

Effects of Sand Acclimation on Burrowing Rate and Siphon Nipping on Growth of Juveniles of the Pacific Geoduck Clam (*Panopea generosa*)

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EFFECTS OF SAND ACCLIMATION ON BURROWING RATE
AND SIPHON NIPPING ON GROWTH OF JUVENILES OF THE
PACIFIC GEODUCK CLAM (*Panopea generosa*)

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ABSTRACT

Liu, W., Pearce, C.M., McKinley, R.S., and Forster, I.P. 2017. Effects of sand acclimation on burrowing rate and siphon nipping on growth of juveniles of the Pacific geoduck clam (*Panopea generosa*). Can. Tech. Rep. Fish. Aquat. Sci. 3226: v + 12 p.

There has been recent interest in developing aquaculture of the Pacific geoduck clam (*Panopea generosa*) in British Columbia, Canada, and an existing productive culture industry for the species in Washington State, USA. Local hatcheries employ different culture systems either with or without sand as a substrate to produce the juvenile clams. The hatchery-reared seed, when planted on the sea bottom, are vulnerable to bottom-feeding predators. Fast burrowing and burial are important for survival after field out-planting. Out-planted individuals may be subject to various types of predation, including siphon nipping, which can reduce their fitness/growth or cause mortality. The effects of sand acclimation on burrowing rate and simulated siphon-nipping on growth and survival of young juveniles were examined in two separate laboratory studies. Results revealed that approximately 40% of the juveniles grown without sand acclimation temporarily lost their burrowing behaviour. Simulated siphon nipping did not significantly affect growth of the juveniles during a 4-week trial. These results suggest that sand acclimation is crucial prior to field out-planting of juvenile geoduck clams to maximize planting success, and that the occasional loss of siphonal tissues by predation would not critically affect the welfare of out-planted juveniles.

RÉSUMÉ

Liu, W., Pearce, C.M., McKinley, R.S., and Forster, I.P. 2017. Effets de l'acclimatation du sable sur le taux de creusement et le piégeage du siphon sur la croissance des juvéniles de la panope du Pacifique (*Panopea generosa*). Can. Tech. Rep. Fish. Aquat. Sci. 3226: v + 12 p.

On s'intéresse depuis quelque temps au développement de l'élevage de la panope du Pacifique (*Panopea generosa*) en Colombie-Britannique, au Canada, et à la production actuelle de l'espèce dans l'État de Washington, aux États-Unis. Les écloseries locales utilisent différents systèmes d'élevage, avec ou sans substrat de sable, pour produire des panopes juvéniles. Lorsqu'elles sont placées sur le fond marin, les semences élevées en écloserie sont vulnérables aux prédateurs qui s'y nourrissent. La survie après ensemencement sur le terrain dépend de la rapidité de l'enfouissement. Les individus transplantés peuvent être soumis à divers types de prédation, y compris la morsure du siphon, qui peut réduire leur valeur adaptative/croissance ou causer la mort. Les effets de l'acclimatation au sable sur le taux d'enfouissement et de la morsure simulée du siphon sur la croissance et la survie des jeunes juvéniles ont été examinés dans le cadre de deux études en laboratoire. Les résultats ont révélé qu'environ 40 % des juvéniles élevés sans acclimatation au sable perdaient temporairement leur comportement fouisseur. La morsure simulée du siphon n'a pas eu d'incidence significative sur la croissance des juvéniles pendant un essai de quatre semaines. Ces résultats donnent à penser que l'acclimatation au sable est essentiel avant l'ensemencement de panopes du Pacifique juvéniles sur le terrain afin de maximiser la réussite de la transplantation, et que la perte occasionnelle de tissus siphonaux par la prédation ne devrait pas avoir d'incidence critique sur le bien-être des panopes juvéniles transplantées.

1.0 INTRODUCTION

The Pacific geoduck clam (*Panopea generosa*) is distributed from Alaska to Baja California (Bernard 1983) and currently supports the most valuable clam fishery on the west coast of North America both in Washington State (WA), USA and British Columbia (BC), Canada (Palazzi et al. 2001; Department of Fisheries and Oceans (DFO) 2012). Aquaculture production of geoduck clams started in WA in the mid-1990s and has increased rapidly, with 612.9 metric tons (MT) of cultured clams, worth US \$18.5 million, harvested in 2010 (Washington Department of Fish and Wildlife 2012). Geoduck farming has become common in WA throughout Puget Sound on private tidelands during the past decade (Washington Department of Natural Resources 2012). In BC, commercial-scale development of geoduck aquaculture has been limited despite significant interest in culturing this species. Currently in BC, there are approximately 40 existing crown-land aquaculture tenures (both intertidal and subtidal) listing geoducks on their licenses, but the number of tenures actually engaged in geoduck aquaculture is unknown (DFO 2012). A harvest of 51.7 MT of farmed geoduck, worth CA \$1.1 million, was nevertheless recorded in 2010 by the British Columbia Ministry of Agriculture (2012). Furthermore, the Underwater Harvesters Association (UHA, the BC geoduck fishery association) has undertaken experimental geoduck fishery enhancement programs since 1995 and has planted 250,000–700,000 hatchery-reared juveniles annually on several crown-land subtidal sites in the Strait of Georgia since 1997 (DFO 2012; UHA 2012).

Juvenile geoducks used for aquaculture or fishery enhancement in BC and WA are currently provided by only a few local hatcheries, where juveniles can be reared in various culture systems either with or without sand as a substrate (Liu and Pearce, personal observations), generally to 5–20 mm shell length before field out-planting (Goodwin and Pease 1989; Davis 2004). When out-planted, hatchery-reared juvenile geoducks are vulnerable to a variety of bottom-feeding predators such as fish, shrimps, crabs, and sea stars. Fast burrowing and burial are therefore likely to be important to survival after field out-planting (Goodwin et al. 1985; Goodwin and Pease 1989). Burial time of hatchery-reared juvenile geoducks averaged approximately 8 min for 5-mm shell length clams and 30 min for 10-mm shell length clams when tested in sand-filled beakers with seawater (Goodwin et al. 1985; Goodwin and Pease 1989). Note that Goodwin et al. (1985) and Goodwin and Pease (1989) did not mention whether or not these juveniles were produced with sand substrate. A culture system without sand – where juveniles are placed directly on various culture vessel bottoms – greatly facilitates routine hatchery maintenance. However, burrowing rates of the juvenile clams subsequently obtained may be negatively affected, as reported for juveniles of the basket cockle (*Clinocardium nuttallii*) (Epelbaum et al. 2011).

Hatchery-reared juvenile geoducks are subject to various types of predation after field out-planting, one of which is siphon nipping (Goodwin and Pease 1989; Beattie 1992; Feldman et al. 2004; Straus et al. 2008). In soft-sediment marine habitats, bottom-feeding fish, shrimps, and sometimes crabs frequently bite off buried clams' siphons exposed above the sediment surface. Such non-lethal loss of siphon tissues to predators can represent a significant energetic input to higher trophic levels of the food chain (De Vlas 1985; Coen and Heck 1991; Arrighetti et al. 2005; Tomiyama and Ito 2006). Simulated siphon nipping has been shown to affect feeding and growth in various bivalve species including the Baltic macoma (*Macoma balthica*) and the

Pacific littleneck clam (*Protothaca staminea*) (Peterson and Quammen 1982; De Vlas 1985; Kamermans and Huitema 1994). Current farming practices in BC and WA use predator exclusion nets (subtidal/intertidal) or PVC tubes (intertidal) to protect planted juvenile geoducks for the first 1–3 years to reduce loss to predation (Davis 2004; Canadian Aquaculture Systems Inc. 2012). These protective devices, however, do not offer complete predator exclusion.

The present laboratory study evaluated the burrowing rate of hatchery-produced juvenile geoducks, with and without sand acclimation, as well as the growth performance of siphon-nipped juveniles. The goal was to help refine existing hatchery strategies and to promote an understanding of the potential implications of siphon nipping by predators on out-planted juvenile geoducks.

2.0 MATERIALS AND METHODS

2.1 EXPERIMENTAL ANIMALS

Broodstock were collected in the Strait of Georgia (DFO Pacific Fisheries Management Areas 14 and 17) in October 2010 and 2011. Mean (\pm SE) shell length and live weight means for 2010 and 2011 were: 157.7 ± 1.87 mm and $1,275.8 \pm 47.0$ g ($n = 47$) and 149.6 ± 2.07 mm and $1,399.3 \pm 44.6$ g ($n = 50$), respectively. Between 20 and 30 animals were laid horizontally on the bottom of a holding tank (L \times W \times H: $1.2 \times 0.9 \times 0.3$ m) and provided with flow-through, sand-filtered and UV-treated seawater (flow rate: $3\text{--}4$ L min^{-1} ; temperature: $8\text{--}12^\circ\text{C}$; salinity: 26–31 ppt). A single-algal diet of *Chaetoceros muelleri* (CM) or *Isochrysis* sp. (Tahitian strain: TISO) was drip-fed at a ration of $4\text{--}6 \times 10^9$ cells $\text{ind}^{-1} \text{d}^{-1}$. Spawning was induced by addition of excessive amounts of TISO to the holding tank (W. Liu, unpublished data). Batches of fertilized eggs obtained from multiple parents were collected and hatched in tanks (L \times W \times H: $1.2 \times 0.9 \times 0.3$ m) at a density of < 30 eggs ml^{-1} and a temperature of $12\text{--}15^\circ\text{C}$. After 48–60 h, newly developed D-larvae (~ 120 μm) were collected and reared in a 300-L cylindro-conical tank at a density of $3\text{--}8$ ind ml^{-1} . The larvae developed into pediveligers ($\sim 350\text{--}380$ μm) after a further 18–20 d and settled in a circular tray (diameter: 36 cm; depth: 6 cm; area: $1,020$ cm^2) with 200–240- μm bottom mesh, floating on the water surface of the cylindro-conical tank. Post-set juveniles were cultured in the mesh tray to various sizes at a density of < 30 ind cm^{-2} (mesh area) before reaching 2 mm shell length and < 4 ind cm^{-2} before reaching 5 mm, prior to use in experiments. Previous work had shown these densities to be suitable for rearing juveniles with no adverse effects (W. Liu, unpublished data).

During the period of larval and post-set juvenile rearing, seawater in the cylindro-conical tank was provided with aeration and fully renewed every 1–2 d with 1- μm filtered and UV-treated seawater ($15.5 \pm 0.4^\circ\text{C}$). The upwelling water motion, generated by aeration, also facilitated water exchange between the inside and outside of the settlement mesh tray during the post-set period. The animals were fed with a bi-algal diet, mixed at a ratio of 1:1 by ash-free dry weight (AFDW), of *Chaetoceros calcitrans* and TISO for the larval stage and of CM and TISO for the post-set stage, with a feeding density of 1–2 and $2\text{--}10 \times 10^4$ equivalent TISO (E-TISO) cells $\text{ml}^{-1} \text{d}^{-1}$, respectively. A photoperiod of 16-h light and 8-h dark was maintained using overhead fluorescent lighting.

2.2 BURROWING RATE OF JUVENILES WITHOUT SAND ACCLIMATION

The burrowing rate of juvenile clams grown on mesh trays without sand acclimation was examined using three different rearing batches of juvenile geoducks obtained from different spawns (mean shell length: 3.9–4.7 mm; Table 1). In each trial, 300 juveniles were spread onto a mesh tray (the same as used in juvenile rearing described above) with a 2-cm layer of freshly prepared medium-grain-sized sand (250–500 μm grain size) on the bottom (*i.e.* the sand tray). The sand was collected from a natural geoduck bed, sorted in the laboratory, washed in boiling water for 30 min, and dried at 110°C. The sand was then washed in 1- μm filtered seawater and allowed to settle before use in the experiment – referred to subsequently as “freshly prepared” sand. The sand tray was placed on the bottom of a holding tank (L \times W \times H: 1.2 \times 0.9 \times 0.3 m) and submerged in static water before introduction of the juveniles. After overnight holding (16–20 h) at 16°C with aeration, numbers of individuals in-sand (shell fully buried or part of the shell including umbo buried below sand surface), half-in-sand (part of the shell including umbo still exposed above sand surface), and on-sand (lying horizontally on sand surface without any part of the body buried and showing no burrowing behaviour) were recorded and expressed as a percentage of the total number of animals recovered. A few animals found outside the sand tray at sampling time, probably due to drifting passively with water movement by aeration, were not counted.

2.3 BURROWING RATE OF JUVENILES AFTER SAND ACCLIMATION

The above experiment revealed that many (38.5%, Table 1) juveniles grown on mesh trays without sand substrate were unable to burrow after overnight holding on sand. Burrowing rate was therefore further tested for juveniles after sand acclimation. A batch of juveniles, from the same spawn, grown on a mesh tray without sand, were transferred to two mesh trays (the same as described above), 500 individuals per tray, when they reached a mean \pm SE shell length of 3.04 \pm 0.05 mm ($n = 50$). One mesh tray contained a 2-cm layer of freshly prepared medium sand (sand tray) on the bottom and the other was left without sand (control tray). Both the sand and control trays were placed in a holding tank (as described above), the former on the tank bottom and submerged in water and the latter afloat on the water surface. Water in the holding tank was held statically at 16°C and was fully changed every 2 d, followed by feeding the bi-algal diet of CM and TISO *ad libitum* ($5\text{--}10 \times 10^4$ E-TISO cells ml^{-1}). At each water change, juveniles in the sand tray were washed out and returned to the tray to allow them to re-burrow after both the sand and the tray were flushed with clean seawater. The control tray was also flushed at the same time. Initial observations showed that most juveniles in the sand tray, after being washed out at each water change, were eventually able to re-burrow into the sand overnight after 4–6 d and in 2 h after 8 d of sand acclimation (indicating improvement of burrowing ability over time after sand acclimation).

The juveniles were acclimated for 14 d (then about 5 mm in mean shell length, see below, and all buried completely in sand) under these conditions before testing for burrowing rate in 14-cm diameter containers, filled with a 2-cm layer of freshly prepared medium sand and 5 cm of overlying seawater. Ten juveniles were removed from both trays and placed in separate containers at the same time. The trial was conducted six times (*i.e.* 60 juveniles per tray) on the same day. Burrowing status was recorded after 5, 10, 15, and 30 min. Numbers of individuals starting to burrow (for 5 min data only, as the clams just positioned their bodies vertically to start burrowing), in-sand, half-in-sand, and on-sand (see above definitions) were recorded for each of

the six trials at each sampling time and data were expressed as a percentage of the 10 juveniles tested for each trial ($n = 6$). Shell lengths of the juveniles in both the sand and control trays were measured after the tests (mean \pm SE shell length: 5.30 ± 0.12 mm and 5.04 ± 0.14 mm, respectively; $n = 50$).

2.4 SIPHON NIPPING

Approximately 200 juveniles from a common batch grown on a mesh tray without sand were placed in an 18-cm diameter container upon reaching a mean shell length of approximately 4 mm. The container had a 2-cm layer of freshly prepared medium sand on the bottom to allow the juveniles to acclimate and burrow. The container was placed on the bottom of a static holding tank (described above) and submerged in water at 16°C with aeration. Most (>90%) juveniles were found to eventually burrow in the sand in about a week. Water in the holding tank was fully changed every 1–2 d and the juveniles were washed out from the container and allowed to re-burrow after the sand was flushed with clean seawater. A bi-algal diet of CM and TISO was offered *ad libitum* ($5\text{--}10 \times 10^4$ E-TISO cells ml^{-1}) after each water change. The juveniles were reared for 4 weeks (then about 7.5 mm in mean shell length, Table 3) before beginning the experiment on siphon nipping, which used 10 individuals for the siphon-nipping treatment and 10 for the intact animal control.

At the start of the experiment, each juvenile was transferred to a separate glass dish (diameter: 6.5 cm) containing a 4-cm layer of freshly prepared medium sand on the bottom and 0.5 cm of overlying seawater. When the juveniles started burrowing minutes later they also extended their siphons, at which time the pigmented siphon tips ($1/4\text{--}1/3$ of the siphon length) were excised quickly using scissors. The 20 glass dishes, with the juveniles burrowed in sand, were then placed randomly on the bottom of a holding tank (as described above), submerged in water, and maintained for 4 weeks before final sampling. The static holding tank was provided with aeration, and the water completely changed ($16.8 \pm 0.3^{\circ}\text{C}$) twice weekly. A bi-algal diet of CM and TISO was offered *ad libitum* ($5\text{--}10 \times 10^4$ E-TISO cells ml^{-1}) after each water change. At the end of week 2, the juveniles were washed out of each Petri-dish and allowed to re-burrow after the dishes were filled with freshly prepared medium sand. For each juvenile, shell length and wet weight were recorded at the start and the end of the experiment, and dry weight and AFDW at the end of the experiment. Dry weight and AFDW were also measured from an additional 10 individuals from the common batch at the start of the experiment. Dry weight was obtained by drying the specimens at 60°C to constant weight and AFDW by weight loss after incineration in a muffle furnace for 5 h at 500°C .

2.5 STATISTICS

The initial trial on burrowing rate with three batches of juveniles without sand acclimation was not a comparative study. Mean burrowing percent at the end of the trial was therefore calculated without statistical analysis. Burrowing rates in the second trial were compared between with-sand and without-sand acclimation treatments for the in-sand rate category only using a one-way ANOVA at each sampling time of 10, 15, and 30 min. One-way ANOVAs were also used to compare the effect of siphon nipping on the various growth traits of the juveniles at the end of the experiment. Data were deemed normal and homogeneous as confirmed by the Kolmogorov-Smirnov test and Levene's test, respectively. Statistical analyses were facilitated using the software NCSS 2007 (NCSS LLC, Kaysville, Utah, USA), with the significance set at $\alpha < 0.05$.

3.0 RESULTS

3.1 BURROWING RATE OF JUVENILES WITHOUT SAND ACCLIMATION

After overnight holding on sand, the three different rearing batches of juveniles grown on mesh trays without sand acclimation showed percentages of in-sand of 28.5–55.4%, half-in-sand of 14.5–18.3%, and on-sand of 27.7–53.2%, yielding means of 45.0%, 16.6%, and 38.5%, respectively (Table 1).

3.2 BURROWING RATE OF JUVENILES AFTER SAND ACCLIMATION

As noted earlier, when the juveniles were placed on the sand tray for acclimation, all of them were able to bury completely within 14 d. With regard to juveniles acclimated with sand for 14 d, 90.0% showed burrowing behaviour after 5 min and 86.0% had already buried in sand within 10 min. This in-sand rate increased to 93.3% and 95.0% after 15 and 30 min, respectively. After 30 min, only 5% of the juveniles were left half-buried in sand and there were no juveniles lying on sand (Table 2).

Only 50.0% of control juveniles without sand acclimation showed burrowing behaviour after 5 min and only 31.7% buried in sand after 10 min. The in-sand rate increased slightly after 15 and 30 min (36.7% and 43.3%, respectively), as with the sand acclimation treatment. After 30 min, 15.0% of the juveniles were half-in sand while 41.7% were still lying on sand, showing no burrowing behaviour (Table 2). A one-way ANOVA indicated that the difference of in-sand percentages between juveniles with and without sand acclimation was significant at each sampling time of 10, 15, and 30 min ($F_{1,10} = 64.1, 76.1, \text{ and } 47.6$, respectively, $P < 0.001$ for all three tests). Furthermore, the on-sand rates changed very little over time for the juveniles without sand acclimation (43.3–40.0% between 10 and 30 min; Table 2).

3.3 SIPHON NIPPING

During the 4-week experiment, shell length of juveniles with siphon nipping increased from 7.53 ± 0.09 to 10.33 ± 0.38 mm (mean \pm SE, $n = 10$), wet weight from 67.8 ± 3.7 to 384.5 ± 52.6 mg, dry weight from 17.0 ± 1.2 to 94.5 ± 12.4 mg, and AFDW from 5.9 ± 0.4 to 44.8 ± 7.1 mg. Values of control animals without siphon nipping followed very similar growth patterns (Table 3), with no significant ($P > 0.05$) differences being found for any of the growth traits assessed between the siphon-nipping and the control treatments at the end of the experiment. No mortality was observed in both control and siphon nipping treatments.

4.0 DISCUSSION

Juvenile geoducks in the present study showed an average burrowing rate of 86.0% in 10 min after sand acclimation. This is consistent with the 8-min burial time of similar-sized (5 mm) juveniles reported by Goodwin et al. (1985) and Goodwin and Pease (1989). Furthermore, significantly more juveniles were able to burrow in sand after sand acclimation than when not previously acclimated to substrate. Similar results have been reported for juvenile basket cockles in the laboratory (Epelbaum et al. 2011). The lower burrowing rate for juvenile geoducks without sand acclimation is largely attributable to the on-sand rate since ~40% of the juveniles just laid horizontally on the sand surface without showing any burrowing behaviour over the 30-min trial (Table 2). The ~40% on-sand rate in that trial corresponds well with the mean rate (38.5%)

obtained in the first trial for the three batches of juveniles without sand acclimation when held on sand overnight (Table 1).

These results suggest that approximately 40% of the juveniles produced on mesh trays without sand acclimation temporarily lost their burrowing behaviour (*i.e.* they could not burrow into sand overnight, but would eventually do so if acclimated on sand for a certain period of time). This lack of burrowing ability after out-planting would increase vulnerability to predation and current drift (even if they were protected by netting). In addition, unburied individuals may encounter unfavorable temperature changes and increased predation (*e.g.* by birds) if exposed to the air at low tide. This may have significant adverse impacts on the success of field out-planting. Acclimating hatchery-reared juvenile geoducks to sand for a certain period of time just before field out-planting may maximize burrowing rate and minimize the potential for juvenile loss. This strategy also has the advantage over that of holding the juveniles in sand throughout the hatchery stage as juvenile geoducks are usually held in hatcheries for several months before they reach a size suitable for out-planting, making the routine hatchery maintenance with substrates rather difficult over a prolonged period. Our initial observation during the present experiments was that all juveniles were able to bury completely in sand after 14 d of sand acclimation when placed on the sand tray. Further examination of the burrowing behaviours of juvenile geoducks in relation to juvenile size, sediment type and grain size (Goodwin and Pease 1989; Tallqvist 2001; de la Huz et al. 2002), and environmental factors such as temperature and salinity (Savage 1976; Nakamura et al. 2005) would help establish the most efficient field out-planting regime for a particular aquaculture or fishery enhancement site. Additional research should help determine the minimum substrate acclimation time required for clams to develop appropriate burrowing behaviours and the effect of juvenile size on this acclimation time.

In the field, sub-lethal predation on burrowing bivalves occurs when fish, shrimps, and sometimes crabs clip a portion of the clams' soft tissue, often the siphon tips, without causing mortality (Peterson and Quammen 1982; Kamermans and Huitema 1994; Smith et al. 1999; Sasaki et al. 2002). Simulated siphon nipping has been shown to reduce the growth rate of bivalves in field enclosures (Peterson and Quammen 1982; Coen and Heck 1991) and the energetic costs of siphon regeneration and reduced feeding time/efficiency may explain the observations (Coen and Heck 1991; Kamermans and Huitema 1994; Heck et al. 2002). However, simulated siphon nipping in the present study did not significantly affect any of the growth traits, evaluated over 4 weeks, of juvenile geoducks. This could be explained by several factors. First, as geoducks possess very long siphons, nipping of the tips may not represent a significant loss of biomass. Siphon nipping in the laboratory was not found to significantly affect various condition factors of *M. balthica* (Pekkarinen 1984). In contrast, siphon removal from half to all is lethal to the long-siphon clam (*Mya truncata*) (Welch and Martin-Bergmann 1990). Second, the excised siphon tips may regenerate quickly (Pekkarinen 1984; De Vlas 1985; Tomiyama and Ito 2006) so that there might not be significant disruption in feeding due to tissue loss. This is yet to be demonstrated for geoducks. Third, siphon nipping pressure in the laboratory, as in the present study, may be far less intensive than that occurring in the field, where an individual clam may experience repeated siphon nipping by predators, possibly losing several siphon tips per day (De Vlas 1985) or being nipped many times over a growing season (Sasaki et al. 2002). As juvenile geoducks are subject to protective devices upon field out-planting, the chance of being frequently nipped by predators is reduced. Results of the present study nevertheless indicate that occasional

loss of siphonal tissues by predation would probably not critically affect the welfare of juvenile geoducks after field out-planting. Further research with geoduck juveniles is required in both the laboratory and field to determine the frequency and severity of siphon nipping of juveniles with predator protection, the impacts of repeated nipping events on clam growth and survivorship, and the effect of siphon nipping on juveniles of various sizes.

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7.0 TABLES

Table 1. Burrowing percentages of various batches of juvenile geoduck clams grown on mesh trays without sand acclimation after overnight holding on a sand tray.

	Shell length (mm)*	In-sand (%)	Half-in-sand (%)	On-sand (%)
Batch 1	3.87 ± 0.11	28.5	18.3	53.2
Batch 2	4.71 ± 0.05	51.0	14.5	34.5
Batch 3	4.15 ± 0.07	55.4	16.9	27.7
Mean		45.0	16.6	38.5

* Mean ± SE ($n = 50$). There were 300 juveniles tested in each batch. See text for definitions of in-sand, half-in-sand, and on-sand percentages.

Table 2. Mean burrowing percentages of juvenile geoduck clams after sand acclimation for 14 days and without sand acclimation, over a period of 30 minutes.

Time (min)	With sand acclimation			Without sand acclimation (control)		
	In-sand (%)	Half-in-sand (%)	On-sand (%)	In-sand (%)	Half-in-sand (%)	On-sand (%)
5 *	90.0 ± 3.7		10.0 ± 3.7	50.0 ± 10.3		50.0 ± 10.3
10	86.0 ± 2.2 ^a	13.3 ± 2.1	0.0 ± 0.0	31.7 ± 6.5 ^a	25.0 ± 4.3	43.3 ± 8.8
15	93.3 ± 2.4 ^a	6.7 ± 2.1	0.0 ± 0.0	36.7 ± 6.1 ^a	23.3 ± 4.2	40.0 ± 8.6
30	95.0 ± 2.2 ^a	5.0 ± 2.1	0.0 ± 0.0	43.3 ± 7.1 ^a	15.0 ± 5.0	41.7 ± 9.5

* Data are those starting to burrow only (*i.e.* the clams just positioned their body vertically at this time).

^a Significant difference for the in-sand percentage between the sand acclimation and control treatments at each sampling time of 10, 15, and 30 minutes as determined by a one-way ANOVA ($F_{1,10} = 64.1, 76.1, \text{ and } 47.6$, respectively, $P < 0.001$ for all three tests). All data are means ± SE ($n = 6$).

The shell length was 5.30 ± 0.12 mm and 5.04 ± 0.14 mm (mean ± SE, $n = 50$) for juveniles with and without sand acclimation, respectively.

Table 3. Mean size of juvenile geoduck clams, with or without (control) siphon nipping, at the start (initial) and end (final) of a 4-week experiment.

	Initial		Final			
	Shell length (mm)	Wet weight (mg)	Shell length (mm)	Wet weight (mg)	Dry weight (mg)	Ash-free dry weight (mg)
Siphon nipping	7.53 ± 0.09	67.8 ± 3.7	10.33 ± 0.38	384.5 ± 52.6	94.5 ± 12.4	44.8 ± 7.1
Control	7.43 ± 0.10	68.6 ± 3.2	10.34 ± 0.48	414.3 ± 50.6	104.9 ± 12.1	51.0 ± 6.7

The initial dry weight and ash-free dry weight were 17.0 ± 1.2 mg and 5.9 ± 0.4 mg, respectively, from an additional sample of 10 juveniles not used in the experiment. All data are means \pm SE ($n = 10$).