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Project Summary

During this pilot project, we met three general objectives: 1) identify west coast sources of local broodstock and propagules for select native clam species, 2) evaluate growth of select clam species under different grow-out conditions (bay and open coast; with and without two types of substrate; with and without other species), and 3) assess shellfish pathogens and toxins that may affect culture of the native clam test species. Despite several native clam species potentially suitable for aquaculture reported as common in published literature and local knowledge accounts in and around the San Diego region, only west coast venus clams, *Chione californiensis* and *C. undatella* (family: Veneridae) were in abundances high enough in 2021 to support collections for experiments. The paucity of clam diversity and abundance was possibly due to a massive red tide and subsequent die offs in the previous year. Clam survival was similar when grown along the coast than in the bay, but growth by weight was slightly greater on the coast. Clam preferences for potential growout substrates were identified in a lab trial. The use of preferred natural fiber and plastic mat substrates in both the coastal and bay systems was linked to higher mortality rates of larger individuals and, although similar abundances of sessile fouling organisms as in containers with no substrate, more maintenance effort to clear the fouling. Clams in expandable mesh bags without substrate had the highest survival rates while those in mesh cages had the highest growth rates, which was likely associated with a larger mesh size on the cages than bags. When grown alone and in the presence of other species- seaweed and/or sea cucumber- clam survival was similar, but growth was faster when grown alone. Abundances of sessile and mobile fouling organisms were however lower in treatments with sea cucumber revealing a potential benefit of lower maintenance effort as well as diversified products from integrated multi-trophic growout. Histological evaluations for the west coast venus clams revealed sexual maturity at 13.5-15 mm length, separate sexes, and, at

least for spring/summer specimens, dribble spawning. Some individuals contained trematodes and procaryotes known to be common in clams in the region. The remaining histological evaluations and other health assessments using qPCR methods are ongoing. The project results will contribute to the long-term goal of expanding farming of native west coast shellfish.

Takeaways from West Coast Venus clam growout, San Diego, Summer-Fall 2021

1. Growout system. Clam growth may be faster on the coast than in the bay, but may come at a cost of higher mortalities and added design and maintenance effort (e.g. durable deployment structure to withstand wave action, removal of fouling from gear and clams).
2. Growout containers. The use of a flexible bag material with the largest feasible mesh size should be considered; artificial substrates and rigid cage material may constrict clams as they grow and add maintenance effort.
3. Broodstock. West coast venus clams are relatively accessible, being abundant in the intertidal and having an extensive geographic range (Baja to Alaska), maturing at a relatively small size (13.5-15 mm), and dribble spawning (low level of constant spawning, at least during the summer).
4. Product. West coast venus clams grew 2.8-3.1 mm in shell length (2.9-3.8 g whole weight) in 3 summer months and, so far, have no known novel parasites.
5. Novel growout. Clams grown out with other trophic levels had similar survival rates and slower growth rates than clams grown alone; addition of taxa, however, conferred other benefits (e.g., reduced cover of fouling, diversification of products).

Rationale

The waters off the west coast are home to a great diversity of native organisms that are well adapted within and across the region and could serve as farmed species. Nonetheless, culture of native bivalves contributes little to overall aquaculture production. West coast commercial aquaculture clam production, for example, is largely focused on the non-native venerid Manila clam (*Ruditapes* (formerly *Venerupis) japonica*) in tidal flats. This is in part due to the long term success of culturing non-native bivalves and the fact that some consider these species now naturalized. However, the intentional expansion of the use of non-natives in new areas is being questioned by many, including regulators, prospective growers and the public. Heightened awareness of this issue comes at a time of renewed interest in aquaculture for food and conservation, associated increase in ocean culture lease sites, and the desire of existing growers to diversify product and/or switch to natives to improve environmental and business sustainability along with public perceptions. Increasing popularity of 'local' food species and the popularity of west coast recreational clamming indicate that there is also a demand for these products.

Our study focused on venerid clams including littlenecks (*Leukoma staminea*), butter clam (*Saxidomus nutalli*), clipped (flat) semele (*Semele decisa*), and venus clams (*Chione californiensis* and *C. undatella*). These taxa are amenable to success in aquaculture because of their desirability as food, geographic availability, and availability of biological and/or culture

information. These taxa have been popular food sources for indigenous people and, in more modern times, the targets of recreational fishing with over 95% of recreational clam harvest in California focused on these species (Chew and Ma 1987, Reilly 2001). Their geographic distributions are broad, with species that range from Alaska to southern Baja California, Mexico (*L. staminea, C. californiensis*) and those found in warmer waters toward the southern end of that range (e.g., *C. undatella*, *S. nuttalli*, *S. decisa*). Using clams with broad distributions provides opportunity for regional sharing of knowledge and seed, and incorporation of warmer water species into our assessments provides information on products that may strengthen climate-resiliency of industry along the west coast as ocean temperatures continue to warm.

Similarities in the phylogeny, biology, and ecology of venerid clams, and existence of well-developed large scale culture techniques for Manila clams and small scale techniques for a few native species in the northwest, including native littlenecks (Toba et al. 2005) and *Saxidomus gigantea* (a congener of S. nuttalli found from central California to Alaska; Cowles 2005) support the potential for advancing the culture of these native clams to a commercial scale (Toba et al. 2005). While we are unaware of any attempts to culture species of Semele clams, two congeners (*S. solida* and *S. chilienne*) are fished in Chile, and thus has been the focus of biological studies (e.g., Brown et al. 2002).

The selected species are found in both estuarine and subtidal habitats - such broad habitat requirements provide the potential to be grown at a variety of aquaculture sites (onshore, tidal, nearshore). All species are near-surface dwellers (not deep burrowing species) living within the top few (5-15) centimeters of stable substrates (Reilly 2001), which consist of a mix of sand and mud, or cobble, gravel and shell (Fitch 1953, Paul and Feder 1973, Reilly 2001).

Project Goal and Objectives

Addressing the long term goal of expanding farming of native west coast shellfish requires evaluations of: 1) Broodstock and propagule (seed) sources (Phase I), 2) Grow-out (Phase II), and 3) Seed production (Phase III). Assessing ease of grow-out is generally quicker and easier than assessing hatchery rearing potential, thus it is a logical starting point. Our goal was, therefore, to contribute to the expansion of aquaculture products on the west coast through the identification of lead candidate native clam species (i.e., assess Phases I & II) that could be the focus of future hatchery efforts (Phase III).

We met this goal through fulfillment of three objectives:
1) Identify west coast sources of local broodstock and propagules for select native clam species
2) Evaluate performance of our select clam species under different grow-out conditions
3) Assess shellfish pathogens and toxins that may affect culturing of our native clam test species.

This order of evaluation will help to determine the locations and types of sites where the species can be grown thereby providing insight into potential seed demand and the need for hatchery production. Conducting shellfish health assessments for these species also is a critical

first step for identifying potential issues that may arise with broodstock husbandry, seed production, growout and the transport of all stages to other areas.

Objective 1. Identify west coast sources of local broodstock and propagules for select native clam species.

For objective 1, we (1.) conducted a literature review, interviewed local experts knowledgeable about the distribution and abundance of native bivalves, and performed semi-quantitative field surveys of intertidal and subtidal areas to validate and expand clam broodstock and seed source locations and relative abundances; and (2.) created four candidate species profiles to provide a summary of basic biological information and appropriateness for aquaculture that can inform growers' decisions surrounding adoption of new species.

1.1. Broodstock and propagule assessments.

The literature and local experts revealed that our initial list of bivalves, venus clams (*Chione* spp.), common littleneck (*Leukoma staminea*), butter clam (*Saxidomus nutalli*) and clipped (flat) semele clam (*Semele decisa*), are generally fairly common in the intertidal and subtidal zones of area bays, especially Mission Bay and San Diego Bay. We conducted our field surveys in and nearby the intertidal areas identified by the literature and local experts. Tidal flats were accessed during lower low tides (≤0.5 ft MLLW) and systematically surveyed for clam presence along three 50-100 m transects located longitudinally to the water's edge at 3 elevations: ~0-0.5', 0.5-1.0, 1.0-1.5') that were raked (2 m x 2 m x 5-10 cm depth) every 10 m with an additional two to three deeper shovel holes (up 35-50 cm deep) dug per transect. If clams were found, raking and digging were concentrated in those areas as needed to collect specimens for the shellfish health assessments (Obj. 3). The subtidal assessments were fewer and overlapped with areas in Mission Bay identified in the literature and by experts.

During field surveys, seed (premature individuals) of butterclam, venus clams, common littleneck, and bentnose clams (*Macoma* spp.) were found in only one or two locations and in low abundances (under ~25 total for each species) in the intertidal and were not found subtidally (may be in part due to poor visibility when disturbing sediments underwater). No adult littleneck clams were found and adults of butterclam and semele clam were found only subtidally (except for one small Semele adult found intertially at the base of riprap). Two venus clams, *C. californiensis* and *C. undatella*, were abundant and together were the only taxa found in several sites and in high enough numbers to be collected and used for the growout trials. Besides our target species, we found mature individuals of other candidates of interest, including several each heart cockle (*Trachycardium quadragenarium*) and calico scallop (*Argopectin ventricosis*), and many Olympia oyster (*Ostrea lurida*).

One explanation for the observed lack of adults and seed of what should have been common bivalve species was an extreme red tide that occurred during spring 2020, just under one year before our field surveys, that caused widespread die offs of marine life. The impact was especially high in embayments where water residence times are relatively high (i.e., not well

flushed) and plankton biomass was exceptionally high due to nutrients and warmer water temperatures (Styles 2020, SDUT 2020).

1.2. Candidate species profiles.

We created four candidate species profiles based on the species that we expected to be relatively common (i.e, *Leukoma staminea*), that we observed that are of conservation interest (*Argopecten ventricosus*), and that were observed to be the most common during winter/spring 2021 and therefore used in our trials (i.e., *Chione californiensis, C. undatella*). These profiles can be found in Appendix 1 and are being adapted for inclusion on California Sea Grant's California Seafood Profiles website, which was recently launched and is undergoing some growing pains! Once on the Profiles site, they will be publicly accessible.

Objective 2. Evaluate growth of select clam species under different grow-out conditions.

For objective 2, we (1.) conducted a venus clam substrate preference trial in the lab, and used that information to (2.) design and conduct a field growout experiment testing growout system location (bay, open coast) and different container and substrate types. Given the challenges associated with finding several of our originally proposed target clam species, we instead (3.) added a trial that tested the performance in venus clams when grown alone and in the presence of other taxa (seaweed and/or sea cucumber).

2.1. Growout substrate preference trial.

Two plant nursery trays (60 x 60 x 5 cm) were divided into four 30x30 cm squares per tray. Clam substrate preference and substrate use frequency were tested using six substrate types (Figure 2.1). Grooves and circular areas were cut in the substrates to open up bigger spaces before being placed in flats. Ten *Chione californiensis* were placed evenly within each of the 30x30 cm sections of the flats on March 4, 2021 and trials were monitored weekly or biweekly from March 6, 2021 through April 29, 2021. The clam sizes included small (0.9-2.2 cm), medium (2.3-3.4 cm) and large (3.5-5.5 cm) clams.

Figure 2.1. The substrates used in the California venus clam substrate trials during March-April 2021. Photos are from Amazon.com.

Substrate preference

Substrate preference was tested in two ways— a choice between four substrate types of varying materials and forms, and a choice between stiff (gray doormat) and soft (green grass) plastic substrates. Clay aggregate could not be included in the preference trials because it was found to be relatively buoyant and needed to be contained in mesh which would have limited access by clams. Coconut coir was also excluded because the dense mat was difficult to cut grooves wide or deep enough to allow medium and large clams to settle (and space was limited). On average, more clams were found on gray plastic door mat and black plastic hen mats than the other substrates (Figure 2.2), possibly due to the higher, stiffer pile than green plastic grass. The black plastic hen mats were less apt to lose pieces of cut or broken bristles (pile) than the gray door mat because the bristles on the black mat were fused at the bottom adding more stability. The smaller clams, of which there were fewer, were the ones generally found on the green nest grass. While straw had intermediate abundances of clams, the clams sat at the top of the straw bundle and did not burrow in.

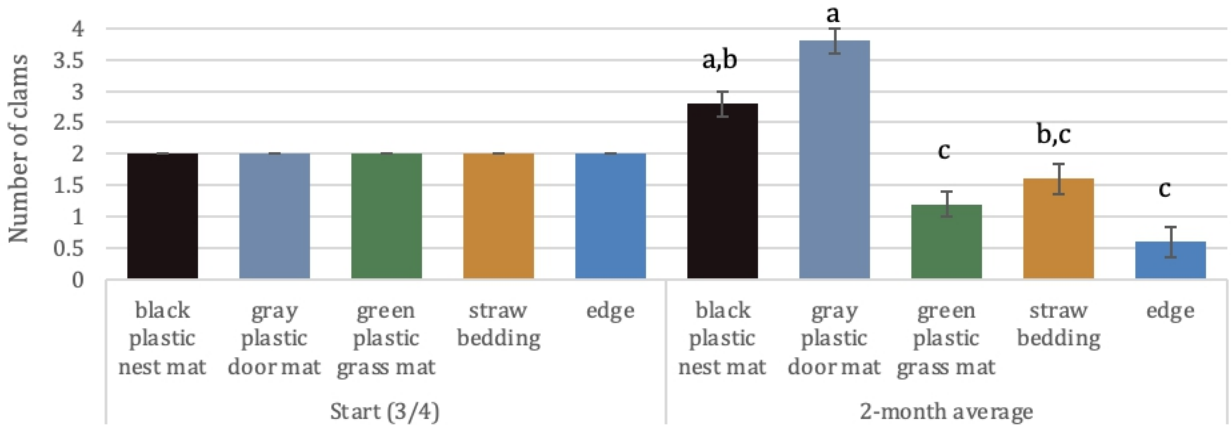


Figure 2.2. Number of California venus clams found on each substrate type and along edges (spaces between substrate strips). Clams (6-8 medium, 2-4 small) were evenly placed across substrates on 3/4/2021 and were relatively mobile with movement observed to within the first two days. Data are number at the start of the trial and average (±1SE) over the two months. ANOVA (blocked by time): substrate: $p<0.001$, $F_4=7.24$; time: $p=0.999$, $F_8=0.049$; n=45. Different letters indicate significance using pairwise Student t-tests, $\alpha=0.05$.

When given the choice between soft and stiff plastic substrates, the smaller clams preferred the softer green grass mat (Figure 2.3). Numbers increased with time, possibly due to more time to find and use substrate, and/or growth of biofilm (on all substrates but greater surface area of green mat may have meant more biofilm). The downside of the soft green grass mat was that the bristles shed when the mat was cut.

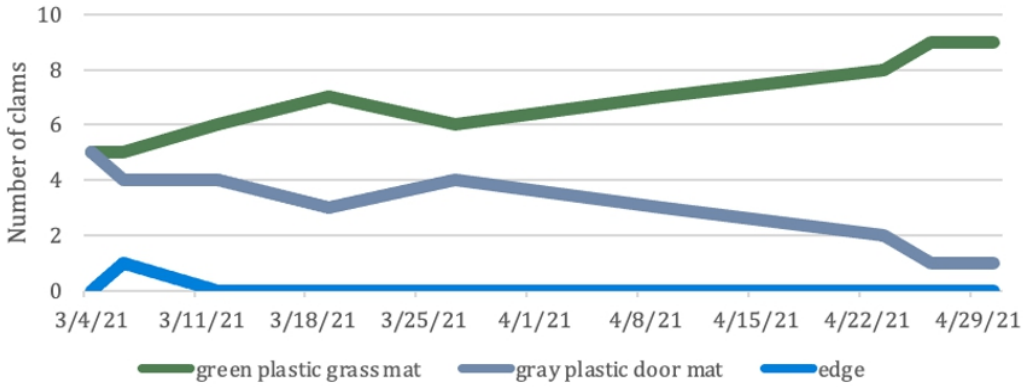


Figure 2.3. Number of clams found on green plastic grass and gray plastic door mats (or along the edges between mats) throughout the trial. Clams (2-3 medium, 7-8 small) were evenly placed across substrates on 3/4/2021 and were relatively mobile, with movement observed within two days. ANOVA (blocked by time) substrate: $p<0.001$, $F_2=51.4$; time $p=1.0$, $F_7=0$; $n=24$. Pairwise student t-tests, $p \leq 0.05$, clam abundance on green>gray>edge).

Substrate use

Substrate use, or the frequency at which clams nestled or burrowed into the substrates, was tested by using one substrate type per section of flat and placing 10 clams— 3-4 clams from each of three size classes—small, medium and large— on to the substrate on March 4, 2021. In general, across all substrate types, more smaller clams burrowed or nestled into the substrate and did so faster than the larger clams (Figure 2.4) indicating a preference or need by smaller clams for some sort of substrate and that the substrates used in this trial may be more suitable for small seed clams than the larger broodstock clams.

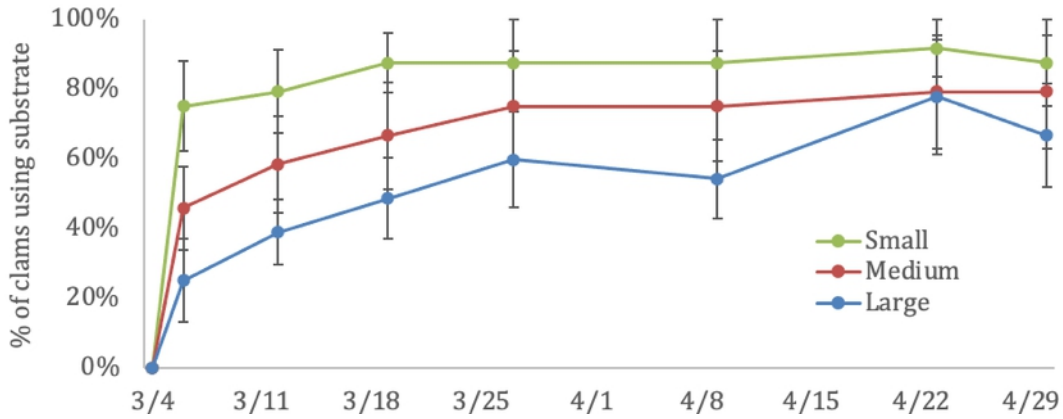


Figure 2.4. Average percent of clams from each of three size classes that used— burrowed or nestled into— the substrates that were used throughout this trial. N= 6 30x30 cm sections of flat per date containing ten clams (3-4 each of small=0.9-2.2 cm; medium=2.3-3.4 cm; large=3.5-5 cm).

Clams on the substrates with a pile—gray door mat, black hen mat, green grass mat— along with the clay aggregate were more apt to burrow or nestle in (Figure 2.5). The coconut coir mat was the least used possibly due to the dense, shorter pile- and lack of wider grooves and spaces. Straw was used with intermediate frequency although clams tended to stay near the top

of the bundle and not burrow into it. The use of straw in the clay aggregate substrate trial further indicated a potential undesirability associated with straw. We put the clams and semi-buoyant clay aggregate in a 30 x 30 x 3 cm mesh box and placed a wad of fine plastic net on top of the clay in ½ the box to stop it from floating. Over the other half of clay, we placed a wad of straw bedding to hold the clay in place. The clams formed a pile at one end of the mesh box on the plastic mesh side for the first week or two; then eventually worked into clay aggregate (especially the medium sized clams), but always stayed beneath the plastic mesh and not the straw (Figure 2.6). This pattern, coupled with the lack of burrowing into straw bundles, indicates a possible aversion to straw or straw extracts.

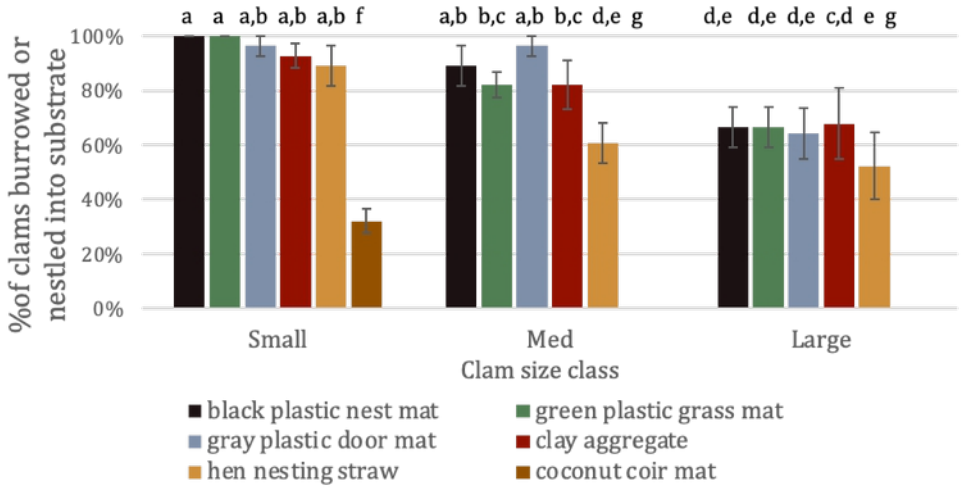


Figure 2.5. Average (±1SE) percent of clams that used (burrowed or nestled into) the substrate. N=8 sampling dates. Two-way ANOVA (clam size, substrate, size x substrate vs residuals of clam frequency (with the effects of time removed): $P<0.001$, $F_{17,108}=35.2$, n=126; clam size $p<0.001$, substrate $p<0.001$, size x substrate $p=0.082$). Different letters indicate significance using pairwise Student t-tests, $\alpha=0.05$.

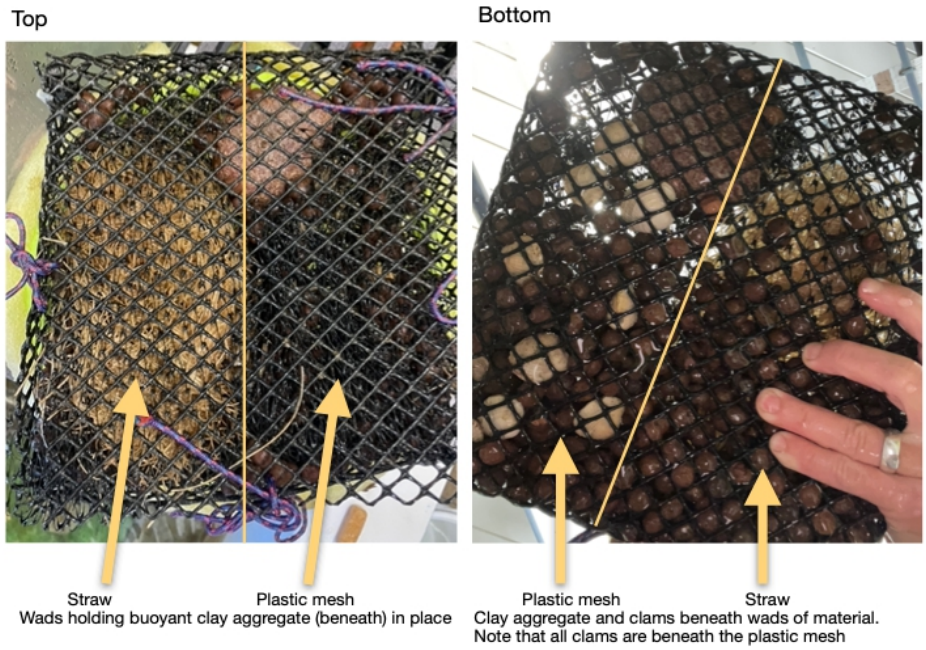


Figure 2.6. Photographs of the top and bottom of the mesh box that held the clay aggregate substrate treatment. Photo taken April 26, 2021, toward the end of the trial but this clam distribution was observed throughout the trial.

Substrate conclusions

California venus clam seemed to prefer substrate over none. The smaller size class of clams chose and tended to most utilize the piled substrates, especially the soft green grass mat which may have offered more appropriate levels of support and spacing for smaller than larger clams. The medium and larger size classes of clams had lower substrate utilization rates than smaller clams which may indicate that substrate is not always required for growout of adults and holding of broodstock, at least over short time periods as in this trial (2 months). In fact, there were no mortalities observed in clams regardless of whether they were burrowed/nestled or not, but a longer-term assessment of the effects of substrate on growth rates is still needed. Medium and large clams more frequently preferred and utilized the higher pile, firmer substrates (black and gray mats) and the clay aggregate over the soft pile (green grass) and dense short pile (coconut coir) substrates. While our hope is to minimize the use of plastics in aquaculture, most of the piled substrates tested and preferred by clams were plastic. The information from these trials informed our growout experiments (Section 2.2).

2.2. Field growout experiment testing growout system location (bay, open coast) and container and substrate types.

Growers, regulators and the public have interest in diversifying aquaculture products with native shellfish species and using suspended cultures to address environmental concerns and help meet growing consumer demand. Raising clams that are native and local eliminates concerns about invasive species and genetic or disease contamination by farmed species (Pranovi et al. 2006, Bert 2007, Padilla 2010), while being filter feeders in a productive estuary avoids the production and waste of feed, and improves water quality (Bert 2007, Bricker et al. 2014). Four species of littleneck clams, smooth chione, wavy chione, common littleneck and Manila clam) make up 95% of the littleneck clam harvest in California (Reilly et al 2001) illustrating desirability of all species, yet only the introduced Manila clam is commercially farmed on the continental west coast of the U.S. (Blue Ocean Institute 2013). Only recently has the commercial culture of a related species of venus clam, *C. fluctifraga*, begun in the Gulf of California, Mexico (Gongora-Goméz et al. 2022). There are many similarities in the phylogeny, biology, and ecology of the native Venerids and the Manila clam (as well as the other cultured bivalves) suggesting that farms raising the Manila clam, mussels and scallops could relatively easily adopt the culture of natives. The range of common littleneck is from Alaska to southern Baja California, Mexico (Fitch 1953, Abbott 1974) while the Chione species tend to be warmer climate species ranging from central California through Mexico and in the Gulf of California (Coan et al. 2000) making them good candidates for climate resilient aquaculture. The introduced range of the Manila clam overlaps extending from British Columbia to southern California (Reilly 2001). Like the Manila clam, the native Venerids usually occur from subtidal to intertidal elevations with peak biomasses between -0.5m to +1.3m (Paul and Feder 1973, Nickerson 1977, Reilly 2001). All are near-surface dwellers living within the top 5-15 cm of stable substrates, which consist of a mix of sand and mud, and sometimes cobble, gravel and/or shell (Fitch 1953, Paul and Feder 1973, Reilly 2001). Venus clams can be found subtidally (i.e., in refuges) to depths of 50 m (Reilly et al. 2001) and two species, *C. californiensis and C. undatella,*

were quite common in intertidal flats at the start of this study and so were chosen as the target species (Figure 2.7).

Figure 2.7. U.S. West Coast Venus clams: Fringed venus clam (*C. undatella* (L) and California venus clam (*Chione californiensis* (R)).

Growth of *C. fluctifraga* in a tidal pond and estuary in northwestern Mexico were higher in enclosures deployed on the benthos compared to those in suspended near the surface where environmental variability (e.g., temperature) was relatively high causing less favorable conditions for growth (Martínez-Córdova et al. 2013). However, benthic deployment not only has greater impact on benthic habitats than suspended systems, but high organic matter content, fine particles, and resulting turbid and/or anoxic conditions in the sediments of some systems, such as estuaries, can contribute to higher mortality rates including mass mortalities (Martínez-Córdova et al. 2013).

Use of artificial substrates in suspended systems has shown promise for the grow out of some benthic clams. For example, clay gravel as substrate improved survival in growout of Manila clams (*Venerupis philippinarum*) when media volume was kept at a relatively low density (Marshall and Dunham 2013). While lab trials using clay gravel prior to this experiment revealed that clay was difficult to use due to its buoyancy, solid but rugous substrates were instead tested in this study. The goal of this project was to determine the performance of native venus clams in various suspended grow out scenarios, including in a bay and on the open coast and in containers with and without two substrate types.

Study sites and Methods

Field experiments. Venus clams for the trials were collected earlier in June 2021 from local tidal flats. Both *C. californiensis* and *C. undatella* were used because they were initially sometimes difficult to tell apart, commonly co-occur in this region (i.e., have similar habitat requirements) and relieved harvest pressure on one or the other species. Assessments of clam species at the end of the trial, once confidence in identifications were high, revealed that *C. californienis* made up 85% and *C. undatella* made up 15% of all clams used.

Grow out trials were conducted on the Port of San Diego's FLUPSY located in San Diego Bay (32.71036° N, -117.17373° W) and on the open coast from the Scripps Institution of

Oceanography pier (32.86692° N, -117.25695° W) in San Diego, California, USA (Figure 2.8) between June 25 to Sept 29, 2021. The FLUPSY was non-operational throughout the trial, therefore the "bay" growout represented ambient flow conditions in a semi-arid, urbanized mid-bay (see Largier 1995, Largier et al. 1997, Chadwick et al. 2004). On the open coast, containers were secured to sturdy, weighted PVC frames (n=4 replicate frames) which were suspended from the pier. In the bay, containers were suspended from PVC frames that were set across the top of the FLUPSY bins (n=4 suspended lines located in one bin).

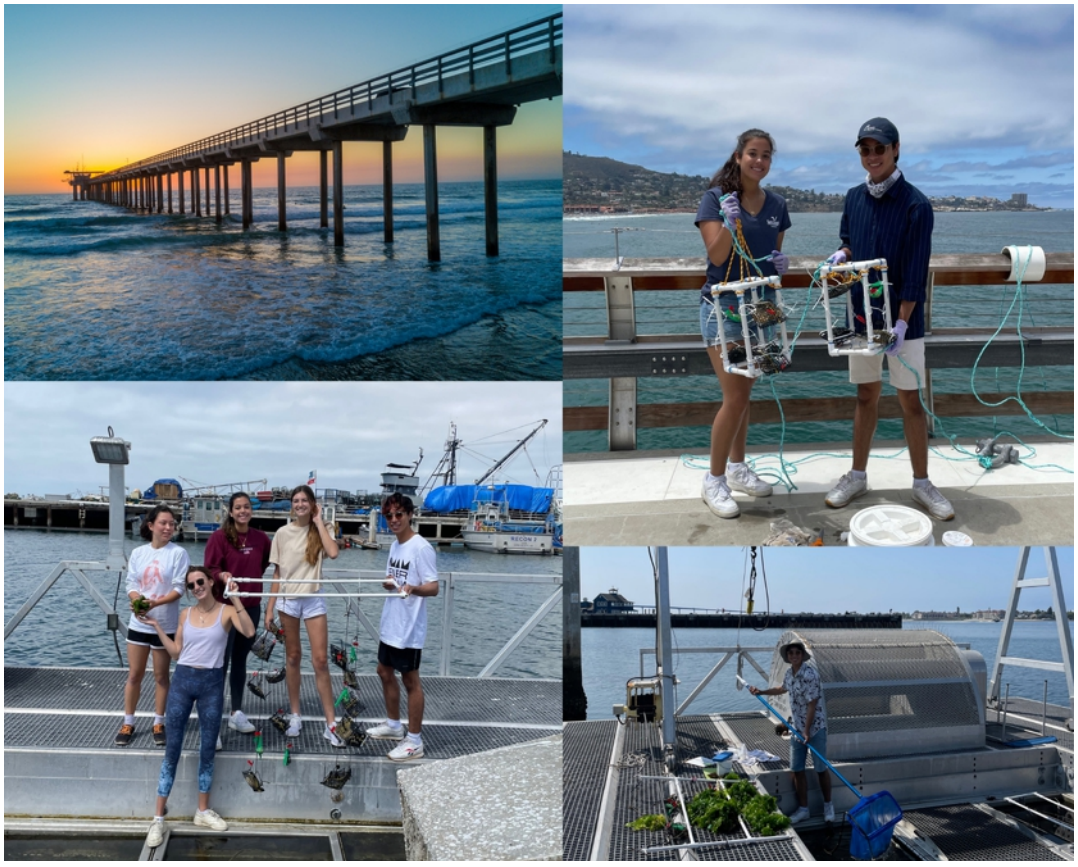
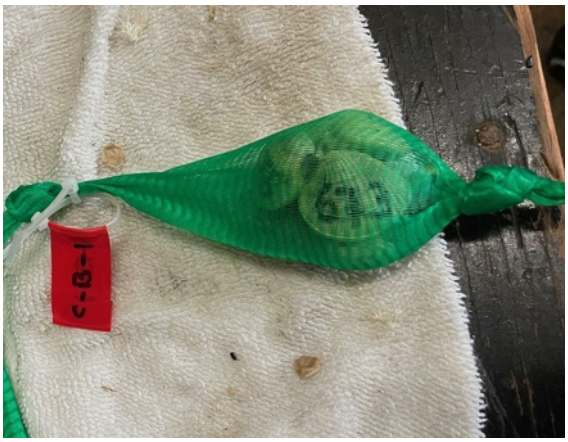


Figure 2.8. Photographs of the trial growout systems. Top row: Open coast- at Scripps Institution of Oceanography Pier where containers were secured to sturdy, weighted PVC frames (n=4 replicate frames) which were suspended from the pier. Bottom row: Bay- Port of San Diego's FLUPSY where containers were suspended from PVC frames that were set across the top of the FLUPSY bins (n=4 suspended lines located in one bin).

Four growout-container treatments were tested (n=4 replicate containers in each system): 3 mm-mesh bags, 6 mm-mesh cages, cages with natural sponge, and cages with black plastic mats; Figure 2.9). Each clam was numbered, measured, weighed, and sorted into four size classes (13-16 mm, 16.1-20 mm, 20.1-25 mm, 25.1-30 mm). Three clams from the two intermediate size classes and 1-2 clams from the smallest and largest size classes were placed in each container for a total of nine per container (Avg±1SD = 21±4 mm length). Dead clams were replaced each week with 7-9 clams per container maintained throughout the 3-month trial.

A. Cage with sponge

B. Bag



C. Cage only

D. Cage with mat

Figure 2.9. Photographs of the container treatments used for clam growout trials: (A.) Cage with natural sponge, (B). mesh bag (no substrate), (C.) cage only (no substrate), (D.) cage with plastic mat (cage not shown). Each container received 9 clams; dead clams were replaced each week with 7-9 clams per container maintained throughout the 3-month trial.

Temperature and light were recorded hourly using Onset Pendant loggers; hourly data were used to calculate daily averages, maximums and minimums which were then averaged across each week for comparison with weekly variables. Weekly assessments of clam mortality (#dead clams per container, dead clam length and height) and water properties (salinity, DO, pH, NO3-) were conducted. Weekly ranked assessments (0-4) of the cover of fouling organisms and the abundances of mobile organisms on and in each container, as well as on the frames, lines (pier) and bin (FLUPSY) were made. Weekly rainfall totals and number of rainy days (≥ trace amounts) were calculated *post hoc* using rainfall data from the nearby San Diego International Airport. Clam whole weight, length, and height were measured at the start and end of the trial, and clam meat wet and dry weights were measured at the end of the trial.

Data Analyses. Relationships between both the growout systems (coast and bay) and container type, and clam mortality and growth (percentage of changes in length, height and weight) over

the 3 month period were explored using two-way ANOVAs with replication, when variables were measured using each individual growout container (e.g., abundance of fouling organisms in each container), and without replication, when one measure of the variable per bin or line was made (e.g., water parameters). Comparison of the sizes (lengths) of surviving clams and those that died in each container treatment were made using Wilcoxon Signed Rank test to elucidate factors contributing to differential survival in the containers.

Comparisons of the end-of-experiment (Sept 2021) morphometric variables (ratios of shell length and both shell and meat weight) for clams grown out in the bay and open coast were made using one-way ANOVAs of the average ratios per container. The morphometric variables of clams used in the experiment were also compared to clams collected from the source tidal flat at the end of the experiment using one way ANOVA; because only 15 tidal flat clams were tested, ten tests were run each using 15 randomly selected clams each from the coast and bay grow out data. Finally, differences in morphometric variables between the two clam species, *C. californienesis* and *C. undatella*, were compared using one-way ANOVAs using clam measurement data pooled across the two growout systems, container treatments, and from a concurrent test of IMTA treatments in the bay system (253 more *C. californiensis* and 40 more *C. undatella*).

Relationships between the weekly environmental variables (water variables, rank cover of fouling organisms, rank abundance of mobile animals) and weekly clam mortalities were tested using forward, stepwise multiple regressions. All data were log (x+1) transformed (numeric data) or arcsin square root transformed (% data) before analysis and all statistical analyses were performed using JMP Pro 16.

Results

Overall mortality rate of clams across treatments in this 3-month trial was 17±3% in the bay and 29±6% on the coast. Weekly mortality rates were consistently, relatively low throughout the 3-month trial (avg±1SE dead per system per week ranged from 0±0 – 0.69±0.24; P=0.085, R^2=0.007, $F_{1,398}$=3.0, n=400).

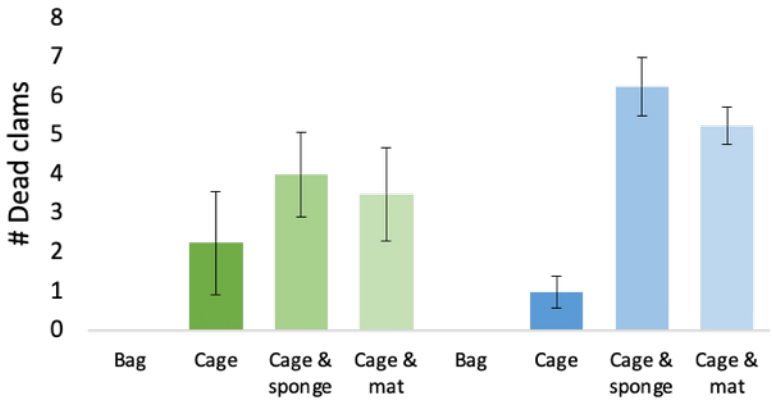


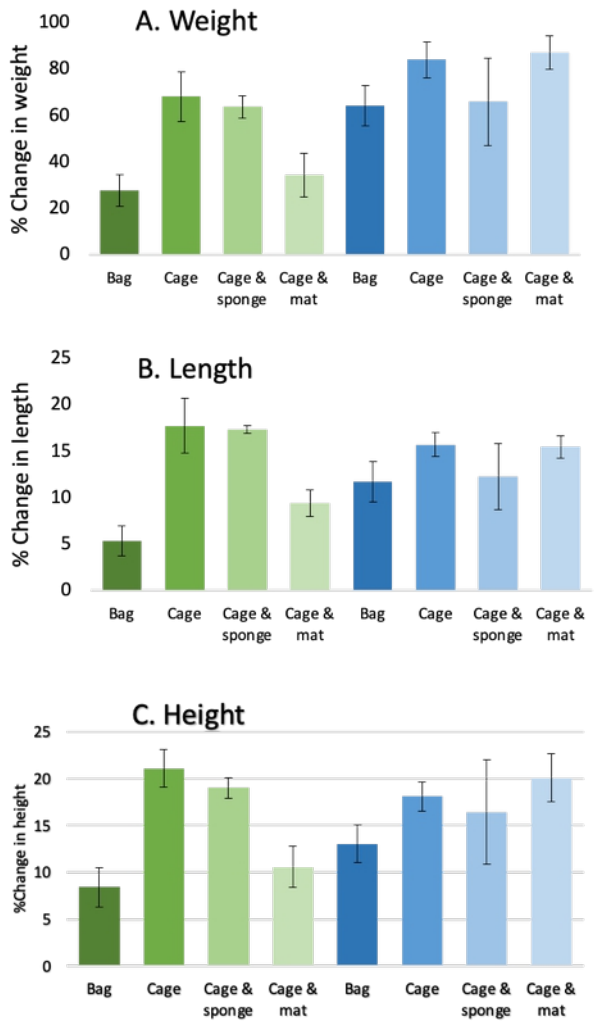
Figure 2.10. Average number of dead clams in each container treatment deployed in the bay (green) and on the open coast (blue) summed from weekly counts over the 3 month trial period (June 25 to Sept 29, 2021). n= 4 replicate containers per treatment each containing 7-9 clams.

Total clam mortality over the 3 months was highest in cages with substrate, lowest in bags where there was no mortality; and was similar between coast and bay (2-way ANOVA: P<0.001, $F_{7,24}$=11, n=32; Figure 2.10). The size (length) of clams that died in containers did not differ from

those that survived except for in the cages that contained plastic mats as substrate where the clams that died were larger than those that survived (P=0.05, S=14, n=8; Table 2.1).

Table 2.1. Average length of clams that survived throughout the trial compared with those that died in each container treatment. N= number of containers that contained dead and surviving clams. Data are from June 25 to Sept 29, 2021.

Treatment	Surviving clam length (mm)	Dead clam length (mm)	P	S	n
Bag	24.4 (±1.2)	n/a	n/a		0
Cage	26.0 (±0.4)	23.3 (±1.5)	0.31	-4	5
Cage + sponge	24.4 (±0.9)	23.6 (±0.4)	0.74	-3	8
Cage + mat	22.7 (±0.2)	25.5 (±1.2)	0.05	14	8
Results of Wilcoxon Signed Rank tests; Lengths are Avg±1SE					



Clam growth, as measured by % change in length, height and whole weight over the 3 month trial, tended to be highest in cages with and without substrate and lowest in bags. While change in whole clam size (length, height) did not differ between coast and bay, whole-weight gain was greatest on the coast (Figure 2.11, Table 2.2). Ratios of whole clam weight to meat weight were also lowest on the coast (Table 2.2).

Figure 2.11. Growth of clams in each container treatment deployed in the bay (green) and on the open coast (blue) measured as (A.) % change in whole weight, (B.) % change in length and (C.) % change in height between the start and end of the 3 month trial period (June 25 to Sept 29, 2021). n= 4 replicate containers per treatment each containing 7-9 clams.

Final (fall) length to whole weight ratios were lower on the coast than in the bay (Table 2.2). Clams collected from source tidal flats at the end of the experiment had ratios of shell length to whole weight, wet meat weight, and dry meat weight that were similar and/or intermediate between the clams grown out in the bay and on the coast (ANOVA length:whole wt: P=0.010-0.664, $F_{2,42}$=0.44-5.18, n=45, length:meat wet wt: P=0.001-0.0385,

$F_{2,42}$=3.76-14.67, n=45; length:meat dry wt: P=0.0.009-0.473, $F_{2,42}$=0.77-5.66 n=45; Figure Length x weight scatter plot; Figure 2.12).

Table 2.2. Results of two way ANOVAs testing relationships between growout system (bay, open coast) and container type on venus clam growth (%change in whole weight, length and height) between July- September 2021 and whole clam weight to meat weight ratios in September 2021. Cont= container treatment.

Independent variable	P	F	df	n	system p	cont p	system X cont p	Pairwise comparisons
%whole weight change	0.003	4	7,24	32	0.001	0.048	0.091	Coast > Bay; Cage ≥ Cage+sponge, Cage+mat ≥ Bag
%length change	0.003	4	7,24	32	0.376	0.004	0.020	Cage ≥ Cage+sponge ≥ Cage+mat ≥ Bag
%height change	0.022	3	7,24	32	0.276	0.019	0.086	Cage, Cage+sponge ≥ Cage+mat ≥ Bag
whole clam weight: meat wet weight ratio	<0.001	14	7,24	32	<0.001	0.058	0.206	Bay > Coast Bag ≥ Cage, Cage+mat ≥ Cage+sponge
whole clam weight: meat dry weight ratio	<0.001	7	7,24	32	<0.001	0.304	0.530	Bay > Coast
clam length: whole weight ratio	<0.001	6	7,24	32	0.002	<0.005	0.011	Bay > Coast Bag, Cage+mat, Cage+sponge > Cage

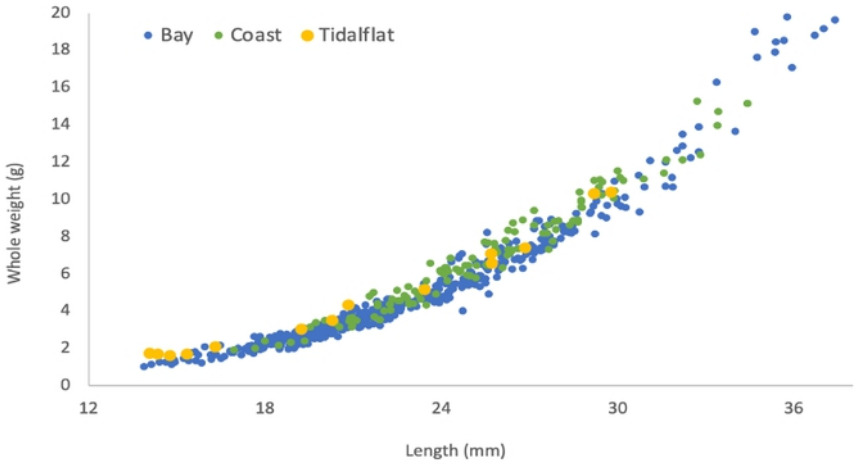


Figure 2.12. The relationship between the length and whole weight of venus clams (*Chione* spp.) from each growout system- open coast and bay- at the end of the trial (25 Sept 2021) and a small sample of clams collected from the source tidal flat (15 Oct 2021).

Venus clam species' morphometric characteristics

C. californiensis had a smaller length to height ratio (avg±1SE: 1.12±0.002) than the more oval *C. undatella* (1.17±0.015; Table 2.3). The two clam species had similar ratios of clam shell length to

whole weight (C.c.: 5.3±0.1, C.u.: 5.3±0.2; Table 2.3). *C. undatella* tended to have similar (or higher but more variable) ratios of length to wet meat weight (C.c.: 36±1, C.u.: 39±3), length to dried meat weight (C.c.: 234±11, C.u.: 300±42), whole weight to wet meat weight (C.c.: 7.2±0.08, C.u.: 7.2±0.3) and whole weight to dried meat weight (C.c.: 45±1, C.u.: 56±6; Table 2.3).

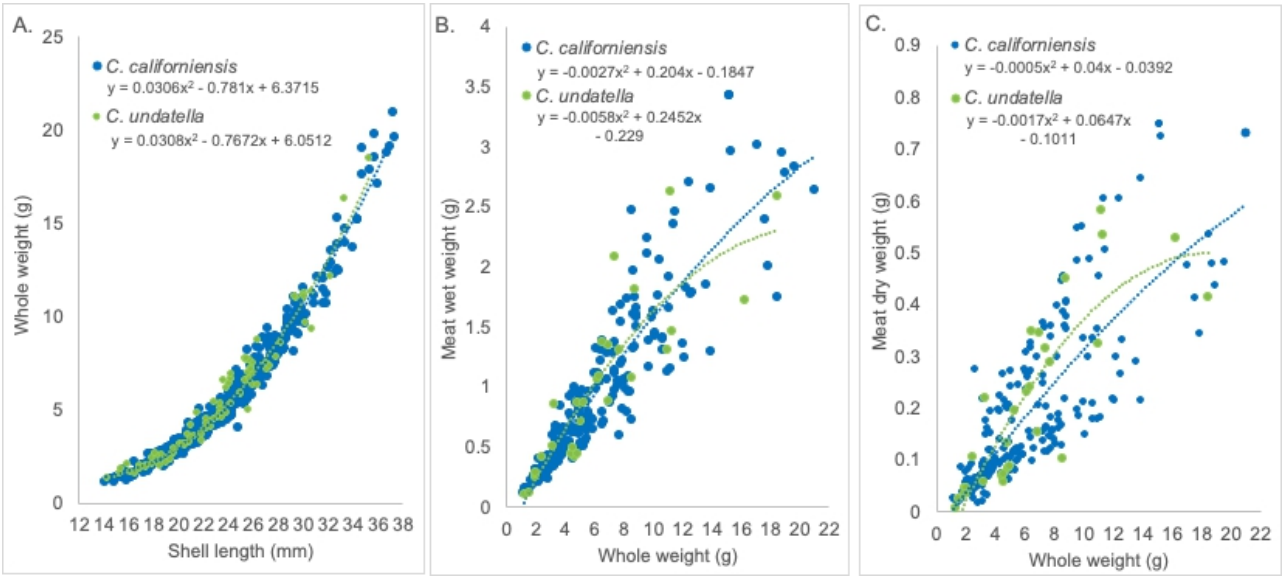


Figure 2.13. Relationships between shell length and whole weight (A.) and whole weight and wet (B.) and dry (C.) meat weights for *Chione californiensis* and *C. undatella* grownout out for 3 months in a bay and on the open coast of San Diego. Data are from September 2021, n=453 (length x wt) and 206 (wt x meat wt) *C. californiensis,* and 85 (length x wt) and 29 (wt x meat wt) *C. undatella*.

Table 2.3. Results of one-way ANOVAs comparing the morphometric variables (ratios of clam shell length, and shell and meat weight) of *Chione californieneis* and *C. undatella*. Data are from September 2021.

Ratio	**P**	**F**	**df**	**n**	**Pairwise comparisons**
Shell length to height	<0.001	93	1,536	Cc=453 Cu=85	Cu > Cc
Shell length to whole weight	0.77	0.1	1,536	Cc=453 Cu=85	Cu = Cc
Shell length to meat wet weight	0.93	0.1	1,233	Cc=206 Cu=29	Cu = Cc
Shell length to meat dry weight	0.62	0.3	1,233	Cc=206 Cu=29	Cu = Cc
Whole weight to meat wet weight	0.73	0.1	1,233	Cc=206 Cu=29	Cu = Cc
Whole weight to meat dry weight	0.54	0.4	1,233	Cc=206 Cu=29	Cu = Cc

Fouling organisms

Sessile fouling organisms including microalgae, macroalgae, spirorbid polychaetes, bivalves (e.g., mussel, oyster) and ectoprocts growing on the clam treatment containers were more abundant on the coast than in the bay, and did not differ between the types of treatment containers (although, cages with substrate took longer to clean since the cages needed to be opened and substrates removed to clean them properly). Abundances of mobile fauna, including sphaeromatid and cirolanid isopods, amphipods, snails, and midge larvae were greater on the coast than in the bay, and higher in the mesh cages with and without substrates than in the bags (fouling; Table 2.2). The relative abundance of mobile animals and cover of sessile organisms fouling containers each week were not strongly correlated with weekly number of clam deaths in the same containers in the bay (mobile: P=0.14, $F_{1,286}$=2.2, n=288; sessile: P=0.013, R^2=0.020, $F_{1,302}$=6.3, n=304) or on the coast (mobile: P=0.77, $F_{1,190}$<0.1, n=192; sessile: P=0.85, $F_{1,190}$<0.1, n=192).

Table 2.2. Results of two-way ANOVAs testing differences in rank abundances of both mobile and sessile fouling organisms between growout systems (open coast and bay) and container treatments (mesh cage, cage with natural sponge, cage with plastic mat, mesh bag). Data are from June 25 to Sept 29, 2021. Cont=container

Independent variable	**P**	**F**	**df**	**n**	**system p**	**cont p**	**system X cont p**	**Pairwise comparisons**
Rank of sessile organisms on containers	0.170	1.4	7,392	400	0.003	0.954	0.781	Coast > Bay
Rank of mobile animals on and in containers	<0.001	14	3,376	384	<0.001	<0.001	0.216	Coast > Bay; Bag < Cage, Cage+sponge, Cage+mat

Water conditions

Over this summer growout trial, the shallower bay growout experienced greater light levels and warmer temperatures, while the coastal waters had greater dissolved oxygen concentrations and pH. Salinity and nitrate concentration did not differ between systems. Throughout the summer weeks, temperatures, light levels, salinity, and nitrate all decreased, dissolved oxygen concentrations were consistent, and pH increased (Table 2.3). Of all the water parameters tested, including temperature, salinity, pH, dissolved oxygen concentration, and nitrate concentration, and rainfall amounts (current and lagged by 1 week), only higher minimum water temperatures on the coast (P=0.086, R^2=0.27, $F_{1,10}$=3.6, n=12) and lower pH levels in the bay (P=0.082, R^2=0.23, $F_{1,12}$=3.6, n=14) were correlated with greater weekly clam mortality numbers.

Table 2.3. Results of two-way ANOVAs (with no replication) testing differences in water properties between growout systems (open coast and bay), and week throughout summer 2021 in San Diego. Testing of water parameters in each individual treatment container was not feasible so measurements were taken weekly at each system (bay, coast). Data are from June 25 to Sept 29, 2021.

Independent variable	**P**	**F**	**df**	**n**	**system p**	**week p**	**Pairwise comparisons**
Light (lux): 12 hr day avg	<0.001	161	2,34	37	<0.001	0.141	Bay > Coast, decreased with week
Temperature (°C) 24-hr avg	<0.001	46	2,31	34	<0.001	<0.001	Bay > Coast, decreased with week
Temperature (°C) 12-hr day avg	<0.001	52	2,31	34	<0.001	<0.001	Bay > Coast, decreased with week
Temperature (°C) 24-hr max	<0.001	71	2,31	34	<0.001	<0.001	Bay > Coast, decreased with week
Temperature (°C) 24-hr min	<0.001	35	2,31	34	<0.001	<0.001	Bay > Coast, decreased with week
Salinity (SSU)	0.042	4	2,34	37	0.153	0.014	Bay = Coast, decrease with week
Nitrate (mg/L)	0.030	4	2,34	37	0.469	0.009	Bay = Coast, decrease with week
Dissolved oxygen (mg/L)	<0.001	26	2,34	37	<0.001	0.586	Coast > Bay, no change with week
pH	<0.001	25	2,34	37	0.007	<0.001	Coast > Bay, increase with week

Discussion

Coast and bay growout systems. Overall venus clam survival rate was 1.7 times lower on the open coast than in the bay where survival rate was 83%. Survival rates were higher than the 46-50% survival rates in warm water (24.1-29.8 ℃) and slightly lower than the 85-90% survival rates in cooler water (12.7-32 ℃) and/or over the longer term of black (or smooth) venus clam (*Chionista fluctifraga*) in the Gulf of California (Martínez-Cordova and Martínez-Porchas 2006, Martínez-Cordova et al. 2013, Góngora-Gómez et al. 2022). The warmest water temperatures in this study were correlated with higher weekly clam mortality on the coast. In the bay, however, weekly mortality numbers were most strongly associated with lower pH levels. While lower pH is often associated with cooler water temperatures (i.e., greater capacity to hold CO_2, lower photosynthetic rates), more acidic conditions occurred during summer in this study, a phenomenon documented in tropical and sub-tropical regions (Kwiatkowski and Orr 2018). Despite this, weekly clam mortality rates were relatively low overall averaging less than one

clam (and often 0 clams) per week per container indicating the potential to grow out these clams in either system.

Despite lower survival rates on the coast, clam growth was similar (shell size) or greater (whole weight) than in the bay with nearly 1.5x more whole weight gain on the coast. The clams grown on the coast were also meatier, with a lower whole weight to meat weight ratio than in the bay, indicating that at least part of the weight gain was due to increases in meat biomass. Growth rates over the three summer months of this study, expressed as growth per day for comparison with other studies (bay: 0.031±0.002 mm height and 0.032±0.003 g/day; coast: 0.033±0.002 mm height and 0.042±0.002 g/day) were within range of growth of the closely related, *Chionista fluctafraga*, cultured in the Gulf of California, Mexico. Growth rates of similarly sized *C. fluctifraga* grown out in the summer in the sandy benthos of the Gulf of California, Sinaloa, Mexico were 0.023±0.009 mm ht/day and 0.063±0.030 g/day (Fig. 4 and Table 3, Góngora-Gómez et al. 2022). *C. fluctifraga* grown for 9 months on the benthos of shrimp-oyster-clam polyculture ponds in Gulf of California, Sonora, MX ranged from 0.043-0.051 g/day (Martínez-Cordova and Martínez-Porchas 2006). Winter shell growth rates of this same species in Sonora Mexico were also similar at 0.032-0.058 mm height/day but weight gain was lower than in the warmer water studies at 0.0004-0.013 g/day (Martínez-Cordova et al. 2013).

Colder water temperatures tend to be associated with slower growth rates (e.g., Martínez-Cordova et al. 2013), therefore the greater weight gain on the cool coast in this study may be attributed to higher dissolved oxygen concentrations (Weber et al. 2009) and pH levels (Ringwood and Keppler 2002) than in the bay. However, abundance of fouling and mobile organisms on the suspended gear (lines and frames), containers, and even clams themselves was also greater on the coast than in the bay (Fitridge et al. 2012). The fouling organisms on the coast consisted mostly of ectoproct bryozoans, red filamentous turf seaweed, the brown slimy filamentous seaweed *Endocarpus* spp. and, less frequently, slipper limpets, barnacles, mussels, tunicates, and the polychaete *Spirorbis* spp., while in the bay fouling was mostly red filamentous turf seaweed, the brown filamentous seaweed *Endocarpus* spp., the polychaete *Spirorbis* spp. and, less frequently, the green sheet seaweed *Ulva* spp., green filamentous seaweed *Chaetomorpha* spp., egg cases (e.g., Navanax) and anemones. Besides variability between sites, the species and biomass of fouling organisms may also change temporally and spatially within sites (Sievers et al. 2014). While there was weak - if any - association between fouling organisms and clam survival and growth in this study, these organisms added significant effort and time to weekly maintenance and cleaning activities which may not be an acceptable trade-off for large-scale production operations (Adams et al. 2011). The placement and type of growout container (e.g., materials, design) should be considered alongside other growout decisions including site location, seasonal timing, species, and equipment structure. For example, while fouling tends to decrease with depth (Claereboudt et al. 1994), the costs involved in cleaning and maintenance of longline equipment as a result of increased weight, drag and exposure to damage may outweigh the benefits of deploying deeper containers (Fitridge et al. 2012).

Suspension and substrates. This study revealed that substrates mimicking the support provided by benthic sediments may not be needed to grow venus clams in suspended culture systems

and may have actually contributed to higher mortality rates. The mesh cages, especially with plastic mat substrates, were more rigid and less expandable than bags which may have constricted larger clams. Further, the substrates associated with higher abundances of fouling and mobile organisms did not appear to influence the survival or growth of clams in this study, yet added significant effort to weekly maintenance. Growth rates were greater in the cages than in the bags, which may have been due to the larger mesh-size of the cages likely allowing better flow and more food. The use of a flexible bag material, with the largest feasible mesh size, should be considered.

Venus clam species. Common venus clam species in Southern California share a large area of distribution between Santa Barbara and the Baja Peninsula (Reilly 2001). However, *C. californesis* and *C. undatella* have a broader latitudinal distribution ranging from Point Conception to Panama (Parker 1949) and also a greater depth distribution ranging from subtidal to depths exceeding 165 ft (Reilly 2001). Similarly, the shell lengths of both species were closely related to their whole weights in this study. The correlation of whole clam weight to wet and dry meat weights revealed that *C. undatella* had similar or slightly higher meat biomass per whole weight than *C. californiensis* up to individuals of 14-16 g, where the meat weight of *C. undatella* leveled off while *C. californiensis* meat biomass continued to increase with clam weight. While the black clam *C. fluctifraga* distribution range from Santa Barabara to the Gulf of California (Góngora-Gómez 2021) is slightly smaller and associated with warmer, shallower waters, increased water temperatures associated with climate change may continue to expand the potential and broaden the range for black clam aquaculture.

2.3. Testing performance in venus clams when grown alone and in the presence of other taxa (seaweed and/or sea cucumber).

The California aquaculture industry is poised to expand and will need to integrate climate change resilience, space use efficiency, production efficiency, and minimized environmental impact into planning, operations, and management. Integrated multi-trophic aquaculture (IMTA), executed in suspended systems, has been proposed to address these challenges. Before implementing IMTA in the state, feasibility assessments of culture and suspended growout for each region and target species are needed. Specifically, California is looking to adopt or increase production of new and emerging species, particularly west coast native species, in order to diversify their products, reduce environmental challenges, and meet public demand (e.g., Neori 2012; Klinger and Naylor 2012). Given growing national and state interest in the culture of clams and seaweeds for food and conservation efforts and the market demand for sea cucumber, we focused our efforts on growing out the venus clams (*Chione californiensis* and *C. undatella*) with *Ulva* spp. (sea lettuce) and *Parastichopus californicus* (California sea cucumber) because of the potential for the of use of these west coast taxa in IMTA in Southern California.

These species all occur intertidally to subtidally, most commonly associated with sandy and/or rocky bottoms from Alaska to Baja California, Mexico. *Ulva* spp. and *Chione* spp. in particular have broad environmental tolerances, occurring from open coasts to estuaries. These species

have already been grown out on small scales along the eastern Pacific (Palzat et al. 2008, Fortune 2013, Shpigel et al. 2017; Walker 2017, Zertuche-González et al. 2021, Góngora-Gómez et al. 2022, MBS 2022), and have been explored for culture elsewhere or have related species (congeners or co-familials) that have been explored for culture (e.g. Sunray venus clam *Macrocallista nimbosa* in Florida [Scarpa et al. 2009], Manila clam *Venerupis japonica* in Washington [Toba 1992], sea lettuce *Ulva lactuca* in Florida [DeBusk et al. 1986], sandfish sea cucumber *Holothuria scabra* in the U.S. Pacific Islands [Ito 2015]). These taxa also have complementary ecological roles that contribute to nutrient cycling, maintenance of water quality, and removal of organic debris accumulations, all of which provide potential for an efficient and productive aquaculture (Fortune 2013).

The seaweed, as a primary producer, converts inorganic nutrients and carbon dioxide to organic carbon, providing not only a food source but also buffering acidification (Tan and Zheng 2020). *Ulva* species, in particular, are efficient at nutrient uptake and, although *Ulva* is commonly grown for nutrient remediation and feed (Cohen and Neori 1991, Bolton et al. 2008), interest in culture for food is growing (Robertson-Andersson 2003). Filter feeders, such as venus clams, remove suspended particulate organics which in turn increases light penetration in the water column and reduces propagules of other organisms, such as fouling organisms (Smaal et al. 2019). Sea cucumbers, like the California sea cucumber, can both filter and deposit-feed, potentially consuming accumulations of organic material on growout substrates and redistributing nutrients for uptake by primary producers (Fortune 2013). Filter feeding is thought to occur when benthic organics are limited, reducing the likelihood of competition with the clams (Bauer et al. 2019). The effectiveness of these interactions for clam production in aquaculture is however uncertain.

Goal

The goal of this trial was, therefore, to test the effectiveness of growing out West Coast venus clams in an IMTA system. We met this goal by addressing the following two objectives:

1. Determine clam mortality and growth when grown in isolation vs with other species
2. Determine whether there are other benefits to including other species

Study site & Methods

Venus clams for the trials were collected earlier in June 2021 from local tidal flats. Both *C. californiensis* and *C. undatella* were used because they were initially sometimes difficult to tell apart, commonly co-occur in this region (i.e., have similar habitat requirements) and relieved harvest pressure on one or the other species. Assessments of clam species at the end of the trial, once confidence in identifiations were high, revealed that *C. californienis* made up 75% and *C. undatella* made up 25% of all clams used.

Grow out trials were conducted on the Port of San Diego's FLUPSY located in San Diego Bay, California, USA (32.71036° N, -117.17373° W) (Figure 2.14) between June 25 to December 10,

2021. The FLUPSY was non-operational throughout the trial, therefore the growout trial represented ambient flow conditions in a semi-arid, urbanized mid-bay (see Largier 1995, Largier et al. 1997, Chadwick et al. 2004). Each of the four treatments (clam, clam seaweed, clam sea cucumber, all three species) were haphazardly assigned to each of four bins (115 cm height, 115 cm length X 115 cm width at the top tapering to 90 cm X 90 cm at the bottom of the bin). A PVC frame with four lines was set across the top of each bin from which the containers holding the clams were hung. Each line held three and, in the second half of the trial, two containers (one 3 mm-mesh bag and two 6 mm-mesh cages, for a total of 12 and, later, 8 replicate containers per treatment [bin]).

Figure 2.14. Growout trials were conducted at the Port of San Diego's FLUPSY located in San Diego Bay, California. Shown is the frame with lines holding suspended clam containers that was laid over the top of each bin.

Midway through the six month trial in September one of the cages was sacrificed, as were 3 clams from each of the other containers. The two bins with sea cucumber treatments received 5 sea cucumbers that were allowed to roam free around the bin. If any of the sea cucumbers escaped- which happened a few times- they were replaced within two weeks and remaining cucumbers were redistributed so that 5 sz. small-medium or 3 sz. large were held in the bins at all times. About 20 L of *Ulva* spp. were added to each bin with a seaweed treatment by tying small clusters along three lines running across the surface water (15-25 cm depth). The *Ulva* declined with the warmer temperatures and was replaced when the volume got down to about 7 L. The replacement *Ulva* was placed toward the bottom of the bins to reduce heat and UV stress.

Each clam was numbered, measured, weighed, and sorted into four size classes (13-16 mm, 16.1-20 mm, 20.1-25 mm, 25.1-30 mm). Three clams from the two intermediate size classes and 1-2 clams from the smallest and largest size classes were placed in each container for a total of nine per container (Avg±1SD = 21±4 mm length). Dead clams were replaced each week with 7-9 clams per container maintained throughout the 3-month trial.

Temperature and light were recorded hourly using Onset Pendant loggers; hourly data were used to calculate daily averages, maximums and minimums which were then averaged across each week for comparison with weekly variables. Weekly assessments of clam mortality (#dead clams per container, dead clam length and height) and water properties (salinity, DO, pH, NO3-) were conducted. Weekly ranked assessments (0-4) of the cover of fouling organisms and the abundances of mobile organisms on and in each container, as well as on the frames and bin were made. Weekly rainfall totals and number of rainy days (≥ trace amounts) were calculated *post hoc* using rainfall data from the nearby San Diego International Airport. Clam whole weight, length, and height were measured at the start, middle, and end of the trial, and clam meat wet and dry weights were measured at the end of the trial.

Relationships between the IMTA treatment and clam mortality and growth (%changes in length, height and weight) were explored using one-way ANOVAs. Relationships between both the IMTA treatments and season and the environmental variables (water parameters, rank cover of fouling organisms, and rank abundance of mobile animals) were tested using two-way ANOVAs with replication, when variables were measured using each individual growout container (e.g., abundance of fouling organisms in each container), and without replication, when one measure of the variable per bin was made (e.g., rank of bin fouling, water parameters). Relationships between the water variables, sessile and mobile fouling organism abundances, and weekly clam mortalities were tested using forward, stepwise multiple regressions with criteria for entry into the model $p \leq 0.05$ and exclusion from the model $p > 0.05$ or $r^2 \leq 0.05$. All data were log (x+1) transformed (numeric data) or arcsin square root transformed (% data) before analysis and all statistical analyses were performed using JMP Pro 16.

Results

Venus Clam Production

Overal clam mortality rate (across IMTA and container treatments) was 17±3% in the summer and 2.5±1.4% in the fall. Clam mortality (total number of dead clams) did not differ among treatments during either the summer or fall (Figure 2.15; Table 2.4). Clam growth, however, was 1.5-3 times faster in the clam only treatment than the other treatments (i.e., change in whole weight, length, and height, Figure 2.16; Table 2.4).

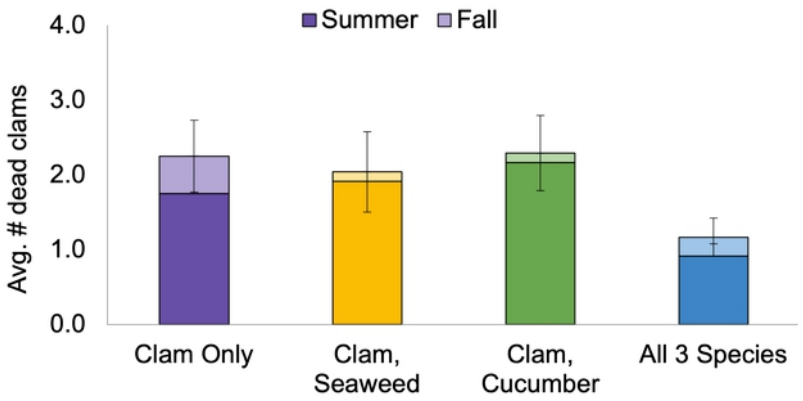


Figure 2.15. Average (±1SE) number of dead clams per suspended container during the summer and fall 2021. SE shows error for the entire time period. N= 12 containers per treatment (bin) in the summer and n=8 containers in the fall.

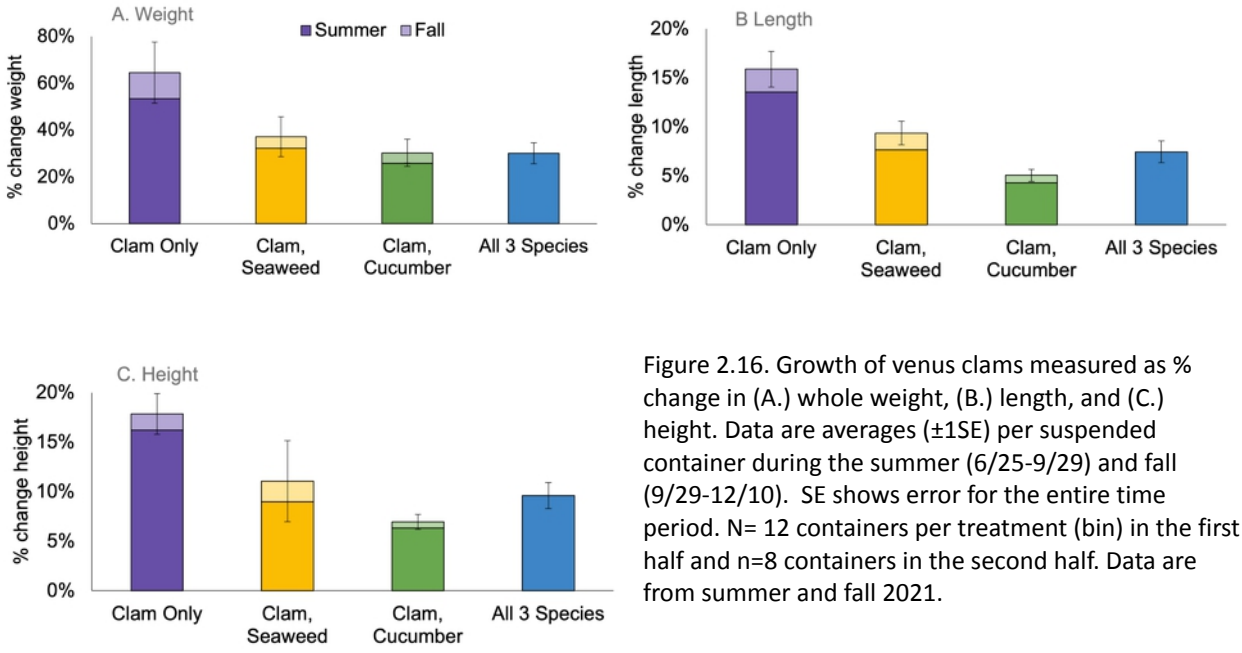


Figure 2.16. Growth of venus clams measured as % change in (A.) whole weight, (B.) length, and (C.) height. Data are averages (±1SE) per suspended container during the summer (6/25-9/29) and fall (9/29-12/10). SE shows error for the entire time period. N= 12 containers per treatment (bin) in the first half and n=8 containers in the second half. Data are from summer and fall 2021.

Table 2.4. Results of one-way ANOVA testing relationships between the IMTA treatments (clam only=C, clam and seaweed=CS, clam and sea cucumber=CC, all three species=CSC) and venus clam mortality and growth variables. Data are from 6/25-9/29/2021 (summer) and 9/29-12/10/2021 (fall).

Independent variable	**Season**	**P**	**F**	**df**	**n**	**Pairwise comparisons**
# dead clams	summer	0.71	0.46	3,44	48	–
	fall	0.89	0.21	3,28	32	–
% change length	summer	<0.001	8.54	3,44	48	C > CS, CC, CSC
	fall	0.29	1.32	3,28	32	–
% change height	summer	<0.001	11.05	3,44	48	C > CS, CC, CSC
	fall	0.31	1.25	3,28	32	–
% change weight	summer	0.001	6.41	3,44	48	C > CS, CC, CSC
	fall	0.068	2.66	3,28	32	C > CS, CC, CSC

Water conditions

Most of the water parameters except dissolved oxygen concentrations changed with season, including decreased light levels, temperatures, salinity, and nitrate concentrations and increased pH from summer to fall (Fig 2.16, Table 2.5). The water parameters did not generally differ however between treatments/bins (Fig 2.16, Table 2.5). Of the water variables tested, including

light penetration, dissolved oxygen concentration, salinity, pH, and nitrate concentration and rainfall (current and lagged by 1 week), only water temperature, in particular the 24-hr minimum temperature, was positively correlated with weekly clam mortality (summed across treatments since there was no treatment effect on mortality rate) (R^2=0.24, P≤0.022, $F_{1,19}$=10, n=21).

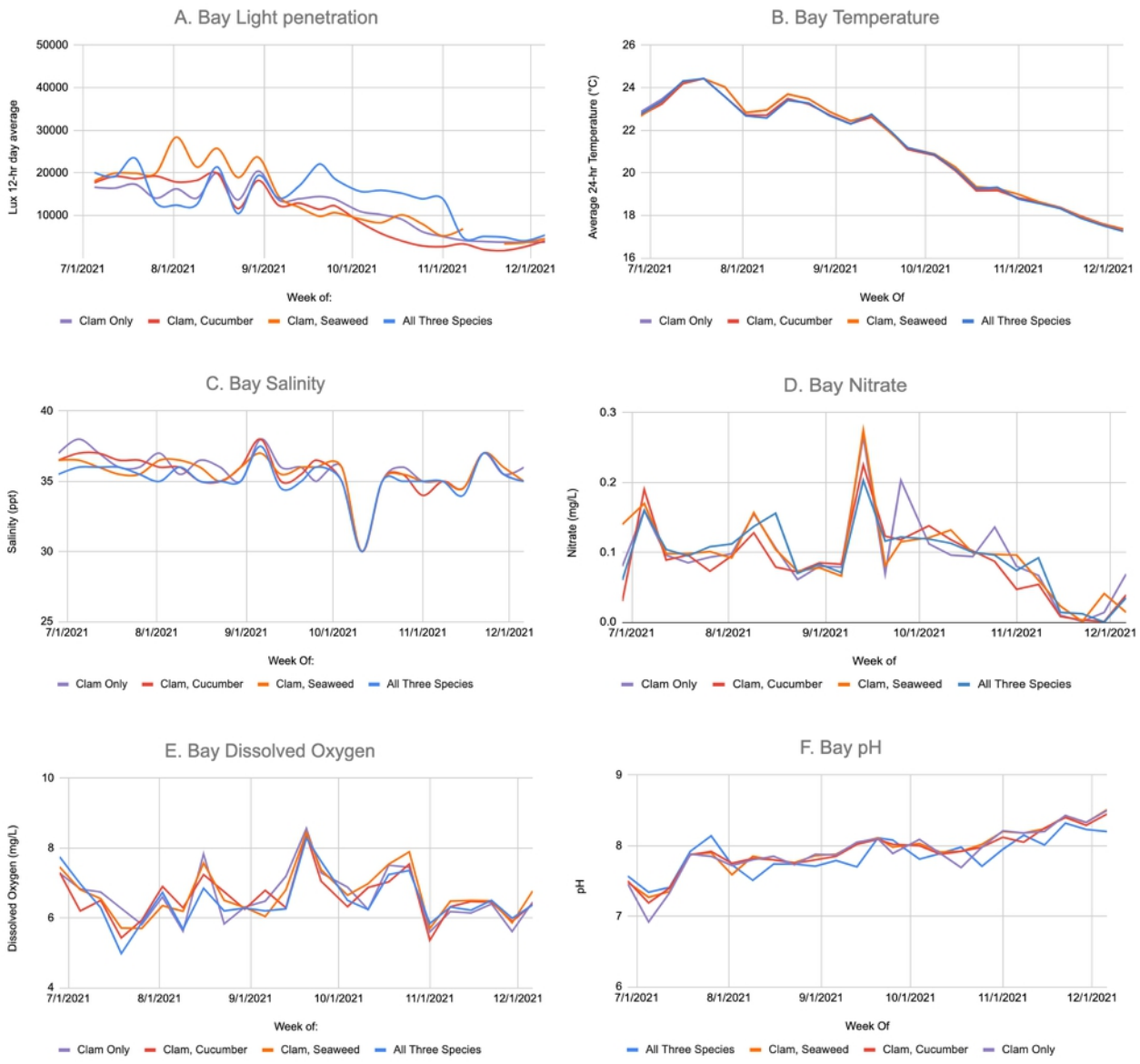


Figure 2.16. Average bay water parameters over time in the four IMTA treatments. Shown are average hourly (A.) daytime light levels (6 am- 6 pm), (B.) 24-hr temperatures, (C.) salinity, (D). nitrate concentration, (E.) dissolved oxygen concentration, and (F.) pH. Data are from June 25 to Dec 10, 2021. N= 1 weekly measure per treatment/bin.

Table 2.5. Results of two-way ANOVAs (with no replication) testing relationships between water properties and both the IMTA treatments (clam only=C, clam and seaweed=CS, clam and sea cucumber=CC, all three species=CSC) and season (summer, fall). Data are from summer and fall 2021. trt = IMTA treatment.

Independent variable	P	F	df	n	trt p	season p	Pairwise comparisons
Light (lux): 12 hr day avg	<0.001	59	4,91	96	0.001	<0.001	CSC,CS ≥ C ≥ CC summer > fall
Temperature (°C) 24-hr avg	<0.001	121	4,89	94	0.982	<0.001	summer > fall
Temperature (°C) 12-hr day avg	<0.001	118	4,89	94	0.910	<0.001	summer > fall
Temperature (°C) 24-hr max	<0.001	92	4,89	94	0.166	<0.001	summer > fall
Temperature (°C) 24-hr min	<0.001	112	4,89	94	0.922	<0.001	summer > fall
Salinity (SSU)	<0.001	6	4,92	97	0.503	<0.001	summer > fall
Nitrate (mg/L)	<0.001	6	4,92	97	0.867	<0.001	summer > fall
Dissolved oxygen (mg/L)	0.722	0.5	4,92	97	0.863	0.252	N.S.
pH	<0.001	13	4,92	97	0.765	<0.001	fall > summer

Fouling organisms

The relative abundance of mobile animals and cover of fouling organisms on the growout containers were greater in the summer than fall, but fouling on the walls and lines of the FLUPSY bins were similar across seasons (Table 2.5).

Table 2.5. Organism ANOVA. Results of two-way ANOVAs testing relationships between the IMTA treatments (clam only=C, clam and seaweed=CS, clam and sea cucumber=CC, all three species=CSC), season (summer, fall) and the organisms associated with the individual growout containers (12 per bin during summer weeks, 8 per bin during fall weeks) and the four FLUPSY bins. Data are from summer and fall 2021. >> = seasonal differences in that variable with the season on line one greater than that on line two. = = there was no seasonal difference. trt = IMTA treatment, seas= season.

Independent variable	P	F	df	n	trt p	seas p	trt x seas p	Pairwise comparisons
Mobile animal abundance (rank)	<0.001	39	7,936	944	<0.001	<0.001	<0.001	S: CSC ≥ CS ≥ CC > C >> F: C, CC, CS, CSC
Sessile organism cover (rank)	<0.001	19	7,983	991	<0.001	<0.001	<0.001	S: C > CS > CC, CSC >> F: CS, CSC > C, CC
Bin fouling cover (rank)	<0.001	7	4,92	97	<0.001	<0.001	N/A	C ≥ CS > CC, CSC

Mobile organisms such as snails, isopods, amphipods, midge larvae, were highest in the treatments with seaweed (CS, CSC) in the summer and there was no difference between treatments (bins) in the fall. The cover of fouling organisms, such as seaweed, bryozoans, and the polychaete *Spirorbus* were highest in the treatments without sea cucumber (C, CS) in the summer and highest in treatments with seaweed in the fall (CS, CSC). However, the cover of fouling organisms on the walls and lines of the bins was lowest in treatments with sea cucumber (CC, CSC) in both seasons (Table 2.5; Figure 2.17). The relative abundance of mobile animals and cover of sessile organisms fouling containers each week were not correlated with weekly number of clam mortalities in the same containers (mobile: $P=0.13$, $F_{1,942}=2.3$, $n=944$; sessile: $P=0.025$, $R^2=0.005$, $F_{1,989}=5.1$, $n=991$).

Discussion

During this 6-month spring/fall trial, there was no cost or benefit to clam mortality in growing out venus clams with seaweed and/or sea cucumber. Clam mortality rates, as revealed by weekly measures, were only associated with warmer water temperatures - in particular, higher average minimum temperatures - and not any of the other water parameters or abundances of fouling organisms. Clam growth was greatest when clams were grown alone compared with other species, causing uncertainty as to why. Water conditions did not vary across treatments (bins) in any consistent way that could explain differences in clam growth. The consistency in water conditions across bins was expected given the open, flow-through system of the FLUPSY (even though it was not operational).

The sea cucumbers in two of the treatments may have competed with the clams for food if they were filter feeding, although the clams in the clam-seaweed treatment (no sea cucumbers) also experienced lower growth rates than when clams were grown alone. The clam-only treatment experienced the lowest abundances of mobile animals and some of the greatest cover by fouling organisms (Figure 2.17) – whether these relationships were part of the cause or effect of clam growth differences, or simply covariates of some unmeasured variable, is uncertain. In the summer, the abundance of mobile animals - mostly invertebrate macrofauna - was highest in treatments with seaweed. These animals were largely taxa that are not known to be predatory or detrimental to clams and were rather herbivore grazers (e.g., small snails), detritivorous and/or scavengers (e.g., cirolanid and sphaeromatid isopods). The greater cover of fouling organisms in the clam-only treatment may have been due to a lack of competition for nutrients and light by the *Ulva* spp. being grown in the other bins (although light levels and nitrate concentrations do not reflect this), absence ofsea cucumbers to clean propagules from the bins and growout gear, and/or lower abundances of mobile fauna to graze on fouling organisms.

Clam-Seaweed-Sea cucumber Clam-only

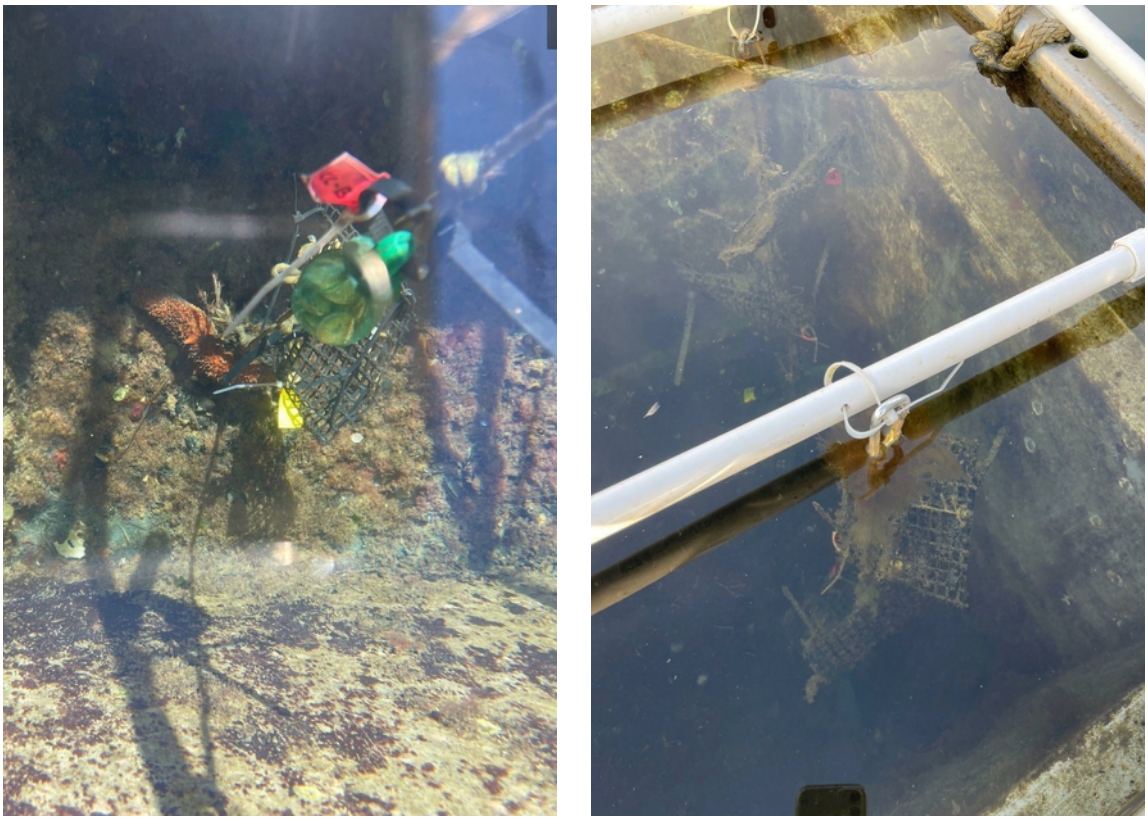


Figure 2.17. Photographs of the bins with IMTA treatments. (L) clam, seaweed, sea cucumber (CSC) and (R) clam only (C). Visible is the cover of fouling organisms (*Endocarpus* spp) in the clam-only treatment on the FLUPSY bin walls of the growout containers.

Despite lower clam growth, there may be other benefits of growing out the venus clams with these other taxa. The reduced fouling of the bins and gear associated with the sea cucumber could reduce maintenance efforts and costs. Further, an IMTA growout approach may allow for diversification of aquaculture products if the associated species are marketable, as in the case of *Ulva* spp., and other seaweeds in addition to sea cucumber. Whether or not the production cost of these additional species might outweigh potential loss in clam production would have to be assessed. Longer-term assessments of clam production in IMTA growout systems particularly spanning the cooler seasons when seaweed and sea cucumber growth and activity may be greater, would also be beneficial in order to more accurately assess production and other environmental benefits.

Objective 3. Assess shellfish pathogens and toxins that may affect culturing of our native clam test species.

For objective 3, we collected broodstock and seed clams from the field and from our pier and bay growout trials (Table 3.1), and submitted them to our collaborating project partners at the

California Department of Fish and Wildlife (CDFW) who are conducting the health assessments. Preliminary histological evaluations for *Chione californiensis* and *C. undatella* found that they have separate sexes and that females are dribble spawners, with gonads in any one clam at various stages of release (maturing gametes, mature gametes, empty follicles.) This is consistent with observations of gametogenesis and spawning at low levels throughout the year with higher intensity in summer and fall (Cardeñas and Aranda 2000). Trematodes and Rickettsiales-like prokaryotic inclusions (RLPs) were present in some of the individuals in this study; each appear to be taxa common in clams in the region. Finally, all but two of the juvenile /seed *Chione* assessed had gonads present indicating that the smallest of the clams collected were already mature (13.5-15 mm length). The remaining histological evaluations and other health assessments using qPCR methods are ongoing.

Table 3.1. Shellfish health assessment samples. Species and numbers of broodstock, seed/juvenile, and growout trial clams collected and submitted during 2021 for shellfish health assessments, including histology and PCR analysis.

Site	Month	Common name	Scientific name	brood-stock	seed/ juvenile	growout trial	Analysis
Mission Bay	March	California venus clam	*Chione californiensis*	26	62	0	histology
	April	Common littleneck	*Leukoma staminea*	0	23	0	histology
	April	butterclam	*Saxidomus nutalli*	0	12	0	histology, qPCR
	April	Bent nose clam	*Macoma* spp.	0	14	0	histology
San Diego Bay	April	California venus clam	*Chione californiensis*	24	0	0	histology
	April	Frilled venus clam	*Chione undatella*	3	0	0	histology
	April	Heart cockle	*Trachycardium quadragenarium*	1	0	0	histology
Mission Bay	July	California venus clam	*Chione californiensis*	28	15	0	qPCR
	July	Frilled venus clam	*Chione undatella*	15	22	0	qPCR
	July	butterclam	*Saxidomus nutallii*	0	7	0	qPCR
San Diego Bay	July	California & frilled venus clam	*Chione californiensis* & *C. undatella*	22	11	0	qPCR
Scripps Pier	Sept	California venus clam	*Chione californiensis*	0	0	35	histology, qPCR
	Sept	Frilled venus clam	*Chione undatella*	0	0	13	histology, qPCR
San Diego Bay FLUPSY	Sept	California venus clam	*Chione californiensis*	0	0	47	histology, PCR
	Sept	Frilled venus clam	*Chione undatella*	0	0	20	histology, PCR

Challenges and Solutions

Two main challenges were encountered during this project period: 1. an apparent decline in the number of individuals from our target species in local bays, and 2. time delays associated with COVID-19 restrictions and subsequent staffing and supply shortages.

While our four candidate species were present in most sites surveyed or mentioned by local experts as being commonly observed, the intertidal numbers within sites were limited. Therefore, we focused our growout trials on *Chione undatella* and *C. californiensis* because they are the only species found in abundances high enough to be feasible for use in the experiments. Instead of testing multiple clam species in growout, we added a multi-trophic growout trial for the venus clams. A few specimens of the diversity of species that were found in tidal flats were collected and used for the shellfish health assessments.

The processes involved in hiring staff, getting permission to access experimental facilities and conduct research, and updating our collecting permit amid COVID-19 restrictions put us behind schedule in the first half of the project and residual staffing shortages led to longer than expected timelines for completing field research, lab analyses, and data entry and analysis. We applied for and were granted a 6-month extension which allowed us to finish Objectives 2 and 3 of the project. However, the staffing shortages at CDFW resulting from COVID-19 continue to impede their ability to complete the histology and qPCR analyses. They have revised their anticipated timeline to needing at least 12 months beyond this project's extended end date of 04/30/22 to complete their evaluations. We re-budgeted the unused supply money that had been allocated to these analyses to salary covering the work added onto the multi-trophic aquaculture experiments, and for assistance with final data analyses and preparation of presentations.

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CANDIDATE SPECIES INFORMATION SHEET

Photo: T.S. Talley

SCIENTIFIC NAME *Chione californiensis*
COMMON NAME California venus clam, banded chione

PHYSICAL DESCRIPTION

- Size: Average shell length 2.9 cm (1.2 in); maximum shell length about 6.4 cm (2.5 in). [1]
- Texture: Radiating bands and closely spaced radial and concentric ridges. [1]
- Color: Variable, ranging from white and ivory to gray to pale pink. [6]

LIFE HISTORY

- Separate sexes (dioecious), with external fertilization via broadcast spawning. [3, 9]
- Actively spawns in warmer months (June to September) with a peak in August when temperatures are near 29°C (84 °F). Gametogenesis is directly related to variations in temperature, with increased spawning frequency with rising seasonal temperature. [3]
- Embryos develop into free-swimming larvae (trochophore, veliger) that resemble a small clam prior to settling. [7]

DISTRIBUTION/HABITAT

- Point Conception (Santa Barbara County, California) to Panama. [1]
- Occurs on flats of sloughs, coves and bays, primarily in the intertidal (as deep as 50 m) where it is the most common clam at some sites along the Pacific coast of Baja California Sur. [2, 3]
- Higher abundance in fine/very fine sand or sand-silt, lower abundance in sediment containing clay. [2]
- Spawning temperatures: ~ 22-30 ℃ (72-86 °F); Spawning is interrupted when temperatures fall below 24°C (75.2 °F). [3]

AQUACULTURE CONSIDERATIONS

- Along with its congener *C. undatella*, this clam has been traditionally targeted and consumed locally along the Gulf of California and the western coast of Baja California Sur; sold fresh and frozen. [3, 6]
- Most extensive research has been conducted in Mexico, with most literature in Spanish. Although biological and culture information for this species is lacking, information is available for *C. undatella* and *C. fluctafraga*.
- Although relatively rare (~0.5% prevalence), can contain parasitic trematodes that impact clam gonads. Trematode in La Paz, Mexico is *Bucephalus sp*. with unidentified species along the Pacific coast. [3, 12]
- May be human health risks associated with eating raw or undercooked seafood.
- Control known predators may be required (e.g., angelfish, *Holocanthus passer*). [11]

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Photo: J. Trausel & F. Slieker [4]
Photo: G. & Ph. Poppe [5]

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CANDIDATE SPECIES INFORMATION SHEET

Photo: T.S. Talley

SCIENTIFIC NAME *Chione undatella*
COMMON NAME Frilled venus clam, wavy chione

PHYSICAL DESCRIPTION

- Size: Average shell length 2.9 cm (1.2 in); maximum shell length about 6.4 cm (2.5 in). [4]
- Shape: Thick, oval shaped shell. [4]
- Color: Variable, ranging from white and ivory to gray to pale pink. Interior, white with purple along the posterior end. [2]
- Texture: radiating bands and very closely spaced concentric ridges. [4]

LIFE HISTORY

- Separate sexes (dioecious), with external fertilization via broadcast spawning. [2]
- Reproductive throughout the year, with peak spawning in late spring or early summer. [2, 9]
- Recruitment occurs over an 8-month period with peak reproduction over a one-month period in the late spring or early summer. [9]
- Shell growth increases with the summer spawning season. [1]
- Embryos develop into free-swimming larvae (trochophore, veliger) that resemble a small clam prior to settling. [7]

DISTRIBUTION/HABITAT

- Point Conception (Santa Barbara County, California) to south America [1, 8]
- Primarily found near mouth of bays, in well-flushed sandy areas, or near and in eelrass beds. [6]
- On flats of sloughs, coves and bays, primarily in the intertidal zone (as deep as 50 m) and in cobble patches. [1, 3]
- Patchy distribution with clams found in clusters distributed irregularly in channels without any known relationship to local substrate variations. [8]

AQUACULTURE CONSIDERATIONS

- Currently cultured in Baja California Mexico; Locally raised and sold in shell at around 0.15 kg (5.3 ounces) whole weight. Expanded culture potential is uncertain as biology is not well known across its range. [2]
- Growth rates and reproductive cycles documented for natural populations and marked/caged experimental animals in Mexico. [2]
- Ideal temperatures for growth between ~17-24℃ (63-75°F). [6]
- Control of a known predator - Muricid snail, *Pteropurpura festiva* - may be required. [5]

PHOTOGRAPHS



Photos: BJ Stacey, some rights reserved (CC BY-NC). [10]

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CANDIDATE SPECIES INFORMATION SHEET

Photo: Dave Cowles [1]

SCIENTIFIC NAME *Leukoma* (formerly *Protothaca*) *staminea*
COMMON NAME Common littleneck

TAXONOMIC DESCRIPTION

- Size: 2.5 - 7.6 cm length; average length is 2.5 - 5.0 cm (~ 1-2 in) [1, 4, 7, 8]
- Shape: Thick oval shell with robust hinge and ventral margin. Elevated umbo facing anteriorly, with heart-shaped impression in front of the umbo. [2, 7]
- Color: Varies by habitat; yellowish gray to gray in sloughs and bays, porcelain-white with wavy brown marks in the open coast. [2, 4]
- Texture: Well-defined hatch marks on dorsal side. Extrusive radiating ribs and less prominent, raised, concentric rings. [2, 7]
- Siphons: Short, black-tipped, fused all the way to the end [1]

LIFE HISTORY

- Few clams are more than 10 years old, with a maximum age of 15 years. [4]
- Legal recreational catch size limit is 3.8 cm (1.6 in) shell length, reached at about 4–5 years old. Southern California clams may reach the minimum legal harvest size sooner than other areas. [4, 8]
- The smallest of the commercially harvested species with an average market size of 5.1 cm (2 in).
- Separate sexes (dioecious) with some hermaphroditism reported. External fertilization via broadcast spawning. [4]
- Females may spawn several times during a season, while individual males release all gametes at once. [4]
- Spawning usually occurs in warmer months, and may be associated with algal blooms. [4]
- Embryos develop into free-swimming larvae (trochophore, veliger) that resemble a small clam prior to settling. Larval (pelagic) duration is 3-4 weeks and larvae are 260–280 µm in length at metamorphosis. [4]

DISTRIBUTION/HABITAT

- From the Aleutian Islands, Alaska to Cape San Lucas in southern Baja California. [1, 7] Common in California including Mission Bay and Tijuana River Estuary connecting Mexico and the United States. [5]
- Resides in middle and low intertidal zones. [8,10]
- Very coarse sediment to fine sand, with higher densities occurring in finer sand. [1, 5] In California, found in sand/mud sediments in bays, sloughs, and estuaries. North of California, on the open coast, found in high density where cobbles overlay sandy bottoms. [7]

AQUACULTURE CONSIDERATIONS

- Tender with a mildly sweet flavor and briny accent. [6]
- Growth is often slow in early years on exposed beaches and becomes more rapid in later years, but the opposite may be true for individuals in protected sites. Growth in protected clam gardens has historically been found to be up to 1.7x faster than in the wild. [4, 12]
- Ancient mariculture systems up to 3500 years old have been identified in the form of coastal Indigenous clam gardens throughout North America. [13]
- Poor digger, and thus does not live in sediments that require frequent digging. Adults typically stay within about 15 cm (6 in.) beneath the surface. [4, 8]
- Ideal conditions for rearing larvae are 10–15˚C and salinity of 32 ppt. [4]

PHOTOGRAPHS



Pacific littleneck clam (*Leukoma staminea*). Left image shows shell color and pattern variation (D. Cowles, [1]), Middle image shows strong ribbed shell, Right image shows the smooth, porcelain-colored interior (J.D. Reynolds, [10].

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CANDIDATE SPECIES INFORMATION SHEET

Photo: T.S. Talley

SCIENTIFIC NAME *Argopecten ventricosus*

COMMON NAME Catarina scallop, Pacific calico scallop, speckled (bay) scallop

TAXONOMIC DESCRIPTION

- Size: Max shell height 9 cm (3.5 in) and length 10 cm (3.9 in). [1]
- Shape: Both valves are convex, but the right valve is slightly more so than the left. Anterior and posterior ears are about equal in size. [1, 11]
- Texture: Shell has about 21 ribs [11]
- Color: Variable. [7]

LIFE HISTORY

- Lifespan: Maximum of about two years. One of the shortest-lived scallops. [5,6]
- Combined sexes (hermaphrodite), with external fertilization via broadcast spawning. [3, 11]
- Reaches sexual maturity at about three to four months old and about 6 cm (2.4 in). [4, 5]
- Two reproductive peaks per year: late winter/early spring and late summer/fall. [11]
- Embryos develop into free-swimming larvae (trochophore, veliger) that resemble a small scallop prior to settling. Larval development takes about 9-10 days. [10]
- Active swimmer throughout life, thus energy-intensive lifestyle. [5]

DISTRIBUTION/HABITAT

- Point Conception (Santa Barbara County, California) to Peru and throughout Gulf of California. [2]
- Sand and mud usually in bays, lagoons and calm offshore waters. Intertidal up to 180 m (591 ft). [1]
- Live within eelgrass beds, a primary substrate. [5]

AQUACULTURE CONSIDERATIONS

- Take is prohibited in California.
- Cultured in Gulf of California for domestic sale in Mexico and export to the United States. [2, 8]
- Small-scale aquaculture production in Ensenada de La Paz, Baja California Sur for enhancing wild populations where the scallop has been overharvested. [13]
- Mature individuals are found year-round in Magdalena Bay, Baja California, thus broodstock are kept in the field instead of in hatcheries. [10]
- Fast growing; Preferred water temperature of ~ 26 °C (79 °F). [4,12]
- Larvae tolerate low concentrations ($0.5x10^5$ CFU ml^{-1}) of *Vibrio alginolyticus*, but high concentrations ($5x10^5$ CFU ml^{-1}) inhibit swimming, feeding and digestion, degrade vela and increase mortality rates. [7]

PHOTOGRAPHS

Photos: T.S. Talley

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