 5 and 10 mm (Price, 2011), while in BC the transfer is delayed until geoduck achieve a shell length of 12–20 mm (Pinfold, 2001).

The size discrepancy prior to field introduction may be attributed to the distinct out-planting techniques employed by Washington and BC industries. Commercial out-planting in Washington is concentrated in Puget Sound and occurs on private intertidal shoreline (Brown and Thuesen, 2011). In contrast, BC aquaculture is smaller-scale and primarily sub-tidal. Out-planting is nearly exclusive to the Strait of Georgia and occurs in government-allotted tenured substrate plots (Hand and Marcus, 2004). Washington intertidal out-planting requires the use of predator exclusion PVC tubing; 1–4 geoduck are placed into a PVC tube that is vertically inserted into the substrate (Price, 2011). For further predator protection, individual netting may be secured over each tube or a collection of tubes may be protected by a large net, anchored in
the substrate (Price, 2011). The netting and PVC tubes are removed within 1–3 years post out-planting and harvest typically occurs within 5–7 years (Price, 2011). In 2010, 613 t of cultured geoduck were harvested in Washington for a landed value of $18.5 million (USD) (Washington Department of Fish and Wildlife, unpublished data).

In BC, sub-tidal out-planting may be mechanized; depending on juvenile size and environmental conditions, technologies developed by UHA and Fan Seafoods Ltd. may deposit 20,000–50,000 geoduck d^{-1} onto the substrate surface (Pinfold, 2001). To maximize survival, the generalized habitat conditions recommended for the out-planting environment suggest a temperature range of 8–18 °C, salinity between 26–31‰, and a water depth of 3–20 m (Pinfold, 2001). Unlike Washington, where geoduck are inserted into the substrate by hand, sub-tidal out-planting success in BC is dependent on predator protection and geoduck burial ability. Spread on the substrate, predator exclusion netting minimizes losses prior to burial, when the exposed geoduck are most vulnerable to morality and predation (Hand and Marcus, 2004; Pinfold, 2001). The netting is estimated to contribute to a 25% survival rate and is used during the first 1–2 years of geoduck growth (Pinfold, 2001). After which, the geoduck’s vertical position in the substrate is considered a sufficient depth for predator protection and the netting is removed; harvest is then estimated to occur between 6 and 9 years post initial out-planting (Heath, 2005).

In addition to sub-tidal culture, a wild stock enhancement programme also functions in the Strait of Georgia, further differentiating BC geoduck management from Washington’s. Funded and operated by the UHA, the organization has out-planted hatchery-reared juveniles into the common marine domain since 1994 (Anonymous, 2013a). From 1997, annual enhancement in the Strait of Georgia varied between 250,000 and 700,000 out-planted juveniles.
(Anonymous, 2013a) with a projected survival rate to harvest between 20 and 80% (James, 2008). Originally developed for the enhancement programme, the sub-tidal dispersion and protection of out-planted juveniles follows the same protocol (*i.e.* mechanized distribution and predator exclusion netting) used in BC geoduck aquaculture. Included in the annual allotted TAC, enhanced stocks contribute to the wild commercial fishery; in 2012 it is estimated the harvest of enhanced product will total 27.7 t (DFO, 2012).

Due to legislative controls, current BC aquaculture production is reduced compared to Washington output; in 2010, 52 t of cultured geoduck were harvested for a landed value of $1.1 million (CAD) (BC Ministry of Agriculture, unpublished data). Established in 1998, the reduced culture activity results from a government policy that restricts the licensing and establishment of new culture plots (Hand and Marcus, 2004). The halted expansion will continue until an appropriate management framework can be implemented (slated for release some time in 2013). Despite the prolonged delay, the aquaculture sector maintains high interest in the eventual commercial expansion of BC geoduck aquaculture (DFO, 2012). At the policy’s termination, it is likely the demand for hatchery produced geoduck will intensify due to the expanded culture area available for out-planting. As current hatchery production is limited (Heath, 2005), the restricted availability of juveniles may bottleneck commercial activity during expansion, impeding aquaculture development and profit. In addition to restricted output, BC hatchery protocols remain ill-defined (Marcus and Hand, 2004) and despite industry pressure, the importation of Washington reared juveniles for out-planting, is prohibited due to genetic and pathogenic concerns (Marcus and Hand, 2004). Thus, development and refinement of BC hatchery protocols may be required to elevate juvenile geoduck seed production to the levels demanded of future commercial aquaculture activity.
1.4 Requirements for geoduck hatchery culture

Critical considerations for generalized hatchery production include culture temperature, salinity, feed type, feed ration, and stocking density. Limited investigations into geoduck culture have examined temperature, salinity, ration quantity, and stocking density effects on larval development or growth (Goodwin, 1973; Marshall, 2012). The seminal hatchery efforts conducted by Goodwin (1973) refined the temperature and salinity requirements of geoduck embryo and larvae, while the recent work of Marshall (2012) examined the combined effect of feed ration and stocking density on larval growth and survival. However, these past optimization efforts in the literature exclude the juvenile culture stage and this omission will likely hinder future BC production attempts, limiting aquaculture expansion. The absence of data necessitates prioritization in pilot investigations, isolating the culture parameters most influential to geoduck growth optimization. Of the above culture parameters, previous bivalve research indicates that temperature and ration may be the most significant factors that dictate growth rate in bivalve juveniles (Beiras et al., 1993; Broom and Mason, 1978; Walne and Spencer, 1974).

1.5 Temperature

Classified as poikilothermic, the geoduck is incapable of internal thermal regulation; its physiological processes are directly influenced by the ambient water temperature. The pervasive importance of environmental temperature is reflected in its ability to dictate the rate of numerous biological reactions in poikilotherms, influencing growth, respiration, feeding, and locomotion activity (reviewed by Hochachka and Somero, 2002). Temperature itself indicates the intensity of molecular activity, termed kinetic energy. Elevated kinetic energy enhances molecular chemical reactivity, increasing the proportion of molecules at or above the minimum energy
level (activation energy) required for a specific reaction (Hochachka and Somero, 2002). The elevated proportion of molecules with an activity level at or beyond the activation energy accelerates the rate of biological reactions that underlie critical physiological processes, such as growth. Increases in temperature will continue to elevate reaction rates until a species-specific upper thermal limit is achieved. Beyond this thermal limit, the excessive kinetic activity can incite molecular damage, inhibit reactions, and possibly incur lethal effects (Hochachka and Somero, 2002). The high temperatures associated with this maximum can disrupt atomic or molecular bonds, modify lipid structure, incite protein denaturation, inactivate enzymes, and inhibit oxygen availability (Kinne, 1970). Following this principle, it is expected that growth in poikilotherms will increase with temperature until an upper thermal maximum is reached, beyond which growth and/or survival may be significantly depressed.

Multiple laboratory studies that have examined bivalve growth under a changing temperature regimen conform to this expected growth response (e.g. Almada-Villela et al., 1982; Kleinman et al., 1996; Laing et al., 1987; Rico-Villa et al., 2009; Sicard, 1999). Exemplified by these growth trends, it is expected that geoduck growth will also increase over a specific thermal range. Goodwin (1973) concluded that 18 °C exceeded the thermal maximum in embryotic geoduck, but postulated that larval and juvenile geoduck would exhibit a wider thermal tolerance. Hatchery observations indicate larvae beyond the straight-hinge stage survive in 17 °C water and successful juvenile growth can occur up to 18 °C (Goodwin and Pease, 1989). However, these reports remain anecdotal and lack confirmation in the scientific literature. If validated, however, utilization of an elevated culture temperature in the juvenile geoduck growth stage may maximize hatchery output and promote financial success. The projected growth
acceleration incited by higher culture temperatures should reduce the required hatchery period, reducing production expenses and enhancing product availability.

1.5.1 Objective 1

Determine the optimal rearing temperature in geoduck culture from the late post-larval stage through the juvenile culture stage.

1.5.2 Hypothesis 1

$H_A$: Water temperature will significantly influence geoduck growth (shell length, DSI, wet weight, SGR, dry weight) and organic tissue accumulation (AFDW). I predict the warmest tested culture temperature, 19 °C, will elicit the fastest growth in post-larval and juvenile geoduck and result in the greatest organic tissue (AFDW) content in juvenile geoduck when compared to lower treatment temperatures.

$H_0$: Temperature will not significantly influence geoduck growth or organic tissue accumulation.

1.6 Feed ration

Feed ration describes the quantity of feed available for consumption in culture. Energy absorbed from the selected feed ration cannot be diverted to shell or tissue growth until maintenance (i.e. respiration, excretion) requirements are exceeded (Thompson and MacDonald, 2006). Beyond this threshold, elevated feed availability is demonstrated to enhance feed consumption and growth in multiple bivalve species (e.g. Beiras et al., 1993; Coutteau et al., 1994a, 1994b; Garcia-Esquivel et al., 2000; Langton et al., 1977; Lu and Blake, 1996; Walne and Spencer, 1974). The significance of this parameter in hatchery culture is emphasized by Walne
and Spencer (1974) who isolated feed ration as the most influential growth determinant in juvenile European flat oyster, *Ostrea edulis* culture (alternative parameters examined included temperature, rearing density, flow rate, and water change frequency). However, indiscriminate elevation of the offered ration quantity can elicit an insignificant or detrimental growth response in the cultured animals (Coutteau *et al.*, 1994a; 1994b; Garcia-Esquivel *et al.*, 2000).

Application of an incorrect feed ration in a hatchery operation can therefore incur substantial operating expenses from the excess feed provision. In addition to the associated growth depression, an excessive feed amount is costly, especially in the juvenile culture stage (Coutteau *et al.*, 1994a; de Pauw, 1981); the live microalgae diet required for the culture period generates high production and maintenance costs (Borwitzka, 1997; Coutteau and Sorgeloos, 1992; Coutteau *et al.*, 1994). To avoid unnecessary algae production, identification of the optimal feeding ration is required in all stages of hatchery culture, but the juvenile stage is the most critical for cost considerations (Coutteau *et al.*, 1994). Juveniles represent the largest biomass in hatchery culture and are the hatchery’s greatest consumers of microalgae (Claus, 1981; Helm, 1990; Manzi and Castagna, 1989). Feed ration optimization in juvenile culture would therefore economize hatchery production, minimizing microalgae usage without compromising animal growth.

However, the optimal feed ration is undefined in juvenile geoduck culture. Establishment of the optimal feed ration for geoduck must also consider shifting feed requirements as the animal increases in size. Indeed, a generalized aquaculture model recommends continued modification of the applied ration level to accommodate the altering dietary requirements of the growing animals (Sedgwick, 1979). Previous bivalve studies (Coutteau *et al.*, 1994a; Urban *et
al., 1983) indicate the necessity of weekly ration adjustments. Identification of the optimal ration for geoduck culture must consider animal growth and apply a shifting-ration regime, isolated to the dietary requirements of each weekly size class.

1.6.1 Objective 2

Determine the optimal ration level of a mixed-algal diet (Chaetoceros muelleri and Isochrysis sp., Tahitian strain, delivered in equal ash-free dry weight combination) to four size classes of juvenile geoduck.

1.6.2 Hypothesis 2

H<sub>A</sub>: Ration optimization will be dependent on the geoduck size. I predict that the optimal ration quantity will shift upwards with each larger size class.

H<sub>0</sub>: Ration optimization will not be dependent on geoduck size.

1.6.3 Hypothesis 3

H<sub>A</sub>: Ration will significantly influence geoduck growth (shell length, DSI, wet weight, SGR, dry weight) and organic tissue accumulation (AFDW). I predict that the ration that elicits the fastest shell growth and wet/dry weight accumulation in each geoduck size class will also contribute to the greatest organic weight sequestration (AFDW).

H<sub>0</sub>: Ration will not significantly influence geoduck growth (shell length, DSI, wet weight, SGR, dry weight) and organic tissue accumulation.
1.7 Limitations in live microalgae

Despite refinement in thermal or dietary conditions, the ubiquitous requirement of live microalgae in bivalve hatchery culture remains a hindrance to economical hatchery production due to cost and culture restraints (Borowitzka, 1997; Guedes and Malcata, 2012). Attempted minimization in live algae usage is a current trend in bivalve hatchery production (Becker, 2004; Borowitzka, 1997; Muller-Feuga, 2000; Spolaore et al., 2006), with prospective dietary substitutions under continued investigation (reviewed in Knauer and Southgate, 1999; Langdon and Önal, 1999; Önal et al., 2005). These alternatives include: preserved microalgae (concentrated microalgae preserved through drying, refrigeration or freezing), bacteria and yeasts, encapsulated artificial diets, and dissolved nutrients (reviewed in Knauer and Southgate, 1999; reviewed in Marshall et al., 2010). Alternative diets have produced varied growth success among various life stages (larval, juvenile, adult) (reviewed by Knauer and Southgate, 1999; reviewed by Marshall et al., 2010) and bivalve species like: clams (Mercenaria mercenaria; Ruditapes decussatus; Tapes philippinarum; T. semidecussata) (Boeing, 1997; Caers et al., 1999; Coutteau et al., 1994a; Epifanio, 1979; Önal et al., 2005; Pérez Camacho et al., 1998); oysters (Crassosterea gigas; C. virginica; Saccostrea commercialis) (Baubin, 2009; Boeing, 1997; Brown and McCausland, 2000; Epifanio, 1979; Knauer and Southgate, 1997a; Nell and Wisely, 1983); mussels (Mytilus edulis; M. galloprovincialis) (Epifanio, 1979; Langdon and Önal, 1999), and scallops (Aequipecten irradians; Argopecten irradians) (Epifanio, 1979; Zhou et al. 1991), but no investigations have been performed in geoduck culture.
1.8 Bivalve nutritional requirements

Selection of a dietary substitute often considers its biochemical composition; this evaluation gives a preliminary indication of the diet’s potential to fulfill the targeted species’ nutritional requirements. The initial biochemical evaluation should examine dietary levels of protein, carbohydrate, lipid, and fatty acid; collectively this content contributes to the diet’s nutritional value (Becker, 2004) and probable success as a feed alternative. Manipulation and exclusion of these dietary components is a common practice in bivalve nutritional research as workers attempt to elucidate their individual importance and required inclusion level through the measured growth response. These studies infer the necessity of the tested component by growth success and, often, tissue evaluation. A summation of the dietary significance of each component will be described as presently evaluated in the bivalve literature.

1.8.1 Total protein

Regardless of species, protein represents the most abundant organic constituent in microalgae; its dry weight typically occupies 12–35% of total organic content and may exceed 60% in select species and culture conditions (Becker, 2004). Similarly, protein is the major biochemical component present in animal tissue (Gabbott, 1976). In the natural environment, it is accumulated by bivalves during periods of low metabolic activity and elevated food abundance (Marin et al., 2003), but it does not undergo the high sequestration activity exhibited by carbohydrates or lipids (Gabbott, 1976). Despite its dominant contribution to the bivalve diet and subsequent tissue composition, the dietary significance of elevated protein remains a disputed component in feed quality assessment, with both beneficial and detrimental effects being attributed to a high inclusion level (Flaak and Epifanio, 1978; Gallager and Mann, 1981; Kreeger
and Langdon, 1993; Langton et al., 1977; Thompson and Harrison, 1992; Uriarte and Farias, 1999; Utting, 1986; Walne, 1973; Wikfors et al., 1992). Therefore, it is inadvisable to examine protein as the sole criterion of dietary value, due to its variable importance among growth studies. However, as demonstrated by Gallager and Mann (1981), Langton et al. (1977), Walne (1973), and Wikfors et al. (1992), high protein inclusion can promote successful growth in clam culture and a similar beneficial effect may be elicited in geoduck.

1.8.2 Total carbohydrate

The dietary benefit of an elevated carbohydrate quantity is similarly disputed in the bivalve nutrition literature. Carbohydrate content comprises 6–23% of a microalga’s dry weight (Becker, 2004) and its nutritional value rests in its utility as an energy or storage molecule (Marin et al., 2003; Whyte et al., 1989). It is readily catabolized as an energy source, permitting dietary-derived protein and lipid to be diverted to biosynthetic processes (Whyte et al., 1989). Surplus carbohydrate is converted to the storage molecule glycogen, the primary energy reserve in adult bivalves (Marin et al., 2003). These glycogen stores are mobilized during gametogenesis and in periods of nutritional duress (Marin et al., 2003).

Dietary carbohydrate inclusion is often manipulated in concomitance with protein. Generally, an increased carbohydrate level permits the synthesis of dietary amino acids into tissue protein and other nitrogenous compounds, as the carbon is selected for energy mobilization (Whyte et al., 1990). Hawkins and Bayne (1991, 1992) indicated the ecological significance of carbohydrate availability in wild mussel populations, suggesting carbon limits animal growth due to its high uptake and turnover rate, while dietary nitrogen is efficiently utilized and recycled in mussel tissue (Hawkins, 1985).
However, as demonstrated by Enright et al. (1986b), in the culture of juvenile *O. edulis*, a dietary carbohydrate surplus cannot be effectively mobilized if the supplied protein level is insufficient. Indiscriminate elevation in the dietary carbohydrate content is therefore irrelevant if unpaired with adequate protein inclusion. Castell and Trider (1974) further discouraged the evaluation of carbohydrate content as a singular determinant of nutritional value. These authors demonstrated that diets with 60–75% carbohydrate content enhanced eastern oyster (*C. virginica*) glycogen production, but did not promote growth. Therefore, like protein, carbohydrate cannot be considered in isolation during dietary assessments. However, its utility as a storage molecule may serve an additional advantage in geoduck culture when individuals are transplanted to the natural environment. Carbohydrate stores sequestered in geoduck tissue during the culture period may promote survival in periods of low phytoplankton availability and contribute to outplanting success. A diet that elicits rapid growth and elevated carbohydrate tissue accumulation may then provide superior nutrition for both the cultured and natural environment.

1.8.3 Total lipid

Lipids typically represent 2–23% of a microalgae’s total organic content (Becker, 2004). Valued for their high energetic storage potential, lipids constitute an important energy reserve in bivalves (Marin et al., 2003). These reserves are utilized during gamete development and in periods of prolonged food scarcity (Beninger and Lucas, 1984; Beninger and Stephan, 1985; Walne, 1970). Stored lipid may also be elevated through glycogen conversion; conversion frequency may exhibit seasonal variation as demonstrated in wild blue mussel (*M. edulis*) populations. Measured lipid reserves maximized in winter, when metabolic demands and food
availability were low, while tissue glycogen peaked in spring and summer when water
temperature and phytoplankton increased (Marin et al., 2003).

The importance of lipid storage for bivalve survival in the natural environment is
demonstrated by Laing and Millican (1986) who cultured O. edulis juveniles with single and
mixed microalgae diets prior to wild outplanting. These authors correlated the lipid accumulation
that occurred in culture with growth and survival success in the natural environment. The best
performing diet, Chaetoceros calcitrans, elicited the highest growth in cultured animals and also
promoted the greatest lipid sequestration. The benefit of lipid accumulation may similarly extend
to out-planted geoducks and support the selection of diets that can maximize lipid reserves in the
culture stage.

However, total lipid content cannot be used as the exclusive evaluator of nutritional
utility when assessing bivalve lipid requirements. This is exemplified by Langdon and Waldock
(1981) who demonstrated that Dunaliella tertiolecta, despite containing the highest lipid content
among tested diets, also elicited the poorest growth in juvenile Pacific oyster (Crassostrea
gigas). The authors concluded that total lipid content did not restrict C. gigas growth in
individuals fed the inferior diet and that an inadequate fatty acid composition contributed to the
nutrient deficiency.

1.8.4 Fatty acid composition

Indeed, bivalve studies emphasize the importance of fatty acids when assessing an
animal’s nutritional requirements and the dietary value of a feed (Enright et al., 1986a, b;
reviewed in Knauer and Southgate, 1999; Langdon and Waldock, 1981; Milke et al., 2004; 2006;
Thompson and Harrison, 1992). A fatty acid is comprised of an aliphatic chain with a carbonyl group on one end and a methyl group on the opposite (Brent and Müller-Navarra, 1997). Its structure can be designated by the nomenclature: X:Yn – Z. The X signifies the number of carbon atoms, Y, the number of double bonds, and Z the position of the first double bond as counted from the methyl end (Brent and Müller-Navarra, 1997). A fatty acid with a singular double bond is classified as a mono-unsaturated fatty acid (MUFA) while one that possesses multiple double bonds is referred to as a poly-unsaturated fatty acid (PUFA) (Brent and Müller-Navarra, 1997).

Of the fatty acids, the inclusion of PUFAs in hatchery diets is given precedence as bivalves demonstrate the limited ability to synthesize these critical compounds de novo (de Moreno et al., 1976; Langdon and Waldock, 1981; Waldock and Holland 1984). These molecules contribute to the maintenance of cell membrane fluidity and their oxidized products (eicosanoids) regulate a suite of critical physiological processes (reviewed in Stanley-Samuelson, 1987, 1994; Brent and Müller-Navarra, 1997). In invertebrates, eicosanoids modulate key functions like ionic transport, immunological responses, and the induction of egg production and spawning (reviewed in Stanley-Samuelson, 1987; 1994).

The pioneering work conducted by Langdon and Waldock (1981) first established the n–3 PUFAs: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as essential fatty acids in oyster nutrition. Absent from the dietary composition of D. tertiolecta, the authors illustrated that the exclusion of EPA and DHA restricted growth in juvenile C. gigas when fed this species. Supplementation of D. tertiolecta with an oyster lipid extract containing EPA and DHA or an exclusive DHA additive resulted in significantly enhanced oyster growth, demonstrating the
dietary significance of the tested PUFAs (Langdon and Waldock, 1981). Further evaluation conducted by Enright et al. (1986 a, b) correlated elevated growth in O. edulis juveniles with increased dietary levels of EPA and DHA, further indicating their nutritional importance.

To achieve adequate dietary EPA and DHA a mixed microalgae diet is typically employed in bivalve culture, rather than a monospecific diet. Algal species differ in EPA and DHA content, with few species (i.e. Pavlova spp.) containing high levels of both (Brown et al. 1997). However, the monospecific utilization of Pavlova spp., in bivalve hatchery culture often incurs growth suppression (reviewed by Marshall et al., 2010), which dissuades its singular dietary application. In juvenile geoduck culture, recent research recommends the bialgal diet: Chaetoceros muelleri (CM) and Isochrysis sp. (Tahitian strain; TISO) delivered in equal AFDW. Compared to a variety of alternative monospecies and mixed species diets, this dietary combination promoted superior geoduck growth through the juvenile stage (W. Liu, unpublished data). CM is a diatom characterized by its rich EPA content (Zhukova and Aizdaicher, 1995; Brown et al. 1997), while TISO is a prymnesiophyte, an elevated source of DHA (Brown et al., 1997).

In addition to EPA and DHA, fatty acids of the n – 6 family (differentiated from the n – 3 group due to the location of the first double bond from the methyl end), specifically docosapentaenoic acid (DPA) and arachidonic acid (AA), exhibited nutritional significance in the culture of post-larval (Placopecten magellanicus and Argopecten irradians) (Milke et al., 2004, 2006, respectively) and juvenile (A. irradians) (Milke et al., 2006) scallops. The accelerated growth elicited by animals fed a DPA and AA enriched diet, supports the nutritional significance of n – 6 fatty acids and their inclusion in scallop culture (Milke et al., 2004; 2006).
These studies indicate that full dietary evaluation cannot only consider EPA and DHA, as unnoted fatty acids may exhibit unrecognized importance in bivalve nutrition.

1.9 Spray-dried live algae substitution

A possible dietary substitute for live phytoplankton includes the heterotrophic species, *Schizochytrium* sp. This species belongs to a group of unicellular marine heterotrophs, termed the thraustochytrids (Lewis *et al*., 1999) which share a close relation to heterokont algae (*i.e.* brown algae and diatoms) (Cavalier-Smith *et al*., 1994). Coupled with its low cost, the manipulation of culture conditions in *Schizochytrium* fermentation can induce high cellular DHA and n – 6 DPA production (Barclay *et al*., 2005), recommending its potential utility as a bivalve feed.

Spray-dried *Spirulina* (genus: *Anthrospira*) is an additional option for live algae replacement. This species is presently valued as a dietary supplement in both human and animal nutrition due to its rich biochemical composition (Habib *et al*., 2008; Hu, 2004; Öthes and Pire, 2001). Classified as a cyanobacterium, *Spirulina* is a multicellular, filamentous algae recognized by its distinctive helical shape (Habib *et al*., 2008; Öthes and Pire, 2001). *Spirulina*’s cultivation and collection is cost efficient and its nutritional value is primarily derived from its high protein content (55–70% by dry weight) (Phang *et al*., 2000). In contrast to *Schizochytrium*, examination of five *Spirulina* strains indicated the absence of DHA, with EPA only present in a singular strain (Öthes and Pire, 2001) and its dietary lipid content much reduced (reviewed in Habib *et al*., 2008).
The dietary incorporation of *Schizochytrium* or *Spirulina* is untested in juvenile geoduck culture. However, the nutritional utility of both spray-dried species has been demonstrated in other bivalves (Baubin, 2009; Boeing, 1997; Langdon and Önal 1999; Önal *et al.*, 2005; Zhou *et al.*, 1991). These studies indicated that partial or full live algal replacement with *Schizochytrium* sp. and/or *Spirulina* can result in similar or enhanced growth when compared to a live algae diet.

For example, Baubin (2009) demonstrated a mixed TISO/Schizochytrium diet (1:1 by DW) elicited superior dry weight accumulation in juvenile *C. gigas* compared to the control TISO diet. This author also indicated live algae (*Thalassiosira nordenskioeldii* mixed with *Schizochytrium* (1:1 by DW) significantly enhanced dry weight and energy stores in juvenile *C. gigas* compared to the control *T. nordenskioeldii* diet (Baubin, 2009). Boeing (1997) concluded 40% *Schizochytrium* sp. diet replacement did not significantly depress growth in juvenile Manila clams (*T. semidecussata*) when compared to individuals fed a full live algae diet of high nutritional value (mixed 1:1 by DW: *Chaetoceros* sp. and *Tetraselmis suecica*). Langdon and Önal (1999) supplemented a 25% live algae ration (mixed *C. calcitrans* and TISO by equal cell amount) with a combined diet of *Spirulina* and *Schizochytrium* sp. (50/50% by wet weight) in juvenile Mediterranean mussel (*M. galloprovincialis*) culture. This diet significantly elevated growth (both in wet and organic weight) when compared to mussels fed the full live algae ration. Complete live algae substitution with a mixture of ground *Spirulina* and *Schizochytrium* sp. (50/50% by wet weight) also produced mussel growth comparable to a live algae diet (TISO), likely indicating the digestibility and nutritional value of the complete spray-dried diet (Langdon and Önal, 1999). Önal *et al.* (2005) concluded the live algae ration (mixed *C. calcitrans* and TISO by equal cell amount) fed to juvenile *T. philippinarum* clams can be reduced by 75% if supplemented with a full ration (equal to full algae ration by dry weight) of *Schizochytrium* sp.
Finally, Zhou et al. (1991) substituted the microalgae (ambient tank algae) diet of bay scallop (*A. irradians*) broodstock with a mixed sun-dried *Spirulina* diet, and concluded that the diet replacement enhanced fecundity and spawning in tested animals.

It is unknown if similar growth enhancement can be elicited by the integration of spray-dried algae in geoduck culture. To examine this, the CM and TISO bialgal diet will serve as a positive control diet, and will be replaced with variant levels of *Schizochytrium* or *Spirulina*. The nutritional divergence between the spray-dried species will provide a wide nutritional range for possible dietary improvement of the live algae control. It was unknown how the supplementation or reduction of specific dietary components (incurred by the spray-dried replacement) would influence geoduck growth. To evaluate this, geoduck growth will be measured under graduated dietary substitutions (0 – 100%). The biochemical composition of the delivered diets will also be examined in the attempt to clarify the possible dietary components most valuable to growth. Tissue analysis will also be conducted on geoduck in this experiment in an effort to discern the dietary influence on tissue composition and compound sequestration.

### 1.9.1 Objective 3

Investigate the feasibility of a partial or complete dietary replacement of the live algae diet with *Schizochytrium* sp. or *Spirulina* substitutes in two size classes of juvenile geoduck.

### 1.9.2 Hypothesis 4

$H_A$: Live algae replacement with up to 50% *Schizochytrium* sp. will influence geoduck growth.
\(H_0\): Live algae replacement with up to 50% *Schizochytrium* sp. will not influence geoduck growth. It is predicted that up to 50% live algae replacement with *Schizochytrium* sp. will not result in significantly different geoduck growth (shell growth, DSI, wet weight, SGR, dry weight) and organic tissue accumulation when compared to the full algae control diet for both size classes.

1.9.3 Hypothesis 5

\(H_A\): Live algae replacement with up to 50% *Spirulina* will influence geoduck growth.

\(H_0\): Live algae replacement with up to 50% *Spirulina* will not influence geoduck growth. It is predicted that up to 50% live algae replacement with *Spirulina* will not result in significantly different geoduck growth (shell growth, DSI, wet weight, SGR, dry weight) and organic tissue accumulation when compared to the full algae control diet for both size classes.

1.9.4 Objective 5

Complete proximate and fatty acid analysis of geoduck tissue. Correlate dietary content with final geoduck composition to identify the dietary influence on tissue content and sequestration.

1.9.5 Hypothesis 6

\(H_A\): Geoduck tissue will reflect the nutritional composition of the delivered diet. It is predicted that a significant positive correlation will exist between proximate components in the diet, select dietary fatty acids and final geoduck tissue composition.

\(H_0\): Geoduck tissue will not reflect the nutritional composition of the delivered diet.
1.10 Summary

My research will address some of the knowledge gaps in current geoduck hatchery protocols. It will be a starting point for subsequent research trials and will contribute to future hatchery guidelines in the eventual standardization of geoduck aquaculture. The economic feasibility of geoduck aquaculture may be further enhanced by the successful substitution of live microalgae diets with spray-dried algae, reducing the operating costs that may restrict successful implementation. Examination of proximate content of geoduck tissue may give an indication of the energy reserves available for consumption when juveniles are transported from the hatchery or nursery to the natural environment, and may indicate the long-term importance of different culture parameters. The establishment of these culture protocols may promote the success of the BC geoduck aquaculture industry, ensuring the supply of high-quality juveniles to prospective aquaculture operations.
2 Temperature and feed-ration optimization in the hatchery culture of juvenile Pacific geoduck clams (*Panopea generosa* Gould, 1850)

2.1 Introduction

Exemplified by the growth trends of multiple bivalve species (e.g. Almada-Villela *et al.*, 1982; Kleinman *et al.*, 1996; Laing *et al.*, 1987; Rico-Villa *et al.*, 2009; Sicard, 1999), temperature represents an important abiotic determinant in poikilothermic growth rate (reviewed by Hochachka and Somero, 2002). Temperature elevation accelerates poikilotherm growth until a species-specific thermal maximum is met; beyond which, growth and/or survival is depressed (Hochachka and Somero, 2002; Kinne, 1970). 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.

This thermal optimum remains undefined for post-larval and juvenile Pacific geoduck clams (*Panopea generosa*), with thermal investigations on this species limited to embryos (Goodwin, 1973). In the seminal study conducted by Goodwin (1973), geoduck embryos were cultured at 6, 10, 14, and 18 °C. Compared to 6 and 18 °C, the 10 and 14 °C cultures produced a higher percentage of normal straight-hinge larvae with a second experiment establishing 16 °C as the upper thermal limit for embryonic geoduck (Goodwin, 1973). However, thermal tolerance in marine invertebrates typically alters during ontogenic development, widening with growth (Kinne, 1970). For example, Tettelbach and Rhodes (1981) indicated that normal development of northern bay scallop (*Argopecten irradians irradians*) embryos into the straight-hinge veliger...
stage occurs within a narrow thermal range (20 – 25 °C), with optimal development (100%) at 20 °C. Larvae of this species, however, displayed diminished thermal sensitivity and demonstrated high survival at 20 (100%), 25 (91%), and 30 °C (93%); in contrast, only 39% of embryos completed normal development at the 30 °C culture temperature (Tettelbach and Rhodes, 1981). This generalized model is further corroborated by geoduck hatchery observations reported by Goodwin and Pease (1989) – high larval survival occurred at 17 °C while juvenile geoduck growth continued up to 18 °C.

The first objective of the present study was to examine the veracity of this anecdotal report and establish the optimal thermal range for post-settlement Pacific geoduck. Four rearing temperatures (7, 11, 15, 19 °C) were tested in the culture of two size classes: late post-larvae and juveniles. This size separation was established to elucidate the influence of geoduck size on thermal sensitivity. An optimal thermal regime customized to the size-dependent requirements of each post-settlement life stage may promote accelerated growth and culture output as the geoduck develop from post-larvae into nursery-ready juveniles.

Growth parameters examined for the juvenile size class (temperature experiment 1) were: shell length (mm), daily shell increment (DSI, µm d⁻¹), individual wet weight (mg ind⁻¹), specific growth rate (SGR), individual dry weight (mg ind⁻¹), and ash-free dry weight (AFDW, % initial). The growth parameters examined for the late post-larval size class (temperature experiment 2) were: shell length (mm) and DSI (µm d⁻¹). Survival rate (%) was also calculated for both experiments.

An additional critical consideration in bivalve culture is feed ration optimization (Beiras et al., 1993; Walne and Spencer, 1974). Designation of an optimal ration level has occurred
under variant definitions [e.g. the level that produces the maximal growth to ration ratio (Brett and Groves, 1979); the level that promotes the highest gross growth efficiency (Thompson and Bayne, 1974; Winter and Langton, 1976)], but for practical hatchery application, the present study will define optimal feed ration as the minimal tested level required for maximal growth (see Liu et al., 2011).

Due to variation in nutritional value, attempted feed ration optimization is dependent on the microalgal species utilized (Coutteau et al., 1994b; Enright et al., 1986a). For Pacific geoduck, recent research has shown that a mixed species diet of the diatom *Chaetoceros muelleri* (CM) and the prymnesiophyte *Isochrysis* sp. (Tahitian strain: TISO), delivered in equal proportion by AFDW, supports the best growth throughout the juvenile stage, compared to a variety of alternative monospecies and mixed-species diets (W. Liu, unpublished data). This diet combination is therefore recommended in geoduck feed manipulation studies and will be used in the present study.

Optimal feed ration levels remain undefined in juvenile Pacific geoduck culture and, coupled with the absence of thermal protocols, the paucity of information culminates in ambiguous BC hatchery protocols (Hand and Marcus, 2004) and limited BC commercial production (Heath, 2005). Therefore, the second aim of the present study was to refine geoduck ration requirements in four juvenile size classes that spanned the latter hatchery period prior to nursery outplanting (mean initial shell length of smallest-size group: 2.34 mm; mean initial shell length of largest-size group: 4.98 mm). The optimal ration was identified for the following growth parameters: shell length (mm), DSI (µm d$^{-1}$), wet weight (mg ind$^{-1}$), SGR, dry weight (mg ind$^{-1}$), and AFDW (% initial). To permit literature comparisons, weight-specific rations: g
(algal organic weight) g\(^{-1}\) (initial geoduck wet weight) week\(^{-1}\) (Laing and Psimopoulous, 1998; Laing, 2000) were calculated at each ration optimum. Survival rate was also determined for each size class.

2.2 Materials and methods

2.2.1 Common protocols for each experiment

2.2.1.1 Experimental animal source and husbandry

Broodstock collection occurred in the Strait of Georgia (DFO Pacific Fisheries Management Areas 14 and 17) in October 2010 and 2011. The mean (±SD) shell length and live weight of broodstock collected in 2010 was: 149.3 ± 12.8 cm and 1.3 ± 0.3 kg (n = 47), respectively; broodstock from the 2011 collection measured: 157.7 ± 14.7 cm and 1.4 ± 0.3 kg (n = 50), respectively. Animals were reared at the Pacific Biological Station (Nanaimo, BC) in indoor holding tanks (L x W x H: 1.2 x 0.9 x 0.3 m) stocked with 20 – 30 individuals. The tanks received seawater (8 – 12 °C, 1-µm sand filtered and UV treated) at 3 – 4 l min\(^{-1}\). A single-algal diet of CM (Chaetoceros muelleri) or TISO (Isochrysis sp. (Tahitian strain)) was drip-fed into the tank at a ration level of 4 – 6 x 10\(^9\) equivalent (by AFDW) TISO cells ind\(^{-1}\) d\(^{-1}\). Standardization to equivalent TISO cells accounts for the weight difference between algal species, and ensures that an equal feed amount (by AFDW) is delivered, despite species or cellular weight variation (Helm and Bourne, 2004).

Excessive provision of the TISO diet induced spawning in ripe broodstock. Fertilized eggs were collected and hatched in holding tanks (L x W x H: 1.2 x 0.9 x 0.3 m) at a density of < 30 eggs ml\(^{-1}\) at a temperature of 12 – 15 °C. After 48 – 60 h, the newly developed D-larvae were
transferred to a 300-l cylindro-conical tank and reared at 3 – 8 ind ml⁻¹. After 18 – 20 d of culture, the developed pediveligers reached settlement stage and were collected from the water column on a 200-µm mesh screen. Collected pediveligers were transferred to a floating tray fitted with a mesh bottom (diameter: 36 cm; base area: 1020 cm²; mesh size: 200 – 240 µm) and reintroduced to the cylindro-conical tank. At shell lengths below 2 mm, the density was maintained at < 30 ind cm⁻² (base area) while at shell lengths above 2 mm the density was maintained at < 4 ind cm⁻² (base area). Geoduck were reared on the mesh tray until the desired experimental size was achieved.

During larval and early-post-settlement culture, complete seawater exchanges in the cylindro-conical tank were conducted every 1 – 2 d. Seawater was 1-µm sand filtered, UV-treated, and maintained at 15.5 ± 0.4 °C (mean ± SE). Constant aeration was provided by 2 or 3 air stones (L x W: 5.08 cm x 2.54 cm) placed on the tank bottom. The aeration activity generated an upwelling water motion which facilitated water exchange between the mesh tray and the tank in the post-settlement culture stage. Through the larval stage, animals were fed a mixed algal diet of Chaetoceros calcitrans (CC) and TISO (in equal AFDW proportions) at a ration level of 10,000 – 20,000 equivalent TISO cells ml⁻¹ d⁻¹. In the post-settlement stage, animals were fed a mixed algal diet of CM and TISO (in equal AFDW proportions) at a ration level of 20,000 – 100,000 equivalent TISO cells ml⁻¹ d⁻¹. A photoperiod of 16:8 h light:dark was maintained for the full culture period.

2.2.1.2 Live algal culture and density determination

Microalgae species were sourced from the Provasoli-Guillard National Center for Marine Algae and Microbiota (East Boothbay Harbor, Maine, USA). The following strains were used:
CM (CCMP 1316), TISO (CCMP 1324), and CC (CCMP 1315) (CC was not used as an experimental diet and was only utilized in larval culture). CM and TISO were grown in batch cultures in 20-L carboys, while CC 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 in the late logarithmic growth phase. Cultures were grown in 1-μm sand filtered, UV-treated seawater enriched with an artificial growth medium, Harrison’s formula (Harrison et al., 1980), modified by the partial substitution of organic phosphates by inorganic phosphates. Algae were used between 4 – 8 d after carboy or flask inoculation. Daily algae culture density was determined prior to feeding with cell counts obtained using a Reichert Bright-Line hematocytometer (Hausser Scientific, Horsham, USA) (slide depth: 0.1 mm).

2.2.1.3 System monitoring

All experiments were conducted indoors at the Pacific Biological Station. Four temperature loggers (HOBO Tidbit v2, Onset Computer Corporation, Bourne, USA) recorded culture water temperature at 5-min intervals (with the exception of temperature in experiment 2, which did not have a temperature logger). In all experiments, container temperature was also monitored daily with a glass thermometer. The salinity (‰) of the incoming seawater source was measured on each water change day with a refractometer (VEE GEE Stx-3, VEE GEE Scientific Inc., Kirkland, USA). Water pH sampling occurred at three intervals throughout each trial or sub-trial. Water samples were collected in 10-ml glass vials and allowed to equilibrate to room temperature before measurement with a pH probe (Orion AquaPro Professional pH/ATC triode, Thermo Fisher Scientific Inc., Waltham, USA). A photoperiod of 16:8 h light:dark was maintained for all experiments.
2.2.1.4. Growth parameters

The following growth parameters were determined for each experiment (with the exception of temperature experiment 2): shell length (measured from the anterior to posterior axis) (mm), DSI (µm d\(^{-1}\)), wet weight (mg ind\(^{-1}\)), SGR, dry weight (mg ind\(^{-1}\)), and AFDW (%) initial). Small geoduck size prohibited weight measurements in temperature experiment 2; therefore, only shell length and DSI were considered. Survival rate was calculated for all experiments.

DSI, SGR, and survival were calculated with the following equations:

\[
\text{DSI (µm d}^{-1}\text{)} = \frac{(\text{final shell length} – \text{initial shell length})}{T}
\]

\[
\text{SGR} = \frac{[\ln(W_2) – \ln(W_1)]}{T}
\]

\[
\text{Survival (\%)} = 100 – \left[\frac{\text{(initial individuals} – \text{final individuals})}{\text{initial individuals}}\times100\right]
\]

where, \( W_2 \) is the final wet weight (mg) of the geoduck seed, \( W_1 \) is the initial wet weight (mg), and \( T \) represents the duration of the experiment (d).

2.2.2 Temperature experiment 1

2.2.2.1 General conditions

Temperature experiment 1 ran for 28 d and tested four temperature treatments (7, 11, 15, 19 °C) in triplicate. Each treatment replicate was represented by 100 randomly chosen geoduck juveniles collected from a common rearing batch. An initial sample of 100 additional geoduck were collected in triplicate; 50 animals were measured to determine shell length, and each
replicate (3) was batch weighed to determine wet weight. Individual geoduck wet weight (mg ind\(^{-1}\)) was calculated by: total group weight / individuals weighed (mean shell length ± SE: 3.22 ± 0.05 mm, \(n = 50\); mean individual wet weight ± SE: 7.04 ± 0.25 mg ind\(^{-1}\), \(n = 3\); all size designations hereafter, with the exception of temperature experiment 2, will denote geoduck size as mean shell length ± SE and mean individual wet weight ± SE). These initial measurements were used in the calculation of treatment DSI and SGR. Geoduck for each replicate were maintained in a PVC container (diameter x L: 10 x 25 cm) fitted with a 300-µm Nitex mesh bottom. PVC containers were held individually in 19-l plastic buckets filled with 18-l of 1-µm sand-filtered and UV-treated seawater of the appropriate treatment temperature. Buckets were covered with lids and gentle aeration was provided by an air stone (L x W 5.08 x 2.54 cm) placed beneath the PVC container, which facilitated water exchange across the mesh bottom.

Buckets were held in water-bath tanks to maintain treatment temperature. To reduce temperature shock at initial stocking, all experimental geoduck were placed into buckets filled with 15 °C (culture temperature of the stocking population) water before transfer into the appropriate water bath. Bucket culture water calibrated to the treatment temperature in < 24 h. Due to differential levels of light exposure between water bath tanks (tanks were stacked in duplicate), a temperature/light logger (HOBO Pendant, Onset Computer Corporation, Bourne, USA) was placed in each treatment bucket to measure light intensity (lux) at day 17. At experiment termination, the light intensity within each empty replicate bucket (covered with its lid) was measured using a digital luxmeter (EC1, B Hagner AB, Solna, Sweden) – two 1-cm diameter holes drilled in the bucket lid permitted observance of the luxmeter readings with the closed lid.
After trial initiation, full water exchanges occurred every second day up to 10 d. At 11 d of culture and onwards, water exchange occurred daily due to increased geoduck biomass. Buckets and lids were cleaned with a 10% bleach solution and rinsed with freshwater during each water exchange. Air stones were also rinsed with freshwater at this time. Geoduck were transferred into clean PVC containers three times during the trial. Due to seasonal acidity (pH<7.8) in the seawater source, 3-ml of 0.5-M NaOH was added to each bucket during water exchanges to adjust system pH to normalized levels (>7.8).

2.2.2.2 Algal feeding

Between days 0 and 10, feed delivery occurred daily with the exception of the 7 °C replicates. Due to reduced feeding activity of the clams, the 7 °C replicates received feed every second day, immediately following water exchange. From 11 d to the trial’s conclusion, all treatments were fed daily after the water exchange. All treatments received an initial ration amount of 100,000 equivalent TISO cells ml⁻¹. Ration quantity was always ad libitum to ensure food availability did not become limiting as geoduck size increased; ration amount was adjusted for each temperature treatment through the qualitative assessment of residual algae. On day 10 the ration quantity delivered to the 15 and 19 °C treatments was increased to 150,000 equivalent TISO cells ml⁻¹ and on day 14 the ration amount fed to the 11 °C treatment was elevated to 150,000 equivalent TISO cells ml⁻¹. Following these adjustments, ration levels remained unchanged for the duration of the experiment.
2.2.2.3 Sampling

With the exception of the 7 °C treatment (which received a smaller feed amount), geoduck bucket biomass was standardized on 14 d and 21 d to account for the large size differences among treatments (11, 15, and 19 °C) fed an identical ration level. Combined with routine samplings (see below), geoduck were removed randomly from each replicate container and discarded to reduce the total live biomass to 1200 mg bucket⁻¹ on 14 d and 1000 mg bucket⁻¹ on 21 d.

Sub-sampling occurred weekly (days 7, 14, and 21). At each sampling time, empty shells were extracted and counted for each replicate to determine survival rate. For shell length and wet weight determination, 15 randomly-selected geoduck were destructively sampled from each replicate. At final sampling (day 28), all geoduck were removed and counted in each replicate. All samples were rinsed three times with 10-mL of 0.5-M ammonium formate for salt removal and transferred to a petri dish. Geoduck were photographed with a digital SLR camera (EOS Digital Rebel XSi, Canon, Meville, USA) mounted on a dissecting microscope. Images were later measured using the imaging software Motic Images Advanced 3.2 (Motic, Xiamen, China) to determine shell length (15 ind replicate⁻¹). Shell length (mm) was measured for each replicate and then averaged within each replicate and treatment for each sampling period. To determine wet weight, geoduck were blotted on a paper towel and group weighed. Individual geoduck wet weight (mg ind⁻¹) was calculated (total group weight / individuals weighed) for each replicate and averaged within each replicate and treatment at each sampling period. Survival rate was calculated for each replicate and then averaged within each treatment for the full trial period.
Sampled geoduck were placed in 50-ml centrifuge tubes and stored at -80 °C prior to lyophilization.

To determine dry weight, frozen geoduck were lyophilized for 48-h in a freeze dryer (FreeZone, Labconco, Kansas City, USA) and then re-weighed. Individual dry weight (mg ind⁻¹) was calculated (total group weight / individuals weighed) for each replicate and averaged within each treatment at sampling. To determine ash at the initial (3 ind replicate⁻¹), final (3 ind replicate⁻¹), and sub-sampled intervals (15 ind replicate⁻¹), lyophilized geoduck were weighed and transferred to a muffle furnace for 4-h at 500 °C and then re-weighed. AFDW was calculated for each replicate and then averaged within each replicate and treatment for each sampling period.

2.2.3 Temperature experiment 2

2.2.3.1 General conditions

Temperature experiment 2 was initiated on day 7 of temperature experiment 1 and concluded on the same day (21 d duration). This trial tested the same treatment temperatures (7, 11, 15, and 19 °C) as temperature experiment 1, but examined a smaller size class. Each treatment replicate (3) was represented by 100 randomly-selected post-larval geoduck (mean initial shell length ± SE: 0.54 ± 0.01 mm, n = 50) collected from a common rearing batch. Treatment DSI was calculated from this initial shell size. Geoduck for each replicate were maintained in 4-l buckets fitted with lids. These containers were filled with 2-l of 1-μm sand filtered and UV treated seawater of the appropriate treatment temperature. Culture water was agitated for 20 s twice d⁻¹ with a glass rod mixer in lieu of an air stone. In the absence of a mesh
containment screen, individuals were too fragile to undergo the continuous aeration of an air stone. Despite reduced water circulation, qualitative observation of residual algae suggested algal clearance continued through the trial duration.

To maintain treatment temperature, containers were held in the same water bath tanks as temperature experiment 1. To reduce temperature shock at initial stocking, placement into the water bath tanks followed the same protocol as temperature experiment 1. Container culture water calibrated to the treatment temperature in < 24 h. Due to differential levels of light exposure between water bath tanks, a digital luxmeter (EC1, B Hagner AB, Solna, Sweden) was used to measure light intensity (lux) directly above each replicate container. At experiment termination, the light intensity within each empty replicate bucket (covered with its lid) was measured with the digital luxmeter.

Complete water exchanges occurred every second day. Again, due to seasonal acidity (pH <7.8), the pH of the source water was adjusted through the addition of 3-ml 0.5-M NaOH into 18-l of seawater of the appropriate treatment temperature. This modified water source (pH >7.8) was used for bucket water exchanges. Buckets and lids were cleaned with detergent and rinsed with hot freshwater at each exchange.

2.2.3.2 Algal feeding

Treatments were fed every second day immediately following water exchange. All temperature treatments received a fixed ration level of 100,000 equivalent TISO cells ml\(^{-1}\) for the trial duration. Qualitative observation of residual algae indicated the ration amount fulfilled satiation requirements of all treatments and replicates at each feeding.
2.2.3.3 Sampling

On day 9, geoduck culture density was reduced to 55 ind replicate$^{-1}$ in treatments 11, 15, and 19 °C to account for the size variation between these treatments and the 7 °C treatment. Sub-sampling occurred weekly (days 7 and 14). At each sampling time, empty shells were extracted and counted for each replicate for determination of survival rate. At sub-sampling, 15 randomly-selected geoduck were destructively sampled from each replicate; at final sampling (21 d) all geoduck were removed and counted in each replicate. Samples were preserved in 4% formalin for later shell length determination. Preserved samples were rinsed three times with distilled water and transferred to a petri dish. Determination of shell length (15 ind replicate$^{-1}$) followed the same protocol as temperature experiment 1. Shell length (mm) was measured for each replicate and then averaged within each replicate and treatment for each sampling period. Survival rate was calculated for each replicate and then averaged within each treatment for the full trial period.

2.2.4 Feed ration experiment

2.2.4.1 General conditions

The feed ration experiment was sub-divided into four 7 d trial periods to examine the ration requirements of four juvenile size classes separated by 810–980-µm shell length increments. In weeks 1 and 2 we tested eight feed rations: 0.0, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, and 64.0 x 10$^6$ equivalent TISO cells ind$^{-1}$ d$^{-1}$ (in triplicate). To reflect geoduck growth, feed rations were shifted upwards at weeks 3 and 4: 0.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, and 128.0 x 10$^6$ equivalent TISO cells ind$^{-1}$ d$^{-1}$ (in triplicate).
Geoduck in the stock population (maintained in the cylindro-conical tank for trial duration) were fed to satiation to ensure mean population size correlated with the growth of experimental animals. Geoduck were taken from this common rearing batch at the weekly stocking intervals. For the week 1 trial, each treatment replicate was represented by 100 randomly-selected geoduck juveniles collected from the common rearing batch. Two-hundred additional geoduck, collected in triplicate, were designated as the initial sample. For each size-class, 50 animals were measured to determine shell length, and each replicate (3) was batch weighed to determine wet weight. Individual geoduck wet weight (mg ind\(^{-1}\)) was calculated by: total group weight / individuals weighed (2.34 ± 0.04 mm, \(n = 50\); 2.96 ± 0.15 mg ind\(^{-1}\), \(n = 3\)).

Due to increased size, each treatment replicate at week 2 was represented by 50 randomly-selected geoduck juveniles. Fifty additional geoduck, collected randomly in triplicate, were designated as the initial sample (3.32 ± 0.04 mm, \(n = 50\); 6.73 ± 0.27 mg ind\(^{-1}\), \(n = 3\)). To reflect the continued size increase, each treatment replicate in weeks 3 and 4 was represented by 25 randomly-selected geoduck. Fifty additional geoduck, collected randomly in triplicate, represented the initial sample at each stocking period (week 3: 4.13 ± 0.04 mm, \(n = 50\); 12.31 ± 0.16 mg ind\(^{-1}\), \(n = 3\); week 4: 4.98 ± 0.04 mm, \(n = 50\); 21.23 ± 0.36 mg ind\(^{-1}\), \(n = 3\)). Initial sample measurements were used in the calculation of treatment DSI and SGR.

Geoduck in each replicate were maintained at 15 °C and held in the same bucket set-up and water source as temperature experiment 1 (although pH did not require modification due to seasonal increase, pH > 7.8). Complete water exchanges followed by feed delivery occurred daily. Buckets, lids, and air stones were rinsed with freshwater at each water exchange and cleaned with 10% bleach solution between trial divisions. Treatment replicates were randomly distributed and held in 15 °C water bath tanks.
2.2.4.2 Sampling

Sampling occurred at the conclusion of each one-week sub-trial and followed the same protocol as temperature experiment 1, with the exception of the geoduck number used to determine shell length (20 ind replicate\(^{-1}\)). Sampling calculations (mean shell length, individual wet weight, survival rate) and sample storage followed the same procedure as temperature experiment 1. Frozen geoduck were lyophilized with the same protocols as temperature experiment 1; determination of dry weight and ash at the initial and final sampling intervals (3 ind replicate\(^{-1}\)) also followed the same procedures. The optimal ration level determined for each size-class growth parameter was designated as the minimal feed amount that elicited the highest growth, beyond which further ration increase did not result in significant growth improvement. To permit literature comparisons, weight-specific ration – g (algae organic weight) g\(^{-1}\) (initial geoduck wet weight) week\(^{-1}\) (as in Laing and Psimopoulous, 1998; Laing, 2000) – was calculated for each optimal ration amount.

2.2.5 Statistics

Variation between culture parameters (salinity, pH, light intensity) was tested with a one-way ANOVA followed by Tukey’s multiple comparison test \((P<0.05)\). The effect of treatment (temperature, feed ration) on intermediate (temperature) and final (temperature and feed ration) geoduck shell length, DSI, wet weight, SGR, dry weight, AFDW, and survival rate \((n = 3\) for each tested parameter) was also examined with a one-way ANOVA followed by Tukey’s multiple comparison test. The significance value was \(P<0.05\) (Temperature treatment 2 only tested shell length, DSI, and survival rate). A normality test (Kolmogorov-Smirnov, \(P<0.05\)) and equal variance test (Levene’s test, \(P<0.05\)) were used to examine data normality and
homogeneity, respectively. In temperature experiment 1, SGR at day 14 was inverse transformed \((x^{-1})\) and dry weight at day 28 was natural log transformed \((\ln(x))\) to assure normality. Survival rate data were arcsine transformed in temperature experiments 1 and 2, but still failed the normality test \((P=0.037)\). In the feed ration experiment, week 1 SGR and AFDW, and week 2 wet weight and SGR failed the equal variance test \((P<0.05)\). Week 2 dry weight data required transformation \((x^{-1})\) to assure normality. Week 1 survival rate data failed normality \((P<0.01)\), despite arcsine transformation. Survival rate remained at 100% for the remainder of the experiment (weeks 2 – 4), eliminating the need for further statistical analysis. Despite a slight failure in some data sets’ normality, we chose to continue data analysis with ANOVA, due to the test’s robust tolerance for departures from normality. Statistical analyses were performed with the statistical software SigmaPlot 12.3 (Systat Software Inc., San Jose, USA).

2.3 Results

2.3.1 Temperature experiment 1

2.3.1.1 System monitoring

Culture conditions for temperature experiment 1 are given in table 2.1. The mean (± SD) temperature recorded for each water bath tank ranged from 7.5 ± 0.2 to 18.6 ± 1.0 (°C) \((n = 8,026; \text{Table 2.1})\). On day 15 there was an overnight temperature drop to 10.6 °C in the 19 °C treatment due to disconnection of a water hose. The temperature was gradually re-adjusted over 24 h. No mortalities resulted from this unexpected temperature drop. Between water bath tanks, mean (± SD) salinity (‰) and pH ranged from 28.1 ± 0.5 to 28.3 ± 0.7 \((n = 27)\) and 8.02 ± 0.07 to 8.09 ± 0.08 \((n = 9)\), respectively (Table 2.1). These values did not significantly differ between

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treatments ($P>0.05$). The mean (± SD) internal light intensity (lux) recorded for each treatment ranged between $0.2 ± 2.8$ (7 °C) to $47.6 ± 33.2$ (15 °C) ($n = 368$; Table 2.1). This variable significantly differed among temperature treatments ($P<0.05$, Table 2.1) The wide SD within treatments resulted from the dark intervals in the 16:8 h light:dark photoperiod. The mean (± SD) light intensity (lux) recorded at experiment’s end for each closed empty bucket replicate ranged between: $28.7 ± 2.5$ (7 °C) and $166.0 ± 45.9$ (15 °C) ($n = 3$; Table 2.1). Final light intensity significantly varied between 7, 11, and 15 °C ($P<0.05$), but did not significantly vary between 11 and 19 °C or 15 and 19 °C (Table 2.1).

2.3.1.2 Growth parameters

Rearing temperature significantly affected all tested growth and survival variables in the larger juveniles. At final sampling (day 28), geoduck held at 19 °C displayed a significantly elevated mean shell length, DSI, individual wet weight, SGR, and individual dry weight, when compared to individuals held at all other temperatures (Fig. 2.1A-E). All of these growth parameters were significantly different among all four temperature treatments on the final sampling day. Although 19 °C geoduck displayed the fastest growth, final AFDW in this treatment was significantly reduced compared to 11 or 15 °C treatments (Fig. 2.1 F). Survival was high in all treatments, ranging from 97.3% to 100.0% on 28 d. Mean survival rate (±SE) was significantly lower at 7 °C ($97.3 ± 0.7\%$) than at 15 or 19 °C ($100.0 ± 0.0\%$), but did not significantly differ from 11 °C ($99.0 ± 0.6\%$).
2.3.2 Temperature experiment 2

2.3.2.1 System monitoring

Culture conditions for temperature experiment 2 are given in table 2.1. The mean (± SD) temperature recorded for each water bath tank ranged from 7.4 ± 0.2 to 18.4 ± 1.1 °C (n = 66; Table 2.1). On day 10 there was an overnight temperature drop to 14.0 °C in the 19 °C treatment due to disconnection of a water hose. The temperature was gradually re-adjusted over 24 h. No mortalities resulted from this unexpected temperature drop. Between water bath tanks, mean (± SD) salinity (‰) and pH ranged from 28.1 ± 0.6 to 28.3 ± 0.5 (n = 22) and 7.99 ± 0.07 to 8.07 ± 0.02 (n = 9; Table 2.1), respectively. These values did not significantly differ between treatments (P>0.05). The mean (± SD) light intensity (lux) measured directly above each treatment container ranged between: 85.6 ± 8.2 (7 °C) and 779.74 ± 102.22 (15 °C); these variables significantly (P<0.05) varied between treatments (n = 27; Table 2.1). Between treatments, the final mean (± SD) light intensity (lux) recorded in each empty container replicate ranged from: 45.0 ± 8.0 (7 °C) and 245.33 ± 43.47 (15 °C) and significantly (P<0.05) varied among treatments (n = 3; Table 2.1).

2.3.2.2 Growth parameters

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 at all sampling days for both shell length and DSI (Fig. 2.2A, B), with parameters significantly elevated at 19 °C for each interval (Fig. 2.2A, B). Survival was high in all treatments and ranged
from 93.0% to 98.0%. The mean survival rate (± SE) was significantly lower at 7 (93.0 ± 0.0%) and 11 °C (95.3 ± 0.7%) than at 15 °C (98.0 ± 0.0%) or 19 °C (97.3 ± 0.3%).

2.3.3 Feed ration experiment

2.3.3.1 System monitoring

Mean temperature and pH (± SD) maintained across all ration treatments for the full trial period (weeks 1 – 4) were: 15.2 ± 0.3 °C (n = 31,562) and 8.30 ± 0.18 (n = 264), respectively. Mean (± SD) ambient salinity and pH of the seawater source were 28.0 ± 0.5‰ (n = 27) and 7.98 ± 0.04 (n = 5).

2.3.3.2 Growth parameters

2.3.3.2.1 Week 1

For the week 1 size class (initial shell length: 2.34 ± 0.04 mm; initial wet weight: 2.96 ± 0.15 mg ind⁻¹, mean ± SE) mean shell length, DSI, individual wet weight, and SGR significantly (P<0.05) increased with ration up to 4.0x10⁶ equivalent TISO cells ind⁻¹ d⁻¹ (Fig. 2.3A-D). Beyond this ration level, increased feed amount did not significantly improve growth in these parameters. At 4.0x10⁶ equivalent TISO cells ind⁻¹ d⁻¹, the converted weight-specific ration equaled 0.151 g g⁻¹ week⁻¹ (Table 2.2) (mean TISO AFDW: 15.99 pg; determined in Chapter 3, Section 3.2.7). In the above parameters, growth typically displayed a decline at rations above 16.0x10⁶ equivalent TISO cells ind⁻¹ d⁻¹. Optimal DW (1.57 ± 0.03 mg ind⁻¹, mean ± SE) occurred at 8.0x10⁶ equivalent TISO cells ind⁻¹ d⁻¹ (Fig. 2.3 E) with the converted weight-specific ration equaling 0.302 g g⁻¹ week⁻¹ (Table 2.2). The optimal ration determined for AFDW
occurred at a further elevated amount of 16.0x10^6 equivalent TISO cells ind\(^{-1}\) d\(^{-1}\) (Fig. 2.3F), or 0.604 g g\(^{-1}\) week\(^{-1}\) (Table 2.2). Survival was high in all treatments, and ranged from 93.7 to 100.0% and was not significantly affected by ration.

### 2.3.3.2.2 Week 2

For the week 2 size class (initial shell length: 3.32 ± 0.04 mm; initial wet weight: 6.73 ± 0.27 mg ind\(^{-1}\), mean ± SE), the optimal ration shifted upwards to 8.0x10^6 equivalent TISO cells ind\(^{-1}\) d\(^{-1}\) for mean shell length, DSI, individual wet weight, and SGR (Fig. 2.4 A-D). The optimal feed level identified for individual dry weight remained at 8.0x10^6 equivalent TISO cells ind\(^{-1}\) d\(^{-1}\) (Fig. 2.4E). Beyond this ration amount, increased feed amount did not significantly improve growth in these parameters. For this ration, the converted weight-specific ration equalled 0.114 g g\(^{-1}\) week\(^{-1}\) (Table 2.2). Compared to the previous growth parameters, optimal AFDW measurements again occurred at an increased ration quantity of 32.0x10^6 equivalent TISO cells ind\(^{-1}\) d\(^{-1}\) (Fig. 2.4F) or 0.456 g g\(^{-1}\) week\(^{-1}\) (Table 2.2). Survival was 100.0% in all treatments.

### 2.3.3.2.3 Week 3

For the week 3 size class (initial shell length: 4.13 ± 0.04 mm; initial wet weight:12.31 ± 0.16 mg ind\(^{-1}\), mean ± SE) shell length, DSI, and individual dry weight significantly increased with elevated ration up to 32.0x10^6 equivalent TISO cells ind\(^{-1}\) d\(^{-1}\) (Fig. 2.5A-B,E). Weight-specific ration converted to 0.291 g g\(^{-1}\) week\(^{-1}\) at this optimal level (Table 2.2). Optimal individual wet weight and SGR growth both occurred at 16.0x10^6 equivalent TISO cells ind\(^{-1}\) d\(^{-1}\) (converted weight-specific ration: 0.146 g g\(^{-1}\) week\(^{-1}\)), indicating the first deviation between shell length and wet weight ration requirements (Table 2.2, Fig 2.5C-D). Optimal ration level for
tissue AFDW shifted further upwards to 64.0x10^6 equivalent TISO cells ind^{-1} d^{-1} at a weight-specific ration of 0.582 g g^{-1} week^{-1} (Table 2.2, Fig. 2.5F). Survival was 100.0% in all treatments.

2.3.3.2.4 Week 4

The optimal ration for both shell growth and DSI for the week 4 size class (initial shell length: 4.98 ± 0.04 mm; initial wet weight: 21.23 ± 0.36 mg ind^{-1}) remained unchanged from the week 3 size-class (32.0x10^6 equivalent TISO cells ind^{-1} d^{-1}) (Figs. 2.5A-B and 2.6A-B). The optimal ration for wet weight re-converged with shell growth requirements at week 4, elevating to 32.0x10^6 equivalent TISO cells ind^{-1} d^{-1} (Fig. 2.6C). The optimal ration for SGR and individual dry weight was 64.0x10^6 equivalent TISO cells ind^{-1} d^{-1} while AFDW increased to 128.0x10^6 equivalent TISO cells ind^{-1} d^{-1} (Fig 2.6D-F). This continued optimization disparity among growth variables suggests maximal organic weight accumulation in geoduck seed requires an elevated feed amount when compared to shell or wet weight growth requirements.

Compared to the previous weeks, the week 4 size class (with the exception of shell length and DSI) displayed the highest weight-specific ration requirements. For the determined ration optimums, 32.0, 64.0, and 128.0x10^6 equivalent TISO cells ind^{-1} d^{-1}, the converted weight-specific ration equalled: 0.169, 0.338, and 0.675 g g^{-1} week^{-1}, respectively (Table 2.2). This trend suggests weight dependent feed requirements generally elevate with geoduck growth. Survival was 100.0% in all treatments.
2.4 Discussion

2.4.1 Temperature

This study was the first to examine growth and survival of post-larval and juvenile Pacific geoduck under different thermal regimens. In both size classes (post-larval and juvenile), temperature significantly affected all tested growth parameters. In agreement with alternative hypothesis 1 (Section 1.5.3), the highest culture temperature of 19 °C promoted accelerated shell, wet weight, and dry weight growth in temperature experiment 1. However, significant variation in shell length (experiment 1) did not emerge between the 15 and 19 °C treatments until the final sampling. At this time, the final mean shell length exhibited by geoduck reared at 15 and 19 °C exceeded the typical shell length range (3.0 – 6.0 mm) that is required for nursery transfer (Pinfold, 2001). The maximal shell length (6.0 mm) recommended for nursery culture was achieved in <21 d for individuals held at both 15 and 19 °C. These treatments did not significantly differ in shell size at this time, which supports a 15 °C culture temperature for late-juvenile production.

The significant reduction in AFDW observed in geoduck held at 19 °C further supports using 15 °C as the recommended culture temperature. This partially rejects alternative hypothesis 1 (Section 1.5.3), which predicted AFDW would elevate with increasing culture temperature. A similar thermal trend was described by Laing et al., (1987) where the organic weight (AFDW) of juvenile Manila (Tapes semidecussata) and hard (Mercenaria mercenaria) clams increased with temperature between 10 and 25 °C, but the highest tested culture temperature (28 °C) significantly inhibited organic accumulation. In the culture of European flat oyster (Ostrea edulis) spat, Walne and Spencer (1974) demonstrated that shell growth surpassed
tissue growth at elevated temperatures. In the present study, the elevated feed requirement incurred by the thermal increase likely suppressed organic weight gain in the 19 °C treatment. Laing (2000) demonstrated that ration requirements increased with rearing temperature in king scallop (Pecten maximus) spat. Scallops grown at the highest culture temperatures (20.0 and 23.0 °C) displayed the highest weight-specific ration requirement and feed consumption compared to all other treatments (5.0, 6.5, 8.0, 10.0, 15.0, 17.0 °C) (Laing, 2000). In the present experiment, it is likely that the delivered feed ration (100,000 – 150,000 equivalent TISO cells ml\(^{-1}\)) could not maintain optimal organic accumulation at 19 °C.

The high feed amount required for sustained organic weight gain in geoduck is further emphasized by the present feed ration experiment in which a re-occurring disjoint in ration requirements between shell/wet weight and organic growth (AFDW) was observed. In each weekly size class, the optimal feed ration for organic weight gain exceeded the optimal level required for shell and wet weight growth by four fold (with the exception of week 3 where the optimal AFDW requirement exceeded shell growth by only two fold). However, the accelerated shell, wet weight, and dry weight growth displayed by the 19 °C geoduck at day 28, suggests the restricted ration amount only inhibited organic growth. Thus, coupled with these thermal-dependent feed requirements, the unnecessary water heating costs incurred by the 19 °C temperature provides additional support for the adoption of the 15 °C temperature when rearing this juvenile size.

However, unlike experiment 1, temperature elicited an immediate growth effect in the smaller geoduck (temperature experiment 2), with significant variation between all thermal treatments occurring after only 7 d (Fig. 2.2 A, B). Geoduck reared at 19 °C showed significantly
accelerated shell growth at all sampling periods, supporting alternative hypothesis 1 (Section 1.5.3). At final sampling (day 21), the mean shell length of 19 °C geoduck exceeded the 15 °C treatment by 315.1 µm. The mean DSI (107.98 µm d\(^{-1}\)) exhibited by 15 °C geoduck at day 21 indicates that 2.9 d are required to recover the growth deficit between the 15 and 19 °C treatments. Selection of the 19 °C culture temperature for the smaller geoduck size class (0.5 – 3.1 mm) may therefore promote elevated geoduck production, shortening the culture period by 3 d. However, the production benefits incurred by the time reduction must balance the required water heating cost, which may incur significant production expenses. In abalone culture, for example, Davis and Carrington (2005) estimated that 1,000 NZD d\(^{-1}\) (~838 USD d\(^{-1}\); September 2013) is required to heat a flow-through system by 8 °C in the grow-out of 1 million juvenile abalone. If, however, the resultant cost increase is outweighed by elevated output, the shifting thermal regimen may support maximized profit in geoduck hatchery production. The 19 °C culture temperature is potentially recommended for the late post-larval stage (initial shell length: 0.5 mm) through the early juvenile culture stage (shell length: 3.0 mm). For the remainder of hatchery culture, rearing temperature should be reduced to 15 °C.

The tested temperature range tightly corresponds with thermal variation in the geoduck’s natural environment. Examination of Fisheries and Oceans Canada SST time series data since 2000 (locations: Chrome Island and Departure Bay – the closest sampling points to the region of broodstock collection) indicated the mean (±SD, n = 12) minimum and maximum SST to be: 7.2 ± 0.8 °C and 18.0 ± 0.8 °C, respectively (Anonymous, 2013c). The growth success exhibited by geoduck held at 19 °C, suggests the thermal maximum of the source population exceeds its typical natural range. Anecdotal Puget Sound (Washington State, USA) observations further indicate the elevated thermal tolerance of wild Pacific geoduck; adult intertidal and shallow
subtidal populations experience periodic temperature elevations up to 21 – 22 °C without apparent mortality in July and August (Goodwin and Pease, 1989). This information suggests that the thermal tolerance of BC Pacific geoduck may resemble that of Washington populations despite genetic (Marcus and Hand, 2004) or habitat variation, and indicate temperature thresholds may be conserved within the species.

2.4.2 Feed ration

The optimal feed ration identified for each growth parameter (i.e. shell length, DSI, wet weight, SGR, dry weight, AFDW) elevated with increasing successive size class (except dry weight in week 2 and shell length and DSI in week 4), which is in agreement with alternative hypothesis 2 (Section 1.6.3). The shifting ration optima highlights the size-dependent alteration that underlies geoduck feed requirements and emphasizes the necessity of frequent ration modification in late-stage hatchery culture. Optimal feed ration was designated as the minimal feed level beyond which further ration elevation did not significantly accelerate growth. Ingestion rate was not measured; growth was used as the sole indicator of feed and satiation requirements (i.e. Liu et al., 2011).

Various growth parameters demonstrated significant reduction beyond the optimal identified ration level. The growth inhibition exhibited at the excessive feed rations may have resulted from elevated ingestion rates incurred as a result of increased food availability (Beiras et al., 1993; Epifanio, 1979; Lu and Blake, 1997; Rico-Villa et al., 2009). Ingestion rate has been demonstrated to increase as a function of available feed ration until a saturation level is achieved (Beiras et al., 1993; Rico-Villa et al., 2009). At saturation, the ingested ration may exceed stomach capacity; partial digestion may occur before the feed is diverted to the mid-gut and
expelled (Winter, 1974). The excess energy required for partial digestion and expulsion of unutilized feed as pseudofaeces may have contributed to the reduced growth displayed at high feed concentrations. A potential alternative response to excessive feed availability was demonstrated by the blue mussel (*Mytilus edulis*) whose gill pumping and filtering ability were inhibited at high algal concentrations (Winter, 1978). This reduced capacity may also occur in geoduck, restricting feed capture, and limiting digestion and growth.

Ration quantity significantly influenced geoduck growth within each size class, supporting the first prediction in alternative hypothesis 3 (Section 1.6.4). With the exclusion of week 3, the optimal ration required for shell and wet weight growth correlated within each size class, indicating similarity in the feed requirements for these parameters. However, at each week the optimal dry weight (excluding week 2) and AFDW ration level exceeded the optimal wet weight ration by two and four fold, respectively, emphasizing the high feed requirement for organic tissue accumulation. This incongruity rejects the second prediction made in alternative hypothesis 3 (Section 1.6.4). It is speculated that an elevated feed ration is required to support continued siphon and mantle growth through the juvenile culture stage. The geoduck is characterized by its large, fleshy siphon and mantle, which cannot be fully retracted into the shell at the adult stage (Goodwin and Pease, 1989). In the present study, external siphon protrusion became evident in sampled geoduck in the week 3 size class and upwards; it is possible that the high energy requirement of tissue growth (Bayne and Hawkins, 1997) at this size raised the optimal AFDW ration requirement.

The largest size class (week 4) displayed the highest weight-specific ration requirements among tested juveniles (excepting shell length and DSI). This trend is unusual in bivalve culture,
where weight-specific feed requirements typically decline with increased animal size (Coutteau et al., 1994a; Liu et al., 2011; Urban et al., 1983). In the 3-week culture of large juvenile eastern oysters (*Crassostrea virginica*), the weight-specific feed ration decreased with each successive week (Urban et al., 1983), while the feeding activity of small (final shell length: 1 – 3 mm) juvenile *M. mercenaria* fed “on demand” demonstrated that larger animals in the second week of culture achieved satiation at a reduced feed amount compared to the initial week (Coutteau et al., 1994a). Liu et al. (2011) cultured five size classes (initial mean shell length: 0.74 – 3.00 mm) of juvenile basket cockle (*Clinocardium nuttallii*) and demonstrated that the optimal weight-specific ration generally declined with each successive week (weeks 1 – 5: 7.1, 1.7, 1.1, 1.0, 1.0 g g⁻¹ week⁻¹, respectively). The adherence of this trend in both large (Urban et al., 1983) and small (Coutteau et al., 1994a; Liu et al., 2011) bivalve juveniles highlights the singularity of weight-specific ration elevation in geoduck. The distinction may again derive from the energetic requirements of geoduck siphon and mantle development (discussed above), resulting in the increased weight-specific feed requirement of size class 4 (mean initial shell length: 4.98 mm).

The benefit of an elevated organic content (AFDW) prior to nursery transfer is unknown in geoduck clams and many other bivalve species. Laing (2000) speculated that a reduced condition index (ratio of dry meat weight to dry shell weight) could inhibit outplanting success in juvenile *P. maximus*. Laing and Millican (1986) demonstrated a correlation between lipid accumulation in the hatchery and subsequent growth and survival in outplanted juvenile *O. edulis*. However, it is unknown if these tissue-related growth benefits are transferrable to outplanted geoduck, and even if so, the optimal AFDW required from hatchery culture to receive these outplanting benefits is further undefined.
This absence of knowledge, coupled with the repeated ration disjoint displayed between measured growth parameters, suggests the application of shell length/wet weight ration optima when assigning weekly feed levels. This selection minimizes algae usage, as dry weight and AFDW optimization required elevated ration levels when compared to shell length or wet weight. Following this recommendation, the following ration levels (equivalent TISO cells ind$^{-1}$d$^{-1}$) should be applied between weeks 1 and 4 of juvenile culture: 4.0x10$^6$ for size class 1; 8.0x10$^6$ for size class 2; 16.0 or 32.0x10$^6$ (wet weight or shell length optimum, respectively) for size class 3; and 32.0x10$^6$ for size class 4.

2.5 Conclusions

Both temperature and feed ration significantly affected growth in cultured Pacific geoduck post-larvae or juveniles, correlating with the initial predictions made in alternative hypotheses 1 and 3 (Sections 1.5.3, 1.6.4). However, AFDW did not maximize with increased culture temperature as predicted in hypothesis 1 (Section 1.5.3). The reduced AFDW displayed in 19 °C geoduck, coupled with the delayed growth acceleration at this temperature, recommends a 15 °C culture temperature for the tested size class (mean initial shell length 3.22 mm; mean initial wet weight: 7.04 mg ind$^{-1}$). For the preceding size class, (shell length 0.54 – 3.1 mm) however, the immediate growth acceleration promoted by the 19 °C temperature supports its potential selection in the earlier hatchery stage. The practicality of this selection is contingent upon cost analysis; the accelerated culture period (shortened by ~3 d) must outweigh the associated heating costs.

Optimal ration levels generally shifted upwards with each successive size class, supporting alternative hypothesis 2 (Section 1.6.3). However, dry weight and AFDW did not
maximize with the ration levels identified for optimal shell and wet weight growth, rejecting the second prediction of alternative hypothesis 3 (Section 1.6.4). As AFDW requirements remain ill-defined in geoduck culture and outplanting, it is recommended that ration selection follow the weekly optima established for shell/wet weight growth.
Table 2.1. Culture conditions (mean ± SD) recorded for temperature experiments 1 (juvenile clams) and 2 (post-larval to juvenile) in the culture of geoduck clams (*Panopea generosa*). Sample sizes (*n*) in temperature experiment 1 were: 8,026 (temperature, °C); 27 (salinity, ‰); 9 (pH); 368 (internal light intensity, lux); and 3 (final light intensity, lux). Final light intensity was recorded inside each closed bucket replicate at experiment termination. Sample sizes (*n*) in temperature experiment 2 were: 66 (temperature, °C); 22 (salinity, ‰); 9 (pH); 27 (external light intensity, lux); and 3 (final light intensity, lux). External light intensity was measured directly above each replicate container; final light intensity was recorded inside each closed container replicate at experiment termination. Within experiments, salinity and pH did not vary between temperature treatments (*P* > 0.05). Different letters indicate significant variation in the light intensity received by temperature treatments (Tukey’s test, *P* < 0.05).

<table>
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<th>7 °C</th>
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<th>15 °C</th>
<th>19 °C</th>
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<td>11.2 ± 0.3</td>
<td>14.8 ± 0.3</td>
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<td>28.1 ± 0.5</td>
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<td>0.9 ± 3.0b</td>
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</tr>
<tr>
<td>Final Light Intensity (lux)</td>
<td>28.7 ± 2.5a</td>
<td>38.7 ± 2.3b</td>
<td>166.0 ± 45.9c</td>
<td>102.3 ± 43.7b,c</td>
</tr>
<tr>
<td><strong>Temperature Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>7.4 ± 0.2</td>
<td>11.2 ± 0.2</td>
<td>14.7 ± 0.4</td>
<td>18.4 ± 1.1</td>
</tr>
<tr>
<td>Salinity (‰)</td>
<td>28.3 ± 0.5</td>
<td>28.3 ± 0.5</td>
<td>28.1 ± 0.6</td>
<td>28.2 ± 0.5</td>
</tr>
<tr>
<td>pH</td>
<td>8.01 ± 0.06</td>
<td>7.99 ± 0.07</td>
<td>8.02 ± 0.06</td>
<td>8.07 ± 0.02</td>
</tr>
<tr>
<td>External Light Intensity (lux)</td>
<td>85.6 ± 8.2a</td>
<td>160.5 ± 11.0b</td>
<td>779.7 ± 102.2c</td>
<td>258.0 ± 18.8d</td>
</tr>
<tr>
<td>Final Light Intensity (lux)</td>
<td>45.0 ± 8.0a</td>
<td>85.7 ± 15.5b</td>
<td>245.3 ± 43.5c</td>
<td>111.0 ± 27.2d</td>
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</tbody>
</table>
Table 2.2. Weight-specific ration determined for optimal ration levels in juvenile Pacific geoduck clam (*Panopea generosa*) culture. Each week is represented by a new experimental group of animals of a different size class. Initial mean (± SE) wet weight of geoduck at weeks 1, 2, 3, 4: 2.96 ± 0.15; 6.73 ± 0.27; 12.31 ± 0.16; 21.23 ± 0.36 mg ind⁻¹, respectively (n = 3). Mean TISO AFDW: 15.99 pg (n = 6). Superscripts identify the optimal ration for each growth parameter (10⁶ equivalent TISO cells ind⁻¹ d⁻¹).

<table>
<thead>
<tr>
<th>Week</th>
<th>SL</th>
<th>DSI</th>
<th>WW</th>
<th>SGR</th>
<th>DW</th>
<th>AFDW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>0.151*</td>
<td>0.151*</td>
<td>0.151*</td>
<td>0.151*</td>
<td>0.302†</td>
<td>0.604‡</td>
</tr>
<tr>
<td>Week 2</td>
<td>0.114†</td>
<td>0.114†</td>
<td>0.114†</td>
<td>0.114†</td>
<td>0.114†</td>
<td>0.456§</td>
</tr>
<tr>
<td>Week 3</td>
<td>0.291§</td>
<td>0.291§</td>
<td>0.146‡</td>
<td>0.146‡</td>
<td>0.291§</td>
<td>0.582¶</td>
</tr>
<tr>
<td>Week 4</td>
<td>0.169§</td>
<td>0.169§</td>
<td>0.169§</td>
<td>0.169§</td>
<td>0.338¶</td>
<td>0.675~</td>
</tr>
</tbody>
</table>

SL = shell length; DSI = daily shell increment; WW = wet weight; SGR = specific growth rate; DW = dry weight; AFDW = ash-free dry weight.

*4.0;
†8.0;
‡16.0;
§32.0;
¶64.0;
~128.0
Figure 2.1. Mean (A) shell length (mm), (B) daily shell increment (μm d⁻¹), (C) wet weight (mg ind⁻¹), (D) specific growth rate, (E) dry weight (mg ind⁻¹), and (F) ash-free dry weight (% initial) of larger juvenile geoduck (*Panopea generosa*) reared at different temperatures (7, 11, 15, 19 °C). Different letters indicate significant differences (Tukey’s test, *P*<0.05) between weekly means. Error bars represent SE. *n* = 3 (except day 0 shell length, *n* = 50).
Figure 2.2. Mean (A) individual shell length (mm) and (B) daily shell increment (μm d⁻¹) of post-larval to juvenile geoduck (Panopea generosa) reared at different temperatures (7, 11, 15, 19 °C). Different letters indicate significant differences (Tukey’s test, \( P < 0.05 \)) between weekly means. Error bars represent SE. \( n = 3 \) (except day 0 shell length, \( n = 50 \)).
Figure 2.3. Mean (A) shell length (mm), (B) daily shell increment (μm d⁻¹), (C) wet weight (mg ind⁻¹), (D) specific growth rate, (E) dry weight (mg ind⁻¹), and (F) ash-free dry weight (% initial) of juvenile geoduck (Panopea generosa) fed different ration amounts (x10⁶ equivalent TISO cells ind⁻¹ d⁻¹) for the week 1 size class. Week 1 initial mean (± standard error) shell length: 2.34 ± 0.04 mm (n = 50); initial mean (± standard error) wet weight: 2.96 ± 0.15 mg ind⁻¹ (n = 3). Different letters indicate significant differences (Tukey’s test, P<0.05) between means. Error bars represent SE and n = 3. Black bars indicate the optimal ration level determined for that growth parameter.
Figure 2.4. Mean (A) shell length (mm), (B) daily shell increment (μm d⁻¹), (C) wet weight (mg ind⁻¹), (D) specific growth rate, (E) dry weight (mg ind⁻¹), and (F) ash-free dry weight (% initial) of juvenile geoduck (*Panopea generosa*) fed different ration amounts (x10⁶ equivalent TISO cells ind⁻¹ d⁻¹) for the week 2 size class. Week 2 initial mean (± standard error) shell length: 3.32 ± 0.03 mm (n = 50); initial mean (± standard error) wet weight: 6.73 ± 0.27 mg ind⁻¹ (n = 3). Different letters indicate significant differences (Tukey’s test, P<0.05) between means. Error bars represent SE and n = 3. Black bars indicate the optimal ration level determined for that growth parameter.
Figure 2.5. Mean (A) shell length (mm), (B) daily shell increment (μm d⁻¹), (C) wet weight (mg ind⁻¹), (D) specific growth rate, (E) dry weight (mg ind⁻¹), and (F) ash-free dry weight (% initial) of juvenile geoduck (*Panopea generosa*) fed different ration amounts (x10⁶ equivalent TISO cells ind⁻¹ d⁻¹) for the week 3 size class. Week 3 initial mean shell length: 4.13 ± 0.16 mm (n = 50); initial mean (± standard error) wet weight: 12.31 ± 0.16 mg geoduck⁻¹ (n = 3). Different letters indicate significant differences (Tukey’s test, P<0.05) between means. Error bars represent SE and n = 3. Black bars indicate the optimal ration level determined for that growth parameter.
Figure 2.6. Mean (A) shell length (mm), (B) daily shell increment (μm d⁻¹), (C) wet weight (mg ind⁻¹), (D) specific growth rate, (E) dry weight (mg ind⁻¹), and (F) ash-free dry weight (% initial) of juvenile geoduck (Panopea generosa) fed different ration amounts (x10⁶ equivalent TISO cells ind⁻¹ d⁻¹) for the week 4 size class. Week 4 initial mean shell length: 4.98 ± 0.16 mm (n = 50); initial mean (± standard error) wet weight: 21.23 ± 0.36 mg ind⁻¹ (n = 3). Different letters indicate significant differences (Tukey’s test, P<0.05) between means. Error bars represent SE and n = 3. Black bars indicate the optimal ration level determined for that growth parameter.
3 Feasibility of live algae dietary substitution with spray-dried *Schizochytrium* sp. or *Spirulina* (*Arthrospira platensis*) in the hatchery culture of juvenile Pacific geoduck clams (*Panopea generosa* Gould, 1850)

3.1 Introduction

Live algae costs and culture instability remain a critical restraint to economical bivalve hatchery production (Borowitzka, 1997; Coutteau and Sorgeloos, 1992; Guedes and Malcata, 2012). On an international scale, live microalgae production may occupy up to 30% of a bivalve hatchery’s operating costs (Coutteau and Sorgeloos, 1992). The culture sensitivity of the microalgae species common to hatchery production contributes to the high cost; these species require specialized, closed-containment environments that minimize contamination and maintain tight abiotic parameters for optimal growth (Borowitzka, 1997; Guedes and Malcata, 2012). Due to seasonal climate variation, these culture containers are often restricted to regulated indoor environments with strict temperature controls and artificial lighting; this maintenance generates high energy costs (Borowitzka, 1997; Guedes and Malcata, 2012).

The variability inherent in microalgae culture represents an additional constraint at all output levels. Unless maintained in an explicit axenic design, the closed culture environment is not sterile (Tredici, 2004) and will remain susceptible to pathogenic contamination, despite reduced risks (Önal *et al*., 2005; Robert and Trintignac, 1997). Contamination inhibits culture productivity and rapid proliferation of an introduced organism can overwhelm the targeted algal species (Richmond, 2004), triggering abrupt culture mortality (Robert and Trintignac, 1997).
Common hatchery expertise is often ill equipped to accommodate culture crashes (Borowitzka, 1997) delaying reestablishment of the required production levels. To safeguard against unexpected feed losses, sustained culture of a surplus algal supply ensures feed security, but further exacerbates the cost of microalgae production.

The high cost and insecurity of live microalgae production therefore urges the adoption of alternative bivalve diets. Over three decades ago, de Pauw (1981) targeted the replacement of live microalgae as a critical advancement in shellfish culture. This dated target remains unfulfilled, however, as live algae reliance persists as the norm in bivalve hatchery culture (Guedes and Malcata, 2012). Delayed realization of this goal supports the continued investigation of potential dietary substitutes and the attempted reduction in live algae usage remains a current trend in bivalve culture (Becker, 2004; Muller-Feuga, 2000; Spolaore et al., 2006). Live algae substitutes investigated in the bivalve literature have included: preserved microalgae (concentrated microalgae preserved through drying, refrigeration or freezing), bacteria and yeasts, encapsulated artificial diets, and dissolved nutrients (reviewed in Knauer and Southgate, 1999; reviewed in Marshall et al., 2010). These alternatives have produced varied growth success among bivalve life stages (larval, juvenile, and adult) and species (clams, mussels, oysters, and scallops) (reviewed by Knauer and Southgate, 1999; reviewed by Marshall et al., 2010), but the feasibility of live algae substitution has remained unexplored in geoduck culture. Identification of a dietary substitute that could elicit similar or enhanced growth when compared to a live algae diet would represent a significant advancement in geoduck hatchery culture and would further support the economic implementation and success of BC geoduck aquaculture.
Two dietary alternatives for potential integration in geoduck culture include: *Schizochytrium* (sp.) and *Spirulina* (*Arthrospira platensis*). *Schizochytrium* is a unicellular, heterotrophic thraustochytrid, closely related to heterokont algae (*i.e.* brown algae and diatoms) (Cavalier-Smith *et al.*, 1994; Lewis *et al.* 1999). *Schizochytrium*’s lipid content is high, and manipulation of its fermentation conditions can induce elevated docosahexaenoic acid (DHA) and n–6 docosapentaenoic acid (DPA) (Barclay *et al.*, 2005). In contrast, *Spirulina* is a multicellular, filamentous cyanobacteria (Habib *et al.*, 2008; Öthes and Pire, 2001). Compared to *Schizochytrium*, its lipid content is much reduced (reviewed in Habib *et al.*, 2008) and polyunsaturated fatty acids like eicosapentaenoic acid (EPA) or DHA are largely absent among strains (Öthes and Pire, 2001). Instead, its nutritional value is mainly derived from its high protein content (55–70% by dry weight) (Phang *et al.*, 2000). The dietary integration of *Schizochytrium* or *Spirulina* is recommended by their low cost, ready accessibility (Borowitzka, 1997; Langdon and Önal, 1999) and previously displayed success in the culture and nutrition of several bivalve species (Baubin, 2009; Boeing, 1997; Langdon and Önal, 1999; Önal *et al.*, 2005; Zhou *et al.*, 1991).

To examine the effect of live algae substitution on juvenile geoduck growth, this study replaced a live algae dietary control with graduated substitution levels (0 – 100%) of *Schizochytrium* (sp.) or *Spirulina* (*A. platensis*). A bialgal diet of *Chaetoceros muelleri* (CM) and *Isochrysis* sp. (Tahitian strain; TISO) combined in equal AFDW was used as positive control. Previous research indicates that this diet elicits superior geoduck growth throughout the juvenile stage compared to a variety of alternative monospecies and mixed species diets (W. Liu, unpublished data). Due to their divergence in nutritional content, the selection of *Schizochytrium* and *Spirulina* provided a wide nutritional range for the potential maintenance or improvement of
the live algae control. It was unknown how the supplementation or reduction of specific dietary components (incurred by the spray-dried replacement) would influence geoduck growth.

Due to potential variation in size dependent digestive ability (Tizon et al., 2013), substitution success was also evaluated in two size classes of juvenile geoduck (trial 1: mean initial shell length: 2.47 ± 0.03 mm; mean initial wet weight: 2.47 ± 0.10 mg ind⁻¹; trial 2: 4.00 ± 0.06 mm; 10.85 ± 0.10 mg ind⁻¹). The size dependent enzymatic variation demonstrated by Tizon et al. (2013) in the adult Angelwing clam (Pholas orientalis) indicated that increased shell size enhanced the digestive activity of proteases, α-amylase, agarase, and CM-cellulase. It was unknown if the size disparity would elicit a similar digestive response in geoduck and induce a differential growth response between size classes.

The following growth parameters were measured for each size class: shell length (mm), daily shell increment (DSI, µm d⁻¹), wet weight (mg ind⁻¹), specific growth rate (SGR), dry weight (mg ind⁻¹), and ash-free dry weight (AFDW, % initial). Survival rate (%) was also measured in both size classes. Proximate (crude protein, carbohydrate, lipid) and fatty acid analyses were conducted on the diets and geoduck tissue (excluding lipid tissue analysis) to elucidate the dietary influence on growth, tissue composition and sequestration activity.

3.2 Materials and methods

3.2.1 Experimental animal source and husbandry

Broodstock were from the same source as described in Chapter 2, Section 2.2.1.1 and broodstock husbandry followed the same protocols described in this section.
3.2.2 System monitoring

System monitoring adhered to the protocols described in Chapter 2, Section 2.2.1.3.

3.2.3 Growth parameters

The following growth parameters were determined for each trial: shell length (measured from the anterior to posterior axis) (mm), DSI (µm d⁻¹), wet weight (mg ind⁻¹), SGR, dry weight (mg ind⁻¹), and AFDW (% initial). Survival rate was calculated for both trials.

DSI, SGR, and survival were calculated with the following equations:

DSI (µm d⁻¹) = (final shell length – initial shell length) / T

SGR = [ln(W₂) – ln(W₁)] / T

Survival (%) = 100 – [(initial individuals – final individuals) / initial individuals x 100]

where, W₂ is the final wet weight (mg) of the geoduck seed, W₁ is the initial wet weight (mg), and T represents the duration of the experiment (d).

3.2.4 General conditions

The algae substitution experiment was sub-divided into two 14-d trial periods to examine the feasibility of live algae replacement in two juvenile size classes. Size classes were separated by a mean initial shell length of 1.53 mm. Both trials used a diet of Chaetoceros muelleri (CM) + Isochrysis sp. (Tahitian strain, TISO) (mixed by equivalent AFDW) as a control. Variant levels of Schizochytrium sp. or Spirulina (A. platensis) replaced (by AFDW) the live algae control in
eight treatments. For both species, the tested substitution levels were: 100/0%, 75/25%, 50/50%, 25/75%, and 0/100% (live algal diet/spray-dried diet).

Stock geoduck seed were maintained in a 300-l cylindro-conical tank for the duration of the two trials. During trial 1, stock geoduck were fed to satiation to ensure mean population growth matched treatment growth, so that when stocked, trial 2 geoduck would match the final treatment size of trial 1. Treatment geoduck were derived from this common rearing batch for the stocking of each trial. In trial 1, each treatment replicate was represented by 100 randomly-chosen geoduck juveniles. Two-hundred additional geoduck collected in triplicate were designated as the initial sample; 50 animals were measured to determine shell length, and each replicate (3) was batch weighed to determine wet weight. Individual geoduck wet weight (mg ind\(^{-1}\)) was calculated by: total group weight / individuals weighed (mean shell length ± SE: 2.47 ± 0.03 mm, \(n = 50\); mean individual wet weight ± SE: 2.47 ± 0.10 mg ind\(^{-1}\), \(n = 3\)). In trial 2, each treatment replicate was represented by 50 randomly-chosen geoduck juveniles. Fifty additional geoduck were collected in triplicate as the initial sample. Again, 50 animals were measured to determine shell length, and each replicate (3) was batch weighed to determine wet weight (mean shell length ± SE: 4.00 ± 0.06 mm, \(n = 50\); mean individual wet weight ± SE: 10.85 ± 0.10 mg ind\(^{-1}\), \(n = 3\)). These initial measurements were used in the calculation of treatment DSI and SGR.

Geoduck for each replicate were maintained in a PVC container (diameter x L: 10 x 25 cm) fitted with a 300-μm Nitex mesh bottom. PVC containers were held individually in 19-l plastic buckets filled with 18-l of 1-μm sand-filtered and UV-treated seawater. Buckets were covered with lids and gentle aeration was provided by an air stone (L x W 5.08 x 2.54 cm).
placed beneath the PVC container, which facilitated water exchange across the mesh bottom. Geoduck were cultured at 15 °C; to maintain treatment temperature, buckets were randomly distributed and held in 15 °C water bath tanks. Water exchange occurred every second day; feed delivery occurred daily. Buckets, lids, and air stones were rinsed with freshwater at each water exchange, and cleaned with 10% bleach solution between stocking intervals.

### 3.2.5 Live algal culture, density determination, and algal feeding

The live algae, CM (CCMP 1316) and TISO (CCMP 1324), were sourced from the Provasoli-Guillard National Center for Marine Algae and Microbiota (East Boothbay Harbor, Maine, USA). Live microalgae husbandry, feed delivery, and density determination adhered to the protocols given in Section 2.2.1.2. Bio-Marine Incorporated (Hawthorne, USA) supplied the spray-dried *Schizochytrium* sp. diet in the product form, AlgaMac-3050. The spray-dried *Spirulina* (*Arthrospira platensis*) diet was supplied by Brine Shrimp Direct (Ogden, USA).

Prior to daily feeding, 1-g of each spray-dried diet was suspended in 500-ml of 15 °C 1-µm sand filtered, UV-treated seawater, and pulsed in a Warring commercial blender for 2 min. The liquefied mixture was filtered through a 50-µm Nitex mesh to eliminate foam and larger debris particles and then diluted to 15-l with 1-µm sand filtered, UV-treated seawater at 15°C. This diluted suspension was used in feeding. Post homogenization, the mean (±SE) cell diameter of *Schizochytrium* sp. was 7.41 ± 0.27 µm and the mean length of a fragmented *Spirulina* cell was 25.20 ± 1.18 µm.

The initial ration amount used in each trial was determined by the previous feed ration experiment (Chapter 2, Section 2.3.3.2). This quantity was set to the optimal ration level.
(determined for wet weight growth) identified for the corresponding size class. Utilization of this size-specific ration attempted to eliminate the excessive provision of live algae. A live algae surplus could compensate for the spray-dried dietary replacement and indicate artificial substitution success at an inflated replacement level. In the first trial, an initial ration level of 4.0x10^6 equivalent TISO cells ind^{-1}d^{-1} was used (feed ration experiment, week 1; Section 2.3.3.2.1). This value was elevated to 6.0x10^6 equivalent TISO cells ind^{-1}d^{-1} on day 7 to accommodate geoduck growth. In the second trial, the initial ration level was established at 16.0x10^6 equivalent T-ISO cells ind^{-1}d^{-1} (feed ration experiment, week 2; Section 2.3.3.2.2) before shifting upwards to 19.0x10^6 cells ind^{-1}d^{-1} on day 7. In both trials, feed was withheld from all treatments on day 13 to allow for gut content purging prior to geoduck tissue analysis (24 h withdrawal).

3.2.6 Sampling

Sub-sampling for shell length and wet weight determination occurred weekly. At this time, empty shells were extracted and counted for each replicate to determine survival rate. For shell length and wet weight determination, 20 (trial 1) or 15 (trial 2) randomly-selected geoduck were destructively sampled from each replicate. At final sampling (day 14), all geoduck were removed from each replicate and counted. All samples were rinsed three times with 10-ml of 0.5-M ammonium formate for salt removal and transferred to a petri dish. Geoduck were photographed with a digital SLR camera (EOS Digital Rebel XSi, Canon, Meville, USA) mounted on a dissecting microscope. Images were later measured using the imaging software Motic Images Advanced 3.2 (Motic, Xiamen, China) to determine shell length (Trial 1: 20 ind replicate^{-1}; trial 2: 15 ind replicate^{-1}). Shell length (mm) was measured for each replicate and
then averaged within each replicate and treatment for each sampling period. To determine wet weight, geoduck were blotted on a paper towel and group weighed. Individual geoduck wet weight (mg ind\(^{-1}\)) was calculated (total group weight / individuals weighed) for each replicate and averaged within each replicate and treatment at each sampling period. Survival rate was calculated for each replicate and then averaged within each treatment for the full trial period. Sampled geoduck were placed in 50-ml centrifuge tubes and stored at -80 °C prior to lyophilization.

To determine dry weight, frozen geoduck were lyophilized for 48-h in a freeze dryer (FreeZone, Labconco, Kansas City, USA) and then re-weighed. Individual dry weight (mg ind\(^{-1}\)) was calculated (total group weight / individuals weighed) for each replicate and averaged within each treatment at sampling. To determine ash at the initial (3 ind replicate\(^{-1}\)), final (3 ind replicate\(^{-1}\)), and sub-sampled intervals (trial 1: 20 ind replicate\(^{-1}\); trial 2: 15 ind replicate\(^{-1}\)), lyophilized geoduck were weighed and transferred to a muffle furnace for 4-h at 500 °C and then re-weighed. AFDW was calculated for each replicate and then averaged within each replicate and treatment for each sampling period.

3.2.7 Algal dry weight and AFDW determination

To determine the cellular dry weight of CM and TISO, a known volume and density of live algae was filtered through a pre-weighed, pre-ashed, 55-mm diameter Whatman GF/A glass microfibre filter. Filters were rinsed three times with 5-ml of 0.5-M ammonium formate to remove salt, transferred to a 60 °C drying oven until a constant weight was achieved, and re-weighed. To determine ash, the dried filters were placed into a muffle furnace for 4 h at 500°C and then re-weighed.
To reflect cellular variation due to culture age, the AFDW \((n = 6)\) of each carboy replicate (3) was measured twice: once at 4 – 6 d post inoculation, and again at 7 – 8 d post inoculation. This time frame encompassed the algal culture age used in feeding, spanning from initial harvest at the late logarithmic growth stage to its final use, immediately prior to the stationary growth stage. Ash of the spray-dried diets was determined after the diets were weighed, inserted into the muffle furnace for 4 h at 500 °C, and then re-weighed \((n = 3)\).

3.2.8 Proximate analysis

3.2.8.1 Live algae preparation

Concentrations of live algae, collected from 3 replicate carboys, were centrifuged for 10 min at 3500 rpm (Sorvall Legend RT+Centrifuge, Thermo Scientific, Waltham, USA). To reflect composite cellular variation due to culture age, each carboy replicate was represented by 5 sub-replicates aged 4 – 6 d post carboy inoculation combined with 5 sub-replicates aged 7 – 8 d post carboy inoculation. Concentrated algae was frozen at -80 °C and then lyophilized for 48 h prior to proximate and fatty acid analysis.

3.2.8.2 Protein, carbohydrate, and lipid analysis

Protein analysis was conducted using a modified Lowry method (Lowry et al., 1951) with a DC Protein Assay kit (Bio-Rad, Hercules, USA). Prior to analysis, the concentrated and lyophilized live algae and spray-dried algae were suspended in 0.5-M NaOH and incubated in a 60 °C water bath for 1 h or until total cellular dissolution. Samples were cooled to room temperature and vortexed immediately before analysis. Preparation of whole lyophilized geoduck followed the same procedure, except samples were crushed in 0.5-M NaOH solution.
prior to incubation. Three replicates for each algal species and one replicate for each geoduck treatment replicate \((n = 3 \text{ treatment}^{-1})\) were analyzed.

Carbohydrate analysis of algae and geoduck tissue used the phenol-sulfuric acid method (Dubois et al., 1956). Prior to analysis, all samples were suspended in 10 ml of 0.5-M \(H_2SO_4\) and hydrolyzed in a 90 °C water bath for 3 h. Samples were cooled to room temperature and vortexed immediately prior to analysis. Three replicates for each algal species and one replicate for each geoduck treatment replicate \((n = 3 \text{ treatment}^{-1})\) were analyzed.

Due to limited sample availability, geoduck tissue was excluded from lipid analysis. Lipid extraction (1:2; \(CHCl_3:MeOH\)) and quantification followed the Bligh and Dyer (1959) method. Samples were centrifuged at 3000 rpm for 5 min (Megafuge 2.0 R, Heraeus Instruments, Hanau, Germany) to enhance lipid separation. Each algae species was represented by 3 replicate samples.

### 3.2.8.3 Fatty acid analysis

FAMEs of algae and geoduck tissue were prepared using the method given by Abdulkadir and Tsuchiya (2008). Three replicates were prepared for each algae species, while geoduck in each feeding replicate were represented by an individual FAME replicate \((n = 3 \text{ treatment}^{-1})\). Nonadecanoic acid (19:0) (Nu-Chek Prep, Inc., Elysian, USA) was used as the internal standard. Following the recommendation of Christie (1973), 30 mg of BHT was added to 400 ml of the hexane solvent to prevent lipid oxidation.

FAMEs were quantified using a Varian 3900 Gas Chromatograph (Agilent Technologies, Santa Clara, USA) with a Chrompack capillary column (CP-Sil 88 for FAME; length x diameter:...
50 m x 0.25 mm; film thickness: 0.25 µm). Samples were injected using the Varian Autosampler (Model: 8400) (Agilent Technologies, Santa Clara, USA) and the CP-1177 injector (Agilent Technologies, Santa Clara, USA). The CP-1177 injector was maintained at 270 °C. An injection volume of 1.0 µl was used for standards and algae samples while an injection volume of 5.0 µl was used for geoduck samples. A split ratio of 10:1 was used. Hydrogen was used as the carrier gas at a constant flow rate of 1.0 ml min\(^{-1}\). The initial oven temperature was held at 80 °C for 1 min. Oven temperature was increased to 160 °C at 15 °C min\(^{-1}\), elevated to 220 °C at 4 °C min\(^{-1}\), and held at 220 °C for 5 min (total run time: 26.33 min). The flame ionization detector operated at 270 °C, with a 25 ml min\(^{-1}\) nitrogen make-up flow, 30 ml min\(^{-1}\) hydrogen flow, and 300 ml min\(^{-1}\) air flow. Data were collected and analyzed using Varian Star Chromatography Workstation 6.41 software (Agilent Technologies, Santa Clara, USA). Standards obtained from Nu-Chek Prep, Inc. were used for peak identification. The following standards were used: GLC-428, GLC-443, GLC-455, GLC-480, GLC-642, and GLC-643.

A signal-to-noise ratio of 5:1 was applied with the noise level monitored before each run. Fatty acid values are given as a percentage of the total detected fatty acid content (comprised of both identified and unidentified values). Total unidentified fatty acid content varied from 4.63 to 18.80 (% total fatty acid) for algae analysis with the mean (± SE) unidentified content equalling 9.45 ± 1.45% (\(n = 12\)). In the geoduck tissue analysis the total unidentified content ranged from 18.38 to 41.49% in trial 1 (mean ± SE: 26.95 ± 1.13%, \(n = 30\)) and from 14.65 to 31.65% in trial 2 (mean ± SE: 21.79 ± 0.93%, \(n = 29\)).
3.2.9 Statistics

Two-way ANOVAs followed by Tukey’s multiple comparison test ($P<0.05$) were used in both trials to examine the effect of algae type ($n = 12$), substitution level ($n = 3$), and their interaction ($n = 3$) on growth parameters (final shell length, DSI, wet weight, SGR, dry weight, and AFDW) and tissue composition (final proximate content and select final fatty acid content). To maintain an orthogonal design, 0% substitution values required duplication in each two-way ANOVA (i.e. the same three replicates served as data for both 0% *Spirulina* and 0% *Schizochytrium* sp. treatments). Substitution levels across algae species were pooled ($n = 6$) if the effect of algal type and/or interaction was not significant (two-way ANOVA, $P>0.05$). Due to its repeated occurrence as an outlier ($P<0.05$, Grubb’s test, $n = 7$), a 75% *Spirulina* replicate (trial 2) was removed in fatty acid tissue analyses. Its removal facilitated the passage of equal variance ($P>0.05$, Levene’s Test).

Variation in the cellular biomass (AFDW) and biochemical composition (protein, carbohydrate, lipid, and select fatty acids) between alga l species was examined with a one-way ANOVA followed by Tukey’s multiple comparison test ($P<0.05$) if significant variation was detected. If the test for equal variance (Levene’s test; see failed tests below) failed ($P<0.05$), the Welch ANOVA followed by the Tamhame test were applied ($P<0.05$). These tests do not assume equal variance among treatments.

Kolmogorov-Smirnov and Levene’s tests ($P<0.05$) were used to examine the normality of distribution and homogeneity of variance of each tested parameter, respectively. To ensure normality, the following data transformations were required in trial 1 for geoduck tissue analysis: power ($x^2$; $18:2n – 6$), and square root ($x^{1/2}$; DPA). To ensure equal variance, the following data
transformations were required in trial 1: reciprocal (1/x; shell length), square root (x^{1/2}; DSI), and cube root (x^{1/3}; total tissue fatty acid). To ensure equal variance, the following data transformations were required in trial 2 for geoduck tissue analysis: power (x^2; carbohydrate, EPA, and ΣMUFA).

Despite various data transformations, normality could not be achieved for the following geoduck analyses (two-way ANOVA) in trial 1: wet weight, SGR, dry weight, Σn – 6 PUFA, and 18:3n – 6. Normality (two-way ANOVA) could not be achieved for carbohydrate, total fatty acid, 20:5n – 3 (EPA), and ΣMUFA in trial 2. In trial 2, ΣSAT failed equal variance (two-way ANOVA). Two-way ANOVAs were continued despite failures in normality or equal variance. For algal comparisons, the following parameters failed the equal variance test: AFDW, 20:5n – 3 (EPA), 22:6 n – 3 (DHA), ΣSAT, ΣMUFA, Σn – 3 PUFA. The Welch ANOVA followed by the Tamhame test was applied for these comparisons.

Mean dietary composition of proximate content, selected fatty acids, and PUFA proportions were also correlated against final geoduck tissue composition in both trials by regression analysis. Algal treatments (Schizochytrium and Spirulina) were separated if regression slopes differed significantly (P<0.05); if non-significant (P>0.05) algal treatments were pooled into one regression. If a component was non-detectable, a value of zero was assumed for the regression analysis.

Statistical analyses (regressions excluded) were performed with the statistical software SigmaPlot 12.5 (Systat Software Inc., San Jose, USA). Regression analyses were performed with the statistical software, GraphPad Prism 6.02 (GraphPad Software, Inc., La Jolla, USA).
3.3 Results

3.3.1 System monitoring

Mean temperature and pH (± SD) for the full trial period, pooled across all treatments and trials measured: 15.82 ± 0.19 °C (n = 33,379) and 8.15 ± 0.14 (n = 324), respectively. Mean (± SD) ambient salinity and pH of the seawater source were 27.73 ± 0.38‰ (n = 27) and 7.93 ± 0.05 (n = 12), respectively.

3.3.2 Geoduck growth

In trial 1 with the smaller juveniles, the interaction effect between algal type and substitution level was not significant for the six measured growth parameters. The main effect of algal type was significant for: shell length, DSI, SGR, and dry weight (Fig 3.1, A–B, D–E). Spirulina treatments exceeded the Schizochytrium treatments for these parameters. The main effect of substitution level was significant in all cases – increased dietary inclusion of either spray-dried species (Schizochytrium or Spirulina) induced a general geoduck growth decline in all measured parameters (Fig. 3.1 A–F). For shell length, DSI (Schizochytrium treatments only), wet weight, and dry weight, growth significantly decreased beyond the 25% substitution level while DSI (Spirulina treatments only) SGR and AFDW significantly declined beyond the 50% level (Fig. 3.1 A–F).

In trial 2 with the larger juveniles, the interaction and main effect of algal type was non-significant for wet weight, SGR, and AFDW, while the interaction term was significant for shell length, DSI, and dry weight. Again, the main effect of substitution level was significant in all cases – increased dietary inclusion of either spray-dried species induced a general geoduck
growth decline in all measured parameters (Fig. 3.2 A–F). When examining the effect of algal type within substitution level, a significant dietary effect was detected for shell length, DSI, and dry weight at the 75% level, and at 50% for dry weight. *Spirulina* exceeded *Schizochytrium* in all cases except at 50% substitution (Fig. 3.2 A–B, E). Geoduck displayed a significant growth decline beyond the 0% substitution level for shell length, DSI, wet weight, and dry weight (Fig. 3.2 A–C, E). A significant decline was displayed beyond the 25% level for SGR (Fig. 3.2 D) and beyond the 75% level for AFDW (Fig. 3.2 E). Survival rate remained high in trials 1 (98.3 – 100.0%) and 2 (99.3 – 100.0%) and did not significantly differ among treatments.

### 3.3.3 Algal biochemical composition

Results of algal biochemical analyses are presented in table 3.1. The mean algal protein content among species ranged between 9.7 and 50.9% (% of AFDW) with content lowest in *Schizochytrium* and highest in *Spirulina*. Carbohydrate composition ranged between 15.4 and 32.2% with *Schizochytrium* containing the highest amount. Lipid content displayed the widest variation among algal species (6.2 – 60.4%), and was highest in *Schizochytrium* and lowest in *Spirulina*.

*Spirulina* contained non-detectable levels of EPA, DHA, AA, and n – 6 DPA, with its mean $\sum n – 3$ PUFA content (0.2% TFA) the lowest among the algal species, and significantly reduced compared to CM or *Schizochytrium*. Mean individual fatty acid levels (%TFA) of EPA (9.9%) and AA (1.1%) were maximized in CM, and significantly exceeded the other three species. *Schizochytrium* contained significantly maximized levels of DHA (21.1%) and n – 6 DPA (8.3%). Content of 18:2n – 6 ranged between non-detectable (*Schizochytrium*) and 14.8% (*Spirulina*); 18:3n – 6 content varied between 0.3% (*Schizochytrium*) and 12.7% (*Spirulina*);
these fatty acids significantly maximized in *Spirulina*. In cumulative content, the live algae species (CM and TISO) displayed significantly elevated $\sum$MUFA levels (15.6 – 44.1%) compared to the spray-dried species (1.0 – 10.0%), with $\sum$MUFA highest in CM and lowest in *Schizochytrium*. In contrast, the mean $\sum n$ – 6 PUFA content of *Schizochytrium* and *Spirulina* (9.3 – 27.5%) significantly exceeded both live algae species (3.2 – 5.4%). This content was highest in *Schizochytrium*. Mean $\sum$SAT content was also significantly elevated in *Schizochytrium* (61.8%) compared to all other species (45.0 – 53.0%). The mean proximate, energetic, and fatty acid composition of the mixed experimental diets are given in Table 3.2. Energy values were estimated using the following proximate conversions: protein and carbohydrate: 3.99 kcal g$^{-1}$; lipid: 8.94 kcal g$^{-1}$.

### 3.3.4 Geoduck biochemical composition

#### 3.3.4.1 Trial 1 with smaller juveniles

##### 3.3.4.1.1 Mean proximate content

Mean proximate content of trial 1’s smaller juveniles is given in table 3.3 for *Schizochytrium* treatments and in 3.4 for *Spirulina* treatments. Protein and carbohydrate tissue content were significantly affected by substitution level, but not algae type. The effect of algal type and substitution level was significant for total fatty acid composition, with a significant interaction between the main effects.

All three components displayed a general decline with increased spray-dried substitution level. Protein composition ranged between 10.5 and 16.0% (% dry weight). This content did not vary significantly between 0 and 75% substitution, but significantly declined at 100%
substitution. Geoduck carbohydrate content was significantly elevated at 0 – 25% substitution (2.5 – 3.1%) compared to 75 – 100% substitution (1.3 – 1.9%). Mean fatty acid tissue content ranged between 0.7 and 1.9%. Schizochytrium treatments significantly exceeded Spirulina treatments in cumulative fatty acid content. Signification variation between the Schizochytrium treatments and the 0% substitution level occurred at 75 and 100% Schizochytrium substitution; geoduck fatty acid content was significantly reduced at these levels. Fatty acid content of Spirulina-fed geoduck significantly declined beyond the 25% substitution level and was minimized at 100% Spirulina substitution. A significant dietary effect was detected at 50 and 75% substitution with Schizochytrium exceeding Spirulina at these levels.

3.3.4.1.2 Mean relative fatty acid content (%TFA)

The mean relative fatty acid composition (%TFA) of trial 1 geoduck tissue is given in table 3.3 for Schizochytrium and 3.4 for Spirulina. Statistical analyses were performed on selected fatty acids: EPA, DHA, 18:2n – 6, 18:3n – 6, AA, n – 6 DPA, ∑SAT, ∑MUFA, ∑n – 3 PUFA, and ∑n – 6 PUFA. Algal type induced a significant treatment effect in all tested fatty acids, and the significant effect of substitution level was detected in: EPA, DHA, 18:2n – 6 (Spirulina only), AA, n – 6 DPA (Schizochytrium only), ∑SAT, ∑MUFA, ∑n – 3 PUFA, and ∑n – 6 PUFA. A significant interaction effect between algal type and substitution level occurred in tissue levels of EPA, DHA, 18:2n – 6, 18:3n – 6, n – 6 DPA, ∑SAT, and ∑n – 6 PUFA.

Between algal types, DHA, AA, n – 6 DPA, ∑SAT, ∑n – 3 PUFA, and ∑n – 6 PUFA were significantly elevated in Schizochytrium-fed geoduck. In contrast, EPA, 18:2n – 6, 18:3n – 6, and ∑MUFA content displayed the opposite trend; these fatty acids were significantly depressed in Schizochytrium treatments compared to Spirulina.
Fatty acids AA, ∑MUFA, and ∑n – 3 PUFA exhibited significant variation between substitution levels, but did not exhibit a significant interaction effect between main effects. Relative AA tissue composition generally increased with elevated spray-dried inclusion (Schizochytrium and Spirulina); tissue levels significantly maximized between 75 – 100% substitution (2.2 – 2.8%; However, tissue levels did not significantly differ between 25 and 75% substitution). Cumulative MUFA content was maximized at 0% substitution (20.5%), before displaying a general decline with elevated Schizochytrium or Spirulina substitution. Cumulative n – 3 PUFA content remained relatively constant among substitution levels. This content minimized at 100% substitution (8.8 – 12.2%), but did not vary between 0 – 25% substitution. In contrast, ∑n – 6 PUFA content maximized between 75 – 100% substitution (5.6 – 8.2%), but did not significantly vary from 0 to 50% substitution.

In the fatty acid tissue content that displayed a significant interaction between main effects (EPA, DHA, 18:2n – 6, 18:3n – 6, n – 6 DPA, ∑SAT, and ∑n – 6 PUFA), Spirulina treatments contained significantly elevated EPA, 18:2n – 6, and 18:3n – 6. Schizochytrium treatments contained significantly elevated DHA, n – 6 DPA, ∑SAT, and ∑n – 6 PUFA. At 25 – 100% substitution, the relative tissue content of EPA and 18:2n – 6 was significantly higher in Spirulina-fed geoduck. Geoduck EPA (7.7 – 8.1%) and 18:2n – 6 (0.3 – 0.9%) content did not display significant variation between 0 and 50% Spirulina substitution, while Schizochytrium treatments exhibited a reduction in tissue EPA beyond 0% substitution. The fatty acid 18:2n – 6 did not significantly vary between Schizochytrium treatments. At 50 – 75% substitution, tissue content of 18:3n – 6 (0.8 – 0.9%) was significantly elevated in Spirulina treatments, but this fatty acid did not significantly vary between substitution levels of either algal species.
For DHA, n–6 DPA, and ∑n–6 PUFA, *Schizochytrium* treatments significantly exceeded *Spirulina* treatments between 25 and 100% substitution. Cumulative SAT content in *Schizochytrium*-fed geoduck exceeded *Spirulina* treatments between 50–100% substitution. Significant tissue variation for DHA, n–6 DPA, and ∑n–6 PUFA did not emerge between algal substitution levels for *Spirulina* treatments, but levels were significantly elevated at 25–100% *Schizochytrium* substitution compared to the 0% level. Fatty acid content significantly maximized at 50–75% (DHA: 8.6–11.1%), 50–75% (n–6 DPA: 4.1–4.5%), and 25–100% substitution (∑n–6 PUFA: 6.7–8.2%). Cumulative SAT content significantly declined at 100% substitution in *Spirulina* treatments, but elevated with *Schizochytrium* replacement. At 50–100% substitution cumulative SAT tissue composition was significantly elevated in *Schizochytrium* treatments, with content maximized (46.5–47.9%) at 75–100% *Schizochytrium* substitution.

**3.3.4.2 Trial 2 with larger juveniles**

**3.3.4.2.1 Mean proximate content**

The mean proximate content for trial 2 (larger juveniles) tissue content is given in table 3.5 for *Schizochytrium* and 3.6 for *Spirulina*. The significant effect of algal type was limited to fatty acid composition. Protein and total fatty acid content of geoduck tissue were significantly affected by substitution level and a significant interaction effect was also detected in cumulative fatty acid composition.

Cumulative fatty acid content was significantly elevated in *Schizochytrium* treatments, and with the exception of this treatment (*Schizochytrium* fatty acid content), all proximate
components displayed a general decline with increased spray-dried substitution. Mean protein composition ranged between 13.3 and 15.2% but did not display a significant variation between 0 and 75% substitution. Mean carbohydrate levels varied between 1.6 and 2.7%, but were not significantly different among the substitution levels. Compared to *Spirulina* treatments, cumulative fatty acid tissue content was significantly elevated at 25 – 100% *Schizochytrium* substitution. Fatty acid composition did not significantly vary between 0 and 100% *Schizochytrium* substitution, but displayed a general decline with increased *Spirulina* substitution.

### 3.3.4.2.2 Mean relative fatty acid content (% TFA)

Relative mean fatty acid content (% TFA) of trial 2 geoduck tissue is given in table 3.5 for *Schizochytrium* and 3.6 for *Spirulina*. Again, statistical analyses were performed on the selected fatty acids: EPA, DHA, 18:2n – 6, 18:3n – 6, AA, n – 6 DPA, ΣSAT, ΣMUFA, Σn – 3 PUFA, and Σn – 6 PUFA. Algal type was a significant source of variation in EPA, DHA, AA, n – 6 DPA, ΣMUFA, Σn – 3 PUFA, and Σn – 6 PUFA, content. Significance in substitution level and the interaction between main effects was detected for all fatty acid comparisons.

Between algal types, the fatty acids: DHA, n – 6 DPA, Σn – 3 PUFA, and Σn – 6 PUFA were significantly elevated in *Schizochytrium* treatments; fatty acids: EPA, AA, and ΣMUFA were significantly elevated in *Spirulina*-fed geoduck. For interaction comparisons, DHA, n – 6 DPA, ΣSAT, Σn – 3 PUFA, and Σn – 6 PUFA were significantly elevated in *Schizochytrium*-fed geoduck. Fatty acids: EPA, 18:2n – 6, 18:3n – 6, AA, and ΣMUFA were significantly higher in *Spirulina* treatments.
At 25 – 100% substitution, tissue content of DHA, n – 6 DPA, $\sum$SAT, and $\sum n$ – 6 PUFA was significantly higher in Schizochytrium treatments compared to Spirulina and at 75% substitution, Schizochytrium’s $\sum n$ – 3 PUFA content was significantly elevated. The fatty acids: DHA (11.9%), n – 6 DPA (5.1%), $\sum n$ – 3 PUFA (16.4%), and $\sum n$ – 6 PUFA (8.3%) maximized at 75% Schizochytrium substitution, and significantly exceeded all other treatments at 50 – 100% substitution. Cumulative SAT tissue content significantly maximized at 100% Schizochytrium substitution (52.6%). In contrast, Spirulina-fed geoduck exhibited elevated EPA (25 – 50% substitution), 18:2n – 6 (25 – 100% substitution), 18:3n – 6 (100% substitution), AA (50 – 100% substitution), and $\sum$MUFA (25 – 50% substitution), and significantly exceeded Schizochytrium treatments at the above substitution levels. Fatty acids: 18:2n – 6, 18:3n – 6, and AA displayed a general tissue elevation with increased spray-dried substitution, with 18:3n – 6 (1.4%) and AA (3.2%) significantly maximized at 100% Spirulina substitution. EPA content (7.7 – 8.1%) maximized between 0 – 50% Spirulina substitution, while $\sum$MUFA content was significantly maximized between 0 – 25% Spirulina substitution (18.8 – 20.3%).

3.3.5 Selected dietary and tissue correlation

3.3.5.1 Trial 1 with smaller juveniles

Regression analyses of selected dietary components and final mean tissue composition in smaller juveniles indicated that significant ($P<0.05$) variation between algal treatments (Schizochytrium and Spirulina) occurred in the tissue sequestration of: protein, carbohydrate, total fatty acid, EPA, $\sum$SAT, and $\sum n$ – 3 PUFA (Fig. 3.3 A–D, H, J). Algal species displayed an inverse relationship in protein, total fatty acid, $\sum$SAT, and $\sum n$ – 3 PUFA tissue accumulation. Tissue protein and $\sum$SAT significantly declined with elevated dietary inclusion in Spirulina.
treatments (Fig. 3.3 A, H). Schizochytrium treatments displayed the opposite trend, but the ∑SAT correlation was insignificant. In contrast, tissue levels of total fatty acid and ∑n – 3 PUFA significantly increased with elevated dietary content in Spirulina fed geoduck, but declined for Schizochytrium treatments (Fig. 3.3 C, J). The Schizochytrium correlation was significant for total fatty acid, but insignificant for ∑n – 3 PUFA.

Tissue levels of carbohydrate and EPA exhibited the same accumulation trend between algal types, but significantly varied in regression slope (Fig. 3.3 B, D). Carbohydrate tissue content significantly declined with increased dietary inclusion in both algal species, but the decline was accelerated in Spirulina-fed geoduck (Spirulina slope: -0.035; Schizochytrium slope: -0.011; Fig. 3.3 B). In contrast, tissue EPA significantly elevated with increased dietary content in both algal treatments, but a steeper slope was displayed for Schizochytrium-fed geoduck (1.23) than Spirulina-fed individuals (0.65) (Fig. 3.3 D). R² values for these regressions ranged between 0.051 (Schizochytrium: ∑n – 3 PUFA) and 0.91 (Schizochytrium: EPA) (Fig. 3.3 A–B, D, H, J).

The remaining regressions did not exhibit significant variation between algal species. With the exception of AA content, pooled regressions indicated the general elevation of tissue content with dietary input (Fig. 3.3 E, G, I, K–L). R² values of these regressions ranged from 0.25 to 0.91, with ∑MONO and ∑n – 6 PUFA correlations exhibiting the highest R² values (0.91 and 0.89, respectively; Fig. 3.3 C, E–G, I, K–L). AA content exhibited the reverse trend; tissue content declined with elevated dietary inclusion (Fig. 3.3 F).
3.3.5.2 Trial 2 with larger juveniles

In the larger juveniles, significant variation between algal types occurred in the mean tissue accumulation of: protein, total fatty acid EPA, DHA, n – 6 DPA, \( \sum SAT \), and \( \sum n – 6 \) PUFA (Fig. 3.4 A, C–E, G–H, K). An inverse relationship between algae species was displayed in protein, total fatty acid, DHA, n – 6 DPA, and \( \sum SAT \) tissue content (Fig. 3.4 A, C, E, G, H). With the exception of total fatty acid, tissue levels of these components were positively correlated with elevated dietary content in *Schizochytrium*-fed geoduck, while increased dietary input suppressed the tissue content in *Spirulina* treatments. Total fatty acid significantly increased with dietary inclusion in *Spirulina*-fed geoduck, but did not significantly vary between *Schizochytrium* treatments (Fig 3.4 C). *Spirulina* protein and n – 6 DPA correlations were also non-significant (Fig. 3.4 A, G). For EPA and \( \sum n – 6 \) PUFA tissue content, algal treatments exhibited a similar accumulation trend, but regressions significantly differed in slope. The *Schizochytrium* treatment exhibited a steeper slope in the EPA regression (Schizochytrium slope: 1.07; Spirulina slope: 0.36), while the *Spirulina* treatment displayed accelerated \( \sum n – 6 \) PUFA tissue accumulation (Schizochytrium slope: 0.11; Spirulina slope: 0.80) (Fig. 3.4 D, K). \( R^2 \) values ranged between 0.16 (*Schizochytrium*: total fatty acid) and 0.93 (*Schizochytrium*: EPA and \( \sum SAT \)) (Fig. 3.4 A, D–E, H, K).

The remaining regressions did not exhibit significant variation between algal species and displayed a general fidelity between dietary input and resultant tissue composition, with the repeated exception of carbohydrate and AA (Fig. 3.4 B, F, I– J, L). Elevated \( \sum \) MONO, and \( \sum n – 3/n – 6 \) PUFA dietary content significantly correlated with increased tissue content with the \( R^2 \) values of these regressions ranging between 0.41 (\( \sum n – 3/n – 6 \) PUFA) and 0.86 (\( \sum \) MONO) (Fig
3.4 G, L). The $\sum n - 3$ PUFA correlation was not significant (Fig 3.4 J). In contrast, carbohydrate and AA tissue composition again exhibited a significant decline with increased dietary input; with $R^2$ values of 0.19 (carbohydrate) and 0.87 (AA) (Fig 3.4 B, F).

3.4 Discussion

3.4.1 Prior spray-dried success

Spray-dried substitution incited a negative growth effect in both geoduck size classes; increased dietary departure from the live algae control resulted in an incremental growth decline, with the slowest growth occurring at complete live algae substitution. This detrimental growth effect largely refutes null hypotheses 4 (section 1.9.2) and 5 (section 1.9.3). However, the corresponding alternative hypotheses predicted a significant growth effect would occur only up to 50% substitution. The significant growth suppression induced beyond 25% (trial 1), and 0% (trial 2) substitution, indicates inaccuracy in both the null and alternative hypotheses (section 1.9.2.; 1.9.3), and rejects 50% substitution as the threshold level of dietary significance.

The results also indicate that the dietary modifications incurred by Schizochytrium or Spirulina substitution failed to elicit the same synergistic growth effects incurred in previous studies (Baubin, 2009; Jaime-Ceballos et al., 2006; Langdon and Önal, 1999). In these studies, enhancement of the live algae diet through Schizochytrium (Baubin, 2009), Spirulina (Jaime-Ceballos et al., 2006), or Schizochytrium/Spirulina (Langdon and Önal, 1999) inclusion significantly increased bivalve (Baubin 2009; Langdon and Önal, 1999) and shrimp (Jaime-Ceballos et al., 2006) growth beyond levels achievable with exclusive live algae provision.
However, these authors failed to identify the nutritional value of their live algae control and it is possible the growth enhancement resulted from the spray-dried improvement of a sub-optimal control diet. In these experiments, diets comprised of a singular algal species were selected as nutritional controls (Baubin, 2009; Jaime-Ceballos et al., 2006; Langdon and Önal, 1999). However, hatchery protocol often dissuades the monospecific utilization of a singular algal species, as mixed diets are often required to achieve adequate EPA and DHA content (Helm and Bourne 2004; reviewed in Marshall 2010). Bivalves demonstrate the limited ability to synthesize these critical PUFAs de novo, so EPA and DHA must be supplied through the diet (Langdon and Waldock, 1981). The dietary inclusion of these fatty acids is associated with elevated bivalve growth (Langdon and Waldock, 1981; Enright et al., 1986 a, b), indicating nutritional significance. The spray-dried integration likely buffered inadequacy in the live algae’s EPA or DHA content, promoting the resultant growth enhancement.

Baubin’s (2009) utilization of an ambient tank bloom species (*Thalassiosira nordenskioeldii*) as the control species in *C. gigas* culture further emphasizes potential nutritional inadequacy in controls selected for dietary replacement. This species is absent from common hatchery usage (Brown *et al.*, 1997; Helm and Bourne, 2004), while previous research recommends the mixed diet: *Isochrysis affinis galbana* and *Chaetoceros calcitrans forma pumilum* for elevated *C. gigas* (larval) growth (Rico-Villa *et al.*, 2006).

### 3.4.2 Live algae biochemical composition

Therefore, unlike the previous studies, the extant superiority of the CM+TISO control diet likely restricted the dietary enhancement available through spray-dried supplementation. Compared to the alternative diets, this superior diet (0% substitution) was characterized by its
moderate protein, carbohydrate, lipid, energy, DHA, n–6 DPA, and Σn–3 PUFA content, its elevated EPA, AA, ΣMUFA, and Σn–3/Σn–6 PUFA content, and reduced ΣSAT and Σn–6 PUFA content.

Of these components, multiple studies indicate that the dietary Σn–3/Σn–6 requirement may be conserved among bivalve species (Enright et al., 1986b; Milke et al., 2006; Webb and Chu, 1983). Escalation of dietary Σn–6 PUFA relative to Σn–3 content often coincides with suppressed growth performance (Albentosa et al., 1994; Enright et al., 1986b; Glencross et al., 2002), and nutritionally superior diets often contain n–3/n–6 ratios between 2.0 and 3.33 (Enright et al., 1986b; Milke et al., 2006; Webb and Chu, 1983).

The present study supported these dietary trends; Σn–3/Σn–6 content maximized in the superior CM+TISO diet (2.9), and then displayed an incremental decline with elevated spray-dried replacement. The worst performing diets (100% Schizochytrium or Spirulina substitution) contained maximal (relative to each spray-dried species) Σn–6 content (100% Schizochytrium: 27.5% TFA; 100% Spirulina: 9.3% TFA) and minimal Σn–3/Σn–6 content (100% Schizochytrium: 0.8; 100% Spirulina: non-detectable). Cumulative n–3/n–6 values similar to 2.9 are associated with improved growth in the bivalve literature (Enright et al., 1986b; Milke et al., 2006; Webb and Chu, 1983). Webb and Chu (1983) indicated algal diets sufficient for larval C. virginica contained dietary Σn–3/Σn–6 ratios between 2.0 and 3.33; and Enright et al. (1986b) recommended dietary Σn–3/Σn–6 content should exceed 2.0 in juvenile O. edulis culture. The best performing diets tested by Milke et al. (2006) in post larval and juvenile A. irradians culture contained an Σn–3/Σn–6 ratio between 2.1 and 3.3. This repeated consistency
suggests the dietary $\sum_{n = 3/n}^{6}$ requirement is conserved among bivalve species, and may be an important nutritional consideration in future geoduck studies.

### 3.4.3 Variation between size classes

In the smaller juveniles, live algae replacement beyond 25% substitution depressed growth in all measured indices, except AFDW. The larger size class exhibited enhanced dietary sensitivity to dried algae substitution. Elevations in dried algae inclusion incrementally reduced geoduck growth at all substitution levels (25 – 100%) for all growth parameters (except AFDW). Diminished tolerance to the dried algae was possibly incurred by an ontogenetic shift in nutritional requirements. The high energy requirement for tissue growth (Bayne and Hawkins, 1997) was likely enhanced in the larger size class due to the pronounced siphon and mantle development that accompanies this size range (see Chapter 2, Section 2.4.2). It is possible this development shifted nutritional resources from shell growth, resulting in the decline of all growth parameters, but AFDW. The nutritional degradation that accompanied spray-dried substitution likely could not fulfill the larger geoduck’s cumulative energetic requirements and resulted in the augmented growth decline. This growth decline also indicates the unlikelihood that larger geoduck exhibit the enhanced digestive activity documented in other larger bivalves (Langton and Gabbott, 1974; Tizon et al., 2013).

### 3.4.4 Variation between spray-dried species

Between spray-dried species, *Spirulina* treatments consistently accelerated geoduck growth compared to *Schizochytrium* diets, despite the species’ general n – 3 (EPA, DHA) and n – 6 (DPA, AA) PUFA deficiency. This growth inequality emerged in trial 1 for growth parameters
of: shell length, DSI, SGR, and dry weight. This inequality also occurred in trial 2; the *Spirulina* treatment significantly exceeded the *Schizochytrium* diet in shell length, DSI, and dry weight at 75% substitution.

In dietary contrast, *Schizochytrium* contained significantly elevated levels of the nutritionally emphasized DHA, compared to all algal species. However, Glencross and Smith (2001) indicated excessive dietary DHA can inhibit growth in the prawn, *Penaeus monodon*, and recommended 4% (TFA) as the optimal inclusion level. *Schizochytrium* diets exceeded this recommendation at all substitution levels (8.6 – 21.1%), while the full live algae diet and *Spirulina* DHA treatment content ranged between non-detectable (100% *Spirulina*) and 4.5 (0% substitution). This may indicate *Schizochytrium*’s elevated PUFA content exceeded geoduck nutritional requirements, and possibly contributed to the growth disparity. The spray-dried variation also indicates that indiscriminate PUFA elevation does not incite an automatic growth benefit, and cautions the sole consideration of these fatty acids in future nutritional and growth analyses.

### 3.4.5 Causes of geoduck growth decline

The generalized growth declined associated with spray-dried substitution likely resulted from the nutritional degradation that accompanied *Schizochytrium* or *Spirulina* integration. The diminished nutritional value of the spray-dried diets may be attributed to factors such as: biochemical composition, cellular size, toxicity, and digestibility (Webb and Chu, 1983).

Dietary biochemical composition demonstrated the greatest nutritional divergence from the live algae diet at full spray-dried substitution. Complete *Schizochytrium* replacement resulted
in the relative escalation of total carbohydrate, total lipid, energy, DHA, n – 6 DPA, ΣSAT, Σn – 3 PUFA, and Σn – 6 PUFA, and the depression of protein, EPA, AA, ΣMUFA, and Σn – 3/n – 6 PUFA. *Spirulina* substitution induced the elevation of total protein, total carbohydrate, ΣSAT, and Σn – 6 PUFA, but depressed total lipid, ΣMUFA, and Σn – 3/n – 6 PUFA content. Complete *Spirulina* replacement also caused EPA, DHA, AA, n – 6 DPA, and Σn – 3 PUFA content to drop to undetectable levels. This enhancement and/or reduction of specific dietary components likely tipped dietary content from the “optimal” biochemical levels (*i.e.* Σn – 3/n – 6 PUFA ratio) present in the CM+TISO diet. The incremental departure from this “optimal” baseline likely created the graduated growth decline that occurred with spray-dried substitution, and culminated in the maximal growth reduction displayed at complete substitution.

Cellular size influences ingestion capacity and efficiency (reviewed in Knauer and Southgate, 1999), and a general size range of 3 – 20 µm is recommended for juvenile and adult bivalves (Defossez and Hawkins, 1997; Haven and Morales-Alamo, 1970; Langdon and Siegfried, 1984). The cellular length of CM (4 – 9 µm), TISO (4 – 8 µm) (NCMA 2013a, b), and the diameter of *Schizochytrium* sp. (mean ± SE: 7.41 ± 0.27 µm) adhered to this range, while *Spirulina*’s mean length (±SE) (25.2 ± 1.18 µm) exceeded it. With the exception of dry weight accumulation in trial 2 (50% substitution), *Schizochytrium*’s reduced cellular size did not promote a beneficial growth response between dried algae types, while the accelerated growth in *Spirulina* treatments (discussed above) suggests the increased particle size did not inhibit growth, despite the size similarity between CM, TISO, and *Schizochytrium*, the growth suppression induced by *Schizochytrium* substitution further suggests cellular size did not incur a major growth effect. Barring the presence of toxic metabolites, examination of final geoduck tissue
composition does suggest that variation in geoduck digestion capacity. This potential variation may have contributed to further divergences in dietary nutritional value.

3.4.6 Digestion

In the current study, it is speculated that the incomplete digestion of spray-dried derived protein and carbohydrate occurred. These components displayed a general disconnect between dietary input and final geoduck composition. Regression correlations indicated that elevated dietary protein content significantly correlated with depressed tissue levels in *Spirulina* treatments, but *Schizochytrium*-fed geoduck displayed the opposite trend. Due to variation in dietary content, the increased protein content was spray-dried derived in *Spirulina* treatments, but live-algae derived in *Schizochytrium* diets. The contrast suggests that the protein’s source (spray-dried vs. live) dictated its digestive capacity and resulted in the reciprocal assimilation trend. Despite its dietary elevation, tissue carbohydrate also demonstrated an incremental decline with dietary content in trial 1 and 2’s regressions. In both algal treatments, spray-dried derived carbohydrate contributed to the dietary elevation that correlated with the tissue decline.

Baubin (2009) further indicated that the exclusive provision of *Schizochytrium* (Alga-Mac 3050 or Roti-Mac) corresponded with the absence of digestive proteases (< 53 kilodaltons) in juvenile *C. gigas* tissue post 60 d of dietary exposure. These individuals displayed significantly depressed growth compared to alternative treatments (Baubin, 2009). Digestive content of the starved individuals (60 d) demonstrated an identical protease deficiency, which suggests the spray-dried diet did not support protein digestion or assimilation and culminated in the production of protein starved individuals, indistinguishable from the starved control (Baubin, 2009). The presence of protease activity in the oysters fed dietary mixtures of *Schizochytrium*
and TISO indicates the potential requirement of live microalgae to initiate protease secretion in bivalves (Baubin, 2009). In the present study, the unvaried tissue protein content between 0 and 75% substitution levels, suggested dietary live algae triggered geoduck protease secretion and digestion, despite reductions in its inclusion amount. Only the complete absence of dietary live algae (100% substitution), incurred significant suppression in protein accumulation under the ANOVA comparison.

The importance of protein sequestration in bivalve growth is emphasized by Bayne and Hawkins (1997) who indicated protein deposition incurred the majority of tissue growth (60 – 72% of total DW) in *M. galloprovincialis*. Bayne (2000) further demonstrated Sydney rock oysters (*Saccostrea commercialis*) selected for high growth (increased feeding, reduced metabolic costs) also displayed elevated protein growth and deposition. The potential inaccessibility of spray-dried derived protein in the present study may have inhibited the tissue deposition required for geoduck growth, contributing to the spray-dried growth reduction.

The value of elevated dietary carbohydrate is debated in bivalve nutrition, and its nutritional utility is often demonstrated to be dependent on concurrent protein availability (Castell and Trider, 1974; Enright *et al*., 1986b; Marin *et al*., 2003). Its nutritional value rests in its utility as an energy or storage molecule (Marin *et al*., 2003; Whyte *et al*., 1989), permitting dietary-derived protein and lipid to be diverted to biosynthetic processes (Whyte *et al*., 1989). Potential inhibition of its assimilation (incurred by its spray-dried source, and inadequacy in accessible protein content), may have resulted in the potential mobilization of dietary protein and lipid for energetic compensation, limiting biosynthesis and growth (Marin *et al*., 2003; Whyte *et al*., 1989).
3.4.7 Geoduck tissue fatty acid composition

Cumulative fatty acid content demonstrated variant assimilation between *Schizochytrium* and *Spirulina* treatments. In trial 1, *Schizochytrium*-fed geoduck demonstrated a reciprocal relationship between dietary and tissue content, while *Spirulina* treatments exhibited a positive correlation between factors. Increased lipid content was spray-dried derived in *Schizochytrium* treatments, which may indicate difficulty in cumulative lipid assimilation in trial 1. However, in trial 2, cumulative fatty acid content did not significantly vary between *Schizochytrium* substitutions, and significantly exceeded the *Spirulina* diets (live-algae derived lipid) between 25 and 100% substitution.

In the correlation of selected fatty acids, both trials and algal species demonstrated general fidelity between dietary input and final tissue composition, which in contrast to protein and carbohydrate, suggests successful digestion and assimilation of individual fatty acids. In trial 1, the fatty acids: EPA, DHA, n – 6 DPA, ∑MUFA, ∑n – 6 PUFA, and ∑ n – 3/n – 6 PUFA demonstrated a significant and positive correlation between components. R² values for these correlations ranged between 0.51– 0.91. In trial 2, the fatty acids: EPA, DHA (*Schizochytrium* only), n – 6 DPA (*Schizochytrium* only), ∑SAT (*Schizochytrium* only), ∑MUFA, ∑n – 6 PUFA, and ∑ n – 3/n – 6 PUFA also demonstrated a significant and positive correlation between components. These R² values ranged from 0.41 to 0.93.

The dietary elevation of spray-dried derived fatty acids also coincided with enhanced geoduck tissue content in both relative (%TFA) and absolute (mg g⁻¹ DW) terms. This further suggests the digestion and integration of spray-dried lipid. For example, *Schizochytrium* contained maximal (P<0.05) DHA and n–6 DPA dietary content among algal species. Tissue
DHA and n–6 DPA content (%TFA) maximized at 75% \textit{Schizochytrium} substitution in both trial divisions, and significantly exceeded the live algae (0% substitution), and \textit{Spirulina} treatments at all substitution levels. Absolute fatty acid content (mg g\(^{-1}\) DW) of DHA and n–6 DPA also elevated in \textit{Schizochytrium} fed geoduck. These values exceeded the absolute fatty acid composition of the initial sample, full live algae, and 75% \textit{Spirulina} treatments between 1.5 – 4.6 fold (DHA), and 3.4 – 8.0 fold (DPA) (unpublished data).

In contrast, \textit{Spirulina} contained maximal (P<0.05) 18:2n–6 and 18:3n–6 dietary content and the relative levels of these fatty acids significantly elevated in \textit{Spirulina} exposed geoduck in trials 1 and 2. In trial 1, the absolute 18:2n–6 tissue content in 100% \textit{Spirulina} geoduck exceeded the 100% \textit{Schizochytrium} treatment by 2.2 fold (unpublished data), while tissue 18:3n–6 content remained undetectable at full \textit{Schizochytrium} substitution. This differential became more pronounced in trial 2, as the absolute (mg g\(^{-1}\) DW) 18:2n–6 and 18:3n–6 tissue content of 100% \textit{Spirulina} geoduck exceeded the complete \textit{Schizochytrium} treatment by 5.4 fold and 3.3 fold, respectively (unpublished data).

3.4.7.1 Geoduck tissue EPA and AA content

The selective sequestration of EPA and AA in \textit{Spirulina}-fed geoduck did, however, exhibit a notable exception to the general fatty acid correlation between dietary and tissue content. \textit{Spirulina} contained undetectable levels of EPA and AA; its elevated inclusion therefore induced an incremental decline in dietary EPA and AA until both were undetectable at complete substitution. Despite dietary reduction or deficiency, relative EPA (trials 1 and 2) and AA (trials 1 and 2) tissue content significantly elevated in \textit{Spirulina} fed geoduck compared to \textit{Schizochytrium} treatments, which contained dietary EPA and AA at all substitution levels. EPA
dietary and tissue correlations also indicated significant slope variation existed between
Schizochytrium and Spirulina treatments. Relative AA tissue content further maximized with
complete (100%) Spirulina substitution in trial 2, and significantly exceeded the tissue content of
all alternative treatments. AA tissue content also displayed a significant negative correlation with
dietary content in trials 1 and 2, and maximized at the lowest dietary level.

However, examination of absolute fatty acid tissue content indicated the absolute decline
of EPA and AA (mg g⁻¹ DW) in Spirulina exposed tissue (unpublished data). This suggests
goduck modulated and conserved relative fatty acid content, but did not alter absolute tissue
composition through fatty acid synthesis. The formation of EPA and AA occurs through the
elongation and desaturation of the precursors, \(\alpha\) linolenic (18:3n – 3) and linoleic (18:2n – 6)
acid, respectively (Tocher, 2003). These precursors were present in all Spirulina diets, but the
absolute decline in tissue EPA and AA, suggests their dietary inclusion did not induce PUFA
synthesis. This corroborates with other bivalve research, that indicated (via radioactive labeling)
absent or limited PUFA synthesis in adult C. virginica (Chu and Greaves, 1991); the yellow
clam, Mesodesma mactroides (de Moreno et al., 1976), and juvenile C. gigas (Waldock and
Holland, 1984).

The selective retention of AA is a common trait exhibited in starved bivalves (Caers et
al., 1998, 1999; Coutteau et al., 1996; Knauer and Southgate, 1997b,c; Pirini et al., 2007). It is
speculated the regulatory importance of AA derived eicosanoids induces its sequestration in
periods of nutritional duress (Caers et al. 1998, 1999). Eicosanoids represent a wide collection of
regulatory signal molecules and in invertebrates, modulate key functions like ionic transport,
immunological responses, and induction of egg production and spawning (reviewed in Stanley-
Samuelson, 1987; 1994). Eicosanoids are derived from both EPA and AA; those that are generated from AA are more biologically active, and incur an inflammatory effect, while EPA products (anti-inflammatory) modulate and dampen activity (Arts and Kohler, 2009; Sargent et al., 1999; Tocher, 2003). PUFA starved individuals in the full Spirulina treatment likely sequestered AA to maintain endocrine processes during dietary privation.

Bivalves also demonstrate the capacity to regulate EPA assimilation, independent of dietary content (Delaporte et al., 2005; Pirini et al., 2007). The relative increased retention of EPA that accompanied dietary Spirulina inclusion and subsequent AA assimilation possibly modulated the AA conversion. Due to its elevated bioactivity, the disproportionate provision of dietary AA can incite excessive inflammatory activity and possibly induce detrimental physiological effects (Okuyuma et al., 1996; Sargent et al., 1999). The EPA elevation possibly ensured eicosanoid production did not induce excessive inflammatory activity (Arts and Kohler, 2009; Sargent et al., 1999; Tocher, 2003). It is possible the collective modulation of EPA and AA maintained growth in Spirulina treatments, and promoted growth comparable or better than Schizochytrium treatments, despite PUFA reduction or exclusion.

### 3.4.8 Geoduck tissue composition

The varied dietary influence on final geoduck tissue composition, inhibited the predictive quality of hypothesis 6 (section 1.9.5), which predicted a positive significant correlation would exist between proximate components in the diet, select dietary fatty acids and subsequent geoduck tissue composition. As discussed, protein content significantly and positively correlated with tissue composition in Schizochytrium treatments, but negatively correlated in Spirulina treatments. Carbohydrate tissue content displayed a significant negative correlation with dietary
content in both spray-dried species, but fatty acid content (especially for *Schizochytrium* treatments) exhibited general fidelity between dietary input and resultant tissue composition. Dietary influence was therefore dependent on the biochemical component and potentially, the geoduck’s differential digestive and sequestration ability.

### 3.5 Conclusions

In general, geoduck growth declined with increased *Schizochytrium* or *Spirulina* substitution. These findings do not support the alternative or null hypotheses given in section 1.9.2 and 1.9.3, which designated 50% substitution as the threshold of dietary significance. Spray-dried integration also did not promote a beneficial growth effect, as demonstrated in previous studies. It is possible the selection of a sub-optimal control diet, resulted in the growth enhancement that occurred with the spray-dried substitution in these studies. In contrast, the positive, superior live algae control used in the present experiment likely restricted additional dietary improvement available through spray-dried substitution.

This superior live algae diet (*Chaetoceros muelleri*, and *Isochrysis* sp. TISO mixed by AFDW) contained moderate protein, carbohydrate, lipid, energy, DHA, n – 6 DPA, and Σn – 3 PUFA content, elevated EPA, AA, ΣMUFA, and Σn – 3/Σn – 6 PUFA content, and reduced ΣSAT and Σn – 6 PUFA content compared to the spray-dried species. This diet was also characterized by a Σn – 3/Σn – 6 PUFA ratio of 2.9, which may be a dietary requirement conserved among bivalve species. Despite the exclusion of important dietary PUFAs (*i.e.* EPA, DHA, n – 6 DPA, and AA), *Spirulina* treatments also consistently outperformed *Schizochytrium* diets, despite the latter’s inclusion of these fatty acids. It is speculated excessive dietary DHA
was associated with the growth decline in *Schizochytrium* and promoted the growth disparity between spray-dried treatments.

The biochemical inadequacy of the spray-dried diets, coupled with the digestive difficulty of specific proximate component possibly promoted the dietary degradation that occurred with live algae replacement. The enhancement or reduction of dietary components through spray-dried substitution likely skewed dietary content from the “optimal" biochemical levels present in the CM+TISO diet, and resulted in the incremental growth decline that maximized at 100% *Schizochytrium* or *Spirulina*. Dietary and tissue correlations also indicate the potential digestive inaccessibility of spray-dried derived protein and carbohydrate, which possibly augmented the growth degradation that accompanied spray-dried substitution.

However, fatty acid dietary and tissue correlations, did demonstrate general fidelity between dietary input and resultant tissue content. This may indicate the successful digestion of spray-dried derived fatty acids. The relative and absolute tissue elevation of DHA and n–6 DPA that occurred with increased dietary content in *Schizochytrium* treatments, along with the 18:2n – 6 and 18:3n – 6 elevation that occurred *Spirulina* treatments further suggests dietary assimilation.

EPA and AA sequestered in *Spirulina*-fed geoduck demonstrated a notable exception to the positive correlations exhibited for other fatty acids, and indicated the selective retention of EPA and AA. This accumulation possibly compensated for the dietary EPA/AA reduction or exclusion that accompanied *Spirulina* substitution. EPA and AA represent important precursors to regulatory signal molecules, the eicosanoids. Despite its general PUFA deficiency, this selective modulation possibly contributed to treatment growth maintenance, promoting the growth of *Spirulina*-fed geoduck above the levels achieved by dietary *Schizochytrium*. 
Due to variation in assimilation, the overall results do not completely support hypothesis 6 (section 1.9.5), which predicted a positive significant correlation would exist between dietary components and subsequent geoduck tissue composition. Resultant assimilation success appeared dependent on the biochemical component, perhaps indicating variant digestive ability.

The economic benefit of a successful live algae substitute encourages continued investigation of dietary alternatives. It is recommended that selected live algae replacements mirror the superior CM+TISO diet in nutritional composition, as the dietary variation incurred by *Schizochytrium* or *Spirulina* substitution did not benefit growth. Concurrent digestibility studies should be applied with growth trials, to further elucidate the digestive accessibility of spray-dried components. If digestive enzymes cannot be activated under exclusive spray-dried provision, then the dietary inaccessibility may render growth suppression, despite similarity to the live algae diet. Overall, the study’s cumulative uncertainty cautions the integration of *Schizochytrium* and *Spirulina* in geoduck culture as further investigations into dietary value and geoduck nutritional and digestive requirements are warranted.
Table 3.1. Cellular biomass and biochemical properties (mean ± SE) of the live (*Chaetoceros muelleri*, *Isochrysis* sp. Tahitian strain) and spray-dried (*Schizochytrium* sp., *Spirulina*) algal species used in experimental substitution diets. Within rows, different letter superscripts indicate significant differences between algae species (*n* = 6, AFDW; *n* = 3, all other parameters (ANOVA test followed by Tukey’s test or Welch ANOVA followed by Tamhane test, *P* < 0.05)). Applied tests are summarized in the statistical analysis column; tests in bold were significant. n.d. = non-detectable. – indicates statistical comparisons were not performed.

<table>
<thead>
<tr>
<th></th>
<th>C. muelleri</th>
<th>Isochrysis sp.</th>
<th>Schizochytrium sp.</th>
<th>Spirulina</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell Biomass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFDW (% dry weight)</td>
<td>Welch</td>
<td>75.0 ± 5.9^a</td>
<td>92.6 ± 0.4^b</td>
<td>91.2 ± 0.0^b</td>
</tr>
<tr>
<td>AFDW (pg cell^-1)</td>
<td>ANOVA</td>
<td>16.8 ± 1.0</td>
<td>16.0 ± 0.8</td>
<td>-</td>
</tr>
<tr>
<td><strong>Proximate Composition (% AFDW)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>ANOVA</td>
<td>28.9 ± 1.5^a</td>
<td>46.2 ± 1.8^b</td>
<td>9.7 ± 0.7^c</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>ANOVA</td>
<td>18.5 ± 2.3^a</td>
<td>15.4 ± 1.8^a</td>
<td>32.2 ± 1.9^b</td>
</tr>
<tr>
<td>Lipid</td>
<td>ANOVA</td>
<td>24.5 ± 1.1^a</td>
<td>34.8 ± 2.9^b</td>
<td>60.4 ± 0.8^c</td>
</tr>
<tr>
<td><strong>Fatty Acid Composition (% of total fatty acid)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>-</td>
<td>16.2 ± 3.1</td>
<td>35.6 ± 3.0</td>
<td>22.1 ± 0.3</td>
</tr>
<tr>
<td>15:0</td>
<td>-</td>
<td>1.2 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.8 ± 0.0</td>
</tr>
<tr>
<td>16:0</td>
<td>-</td>
<td>26.6 ± 2.5</td>
<td>10.2 ± 0.2</td>
<td>38.4 ± 0.2</td>
</tr>
<tr>
<td>17:0</td>
<td>-</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>18:0</td>
<td>-</td>
<td>0.9 ± 0.2</td>
<td>0.1 ± 0.0</td>
<td>0.5 ± 0.0</td>
</tr>
<tr>
<td>14:1</td>
<td>-</td>
<td>0.2 ± 0.0</td>
<td>0.7 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>15:1</td>
<td>-</td>
<td>0.6 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td>0.4 ± 0.0</td>
</tr>
<tr>
<td>16:1</td>
<td>-</td>
<td>30.9 ± 1.9</td>
<td>3.0 ± 0.4</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>17:1</td>
<td>-</td>
<td>2.1 ± 0.4</td>
<td>0.6 ± 0.0</td>
<td>n.d.</td>
</tr>
<tr>
<td>18:1n - 9(t)</td>
<td>-</td>
<td>4.9 ± 0.1</td>
<td>0.4 ± 0.3</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>18:1n - 9</td>
<td>-</td>
<td>0.9 ± 0.1</td>
<td>8.0 ± 1.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>20:1n - 9</td>
<td>-</td>
<td>0.3 ± 0.0</td>
<td>2.9 ± 0.4</td>
<td>n.d.</td>
</tr>
<tr>
<td>22:1n - 9</td>
<td>-</td>
<td>n.d.</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>18:3n - 3</td>
<td>-</td>
<td>n.d.</td>
<td>5.6 ± 0.4</td>
<td>n.d.</td>
</tr>
<tr>
<td>20:5n - 3 (EPA)</td>
<td>Welch</td>
<td>9.9 ± 0.7^a</td>
<td>0.6 ± 0.1^b</td>
<td>1.3 ± 0.0^c</td>
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<tr>
<td>22:5n - 3 (DPA)</td>
<td>-</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>22:6n - 3 (DHA)</td>
<td>Welch</td>
<td>0.8 ± 0.1^a</td>
<td>8.2 ± 1.3^a</td>
<td>21.1 ± 0.3^c</td>
</tr>
<tr>
<td>18:2n - 6</td>
<td>ANOVA</td>
<td>0.5 ± 0.0^a</td>
<td>2.9 ± 0.0^b</td>
<td>n.d.</td>
</tr>
<tr>
<td>18:3n - 6</td>
<td>ANOVA</td>
<td>2.6 ± 0.2^a</td>
<td>0.4 ± 0.0^b</td>
<td>0.3 ± 0.0^b</td>
</tr>
<tr>
<td>20:4n - 6 (AA)</td>
<td>ANOVA</td>
<td>1.1 ± 0.2^a</td>
<td>0.6 ± 0.5^b</td>
<td>0.5 ± 0.0^b</td>
</tr>
<tr>
<td>22:5n - 6 (DPA)</td>
<td>ANOVA</td>
<td>n.d.</td>
<td>1.9 ± 0.4^a</td>
<td>8.3 ± 0.1^b</td>
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<td>ΣSAT</td>
<td>Welch</td>
<td>45.0 ± 4.6^a</td>
<td>46.5 ± 3.2^a</td>
<td>61.8 ± 0.4^b</td>
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<tr>
<td>ΣMUFA</td>
<td>Welch</td>
<td>44.1 ± 3.4^a</td>
<td>15.6 ± 0.2^b</td>
<td>1.0 ± 0.1^c</td>
</tr>
<tr>
<td>Σn – 3 PUFA</td>
<td>Welch</td>
<td>10.3 ± 0.6^a</td>
<td>14.6 ± 1.6^a,b,c</td>
<td>22.7 ± 0.3^b</td>
</tr>
<tr>
<td>Σn – 6 PUFA</td>
<td>ANOVA</td>
<td>3.2 ± 0.9^a</td>
<td>5.4 ± 0.4^a</td>
<td>27.5 ± 0.2^c</td>
</tr>
</tbody>
</table>

Statistical Analysis: Welch ANOVA, ANOVA.
Table 3.2. Proximate, energetic, and fatty acid composition of each experimental diet arranged by increasing dried algae inclusion. Values were calculated from the means determined for individual algae species, adjusted for substitution level and algal composition. The 0% substitution level is represented by a full live algae diet of *Chaetoceros muelleri* and *Isochrysis* sp. (Tahitian strain) mixed in equal AFDFWs; *Schizochytrium* (Sch.) or *Spirulina* (Sp.) replaced this live bi-algal diet at variant inclusion levels (25 – 100%). Energy values were estimated using the following proximate conversions: protein and carbohydrate: 3.99 kcal g⁻¹; lipid: 8.94 kcal g⁻¹ n.d. = non-detectable.

<table>
<thead>
<tr>
<th></th>
<th>0% Sch.</th>
<th>0% Sp.</th>
<th>25% Sch.</th>
<th>25% Sp.</th>
<th>50% Sch.</th>
<th>50% Sp.</th>
<th>75% Sch.</th>
<th>75% Sp.</th>
<th>100% Sch.</th>
<th>100% Sp.</th>
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<tbody>
<tr>
<td><strong>Dietary Proximate Composition (mg g⁻¹ AFDW)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>375.7</td>
<td>305.9</td>
<td>409.0</td>
<td>236.1</td>
<td>442.4</td>
<td>166.3</td>
<td>475.7</td>
<td>96.5</td>
<td>509.1</td>
<td></td>
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<tr>
<td>Carbohydrate</td>
<td>176.0</td>
<td>212.5</td>
<td>186.4</td>
<td>249.1</td>
<td>196.8</td>
<td>285.6</td>
<td>207.2</td>
<td>322.1</td>
<td>217.6</td>
<td></td>
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<tr>
<td>Lipid</td>
<td>296.9</td>
<td>373.7</td>
<td>238.1</td>
<td>450.6</td>
<td>179.4</td>
<td>527.5</td>
<td>120.6</td>
<td>604.3</td>
<td>61.9</td>
<td></td>
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<tr>
<td>Total Fatty Acid</td>
<td>165.3</td>
<td>217.3</td>
<td>145.6</td>
<td>269.4</td>
<td>126.0</td>
<td>321.5</td>
<td>106.3</td>
<td>373.6</td>
<td>86.7</td>
<td></td>
</tr>
</tbody>
</table>

|                  |     |        |          |         |          |         |          |         |           |          |
| **Dietary Energy (cal g⁻¹ AFDW)** |     |        |          |         |          |         |          |         |           |          |
| Total Energy     | 4.9 | 5.4    | 4.5      | 6.0     | 4.2      | 6.5     | 3.8      | 7.1     | 3.5        |          |

|                  |     |        |          |         |          |         |          |         |           |          |
| **Fatty Acid Composition (% of total fatty acid)** |     |        |          |         |          |         |          |         |           |          |
| 14:0             | 25.9| 24.9   | 19.5     | 24.0    | 13.1     | 23.0    | 6.7      | 22.1    | 0.2        |          |
| 15:0             | 0.8 | 0.8    | 0.8      | 0.8     | 0.7      | 0.8     | 0.7      | 0.8     | 0.7        |          |
| 16:0             | 18.4| 26.5   | 23.4     | 34.6    | 28.4     | 42.7    | 33.4     | 50.8    | 38.4       |          |
| 17:0             | 0.2 | 0.2    | 0.2      | 0.1     | 0.3      | 0.1     | 0.3      | 0.1     | 0.3        |          |
| 18:0             | 0.5 | 0.5    | 0.6      | 0.5     | 0.7      | 0.5     | 0.8      | 0.5     | 0.9        |          |
| 14:1             | 0.4 | 0.3    | 0.4      | 0.2     | 0.3      | 0.2     | 0.2      | 0.1     | 0.2        |          |
| 15:1             | 0.5 | 0.5    | 0.9      | 0.4     | 1.4      | 0.4     | 1.8      | 0.4     | 2.2        |          |
| 16:1             | 16.9| 12.8   | 14.2     | 8.6     | 11.5     | 4.4     | 8.7      | 0.3     | 6.0        |          |
| 17:1             | 1.4 | 1.0    | 1.1      | 0.7     | 0.9      | 0.3     | 0.7      | n.d.    | 0.4        |          |
| 18:1n – 9(t)     | 2.6 | 2.1    | 2.0      | 1.5     | 1.3      | 0.9     | 0.7      | 0.3     | n.d.       |          |
| 18:1n – 9        | 4.5 | 3.4    | 4.0      | 2.3     | 3.5      | 1.2     | 3.0      | 0.1     | 2.6        |          |
| 20:1n – 9        | 1.6 | 1.2    | 1.2      | 0.8     | 0.8      | 0.4     | 0.4      | n.d.    | n.d.       |          |
| 22:1n – 9        | n.d.| 0.1    | n.d.     | 0.1     | n.d.     | 0.1     | n.d.     | 0.1     | n.d.       |          |
| 18:3n – 3        | 2.8 | 2.1    | 2.2      | 1.4     | 1.5      | 0.7     | 0.8      | n.d.    | 0.2        |          |
| 20:5n – 3 (EPA)  | 5.2 | 4.3    | 3.9      | 3.3     | 2.6      | 2.3     | 1.3      | 1.3     | n.d.       |          |
| 22:5n – 3 (DPA)  | n.d.| 0.1    | n.d.     | 0.2     | n.d.     | 0.2     | n.d.     | 0.3     | n.d.       |          |
| 22:6n – 3 (DHA)  | 4.5 | 8.6    | 3.4      | 12.8    | 2.2      | 16.9    | 1.1      | 21.1    | n.d.       |          |
| 18:2n – 6        | 1.7 | 1.3    | 5.0      | 0.9     | 8.2      | 0.4     | 11.5     | n.d.    | 14.8       |          |
| 18:3n – 6        | 1.5 | 1.2    | 4.3      | 0.9     | 7.1      | 0.6     | 9.9      | 0.3     | 12.7       |          |
| 20:4n – 6 (AA)   | 0.8 | 0.7    | 0.6      | 0.7     | 0.4      | 0.6     | 0.2      | 0.5     | n.d.       |          |
| 22:5n – 6 (DPA)  | 1.0 | 2.8    | 0.7      | 4.6     | 0.5      | 6.4     | 0.2      | 8.3     | n.d.       |          |
| ΣSAT             | 45.8| 49.8   | 47.6     | 53.8    | 49.4     | 57.8    | 51.2     | 61.8    | 53.0       |          |
| ΣMUFA            | 29.9| 22.7   | 24.9     | 15.5    | 19.9     | 8.3     | 14.9     | 1.0     | 10.0       |          |
| Σn – 3 PUFA      | 12.5| 15.0   | 9.4      | 17.6    | 6.3      | 20.1    | 3.3      | 22.7    | 0.2        |          |
| Σn – 6 PUFA      | 4.3 | 10.1   | 5.5      | 15.9    | 6.8      | 21.7    | 8.0      | 27.5    | 9.3        |          |
| Σn – 3/Σn – 6 PUFA| 2.9 | 1.5    | 1.7      | 1.1     | 0.9      | 0.9     | 0.4      | 0.8     | n.d.       |          |
Table 3.3. Trial 1 initial (day 0) and final (day 14) proximate and fatty acid composition of juvenile geoduck tissue (mean ± SE). The 0% substitution level is represented by a full live algae diet of *Chaetoceros muelleri* and *Isochrysis* sp. (Tahitian strain) mixed at equal AFDW; *Schizochytrium* (Sch.) replaced this live bi-algal diet at variant inclusion levels (25 – 100%). Total fatty acid (% dry weight) is represented by total measured content. Two-way ANOVAs (**P**<0.05) were used to determine the effect of algae type (A), substitution level (S), and their interaction (AxS) on final tissue composition. Detection of a significant interaction effect between dietary algae species and substitution level (n = 3) is denoted by *. Different letters within rows indicate significant differences between substitution levels (two-way ANOVA, Tukey’s test, **P**<0.05, n = 3). Substitution levels across algae species were pooled (n = 6) if the effect of algal type and/or interaction was not significant (**P**>0.05). These results are summarized in the statistical analysis column. Letters (A, S, AxS) in bold indicate that parameter induced a significant treatment effect.

<table>
<thead>
<tr>
<th>Statistical Analysis</th>
<th>Initial</th>
<th>0%</th>
<th>25% Sch.</th>
<th>50% Sch.</th>
<th>75% Sch.</th>
<th>100% Sch.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximate Composition (% DW)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein A, S, AxS</td>
<td>14.0 ± 0.6</td>
<td>15.7 ± 0.6a</td>
<td>15.4 ± 0.3a</td>
<td>14.2 ± 1.0a</td>
<td>12.9 ± 0.9a</td>
<td>10.5 ± 0.4b</td>
</tr>
<tr>
<td>Carbohydrate A, S, AxS</td>
<td>2.1 ± 0.1</td>
<td>3.1 ± 0.2a</td>
<td>2.5 ± 0.1ab</td>
<td>2.5 ± 0.1bc</td>
<td>1.9 ± 0.2d</td>
<td>1.3 ± 0.1d</td>
</tr>
<tr>
<td>Total Fatty Acid A, S, AxS</td>
<td>1.3 ± 0.0</td>
<td>1.9 ± 0.2a</td>
<td>1.7 ± 0.0a</td>
<td>1.6 ± 0.1ab</td>
<td>1.4 ± 0.1b</td>
<td>0.8 ± 0.0c</td>
</tr>
<tr>
<td><strong>Fatty Acid Composition (% of total fatty acid)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0 -</td>
<td>2.6 ± 0.1</td>
<td>5.8 ± 2.6</td>
<td>3.0 ± 0.15</td>
<td>3.7 ± 0.7</td>
<td>2.6 ± 0.4</td>
<td>0.8 ± 0.8</td>
</tr>
<tr>
<td>15:0 -</td>
<td>0.7 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.8 ± 0.0</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>16:0 -</td>
<td>21.1 ± 0.3</td>
<td>22.4 ± 1.6</td>
<td>26.5 ± 1.4</td>
<td>28.6 ± 1.0</td>
<td>33.6 ± 1.4</td>
<td>32.4 ± 0.7</td>
</tr>
<tr>
<td>17:0 -</td>
<td>0.2 ± 0.0</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>0.4 ± 0.3</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>18:0 -</td>
<td>8.4 ± 0.0</td>
<td>8.0 ± 0.5</td>
<td>8.8 ± 0.0</td>
<td>8.8 ± 0.5</td>
<td>10.0 ± 0.4</td>
<td>11.4 ± 0.9</td>
</tr>
<tr>
<td>20:0 -</td>
<td>0.5 ± 0.0</td>
<td>n.d</td>
<td>0.3 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>15:1 -</td>
<td>2.0 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>0.6 ± 0.0</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>16:1 -</td>
<td>10.4 ± 0.1</td>
<td>14.3 ± 1.6</td>
<td>11.6 ± 0.2</td>
<td>8.4 ± 0.5</td>
<td>4.7 ± 0.2</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>18:1n-9 -</td>
<td>5.3 ± 0.1</td>
<td>3.7 ± 0.3</td>
<td>3.4 ± 0.1</td>
<td>3.0 ± 0.1</td>
<td>2.7 ± 0.0</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>20:1n-9 -</td>
<td>0.6 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0.4 ± 0.1</td>
<td>n.d</td>
</tr>
<tr>
<td>18:3n-3 -</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>20:5n-3 (EPA) A, S, AxS</td>
<td>7.7 ± 0.1</td>
<td>8.0 ± 0.3a</td>
<td>6.3 ± 0.6b</td>
<td>5.0 ± 0.1bc</td>
<td>4.0 ± 0.2cd</td>
<td>3.1 ± 0.1d</td>
</tr>
<tr>
<td>22:5n-3 (DPA) -</td>
<td>0.3 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.0</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>22:6n-3 (DHA) A, S, AxS</td>
<td>5.6 ± 0.1</td>
<td>5.6 ± 0.5a</td>
<td>8.6 ± 0.6b,c</td>
<td>10.1 ± 0.6bc</td>
<td>11.1 ± 0.9bc</td>
<td>8.3 ± 0.4c</td>
</tr>
<tr>
<td>18:2n-6 -</td>
<td>0.7 ± 0.0</td>
<td>0.4 ± 0.2</td>
<td>0.5 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>18:3n-3 -</td>
<td>0.4 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td>n.d</td>
</tr>
<tr>
<td>20:4n-6 (AA) A, S, AxS</td>
<td>1.8 ± 0.0</td>
<td>1.8 ± 0.1a</td>
<td>2.1 ± 0.0ab</td>
<td>2.1 ± 0.1ab</td>
<td>2.3 ± 0.1bc</td>
<td>2.8 ± 0.2c</td>
</tr>
<tr>
<td>22:4n-6 -</td>
<td>0.5 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>22:5n-6 (DPA) A, S, AxS</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.1a</td>
<td>3.1 ± 0.1b</td>
<td>4.1 ± 0.3cd</td>
<td>4.5 ± 0.4e</td>
<td>3.2 ± 0.2bc</td>
</tr>
<tr>
<td><strong>ΣSAT</strong> A, S, AxS</td>
<td>33.6 ± 0.2</td>
<td>36.9 ± 0.8a</td>
<td>39.7 ± 1.3ab</td>
<td>42.7 ± 0.9bc</td>
<td>47.9 ± 1.5a</td>
<td>46.5 ± 0.2cd</td>
</tr>
<tr>
<td><strong>ΣMUFA</strong> A, S, AxS</td>
<td>19.4 ± 0.2</td>
<td>20.5 ± 1.7a</td>
<td>17.0 ± 0.3ab</td>
<td>13.1 ± 0.3bc</td>
<td>8.4 ± 0.2cd</td>
<td>6.4 ± 0.4d</td>
</tr>
<tr>
<td><strong>Σn – 3 PUFA</strong> A, S, AxS</td>
<td>13.6 ± 0.2</td>
<td>14.0 ± 0.8ab</td>
<td>15.2 ± 1.2ab</td>
<td>15.7 ± 1.2a</td>
<td>15.7 ± 1.1a</td>
<td>12.2 ± 0.7b</td>
</tr>
<tr>
<td><strong>Σn – 6 PUFA</strong> A, S, AxS</td>
<td>4.5 ± 0.0</td>
<td>4.8 ± 0.3a</td>
<td>6.7 ± 0.2bc</td>
<td>7.5 ± 0.3bc</td>
<td>8.2 ± 0.6e</td>
<td>7.3 ± 0.8b</td>
</tr>
</tbody>
</table>

n.d. = non-detectable; – indicates statistical comparisons were not performed.
**Table 3.4.** Trial 1 initial (day 0) and final (day 14) proximate and fatty acid composition of juvenile geoduck tissue (mean ± SE). The 0% substitution level is represented by a full live algae diet of *Chaetoceros muelleri* and *Isochrysis* sp. (Tahitian strain) mixed at equal AF DW; *Spirulina* (Sp.) replaced this live bi-algal diet at variant inclusion levels (25 – 100%). Total fatty acid (% dry weight) is represented by total identified content. See Table 3.3 for explanation of statistical analyses and superscript notation.

<table>
<thead>
<tr>
<th>Proximate Composition (% DW)</th>
<th>Statistical Analysis</th>
<th>Initial</th>
<th>0%</th>
<th>25% Sp.</th>
<th>50% Sp.</th>
<th>75% Sp.</th>
<th>100% Sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>A, S, AxS</td>
<td>14.0 ± 0.6</td>
<td>15.7 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.0 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.5 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.6 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.1 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>A, S, AxS</td>
<td>2.1 ± 0.1</td>
<td>3.1 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 0.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.3 ± 0.1&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.9 ± 0.2&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>1.8 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total Fatty Acid A, S, AxS</td>
<td>1.3 ± 0.0</td>
<td>1.9 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.7 ± 0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

**Fatty Acid Composition (% of total fatty acid)**

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Initial</th>
<th>0%</th>
<th>25% Sp.</th>
<th>50% Sp.</th>
<th>75% Sp.</th>
<th>100% Sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>-</td>
<td>2.6 ± 0.1</td>
<td>5.8 ± 2.6</td>
<td>3.3 ± 1.2</td>
<td>1.9 ± 0.3</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>15:0</td>
<td>-</td>
<td>0.7 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.0</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>16:0</td>
<td>-</td>
<td>21.1 ± 0.3</td>
<td>22.4 ± 1.6</td>
<td>25.0 ± 0.8</td>
<td>22.4 ± 0.6</td>
<td>20.5 ± 0.5</td>
</tr>
<tr>
<td>17:0</td>
<td>-</td>
<td>0.2 ± 0.0</td>
<td>n.d.</td>
<td>0.3 ± 0.0</td>
<td>0.4 ± 0.3</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>18:0</td>
<td>-</td>
<td>8.4 ± 0.0</td>
<td>8.0 ± 0.5</td>
<td>8.3 ± 0.7</td>
<td>9.6 ± 0.7</td>
<td>10.4 ± 0.1</td>
</tr>
<tr>
<td>20:0</td>
<td>-</td>
<td>0.5 ± 0.0</td>
<td>n.d.</td>
<td>0.4 ± 0.2</td>
<td>0.8 ± 0.0</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>20:1n – 9</td>
<td>-</td>
<td>5.3 ± 0.1</td>
<td>3.7 ± 0.3</td>
<td>3.5 ± 0.1</td>
<td>2.8 ± 0.0</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>20:1n – 9</td>
<td>-</td>
<td>0.6 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td>0.6 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>18:3n – 3</td>
<td>-</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.9 ± 0.3</td>
<td>n.d.</td>
</tr>
<tr>
<td>20:5n – 3 (EPA)</td>
<td>A, S, AxS</td>
<td>7.7 ± 0.1</td>
<td>8.0 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.2 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.3 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>22:5n – 3 (DPA)</td>
<td>-</td>
<td>0.3 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>22:6n – 3 (DHA)</td>
<td>A, S, AxS</td>
<td>5.6 ± 0.1</td>
<td>5.6 ± 0.5</td>
<td>4.5 ± 0.2&lt;sup&gt;’&lt;/sup&gt;</td>
<td>5.4 ± 0.4&lt;sup&gt;’&lt;/sup&gt;</td>
<td>5.2 ± 0.4&lt;sup&gt;’&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:2n – 6</td>
<td>A, S, AxS</td>
<td>0.7 ± 0.0</td>
<td>0.4 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:3n – 6</td>
<td>A, S, AxS</td>
<td>0.4 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.8 ± 0.0&lt;sup&gt;’&lt;/sup&gt;</td>
<td>0.9 ± 0.1&lt;sup&gt;’&lt;/sup&gt;</td>
</tr>
<tr>
<td>20:4n – 6 (AA)</td>
<td>A, S, AxS</td>
<td>1.8 ± 0.0</td>
<td>1.8 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8 ± 0.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.1 ± 0.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.2 ± 0.1&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>22:4n – 6</td>
<td>-</td>
<td>0.5 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>22:5n – 6 (DPA)</td>
<td>A, S, AxS</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.7 ± 0.0&lt;sup&gt;’&lt;/sup&gt;</td>
<td>0.8 ± 0.1&lt;sup&gt;’&lt;/sup&gt;</td>
<td>0.8 ± 0.1&lt;sup&gt;’&lt;/sup&gt;</td>
</tr>
<tr>
<td>∑SAT</td>
<td>A, S, AxS</td>
<td>33.6 ± 0.2</td>
<td>36.9 ± 0.8&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>37.8 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.6 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.3 ± 0.6&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>∑MUFA</td>
<td>A, S, AxS</td>
<td>19.4 ± 0.2</td>
<td>20.5 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.2 ± 0.6&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>17.5 ± 0.4&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>12.8 ± 0.9&lt;sup&gt;c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>∑n – 3 PUFA</td>
<td>A, S, AxS</td>
<td>13.6 ± 0.2</td>
<td>14.0 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.3 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.0 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.8 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>∑n – 6 PUFA</td>
<td>A, S, AxS</td>
<td>4.5 ± 0.0</td>
<td>4.8 ± 0.3</td>
<td>4.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1 ± 0.2</td>
<td>5.6 ± 0.3&lt;sup&gt;’&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

n.d. = non-detectable; – indicates statistical comparisons were not performed.
Table 3.5. Trial 2 initial (day 0) and final (day 14) proximate and fatty acid composition of juvenile geoduck tissue (mean ± SE). The 0% substitution level is represented by a full live algae diet of Chaetoceros muelleri and Isochrysis sp. (Tahitian strain) mixed at equal AFDW; Schizochytrium (Sch.) replaced this live bi-algal diet at variant inclusion levels (25 – 100%). Total fatty acid (% dry weight) is represented by total identified content. See Table 3.3 for explanation of statistical analyses and superscript notation.

<table>
<thead>
<tr>
<th></th>
<th>Statistical Analysis</th>
<th>Initial</th>
<th>0%</th>
<th>25% Sch.</th>
<th>50% Sch.</th>
<th>75% Sch.</th>
<th>100% Sch.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximate Composition (% DW)</strong></td>
<td></td>
<td>Initial</td>
<td>0%</td>
<td>25% Sch.</td>
<td>50% Sch.</td>
<td>75% Sch.</td>
<td>100% Sch.</td>
</tr>
<tr>
<td>Protein</td>
<td>A, S, AxS</td>
<td>16.4 ± 0.7</td>
<td>15.2 ± 0.2a</td>
<td>15.0 ± 0.5a</td>
<td>15.2 ± 0.4ab</td>
<td>14.9 ± 0.2ab</td>
<td>13.3 ± 0.2b</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>A, S, AxS</td>
<td>3.9 ± 0.2</td>
<td>2.4 ± 0.0</td>
<td>2.5 ± 0.2</td>
<td>2.1 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>Total Fatty Acid</td>
<td>A, S, AxS</td>
<td>3.1 ± 0.1</td>
<td>1.9 ± 0.1a</td>
<td>2.2 ± 0.2a</td>
<td>2.2 ± 0.0a</td>
<td>2.0 ± 0.1ab</td>
<td>1.7 ± 0.1b</td>
</tr>
</tbody>
</table>

**Fatty Acid Composition (% of total fatty acid)**

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>12.0 ± 0.3</td>
<td>8.9 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>11.6 ± 1.3</td>
<td>11.5 ± 0.9</td>
<td>12.2 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>15:0</td>
<td>0.7 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.8 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.9 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>22.9 ± 0.3</td>
<td>21.2 ± 0.3</td>
<td>24.4 ± 0.6</td>
<td>26.8 ± 0.7</td>
<td>28.1 ± 0.6</td>
<td>30.8 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>17:0</td>
<td>n.d.</td>
<td>0.4 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.4 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>6.0 ± 0.1</td>
<td>8.8 ± 0.4</td>
<td>8.0 ± 0.4</td>
<td>7.5 ± 0.1</td>
<td>8.0 ± 0.5</td>
<td>8.5 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>20:0</td>
<td>0.4 ± 0.0</td>
<td>1.7 ± 0.0</td>
<td>0.6 ± 0.4</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>14:1</td>
<td>0.8 ± 0.0</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.0</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>15:1</td>
<td>1.5 ± 0.0</td>
<td>1.3 ± 0.0</td>
<td>1.1 ± 0.1</td>
<td>0.4 ± 0.3</td>
<td>0.8 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>16:1</td>
<td>15.6 ± 0.4</td>
<td>13.9 ± 0.5</td>
<td>11.8 ± 1.0</td>
<td>10.1 ± 0.2</td>
<td>6.9 ± 0.0</td>
<td>5.0 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>18:1n-9</td>
<td>2.5 ± 0.2</td>
<td>2.0 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.2 ± 0.0</td>
<td>1.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>20:1n-9</td>
<td>0.6 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>18:3n-3</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.2 ± 0.0</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>20:5n-3</td>
<td>A, S, AxS</td>
<td>6.7 ± 0.2</td>
<td>7.7 ± 0.1a</td>
<td>5.7 ± 0.1b</td>
<td>4.8 ± 0.1b</td>
<td>4.1 ± 0.1b</td>
<td>3.3 ± 0.1b</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>A, S, AxS</td>
<td>0.3 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.4 ± 0.0</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>A, S, AxS</td>
<td>5.1 ± 0.3</td>
<td>4.8 ± 0.1a</td>
<td>7.7 ± 0.6b</td>
<td>9.7 ± 0.4c</td>
<td>11.9 ± 0.9g</td>
<td>9.3 ± 0.2bc</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>A, S, AxS</td>
<td>0.8 ± 0.0</td>
<td>0.5 ± 0.0a</td>
<td>0.4 ± 0.0b</td>
<td>0.3 ± 0.0c</td>
<td>0.2 ± 0.0c</td>
<td>0.1 ± 0.0d</td>
</tr>
<tr>
<td>18:3n-6</td>
<td>A, S, AxS</td>
<td>0.7 ± 0.0</td>
<td>0.5 ± 0.0a</td>
<td>0.4 ± 0.0b</td>
<td>0.3 ± 0.0c</td>
<td>0.2 ± 0.0d</td>
<td></td>
</tr>
<tr>
<td>20:4n-6</td>
<td>A, S, AxS</td>
<td>1.3 ± 0.1</td>
<td>1.9 ± 0.1a</td>
<td>1.9 ± 0.1a</td>
<td>1.9 ± 0.0b</td>
<td>2.1 ± 0.0b</td>
<td>2.4 ± 0.1b</td>
</tr>
<tr>
<td>22:4n-6</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.6 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>22:5n-6</td>
<td>A, S, AxS</td>
<td>0.9 ± 0.0</td>
<td>1.5 ± 0.2a</td>
<td>3.4 ± 0.5b</td>
<td>4.2 ± 0.4bc</td>
<td>5.1 ± 0.2c</td>
<td>4.4 ± 0.4bc</td>
</tr>
<tr>
<td>∑SAT</td>
<td>A, S, AxS</td>
<td>42.1 ± 0.3</td>
<td>41.8 ± 0.8a</td>
<td>44.5 ± 0.4ab</td>
<td>46.9 ± 0.7bc</td>
<td>48.5 ± 0.3c</td>
<td>52.6 ± 0.8d</td>
</tr>
<tr>
<td>∑MUFA</td>
<td>A, S, AxS</td>
<td>21.2 ± 0.3</td>
<td>18.8 ± 0.4a</td>
<td>16.2 ± 0.9ab</td>
<td>13.4 ± 0.1bc</td>
<td>10.2 ± 0.1c,d</td>
<td>7.8 ± 0.6d</td>
</tr>
<tr>
<td>∑n-3 PUFA</td>
<td>A, S, AxS</td>
<td>12.1 ± 0.5</td>
<td>12.9 ± 0.1a</td>
<td>14.1 ± 0.7ab</td>
<td>14.9 ± 0.4ab</td>
<td>16.4 ± 1.0b</td>
<td>13.3 ± 0.3d</td>
</tr>
<tr>
<td>∑n-6 PUFA</td>
<td>A, S, AxS</td>
<td>4.0 ± 0.1</td>
<td>5.3 ± 0.2a</td>
<td>6.7 ± 0.6b</td>
<td>7.3 ± 0.4b</td>
<td>8.3 ± 0.2c</td>
<td>7.7 ± 0.5bc</td>
</tr>
</tbody>
</table>

n.d. = non-detectable; – indicates statistical comparisons were not performed.
### Table 3.6

Trial 2 initial (day 0) and final (day 14) proximate and fatty acid composition of juvenile geoduck tissue (mean ± SE). The 0% substitution level is represented by a full live algae diet of *Chaetoceros muelleri* and *Isochrysis* sp. (Tahitian strain) mixed at equal AFDW; *Spirulina* (Sp.) replaced this live bi-algal diet at variant inclusion levels (25 – 100%). Total fatty acid (% dry weight) is represented by total identified content. See Table 3.3 for explanation of statistical analyses and superscript notation.

<table>
<thead>
<tr>
<th>Statistical Analysis</th>
<th>Initial</th>
<th>0%</th>
<th>25% Sp.</th>
<th>50% Sp.</th>
<th>75% Sp.</th>
<th>100% Sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximate Composition (% DW)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein A, S, AxS 16.4 ± 0.7</td>
<td>15.2 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.4 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.6 ± 0.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>14.7 ± 0.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>14.5 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate A, S, AxS 3.9 ± 0.2</td>
<td>2.4 ± 0.0</td>
<td>2.7 ± 0.1</td>
<td>2.4 ± 0.4</td>
<td>2.3 ± 0.4</td>
<td>1.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Total Fatty Acid A, S, AxS 3.1 ± 0.1</td>
<td>1.9 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.4 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3 ± 0.2&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>0.8 ± 0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

| **Fatty Acid Composition (% of total fatty acid)** |
|-----------|---------|---------|---------|---------|---------|---------|
| 14:0 - | 12.0 ± 0.3 | 8.9 ± 0.2 | 4.0 ± 2.1 | 1.5 ± 0.1 | 0.8 ± 0.1 | n.d. |
| 15:0 - | 0.7 ± 0.0 | 0.7 ± 0.0 | 0.7 ± 0.0 | 0.7 ± 0.1 | 0.7 ± 0.1 | 1.3 ± 0.0 |
| 16:0 - | 22.9 ± 0.3 | 21.2 ± 0.3 | 23.3 ± 1.0 | 21.0 ± 0.8 | 18.7 ± 3.7 | 19.7 ± 0.4 |
| 17:0 - | n.d. | 0.4 ± 0.2 | 1.0 ± 0.1 | 1.4 ± 0.1 | 1.9 ± 0.1 | 0.2 ± 0.2 |
| 18:0 - | 6.0 ± 0.1 | 8.8 ± 0.4 | 9.2 ± 0.7 | 9.7 ± 0.5 | 9.6 ± 1.7 | 12.4 ± 0.1 |
| 20:0 - | 0.4 ± 0.0 | 1.7 ± 0.0 | 1.3 ± 0.4 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.9 ± 0.0 |
| 14:1 - | 0.8 ± 0.0 | 1.0 ± 0.1 | 1.1 ± 0.1 | 1.2 ± 0.1 | 1.2 ± 0.2 | 1.7 ± 0.0 |
| 15:1 - | 1.5 ± 0.0 | 1.3 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0.1 | 0.8 ± 0.3 | 0.7 ± 0.4 |
| 16:1 - | 15.6 ± 0.4 | 13.9 ± 0.5 | 14.8 ± 0.8 | 14.6 ± 0.8 | 10.6 ± 1.0 | 6.5 ± 1.9 | 5.6 ± 0.3 |
| 18:1n-9 - | 2.5 ± 0.2 | 2.0 ± 0.1 | 2.3 ± 0.1 | 2.4 ± 0.2 | 1.8 ± 0.5 | 1.9 ± 0.0 |
| 20:1n-9 - | 0.6 ± 0.0 | 0.6 ± 0.0 | 0.7 ± 0.0 | 0.9 ± 0.1 | 0.9 ± 0.1 | 1.2 ± 0.0 |
| 18:3n-3 - | n.d. | n.d. | n.d. | 0.5 ± 0.0 | 0.5 ± 0.0 | 0.6 ± 0.0 |
| 20:5n-3 (EPA) A, S, AxS 6.7 ± 0.2 | 7.7 ± 0.1<sup>a,b</sup> | 8.0 ± 0.2<sup>a,b</sup> | 8.1 ± 0.3<sup>a</sup> | 6.5 ± 1.0<sup>b</sup> | 6.0 ± 0.1<sup>b</sup> |
| 22:5n-3 (DPA) - | 0.3 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.0 | 0.7 ± 0.0 |
| 22:6n-3 (DHA) A, S, AxS 5.1 ± 0.3 | 4.8 ± 0.1 | 4.8 ± 0.1<sup>*</sup> | 5.7 ± 0.6<sup>*</sup> | 5.0 ± 0.3<sup>*</sup> | 6.2 ± 0.2<sup>*</sup> |
| 18:2n-6 A, S, AxS 0.8 ± 0.0 | 0.5 ± 0.0<sup>a</sup> | 0.8 ± 0.0<sup>a,b</sup> | 0.9 ± 0.1<sup>a,b</sup> | 0.3 ± 0.3<sup>a,b</sup> | 1.4 ± 0.0<sup>b</sup> |
| 18:3n-6 A, S, AxS 0.7 ± 0.0 | 0.5 ± 0.0<sup>a</sup> | 0.4 ± 0.2<sup>a</sup> | 0.5 ± 0.2<sup>a</sup> | 0.7 ± 0.2<sup>a</sup> | 1.4 ± 0.1<sup>b</sup> |
| 20:4n-6 (AA) A, S, AxS 1.3 ± 0.1 | 1.9 ± 0.1<sup>a</sup> | 2.0 ± 0.1<sup>a,b</sup> | 2.4 ± 0.1<sup>b,c</sup> | 2.6 ± 0.3<sup>c</sup> | 3.2 ± 0.1<sup>d</sup> |
| 22:4n-6 - | 0.4 ± 0.0 | 0.6 ± 0.0 | 0.7 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.0 | 1.1 ± 0.0 |
| 22:5n-6 (DPA) A, S, AxS 0.9 ± 0.0 | 1.5 ± 0.2<sup>a,b</sup> | 0.9 ± 0.1<sup>b</sup> | 1.5 ± 0.5<sup>a,b</sup> | 1.0 ± 0.1<sup>a,b</sup> | 2.5 ± 0.4<sup>a</sup> |
| ∑SAT A, S, AxS 42.1 ± 0.3 | 41.8 ± 0.8<sup>a</sup> | 39.5 ± 0.1<sup>a,b</sup> | 35.0 ± 0.8<sup>a,b</sup> | 31.6 ± 6.7<sup>b</sup> | 34.4 ± 0.7<sup>b</sup> |
| ∑MUFA A, S, AxS 21.2 ± 0.3 | 18.8 ± 0.4<sup>a,b</sup> | 20.3 ± 0.8<sup>a</sup> | 16.6 ± 1.0<sup>b</sup> | 11.1 ± 2.4<sup>c</sup> | 11.2 ± 0.4<sup>c</sup> |
| ∑n-3 PUFA A, S, AxS 12.1 ± 0.5 | 12.9 ± 0.1 | 13.2 ± 0.5 | 14.7 ± 1.0 | 12.6 ± 1.4<sup>c</sup> | 13.6 ± 0.3 |
| ∑n-6 PUFA A, S, AxS 4.0 ± 0.1 | 5.3 ± 0.2<sup>a</sup> | 5.0 ± 0.1<sup>a</sup> | 5.8 ± 0.6<sup>a</sup> | 5.5 ± 0.2<sup>a</sup> | 9.7 ± 0.3<sup>b</sup> |

n.d. = non-detectable; – indicates statistical comparisons were not performed.
Figure 3.1: Mean (A) individual shell length (mm), (B) daily shell increment (μm d⁻¹), (C) wet weight (mg ind⁻¹), (D) specific growth rate, (E) dry weight (mg ind⁻¹), and (F) ash-free dry weight (% initial) of juvenile geoduck at final sampling (14 d; trial 1). Geoduck were reared on a live algae diet (CM+TISO delivered in equal AFDW proportion) substituted with variant levels of the dried algae, *Schizochytrium* sp. or *Spirulina* (*Arthrospira platensis*). No significant interaction effects were detected between dietary algae species and substitution level (two-way ANOVA, P>0.05, n = 3). Algal type was a significant source of variation for: shell length, DSI, SGR, and dry weight (two-way ANOVA, P<0.05, n = 12). Different letters indicate significant differences between substitution levels (two-way ANOVA, Tukey’s test, P<0.05, n = 3). Substitution levels across algae species were pooled (n = 6) if the effect of algal type was not significant (P>0.05; n = 12). Error bars denote standard error.
Figure 3.2: Mean (A) individual shell length (mm), (B) daily shell increment (μm d⁻¹), (C) wet weight (mg ind⁻¹), (D) specific growth rate, (E) dry weight (mg ind⁻¹), and (F) ash-free dry weight (% initial) of juvenile geoduck at final sampling (14 d; trial 2). Feeding treatments are described in figure 3.1. A significant interaction effect was detected between dietary algae species and substitution level (two-way ANOVA, P<0.05, n = 3) for shell length and dry weight. A significant dietary effect (Tukey’s Test, P<0.05, n = 3) was detected at 50% (wet weight) and 75% (shell length, wet weight) denoted by axis *. Different letters indicate significant differences between substitution levels (two-way ANOVA, P<0.05, Tukey’s test; n = 3) Substitution levels were pooled if the effect of algal type or the interaction effect was not significant (P>0.05; n = 12, n = 3, respectively). Error bars denote standard error.
Figure 3.3. Trial 1 mean dietary composition of (A) protein (mg g\(^{-1}\) AFDW), (B) carbohydrate (mg g\(^{-1}\) AFDW), (C) total fatty acid (mg g\(^{-1}\) AFDW), (D) EPA (%TFA), (E) DHA (%TFA), (F) AA (%TFA), (G) n – 6 DPA (%TFA), (H) \(\sum\) SAT (%TFA), (I) \(\sum\) MUFA (%TFA), (J) \(\sum\) n – 3 PUFA (%TFA), (K) \(\sum\) n – 6 PUFA (%TFA), and (L) \(\sum\) n – 3/\(\sum\) n – 6 PUFA (%TFA) (X; calculated from mean values in Table 3.2) correlated against the same parameters measured in geoduck tissue (%DW for proximate components, %TFA for fatty acids) after 14 d of experimental dietary exposure (Y; trial 1). Error bars represent standard error.
Figure 3.3. (continued). Trial 1 mean dietary composition of (A) protein (mg g\(^{-1}\) AFDW), (B) carbohydrate (mg g\(^{-1}\) AFDW), (C) total fatty acid (mg g\(^{-1}\) AFDW), (D) EPA (% TFA), (E) DHA (% TFA), (F) AA (% TFA), (G) n-6 DPA (% TFA), (H) \(\Sigma\)SAT (% TFA), (I) \(\Sigma\)MUFA (% TFA), (J) \(\Sigma\)n-3 PUFA (% TFA), (K) \(\Sigma\)n-6 PUFA (% TFA), and (L) \(\Sigma\)n-3/\(\Sigma\)n-6 PUFA (% TFA) (X; calculated from mean values in Table 3.2) correlated against the same parameters measured in geoduck tissue (%DW for proximate components, %TFA for fatty acids) after 14 d of experimental dietary exposure (Y; trial 1). Error bars represent standard error.
Figure 3.4. Trial 2 mean dietary composition of (A) protein (mg g\(^{-1}\)AFDW), (B) carbohydrate (mg g\(^{-1}\)AFDW), (C) total fatty acid (mg g\(^{-1}\)AFDW), (D) EPA (%TFA), (E) DHA (%TFA), (F) AA (%TFA), (G) n-6 DPA (%TFA), (H) ∑SAT (%TFA), (I) ∑MUFA (%TFA), (J) ∑n-3 PUFA (%TFA), (K) ∑n-6 PUFA (%TFA), and (L) ∑n-3/∑n-6 PUFA (%TFA) (X; calculated from mean values in Table 3.2) correlated against the same parameters measured in geoduck tissue (%DW for proximate components, %TFA for fatty acids) after 14 d of experimental dietary exposure (Y; trial 2). Error bars represent standard error.
Figure 3.4. (continued). Trial 2 mean dietary composition of (A) protein (mg g⁻¹ AFDW), (B) carbohydrate (mg g⁻¹ AFDW), (C) total fatty acid (mg g⁻¹ AFDW), (D) EPA (% TFA), (E) DHA (% TFA), (F) AA (% TFA), (G) n-6 DPA (% TFA), (H) ΣSAT (% TFA), (I) ΣMONO (% TFA), (J) Σn-3 PUFA (% TFA), (K) Σn-6 PUFA (% TFA), and (L) Σn-3/Σn-6 PUFA (% TFA) (X; calculated from mean values in Table 3.2) correlated against the same parameters measured in geoduck tissue (%DW for proximate components, %TFA for fatty acids) after 14 d of experimental dietary exposure (Y; trial 2). Error bars represent standard error.
4 Conclusions

4.1 Thesis objectives

This thesis attempted to refine culture conditions in the hatchery production of the juvenile Pacific geoduck clam (*Panopea generosa*), a high value commercial species. The geoduck is an attractive candidate for BC aquaculture production, but BC hatchery protocols remain ill-defined (Marcus and Hand, 2004), and current aquaculture knowledge excludes post-settlement juveniles in the published literature. Due to the absence of data, this research attempted to isolate the parameters most influential to geoduck growth: temperature and feed ration (chapter 2; Beiras *et al*., 1993; Broom and Mason, 1978; Walne and Spencer, 1974). In chapter 3, the feasibility of live algae dietary substitution was explored with the commercial spray-dried species, *Schizochytrium* spp. and *Spirulina* (*Arthrospira platensis*). The importance of this investigation is highlighted by the critical cost and stability constraints imposed by live algae reliance in current bivalve production (Borowitzka, 1997; Coutteau and Sorgeloos, 1992; Robert and Trintignac, 1997). Successful adoption of a spray-dried substitute would economize geoduck culture through cost reduction and enhanced feed accessibility.

To quantify treatment success, each experiment (except temperature experiment 2) examined geoduck growth (shell length, DSI, wet weight, SGR, dry weight, AFDW) and survival under culture manipulation. Temperature and feed ration treatments received a bi-algae diet of *Chaetoceros muelleri* and *Isochrysis* sp. mixed by equal AFDW. This diet was replaced by variant levels of *Schizochytrium* or *Spirulina* during live algae substitution (chapter 3).
4.2 Chapter 2: Temperature and feed ration optimization

The temperature trial examined four temperatures (7, 11, 15, 19 °C) in the culture of juvenile (temperature experiment 1) and post-larval (temperature experiment 2) geoduck. Treatments closely corresponded with the natural thermal range encountered by wild BC geoduck (Anonymous, 2013c). In agreement with alternative hypothesis 1 (section 1.5.3), temperature elicited a significant growth effect \((P<0.05)\) in all tested juvenile and post-larval growth parameters, and significantly elevated shell length, DSI, wet weight, SGR, and dry weight in juvenile geoduck reared at 19 °C (post 28 d). However, AFDW significantly declined in 19 °C geoduck compared to 11 or 15 °C treatments, which did not comply with the remaining prediction made in hypothesis 1 – increased temperature would enhance organic weight accumulation. It is speculated the elevated feed requirements incurred by the temperature increase suppressed organic weight accumulation, and produced the AFDW differential. The significant growth promotion at 19 °C also did not emerge until the 28th d of culture, at which time treatment geoduck exceeded the maximal shell size (6.0 mm) used in out-planting. Juveniles achieved this size in <21 d in both 15 and 19 °C treatments; these treatments did not display significant size variation at this time. The 15 °C culture temperature is therefore recommended for late juvenile production (shell length: 3.2 mm – 6.0 mm). In contrast, the post-larval and early juvenile size class displayed immediate (post 7 d) growth (shell length and DSI) acceleration at 19 °C, and significantly exceeded all other treatments in final sampling size (21 d). Compared to the next highest performing treatment (15 °C), 19 °C shortened the culture period by 2.9 d. This recommends the potential adoption of the 19 °C culture temperature in the earlier hatchery stage, if the benefit of the growth acceleration can outweigh resultant heating
costs. Survival significantly declined at 7 °C in both size classes, but remained above 93% in all treatments.

The feed ration trial aimed to identify optimal feed ration requirements in four juvenile size classes (sizes 1 – 4 mean initial shell lengths: 2.34; 3.32; 4.13; 4.98 mm). Size classes 1 and 2 tested the dietary rations: 0.0, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0x10^6 equivalent TISO cells ind⁻¹ d⁻¹; size classes 3 and 4 examined: 0.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, 128.0x10^6 equivalent TISO cells ind⁻¹ d⁻¹. The dietary exposure lasted 7 d for each size division (cumulative treatment period: 28 d). This experiment defined optimal feed ration as the minimal feed amount beyond which further dietary elevation did not promote significant growth acceleration. In conjunction with alternative hypothesis 2 (section 1.6.3), the optimal ration quantity determined for each growth parameter (i.e. shell length, DSI, wet weight, SGR, dry weight, AFDW) shifted upward with each successive size class (exception: shell length and DSI in week 4), suggesting the size dependent modification in geoduck feed requirements.

The significant growth effect elicited by ration quantity in each tested growth parameter also agrees with the first predication made in alternative hypothesis 3 (section 1.6.4). However, a single ration level did not incite an identical growth response among collective indices, refuting the hypothesis’ second predication. In general, the ration amount identified for optimal shell and wet weight growth correlated within each size class, but the optimal dry weight and AFDW requirements exceeded the optimal wet weight ration by two and four fold, respectively. The discrepancy suggests the energetic requirements of organic weight accumulation exceed those of shell or wet weight. It is speculated the high energy requirement associated with tissue growth (enlargement of the geoduck mantle and siphon; Bayne and Hawkins, 1997) elevated the optimal
AFDW ration requirement, and induced the resultant optimization inequality. As the benefit of an elevated AFDW is unknown in geoduck culture and outplanting, this research recommends selection of shell/wet weight ration optima when establishing the late hatchery feeding regime. This selection minimizes the required feeding amount, promoting production economization. By this recommendation, the following rations ($10^6$ equivalent *Isochrysis* cells ind$^{-1}$ d$^{-1}$) should be applied between tested geoduck size classes: 4.0 (size class 1); 8.0 (size class 2); 16.0 or 32.0 (shell length or wet weight optimum, respectively; size class 3); and 32.0 (size class 4). Survival rate did not significantly vary between treatments, and remained above 93.4% among all size classes.

4.3 Chapter 3: Live algae substitution

This experiment substituted (by AFDW) the CM+TISO live algae diet with variant levels (0 – 100%) of the spray-dried species, *Schizochytrium* sp., and *Spirulina* (*Arthrospira platensis*). Substitution success was examined in in two geoduck size classes (size class 1: mean initial shell length: 2.47 mm; size class 2: 4.00 mm). In general, geoduck growth (all parameters) declined with elevated spray-dried substitution. Measured growth (shell length, DSI, wet weight, SGR, and dry weight) did not display significant variation between 0 – 25% substitution levels in the smaller size class, but larger geoduck (trial 2) exhibited enhanced dried algae sensitivity; substitution levels beyond 0% (full live bi-algae diet) induced significant growth reduction in all parameters (except AFDW). The growth findings do not support the alternative or null hypotheses given in section 1.9.2 and 1.9.3. These hypotheses designated the 50% substitution level as the dietary threshold beyond which, significant growth effects would occur. Geoduck survival did not display significant variation between dietary treatments.
The results indicate the absence of the synergistic live/spray-dried growth response elicited in previous bivalve studies (Baubin, 2009; Jaime-Ceballos et al., 2006; Langdon and Önal, 1999). It is speculated the authors’ selection of a sub-optimal live algae control promoted the growth enhancement incurred by spray-dried substitution. In contrast, the present study’s utilization of a positive control diet (CM+TISO) likely the restricted the dietary improvement available through spray-dried integration. Compared to *Schizochytrium* and *Spirulina*, the superior live algae combination was characterized by moderate protein, carbohydrate, lipid, energy, DHA, n – 6 DPA, and Σn – 3 PUFA content, elevated EPA, AA, ΣMUFA, and Σn – 3/Σn – 6 PUFA content, and reduced ΣSAT and Σn – 6 PUFA content. This diet also contained a Σn – 3 PUFA/ Σn – 6 PUFA ratio of 2.9; ratio values similar to 2.9 are associated with improved growth in the bivalve literature (Enright et al., 1986b; Milke et al., 2006; Webb and Chu, 1983), which may indicate conservation of this dietary requirement between bivalve species. This ratio displayed an incremental decline with increased spray-dried substitution, with the worst performing diets (100% *Schizochytrium* and *Spirulina*) containing the lowest relative levels.

*Schizochytrium* exposed geoduck demonstrated a significant growth reduction compared to *Spirulina* treatments, despite the latter’s deficiency in important dietary PUFAs (*i.e.* EPA, DHA, n – 6 DPA, and AA). It is suggested that the surfeit of DHA in *Schizochytrium* treatments contributed to the spray-dried growth disparity. Glencross and Smith (2001) indicated excessive dietary DHA can inhibit growth in the prawn, *Penaeus monodon*, and recommended 4% (TFA) as the optimal inclusion level. *Schizochytrium* diets exceeded this recommendation at all substitution levels (8.6 – 21.1%). The results indicate indiscriminate elevation of important dietary PUFAs does not elicit an automatic growth enhancement, and cautions the exclusive examination of these PUFAs in future geoduck nutritional studies.
The geoduck growth decline that occurred with spray-dried replacement likely resulted from the biochemical inadequacy of the spray-dried diets, coupled with the digestive difficulty of specific proximate components. The enhancement or reduction of dietary components through spray-dried substitution likely skewed dietary content from the “optimal" biochemical levels present in the CM+TISO diet, and resulted in the incremental growth decline that maximized at 100% *Schizochytrium* or *Spirulina*. Dietary and tissue correlations also indicate the potential digestive inaccessibility of spray-dried derived protein and carbohydrate, which possibly augmented the growth degradation that accompanied spray-dried substitution. Protein sequestration is demonstrated to contribute to the majority of tissue growth in bivalve species (Bayne and Hawkins 1997), while carbohydrate deposition permits dietary-derived protein and lipid to be diverted to biosynthetic processes, such as growth (Whyte *et al.*, 1989). Potential limitation in tissue deposition and energy availability (Bayne and Hawkins, 1997; Marin *et al.*, 2003) may therefore have augmented the growth suppression that accompanied *Schizochytrium* or *Spirulina* inclusion.

However, the fatty acid dietary and tissue correlations did demonstrate general fidelity between dietary input and resultant tissue content. This may indicate the successful digestion of spray-dried derived fatty acids. The relative and absolute tissue elevation of DHA and n – 6 DPA that occurred with increased dietary content in *Schizochytrium* treatments, along with the 18:2n – 6 and 18:3n – 6 elevation that occurred *Spirulina* treatments further suggests dietary assimilation.

EPA and AA sequestration in *Spirulina*-fed geoduck demonstrated a repeated exception to the positive correlations exhibited for other fatty acids, and indicated the selective retention of EPA and AA. This accumulation possibly compensated for the dietary EPA/AA reduction or
exclusion that accompanied *Spirulina* substitution. EPA and AA represent important precursors to regulatory signal molecules, the eicosanoids; their physiological importance likely promoted relative accumulation. Despite its general PUFA deficiency, this selective modulation possibly contributed to relative growth maintenance in *Spirulina* treatments, promoting the growth of *Spirulina* exposed geoduck above the levels achieved by dietary *Schizochytrium*.

Due to variation in assimilation, the overall results do not completely support hypothesis 6 (section 1.9.5), which predicted a positive significant correlation would exist between dietary components and subsequent geoduck tissue composition. Resultant assimilation success appeared dependent on the biochemical component, perhaps indicating variant digestive ability. Overall, the results discourage the dietary integration of *Schizochytrium* or *Spirulina* substitutes in juvenile geoduck hatchery culture as the dietary combination of CM+TISO remains superior.

### 4.4 Recommendations for future research

Future research recommendations center on the algae substitution trial. Geoduck growth performance in this trial confirmed the superiority of the CM+TISO diet, as nutritional modification by spray-dried inclusion did not incite a beneficial growth effect. Therefore, it is recommended future spray-dried formulations mirror CM+TISO’s nutritional composition, so that spray-dried inclusion does not modify dietary content from the established “optima” provided by exclusive CM+TISO delivery.

Explicit examination of ingestion and digestion of spray-dried algae did not occur in this study; geoduck ingestion and digestion capacity was inferred by treatment variation in final tissue composition. This ambiguity warrants future study to establish the digestive accessibility
of selected dietary alternatives. Alternatives may mimic the CM+TISO in biochemical content, but still elicit inferior growth if the diet is inaccessible through initial ingestion or subsequent digestion. Examination of ingestion capacity and enzyme digestion is recommended under the exclusive provision of the spray-dried substitute. Failed ingestion or enzymatic activation would suggest restricted digestion and tissue assimilation. This latter difficulty may be addressed through the external enzymatic digestion. Application of digestive enzymes to the spray-dried diet prior to feed delivery may pre-digest the diets, and permit nutritional assimilation, compensating for prior digestive inadequacy. This may supplement growth to levels achievable by exclusive live-algae provision. This occurrence would re-affirm previous digestive inaccessibility, and its likely contribution to growth suppression.

The overall economic benefit derived from spray-dried integration encourages its further investigation, dictating the necessity of continued research in geoduck dietary and digestive requirements. Although dietary integration of *Schizochytrium* and *Spirulina* is not recommended in geoduck culture, the general refinement of culture conditions elucidated by the thesis, contributes to the generalized knowledge of the species’ physiology, and may result in the enhancement of BC’s juvenile geoduck hatchery production.
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