Preface

This manual has been written to disseminate clam hatchery and nursery information to the shellfish farming industry of British Columbia. It is based on work done at Innovative Aquaculture's facility, Skerry Bay, Lasqueti Island, and at Redonda Sea farms, Squirrel Cove, Cortez Island. Partial funding for the research and subsequent publication of this manual was through the assistance of the B.C. Science Council.

The authors would like to thank all those who shared information and insights on the hatchery production, setting, and nursery rearing of Manila clams, both in North America and throughout Europe.
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Introduction
This manual describes manila clam hatchery and nursery methods developed over many years by aquaculturists all over the world who have shared their knowledge and experiences with us in one form or another. Clam farming in British Columbia is presently in a dynamic growth stage that we expect to continue for many years. The purpose of this manual is to give clam farmers an understanding of clam seed production methods so that they will better understand the animals they are producing. We are not recommending all of these techniques to all growers as the risks of rearing clams from spawn to market are too great for all but the largest companies to take on. However, growers who wish to buy smaller seed, or pediveliger larvae (and assume larger risks than buying ready-to-plant seed) will find many useful ideas here and be able to use this as a guide for adapting these methods to their own sites. Also people who are into self-abuse may want to try their hand at larval rearing.

Manila clams are native to Japan between latitudes 25°N and 45°N. They were accidentally introduced to British Columbia with Japanese oyster seed and the first recorded sighting of them was in Ladysmith Harbour in 1936. They spread quickly throughout the Strait of Georgia and are now found in many bays along the West Coast of Vancouver Island and Central Coast area of mainland B.C.
Manila clams have been cultured in Japan for over a thousand years. Records dating from the year 746 AD show clams being transplanted from seed areas to growout sites. Ironically Oyster culture was started in Japan in 1673 when a clam farmer decided to culture oysters on the bamboo fence around his farm. Japanese clam farming has continued to use the simple technique of moving wild clam seed from high density areas to lower density grow out ground. Clam farming has reached a much technologically higher level of development in North America and Europe.

In recent years interest in clam farming in British Columbia has accelerated because the profit margin has improved radically as the price of Manila clams has quadrupled. The economics of shellfish farming in B.C. has also improved through the implementation of the polyculture of oysters with clams.

Naturally breeding populations of Manila clams can be found on many of the beaches of the B.C. coast.

Besides Manila clam hitch-hikers, the Japanese oyster drill, the flat worm, a parasitic copepod, Japanese weed, and the wood-borer all arrived with shipments of Pacific oyster seed.
Historically a major constraint in B.C. to clam farming has been a federal policy which acted to promote a "wild fishery", rather than a farmed product. The jurisdictional dispute between the Federal Department of Fisheries and Oceans and the B.C. Ministry of Agriculture, Fisheries and Food seems to be resolved. Now we can deal with the real problems of disease, predators, and storm damage.

**General Biology**

In the scientific literature the manila clam is often referred to by various genus (Tapes, Venerupis and Ruditapes) and species names (semi-decussatus, japonicus and philippinarum) and combinations thereof. In British Columbia Tapes philippinarum (Adams and Reeves) is currently the most commonly used Latin name.

**Life Cycle**

The life cycle of the manila clam is typical of the more than 50 clam species inhabiting British Columbia. The sexes are separate and sexual maturity is generally attained when the clams are
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about 20 mm. In the spring, as the water temperatures begin to warm, the gonads of the male and female clams begin to ripen. Once the individual clams are ripe, some stimulus, often a rapid rise in temperature or exposure to the spawn of another clam will trigger spawning. Eggs and sperm are released into the water where fertilization occurs. In British Columbia natural spawnings occur from May through October. The fertilized eggs develop into straight-hinge, free swimming larvae within 24 hours. This 90μ shelled larva is called a veliger larva because of its velum with which it swims and eats. The microscopic clam feeds on phytoplankton of a size range of 2 to 20μ. This veliger stage lasts for about two weeks, during which it grows to approximately 200μ. At this point it becomes a pediveliger and both crawls with its foot and swims with its velum looking for a suitable habitat for adult growth. As the tiny clam grows larger it hangs onto substrate by byssal threads similar to that generated by a mussel. This gives them some protection against being washed away by waves or currents.

Manila Clams and Native Little-neck Clams in B.C. have a range that overlaps, so are only separate at the extremes.

Natural Habitat

It's preferred growing ground is higher in the intertidal zone than most clams, in sand-gravel substrate. It also lives at a relatively shallow depth within the ground, so there is little competition for
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Netting helps to protect juvenile clams from predation.

Manila clams exhibit growth checks that can be used for determining the age of individuals.

space with native clams that occur mainly at lower tidal levels and dig deeper into the beach. Because of the Manila's preference for a higher and shallower home it can be killed by extremes of temperatures. Large area die-off have been reported during severe winters, resulting in areas of intertidal beach littered with empty shells.

**Predators**

T. philippinarm is a favorite food of birds, fish, starfish, crabs, moon snails, as well as people. The extent of the bird problem will vary between sites. Crows and gulls often discover the value of picking their own clams and can become a major problem on specific beaches. The use of predator netting will make it difficult for diving ducks as well as the perch and flatfish to access the clams under the netting. Predator netting will also discourage crabs, and starfish on the beach, but since both crabs and starfish have a larval free-swimming form, predation problems can occur for small seed being held in trays. It is far easier to control predators in the hatchery and nursery situation than on the beach.

**Broodstock**

Selection

Manila clams exhibit growth characteristics and shell colour variations that are genetically related. Through selective breeding in hatcheries breeds can be developed that will grow faster and have distinct markings. When the farmed clams have a distinct different appearance it will discourage stealing and piracy off farms.
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Broodstock selection has been based on the assumption that faster growing clams will produce offspring that will have growth rates comparable to those of the parent stocks. At Innovative Aquaculture we select larger animals that have widely spaced growth checks in the shells (indicative of rapid growth), but are in the 3 to 5 year old range. We avoid stunted or old clams.

Conditioning Systems

There are two main options for conditioning systems for Manila clams; either flow-through systems or static water systems in which the clams are maintained in a volume of heated water that is changed on a regular basis. We prefer the static water system for several reasons:

- It is easy to monitor and determine if there was an unintentional spawning in the system
- It is less difficult to maintain constant temperature control.
- It is not necessary to heat water on a continual basis.

In a static water conditioning system algae and air are constantly added while the temperature is held at a constant 18°C.
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* The tanks must be cleaned on a regular basis which involves a complete water change anyway
* The consumption rate of algae is more easily monitored.

In a flow through conditioning system enriched, heated seawater is consistently pumped through the holding tanks.

Temperature
Broodstock clams should be acclimatized slowly to increasing water temperatures within the system. We normally adjust the temperatures upward over a one week period to avoid the stress of a significant temperature variation (this is especially important during the winter when the temperature variant between ambient and conditioning, is the greatest). We tested different temperatures, both for the time to condition and stress on the animals, and determined the optimum temperature for our conditioning system is 18 °C.

Feeding
Unlike the Pacific oyster Crassostrea gigas, Manila clams do not have a glycogen layer to convert to gonadal tissue as the conditioning process progresses. The quality and quantity of eggs and sperm produced is directly related to the quality and quantity of algae fed to the conditioning animals. It is essential to
supplement any naturally occurring algal species within the incoming water, with the addition of large amounts of cultured algae (especially during those times of low natural productivity). We use a combination of cultured species which include 3H, Nannochloropsis oculata, Chaetoceros gracilis, Tahitian isochrysis, and Isochrysis galbana. The feeding system is an automatic pulse feeder that is adjusted to provide to the broodstock animals approximately 1 litre per pound of clam biomass per hour of densely cultured algae (the cell counts vary depending upon the species combination within the food mixture). This may occasionally prove to be more food than is necessary and will result in an increased production of pseudo feces, however, underfeeding can result in slower conditioning times and a reduction in the quantity of gonadal tissue production; or in cases where the animals are starved, result in no conditioning at all.

To check on the progress of conditioning a small slit in the gonadal region will release gametes for examination in a sacrificed clam.

Duration
The length of time necessary to condition clams varies with the season. During the winter, clams will condition over a 6 to 9 week period, however, they often prove to be more difficult to spawn and exhibit lower rates of fertilization when conditioned outside of their normal breeding cycle. As summer
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Variation between eggs and sperm is noticeable to the keen observer

approaches, the time spent within the conditioning system decreases. This is due mainly to the natural state of the gonads at the time of introduction into the system. During the summer, when they are naturally ripe, we can often remove clams from the beach and spawn them without any further need for conditioning.

Assessment Methods
To determine the gonadal condition of the broodstock animals, a gross examination of a sacrificed animal will, with experience, be all that is necessary. This involves cutting into the gonadal layer and generally noting the softness and the fullness of this tissue as you cut through the layers. A microscopic examination should confirm the presence of well formed eggs or motile sperm when seawater is added to the slide. With experience, no animals need to be sacrificed as "knowing your system and the time needed to condition" will result in easy spawning. Males tend to condition faster than females so a ripe male is not necessarily indicative of fully conditioned broodstock.

Spawning
Manila clams are either male or female and the sexual distribution within any given group should be approximately 1:1. The animals release their eggs and sperm into the water column (in response to certain cues) where the eggs are fertilized external to the parent animals. The eggs and sperm, once released, are forced out with the exhalent water through the exhalent siphon. The 70 µ eggs appear granular and individuals can be seen by the naked eye as they disperse and sink in the water column. The
Thermal manipulation is a key factor in spawning induction

Induction
There are many different methods to induce spawning in Manila clams, and generally speaking; they are easily induced provided the broodstock are well conditioned. It is essential that the animals be fully ripe or the efforts expended will result in very little or nothing. In the first step broodstock should be fed heavily (we normally use a concentration of 3.5 million cells per milliliter of 3H, or 14 million cells per milliliter of Nannochloropsis oculata) at a temperature of about 18 °C -- the clams are allowed to clear the water before the next step is initiated. The spawning trough should be drained and then filled with warm water (between 25° and 30 °C). At this point, the addition of gonadal extract (eggs or sperm) will normally bring on a spawning. If however, after 20 minutes, the clams are not spawning, the trough should be drained and filled with cold water (10 °C). The thermal manipulation and addition of sperm should be attempted once more. If the clams do not spawn, it indicates that the animals are not fully ripe and should be conditioned longer.
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Manila clams tend to spawn over an extended period of time with the males being the first to start spawning within the group (it often takes 2 hours for a spawning to be completed). The animals do not all spawn at once, but instead appear to be stimulated by the presence of eggs and sperm in the water.

**Mass Spawning**
A mass spawning is the most simple method of collecting eggs and sperm for larval rearing. However, it is also the least controlled, so the most likely to experience problems. It is, as the name implies, a spawning of all the animals "en mass". One of the main problems with this method is a condition called polyspermy. If the sperm to egg ratio is too great, the eggs can become over fertilized and larval development will be adversely affected (i.e. the larvae are usually malformed and die early).

**Strip Spawning**
The animals are opened and their sex is determined microscopically before the animals are stripped of their gonadal tissue. The eggs are washed and retained in a bucket of seawater (25°C) prior to fertilization. The Manila clam does not respond well to strip spawning - - this method has not proven to be successful.

**Segregated Spawning**
A segregated spawning involves separating the males and females as they begin to spawn. This method requires quick and accurate assessment of the sex of the individual animals as they begin to release their gametes. The animals are allowed to spawn in their separate containers and the eggs are cleaned and placed in a bucket of 25°C water for fertilization.
Keeping the males and females separate means that you are in control of fertilization.

The method is time consuming, but generally successful.

Semi-controlled (or Quasi-mass) Spawning
A semi-controlled spawning is somewhere between a mass spawning and a segregated spawning, in that the clams are allowed to spawn in a trough, some of the males are removed to reduce the sperm to egg ratio, and the water containing the remaining gametes is pumped into a larval rearing tank. As the water containing the gametes is pumped into the larval tank, the water is continually replaced in the trough so the whole process approaches a flow-through spawning situation. This is the system that we have adopted since it requires less effort and is highly successful.

Fertilization

Ideally, the sperm to egg ratio should be at least 10:1 for proper fertilization of the eggs and for consistent production of high quality larvae. The temperature should be in a range of 23° to 28°C. If temperatures are too low, the fertilization rate drops significantly but if the temperatures are too high, the gametes are destroyed and bacterial growth is promoted.
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The fertilization process, once completed, results in a varying rate of development among individual animals. The trochophore larval development is affected by both the rearing density and the water temperature, so that the successful development to "D"-Stage, or straight hinge, is influenced by these factors. Normally all animals have progressed to "D"-Stage after 24 hours at 24°C.

Fertilization to straight hinge ("D"-stage) can take up to 24 hours.
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Hatchery Methods

Water Quality and Treatment

Source Selection

Water, for use in a bivalve hatchery, must be of a consistently high quality and free from toxins (man-made or natural). It should have a salinity within the 20 to 35 ppt range, and contain as little particulate organic and inorganic material as possible. The source of the water used should be far removed from industry and human contamination. The intake should be placed well below the surface, both to avoid the greater salinity fluctuations, and also to avoid any floating contaminants such as petrochemicals and plastics. Positioning the intake below the thermocline has advantages and disadvantages. The advantages include: water clarity (because of a lower algae content, filtration is simplified), stability of salinity (little mixing provides a more consistent salinity), fewer bacteria (both in species diversity and population numbers), and a reduced chance of toxic contamination (most toxins are associated with surface water). The disadvantages include less natural food in the water, and lower temperatures, with therefore greater heating costs. The ideal for a hatchery is access to both sources, whereby the water can be taken from either deep or shallow depths depending on the current water quality within the source areas.

Filtering

Incoming water should be filtered to 5 microns for use in the larval rearing tanks. Normally the first filter is a sand filter. The water then passes through another system (cartridge filters), before passing through the final bag filters and either into a reservoir.
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or into the larval rearing tanks. Excessive amounts of particulate matter in the water will cause problems with blocked filters that often result in water-stoppage or overflow conditions, causing a loss of filtration.

Heating

Because in-hatchery larval rearing usually occurs at times outside the natural production cycle, the ambient water conditions fall below those required for the optimal growth of Manila clam larvae. This means that energy must be added, in one form or another, to raise the incoming water temperature to 23 °C or warmer. The most cost effective method is through the use of solar panels in conjunction with a water reservoir. A boiler is then used to supplement the system on days when the solar energy levels are low. Another method to efficiently pre-heat the incoming water is with a titanium heat exchanger (it recovers the heat energy from the out-going water and transfers that energy to the incoming water). Many hatcheries use electric immersion heaters to heat, or to maintain the temperature within the larval rearing tanks. However, temperature loss can be greatly minimized simply by insulating the individual tanks.

An energy efficient heating system should incorporate several energy sources
Degassing
Supersaturation is a common problem associated with the heating of water and especially with air leaking into the system. This can occur around pump or pipe connections, wherever air is forced into the system and mixed under pressure. Supersaturated water will release these gasses when it reaches an area of less pressure, such as an open larvae tank. Supersaturated water has been proven to cause problems in aquatic animals (like trout and salmon) and may present problems to bivalve larvae. Cold water has the capacity to carry higher levels of dissolved gases such as oxygen, and nitrogen, however, as the water warms, the carrying capacity of the same water decreases and much of the dissolved gas is released to form bubbles in the system. The bubble formation can be a problem when they collect under setting or upweller screens. It invariably causes blocked screens and sometimes overflows. Degassing can be done in several ways including: allowing the water to "stand" for 24 hours in a larvae tank or a reservoir before adding larvae, passing it through filter bags, or over a series of rings or across a degassing board.

Hygiene

Routine methods for sterilization of equipment

Larval rearing tanks are cleaned using a weak solution of chlorine bleach and water (about 1 capful per 10 litre bucket) each time the water is changed (some hatcheries use soap rather than bleach). We also use a mild solution of muriatic acid to remove stains and to sterilize the interior as required. All siphons and plastics, regularly used in the larval rearing process, are stored in a mild concentration of
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Pushing the cleaning “Pig” through the hose.

Batch culture system

clorine, while the screens are normally rinsed in a bleach solution after each use. The hatchery plumbing is chlorinated systematically to kill bacteria and algal growth. Hoses and pipes are further cleaned using a "pig" pushed through to physically scrape any materials clinging to the interior of the equipment. A "pig" is usually made by tying a scouring pad and a piece of cloth together and pushing it through the pipe by water pressure or a rod.

**Frequency of Sterilization:**
The sterilization program for the hatchery should be set-up and instituted to accommodate the specifics of each hatchery. This regime will vary and may also incorporate extra measures to assist when problems arise.

**Algae Culture Methods**

Algae production is a very important component of the hatchery system. Large quantities are needed on a daily basis to feed larvae, condition broodstock, and as food for the setting larvae and spat. Each hatchery develops a system suitable for their own conditions and requirements, however the basics of algae culture are the same, no matter what system is employed. Most of the species cultured were isolated from tropical water and so have fundamental requirements which differ from our local algae. These species have been studied extensively for nutritional value, digestibility, and for algal growth characteristics.

There are two basic types of single-celled algae cultured in the hatchery, a diatom (a plant with a shell of silica), and a naked flagellate (a plant without a cellulose cell wall and that has a motile tail-like structure and the ability to swim). Their culture
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requirements and growth characteristics vary as does their nutritional value and digestibility. Most hatcheries have found that growing a combination of species that includes both flagellates and diatoms provides a more nutritionally complete diet formulation for the larvae.

**Nutrient Solution**

Growing single-celled algae is like growing other plants -- if you want fast growth of a healthy plant, certain conditions must be met. Algae requires sufficient light for photosynthesis, minerals, vitamins, trace metals, carbon dioxide, and correct amounts of nutrients to support rapid growth. The formulation of a nutrient solution which meets the fundamental requirements of all species (flagellates and diatoms) is at the right:

**Sodium metasilicate** is an additional nutrient requirement for diatoms. It is used by diatoms in the production of a scilaceous test or shell.

When growing large cultures of unicellular algae, the use of "agricultural grade" nutrients for the initial

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**Nutrient Solution**

1350 grams of Sodium Nitrate
120 grams of Sodium Phosphate (NaH2PO4)
22 grams of Ferric Sequestrene
44 grams of Ferric Ammonium Citrate

Combine the above with 20 l of hot (80 °C) fresh water to dissolve. Cool, then add 10ml of trace mineral solution and 20ml of vitamin solution.

**Trace Mineral Solution:**

18 grams of Manganese Chloride (MnCl2·4H2O)
2.2 grams of Zinc Sulfate (ZnSO4·7H2O)
1.0 gram of Copper Sulfate (CuSO4·5H2O)
1.0 gram of Cobalt Chloride (CoC12·6H2O)
0.7~ gram of Sodium Molybdate (Na2MoO4)

Combine minerals and add fresh water to 200 ml.

**Vitamin Solution:**

20 grams of Thiamine HCL (Vitamin B1)
0.1 gram of Biotin (Vitamin H)
0.1 gram of Cyanobalamine (Vitamin B12)

Add vitamins to 200 ml of sterilized fresh water and store frozen to prevent degradation of the vitamins.

**Silicate Solution:**

540 grams of Sodium Metasilicate

Dissolve in 20 l of hot (80°C) fresh water.
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Innoculant cultures can be obtained from research labs and other hatcheries where sterile collections are obtained.

Innovative Aquaculture Products hatchery.

The glassware used for algae culture comes in several sizes - everything from a screw-top test tube to a 20 litre carboy. The preparation of the glassware involves cleaning with a soap/warm water solution, or in the case of mineral deposits or difficult residues, a mild solution of muriatic acid can be used to clean the glass.

**Parent Stock and Starter Cultures**

To begin with, sterile algae cultures (bacteria-free, or at least bacteria reduced) are maintained in small quantities to be used as the starter cultures within the hatchery. Each hatchery maintains its own cultures after first obtaining a "start" from research facilities, algae laboratories, or another hatcheries' parent stock. This new culture becomes the parent stock from which the hatchery algae is produced. To produce large volumes of clean, fast-growing, good quality algae, the parent stocks must be kept healthy and clean.

**Sterilization of Culture Media**

The growing medium (seawater with nutrients and silicates added) is sterilized by autoclaving the individual flasks containing the media for 15 minutes at 15 psi. The flasks (usually 150 ml or 350 ml) are then ready for inoculation with one of the various formulations necessary to improve the cost-effectiveness of the algae production (i.e. bulk prices are more reasonable). The algae is maintained in the hatchery within its own special, segregated area away from the larval rearing area. This arrangement helps to minimize the risk of culture contamination and also reflects different conditional requirements for plants (i.e. temperature control, light levels, and a clean growing environment).
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species being cultured. It is crucial to maintain sterile conditions for the transfer of algae as any contaminants will quickly takeover the culture media and cause production failures.

Batch Culture Systems
The most common form of large-scale algae production is batch culture. This consists of adding small, dense cultures of unicellular algae to larger containers of sterilized water (with nutrients added); growing these cultures to a high density; before transferring to a larger culture container, or harvesting completely. It is a simple, effective way to produce algae.

Within this system, usually only the flasks are sterilized using the autoclave process. The carboy, which is the following container, is filled with chlorinated water (20 ppm), and allowed to stand for at least 6 hours before neutralizing the chlorine using sodium thiosulphate. Nutrients are added (and silicates when necessary) at the time of inoculation. The carboys are aerated, both to deliver carbon dioxide to the cells, and to keep the cultures moving.
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so that all cells have access to the light. The carboys are then used to start larger cultures, either columns or open tanks, which have been chlorinated/de-chlorinated.

Semi-continuous Systems
The semi-continuous system is based on the assumption that a culture can be maintained in logarithmic phase of growth through continuous harvesting by replacing the volume removed with new culture medium. The system itself can be open (open tanks) or closed (bag culture) and the production levels and the length of time at which the cultures can be maintained varies significantly between species and between culture techniques. The open cultures tend to experience bacterial and protozoan contamination problems much more frequently than the bag system. The bag system depends on pasteurized water rather than chlorination to achieve a sterile supply for renewing the harvested bags.

Lighting
Light is essential for the growth of all algal cultures. Unicellular algae can be cultured using artificial lighting such as fluorescent tubes or the more intense metal Halide systems; however, the most cost-effective light to use is sunlight. Natural sunlight can be supplemented with the use of artificial light to increase production during those times of year when the natural light levels are low. Direct sunlight should be avoided and care must be taken to shade the cultures after the initial inoculation.

Temperature
Temperature also plays a role in the growth rate of the various species. We grow our algae using natural light and ambient temperatures (15° to 25° C.) so we
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see tremendous variations in the production levels between late spring (our greatest level of production) and the middle of winter (our lowest level of production). Not only are the algae growing slower, but the maximum densities are reduced. Water temperature causes significant growth variation -- if it's too cold (5° to 8°C) they appear to be in hibernation, while if it's too hot, the individual cells die. The upper lethal level varies between species but generally is about 25°C. Where as the lower temperatures are not lethal, algae merely cease to grow.

pH
The pH of algae cultures will increase as cell density increases within the individual culture containers. Problems will occur if the pH exceeds 8.5 (either a slow-down of the growth rate or an algal crash within the tank). To maintain the optimum pH of 7.2 to 8.2, injection of carbon dioxide at a rate of 4% with the air supply is essential, although having a resting period, "darkness", is also an effective control of pH. Because we utilize mainly natural lighting, we have found that the use of CO₂ is necessary only during the fastest production times in the late spring and summer.

Counting and Analysis of Cultures:
To determine the density of cultured algae, it is necessary to count the cells in a known volume. This can be done in several ways. The most time-consuming is through the use of a haemacytometer, a microscope slide that holds a specific volume of liquid and has a grid marked on the slides' surface. It is necessary to count individual cells within the grids and mathematically extrapolate the numbers. The use of a colorimeter is also time-consuming and not simple. It reads the density of the chlorophyll that
A COULTER COUNTER is a fast and easy method for counting cells to determine culture density.

Plating algae for bacterial analysis is normally done on agar plates in petri dishes and incubated for 24 hours.

must then be translated into a number, reflective of cells per ml. We use a Coulter Counter which quickly and accurately counts the particles of a specific size, within the sample.

The cultures are routinely monitored under a compound microscope to look at the cell condition and for contaminants like protozoan or unwanted algae species.

Bacterial Assessment
To assure low levels of bacteria within the algae cultures, they are tested regularly by plating with TCBS agar for the presence of bacteria. Most bacteria do not pose a threat to the quality of the food. However, large numbers of bacteria (any species) and the presence of Vibrio spp. is cause for concern.

Culture Density
Densities of cultures vary significantly between species since the larger cells attain much lower numbers at maximum density than those species with smaller cells. For example, under normal light and growing conditions, the maximum cell density at which we normally culture 3H is 3 to 3.5 million cells per milliliter, while the Nannochloropsis oculata
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would be cultured at a density of 15 to 18 million cells per millilitre.

Toxicty
Algae produce metabolites as they grow and the food value or quality decreases as the algae enters the end of its growth phase. For this reason, "old" cultures are not as desirable for use as larval food, as are algal cultures in their log-phase of growth. Algal metabolites can pose problems for clam larvae so it is important to recognize the toxicity associated with older, very dense cultures of algae. We have always felt that larval problems are often attributable to algae quality problems -- so if the algae looks bad, or smells bad, throw it away!

Algae Paste Production

Algae paste is made by centrifuging large quantities of cultured algae through a continuous flow clarifier. The resulting paste is then mixed with preservatives and stored in air-tight containers at a temperature of between 5 and 8°C. The centrifuging process actually eliminates bacteria and metabolites within the culture along with the water in which the cells have grown, thus creating a product that has few bacteria, but all of the nutritional value of the original cells in water. The development of algae paste has proven to be an great advantage for both harmonizing the algae supply with the hatchery production cycle, and in creating a high quality food that can be easily transported and stored for use at setting facilities. Because a variety of species can be centrifuged, pastes can be combined to give formulations for specific purposes: such as early larval rearing, or for setting, or even for the newly metamorphosed animals.

When in doubt, dump it out!

To reconstitute algae paste, wash through a bong screen.
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The preserved paste is easily suspended in water by washing the paste through a 202µ screen into a bucket. The cell counts will vary between paste formulations and between species but approximate numbers (cells per microlitre) are available from the paste producers.

Two different centrifuge models for making algae paste: wine clarifier on left and cream separator on right.
Larval Rearing Methods

Rearing Temperatures

The optimum larval rearing temperature for manila clams is 23°C, although the animals can be grown successfully at temperatures ranging from 18 to 28°C. This temperature allows for a good growth rate while helping to minimize the risk of increased bacterial growth (associated with higher temperatures) within the larval rearing environment.

Water Changes

The water within larval rearing tanks is changed regularly, both to remove the larvae from water contaminated with their own metabolic wastes and to clean the tank surfaces. Every three days the water is removed from the tank and filtered through different sized mesh screening to catch the larvae. The larvae are then returned to another tank that has been cleaned and contains clean, filtered seawater. As metamorphosis approaches, the water changes are done daily to remove any animals that have become pediveligers.

Continuous Flow

A system can be set up to allow for the constant exchange of water within the larval rearing tank. This constant influx of "new" water influences the density at which the animals can be cultured. The total number of larvae within a 4,000 litre tank can be significantly increased by this process, however, the total amount of water required to raise the larvae remains the same. The flow-through system requires daily cleaning of the tanks to minimize bacterial problems, increases the total number of animals.
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cultured within a given tank volume, but appears best suited to the smaller conical shaped tanks commonly used in Europe.

The system itself, consists of an inflow pipe carrying water that is preheated to 23°C and contains fresh cultured algae (approximately 10 to 20 thousand cells per milliliter). The outflow is through a large "banjo" screen that allows the water to pass through, but not the larvae. This screen must be monitored frequently since the larvae will tend to clog the screen and create overflows. The mesh size is also an important factor as the larger meshes are less likely to experience clogging problems and each "banjo" filter must be designed to be sufficiently large to accommodate the flow requirements for the tank.

Continuous flow larval culture allows for much higher culture densities.

Rearing Density

Although we successfully raised Manila clam larvae at a density of 10 animals per milliliter, the optimum density is in the range of 2 to 5 larvae per milliliter. The higher densities may cause increased stress which, in conjunction with other factors, will cause larval mortalities.

Feeding

The food requirements for the larvae will vary with their increasing size and with water conditions. The larvae are sensitive to water quality, temperature, and larval density and demonstrate this sensitivity through food consumption. Larvae not clearing the water (indicative of not eating) is one of the primary indicators of a problem within the larval rearing tank. The problem can be as simple as the temperature being too low, or as complex as an algae bloom in the

Feeding a varied diet of algae species will improve larval health.
Manila Clams: Hatchery and Nursery Methods

Shell growth is used to measure growth.

bay affecting the larvae inside the hatchery. The feed rate must always be adjusted to the current rate of consumption by each individual group of larvae. We normally feed at a rate that will achieve 30 to 50 thousand algae cells per milliliter of water within the tank.

Algal species fed vary with the size of the larvae but normally we add larger species of algae to their diet as the larvae attain the 150µ size. The larvae receive their "first food" on their second day in the larvae tank (food should be available to the "D"- Stage larvae). The first food consists of a mixture of the smaller flagellate species: Nannochloropsis oculata, Tahitian isochrysis, and Isochrysis galbana (a small diatom like Chaetoceros calcitrans can also be fed at this stage). The larger diatoms, 3H and Chaetoceros gracilis are added to the food mixture as the larvae reach 150µ.

Growth Rates
The larvae usually grow at an average rate of 10µ, shell-length increase, per day. This will vary between larval groups and within the seasonal time frame, but the larvae should become pediveliger 10 to 14 days post fertilization. The "D"- Stage larvae are approximately 100µ in length and attain an average shell-height of 180 to 210µ at the pediveliger stage.

Counting and Sizing Larvae
We use two methods to measure the clam larvae: (1) they are measured for shell-length under the microscope (the longest length of the shell edge), and (2) the larvae are washed through various sized screens so the largest mesh on which the larvae are retained represents a larval size. For example, the
Manila Clams: Hatchery and Nursery Methods

Two day old larvae that are 100µ in length are held on a 55µ screen while a pediveliger larvae that is 210µ long will stay on a 155µ screen.

To count larvae, they must be evenly distributed throughout a bucket of seawater (10 liters) before removing a sub-sample. At least three samples should be taken and counted, then averaged to give an estimate of the total number of larvae within the bucket. We have developed a counting system based on volume related to the larvae's screen size, which considerably shortens the time necessary to determine the larval numbers for a given group.

Disease Problems

There are no known diseases specific to larval Manila clams, however, bacteria appear to cause problems within the larval rearing cycle. The Vibrio spp. cause the greatest "ill-effect" on the larvae, often being responsible for massive mortalities among larval groups. The only control for Vibrio bacteria is hatchery management (keeping the plumbing and food-algae clean). The larvae can be treated with a mild chlorine dip to kill bacteria clinging externally to the shells, or the rearing water can be treated using an anti-bacterial drug. The use of drugs in the water should be avoided not only because of the increased cost of production, but because it is normally "too late" when the treatment is instigated.
Metamorphosis

Determining Competency to set

For reasons unknown, perfectly good manila clam larvae can vary significantly in size when competent to set and metamorphose. This naturally occurring variation between larval groups makes it necessary to generalize and use approximate size ranges and times. Normally, the screen size we use to "catch" setting-size pediveligers is 150 μ, however, this can range from 130 to 165 μ as some clams are ready to set at a smaller size, while others do not reach the pediveliger stage until they are much larger. We have not yet been able to prove that larger is better.

During larval culture, the clams are viewed daily under a microscope to monitor physical changes indicative of approaching metamorphosis. The shell length should be in the range of 180 to 220 μ; the colour, a golden brown; and foot activity should be evident. Unlike oysters at setting size, clams do not have an eyespot.

When clam larvae are close to setting, their typical activities are expressed by their pediveliger lifestyle; alternating swimming within the water column, then dropping to the bottom and using their foot to crawl across the substrate. This pediveliger period can be protracted as the animals approach metamorphosis, lasting from a few days to almost two weeks (water temperature seems to play the largest role in time spent as a pediveliger; lower temperatures increase the time necessary to complete metamorphoses).

At the time of metamorphosis, the larval clam completely loses its ability to swim, because the velum is shed. However, the animal is still mobile.
Manila Clams: Hatchery and Nursery Methods

Clams move frequently in their search for the perfect home.

Too many pediveligers on the setting screen causes low set survival.

through the use of its foot, and unlike oysters, retains the foot after metamorphosis is completed. The clam uses a byssal thread (like a mussel) to attach itself to the substrate (either a grain of sand, shell or the bottom of a setting screen). Often the newly "set" clams don't like their chosen position, so they sever their connecting thread and crawl to a new location where they reattach to the substrate with a newly generated byssal thread.

Factors Affecting Success
There are many known factors that influence the success of a set.

Larval Density
The larval density (number of clams per square cm. of screen space, or per ml. of water) within the setting system, must be optimal for that system. These numbers can vary significantly between systems, however 150 to 200 per sq. cm. is the approximate number for downwellers.

Temperature
Setting water temperatures should be similar to the larval rearing temperatures or slightly lower (20 to 24°C). The water temperature not only affects the
Manila Clams: Hatchery and Nursery Methods

metabolic rate of the animal, but affects the growth of other organisms within the setting environment, such as, algae and bacteria.

**Bacterial Loading**
Large numbers of bacteria within the setting system, can adversely effect the larvae. Most bacteria in small numbers are harmless. However, explosive growth of some species, especially Vibrio spp., can act to inhibit the successful metamorphosis of the pediveliger clams and may result in high mortalities among the setting larvae. Setting clams seem to be more sensitive to this than larvae or juveniles.

**Fouling**
The shells which may become fouled by diatoms can also affect on setting success. It is a problem which varies as the season progresses and requires the daily cleaning of the pediveligers (i.e. washed with a gentle spray of saltwater).

**Algal blooms**
Explosive increases in algal cell numbers, both within the setting system and in the local environment, can cause a slowdown in the growth rate; severe blooms may even foul the velum and result in high mortalities among the pediveligers.

**Feeding**
The addition of food in the form of fresh cultured algae or algae paste is necessary to ensure the availability of a nutritious diet for the animals (even waters rich in natural phytoplankton may not have algal cells small enough to be of value to the pediveliger larvae). Recent research suggests that pediveligers use both their foot and their velum to feed during this phase of metamorphosis.
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Transportation of Larvae
Pediveliger clam larvae are prepared for shipment by the hatchery using the same methods as for transporting oyster larvae. The animals are screened upon removal from the larval rearing tank, counted, and placed on the shipping material, (nytex or paper coffee filter) before being wrapped in several layers of moist toweling. This bundle is placed in a well insulated shipping box, along with an ice pack which should not come into direct contact with the larva.

Clam larvae are shipped in insulated containers to maintain a temperature of not more than 8°C.

As with oyster larvae, temperature of the larval ball during transport can affect the viability of the larvae. Temperatures should not exceed 8°C or fall below freezing.

The time in transport (i.e. number of days the larvae are out of water) also affects larval competency. In a recent experiment, clam larvae were kept under refrigerated conditions for 17 days all animals were dead after 15 days in the refrigerator. Their condition
Manila Clams: Hatchery and Nursery Methods

It’s important that larvae be put in the water as soon as possible. Don’t forget it!

deteriorated progressively, and most rapidly after the first 5 days. We always recommend that larvae should be returned to the water as soon as is possible upon receiving the shipment, but one or two days out of the water, if maintained at 4 - 5°C, should have no detrimental effect on the success of the set.

Setting Methods (Micro-Nursery Stage)

Types of Systems
There are several general systems for setting clam larvae and growing them through this extremely delicate stage. Most hatcheries hold them on nylon screens in a flow through or recirculating system with frequent water changes.

**Water Tables**

Water tables are shallow-sided tanks, made of smooth non-toxic material. The pediveliger larvae are put directly in the sea water on the table and allowed to crawl and swim on it. The table can be used either with continuously flowing water (necessitating a collecting screen at the outflow to prevent larval loss), or with standing water that is changed twice or more per day. Water tables provide a large surface area to water ratio to accommodate the pediveliger's characteristic lifestyle of swimming then crawling on the bottom surfaces.

**Water tables stacked like bunk beds are lined with setting screens.**

A recirculating air-lift downweller is a good way to set moderate numbers of clam pediveligers.

**Setting screens can be constructed using NITEX screen on wood frames that are finished with fiberglass and gel-coat.** While stretching the screen, cover with a damp cloth to ensure a tight screen when completed. Always start at the centre working on opposite sides of the frame as you staple the NITEX in place.
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Downwellers

Downwellers are screened chambers that are used to hold larvae or seed with a flow of water passing downward past the animals and through a screen at the bottom. When using a downwelling flow for setting pediveliger clams, the larvae are added to the screened units (screen size 120 to 130 µ) at a density of approximately 150 - 200/sq. cm. of screen surface area and the flow rate should be adjusted to 1 litre/min/million pediveligers.

Setting Screens
Setting screens are really shallow-lipped downwellers. Commonly used styles vary both in size and shape as well as material make-up. The screen itself is 120 to 130 µ. mesh size, while the frame is commonly constructed of PVC pipe, fiberglass, or from wood which is then fiber-glassed in various sizes to fit the particular tank they are being floated or suspended in. The larvae are introduced to the screens at a density of approximately 150-200/sq. cm. of surface area and a

The screens are cleaned frequently using a gentle salt-water spray.
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The downwelling flow of water is adjusted to 1 litre/min/million larvae.

Our preference is the square shaped setting screens that we use on water tables. The advantages in a square or rectangular shaped glass-over-wood screen used in conjunction with the water tables are:

* They float, making the depth of water within the table unimportant and the screen can be easily kept above the underlying bottom of the water table.
* Pouring larvae from the screen to another container is simpler from a corner, rather than from the lip of a cylinder.
* Storage of trays is convenient.

The floating beach can be easily constructed using wood with styrofoam floatation, but the wood needs to be protected from wood borers, either with a coating or by periodically removing the “beach” from the water for a drying period.

A floating beach with removable screens at each end. The screen size can be increased as the clams get larger.
Manila Clams: Hatchery and Nursery Methods

Floating Beaches
The floating beach is a "low-tech" method that can be used in remote locations where electricity for pumping water constantly is unavailable. They are commonly constructed of wood (covered with fiberglass), the unit has two open ends fitted with removable screens and is filled with a 1 to 2 inch layer of sand. It should be anchored in a protected area with good tidal flow to ensure adequate exchange of water for the introduction of food and the elimination of metabolic wastes.

Pediveliger clam larvae are introduced to this system at approximately 1.5 million/sq. m. of gravel area. This beach can double as a nursery area for the clams by replacing the 130 µ end screens with larger mesh (approx. 1000 µ) after the larvae have metamorphosed (about 2 weeks post introduction). Because the system is not easily monitored, set success is difficult to determine until the clams are approaching a larger seed size. This system is still quite experimental and appears to work better as a nursery system than a setting system.
Manila Clams: Hatchery and Nursery Methods

Oyster Larvae Setting Tanks
Setting tanks can also be used as a "low-tech" alternative for setting pediveliger clams. A thin layer (1 in. or less) of #20 silica sand is spread on the bottom of the tank; the tank is then filled with filtered (10 µ) sea water and the pediveliger larvae are added at a rate of 150/sq. cm. (tank bottom area). Water should be changed once or twice per week (using a screen size 130 µ to catch the pediveligers, while the larvae are still swimming) and food in the form of algae paste is added. The amount of food to add depends on the number of animals present, the water temperature, and their clearance rates (how fast the algae disappears from the water). Since feeding is based on "tank specific" factors, food should be added when the water is clear (at a rate of approximately 20,000 to 50,000 cells per ml).

Another option for setting manila clam larvae in oyster setting tanks is to use floating screens and an air-lift to create a re-circulating downwelling current of water across the screens within the tank.

Setting Techniques
The pediveliger clam larvae are shipped from the hatchery in a well insulated shipping container with frozen ice packs. The temperature of the larval ball on arrival at the remote setting site should be between 4 and 8°C. To evaluate the condition of the larvae place a small sample of the larvae on a slide under a microscope (add a drop or two of saltwater). The larvae should soon become active, demonstrating crawling and swimming activity within the water. The shell height should be between 180 and 220µ., the gut full, and the velum healthy and covered with hair-like cilia. If you see empty shells and ciliated protozoan, there could be a problem with the larvae.
Manila Clams: Hatchery and Nursery Methods

An oyster setting tank can be converted to clam setting by using floating screens and air lifts.

Clam larvae are concentrated into a ball by washing them onto a screen.

Count the larvae, not only to ensure that the correct number has arrived from the hatchery, but to make for easier division of the larval ball. Counting can be done by: adding the larvae to a plastic bucket with ten litres of sea water, mixing gently to distribute the animals throughout the water column; with a pipette take 3 - 0.5 ml samples; count the larvae on a microscope slide. Using the mean of the samples, the total number can be determined by dividing 10000 ml (10 litres) by 0.5 and multiplying the resulting number by the mean of the samples. This number reflects the number of larvae in the original 10 litre bucket.

We have found that by volume, 3 ml. of pediveliger clam larvae contains 1 million clams and weighs 2.75 gm. Because clam larvae are much smaller than oyster larvae at setting size, the volume of larvae to add to any of the setting systems is also smaller.

If using individual setting screens, the larval ball is divided into the appropriate number/volume to add to
Manila Clams: Hatchery and Nursery Methods

each. Care must be taken to not allow the larvae to dry out or become too warm. Add the pediveligers to the water immediately and make sure the animals are fed, with the addition of algal paste.

Substrate vs. No Substrate
Several methods can be used when using downwelling screens for setting pediveliger clam larvae. We have found that there is no difference in the set success between using substrate [ground shell (size 200 µ), sand (size 200 µ)], or no substrate is used. However, differences in handling, maintenance, and estimating success become obvious. The use of a substrate within the screen creates more surface area for the pediveligers to crawl and feed on. It acts to keep the screen from clogging with algae as the water downwells through the system, thus requiring less frequent cleaning to avoid overflow when the screen clogs. The negative aspects of using a substrate in the setting screens stems from an inability to assess the success or failure of a set until the animals have achieved a size large enough to be screened away from the material. This screening process is complex because the animals are attached to particles by byssus. Counting and monitoring the animals is made more difficult through the addition of substrate but production is easier.

Nutrition & Temperature
As has been previously discussed, the addition of food to the setting system is essential to achieve healthy clam seed from the pediveliger larvae. The amount of naturally occurring algae within any systems' intake will vary between sites as well as between time of year at the same site. Similarly, the nutritional value of this algae will vary and cell size
Manila Clams: Hatchery and Nursery Methods

may be too large for the clam larvae to utilize. Fresh algae, or algae paste must be added to the setting system to provide a reliable food source of the correct size for the animals. Several species are routinely grown for use with setting clams. These include:

* **Chaetoceros gracilis** (a diatom with spines that may cause screen clogging problems).
* **Thalassiosira pseudonana**, or 3H, (a diatom - the most common specie used for algae paste).
* Tahitian **isochrysis** (a flagellate, an excellent larval food).
* Isochrysis galbana (a flagellate with the same characteristics as T.iso).
* **Chaetoceros meulleri** (a diatom often referred to as "chagra").
* **Chaetoceros calcitrans** (a diatom small enough for early-stage larvae).
* **Nannochloropsis oculata** (a flagellate 2-3 m in size).
* **Skelotenema spp.** (diatoms commonly used in Europe).

A food mixture of several species (combining both flagellates and diatoms) offers a better and more complete diet for the pediveliger clams.

Feeding rates are arbitrary and should be adjusted to reflect the seasonal availability of natural algae within the water. The pediveligers should be fed at a rate of between 20,000 and 50,000 cells per ml. of water, if using standing water, and at a rate of 10,000 cells per ml. of flowing water. Obviously, a recirculating system makes more efficient use of both algae and heating, but can create greater risk to the health of the setting larvae. Both heat and algae can be partially recovered from the flow-through system.

“I’ll have a double order of 3H, a side of Nanno and an extra T. iso.”
Manila Clams: Hatchery and Nursery Methods

with the expedient use of a heat exchanger and by using the outflow water to grow small seed.

Water temperature at setting plays a large role in the length of time it takes for the pediveliger larvae to metamorphose; growth slows with decreasing temperatures. The larvae are tolerant of temperature fluctuations, however, we have found that the optimal temperature to maintain for setting is 23 °C. Clam larvae will die at temperatures over 30 °C, and when temperatures are below 16 °C, growth slows and the animals continue as a pediveliger for what seems like forever.

Flow Rate/Water Change
Each system has different requirements of water use. Essentially, a flow-through system requires approximately the same volume of water per larve as a standing water system; the difference lies in the method of water exchange or replacement. In a standing water system, the water is changed twice per day as two separate and complete water changes. Within the flow-through system, the flow rate is adjusted to approximately 2 complete water changes per day.

Maintenance and Handling
The setting larvae have specific basic requirements to maintain health and promote successful metamorphosis. It is essential that the system be kept clean. The animals must be cleaned daily with a gentle spray of saltwater to remove diatom fouling and to wash away metabolic wastes. The screens must be cleaned daily, or more frequently if they are clogging and blocking the flow of water across the screen. Anytime the animals are removed from the water, care must be taken to ensure they do not dry out or become too warm. (Telephones can kill clams.)
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If it becomes necessary to keep the animals out of the setting environment for more than 10 to 15 minutes, the pediveligers or set clams must be placed on nytex screening, wrapped in moist toweling, and placed in the refrigerator until they can be returned to the system.

The animals should be examined regularly under a microscope to check for growth, development, and shell condition (external fouling). As they approach the "nursery" stage, their increasing size facilitates the separation of the larger, healthier, faster-growing animals from the dead and the "runts" which should be discarded. By moving the animals to larger screens as their increased size warrants, the problem of screen clogging becomes much less of a dilemma.

Estimating Success
To determine the success or failure of any setting venture, it is necessary to decide a cut-off point for the "setting" as opposed to the "nursery rearing" so that a statistical measure can be created which in turn can be used as a comparative standard against other sets carried out at the facility. This point can be based on a time frame (e.g. 10 days after introduction to the setting facility) or may reflect a measure of animal size (e.g. total number of animals from a particular set to reach 400 µ.). It is essential to be consistent.

Once a method for determining "success" has been resolved, the actual statistical analysis is straightforward. The animals are counted by taking volumetric sub-samples.

EXAMPLE
10 days post introduction to the setting screen the animals are washed, screened through a large mesh
Develop your own standards to evaluate your clam sets. Keep accurate records.

screen (500 µ to remove any larger material) onto a 130 µ screen. The total volume is measured in a graduated cylinder. Three samples are taken then counted on a prepared slide (being sure to include only live animals as a part of the final count). The total volume is divided by the sample volume and then multiplied by an average of the samples

Total larvae added to system = 250000 animals
Total volume of the post set clams = 12 ml.
Sample #1 = 0.01 ml = 160 animals
Sample #2 = 0.01 ml = 155 animals
Sample #3 = 0.01 ml = 171 animals

To find the total live animals in the total volume of post set clams:

Add sub samples and average 160+155+171 / 3 = 162 animals per 0.01 ml
Divide the total volume by 0.01, then multiply that number by the 162 animals in the sample.
12ml/0.01 = 1200 1200 X 162 = 194400 live animals
To find the percent survival or "success":
Divide total live animals by the total number initially added to the system and multiply by 100 %
194400/250000 X 100 = 77.8%
Manila Clams: Hatchery and Nursery Methods

Rearing To Out-Plant (Nursery Methods)

Upwellers/Downwellers

Recirculating Downwell
A recirculating downwell system involves the reuse of water within a tank. Water is lifted from the holding tank and passed down through a screen supporting seed, thus creating a recirculating downwelling current. The water must be changed on a regular basis (frequency is determined by the stocking density relative to the size of the tank) and food added. This system is only feasible for use with very small clam seed as the food and tank requirements are too great to be cost effective for larger clams (seed should be smaller than 500 microns).

The stocking density will be based on flow rate and quantity of algal cells available to the clams. For example, if the maximum flow rate is 40 litres per minute, and the algae is added to maintain an average of 50,000 cells per millitre, the total volume of clam seed to stock in the system would be approximately 3 litres.

Maintenance
It is essential to keep the screens clean as any clogging will affect the water flow rates and ultimately growth rates of animals in the system. The screens should be cleaned at least once per day and the clams should be size graded to ensure the animals are all of a similar size. If clams of different sizes are kept in the same unit, the larger clams out-compete the smaller clams and retard their growth.
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Handling
Although clams have a protective shell, it's important to remember that the shell will crack or break if they are not handled with care (this is especially true as the size increases). Often the animals can repair minor damage, however any opening to the body is an opportunity for bacteria or predation.

Growth Rates
It is important to recognize that many factors combine to influence growth of clam seed in a nursery. Most factors, such as food availability, water temperature, or salinity, are difficult or impractical to control so the grower must rely on the ability to manipulate the factors which can be controlled to optimize the growth rate. Stocking densities or flow rates (which are interrelated) can be manipulated to increase the growth rate of individual clam seed within a system. Generally, we expect our seed to double in volume every 7 to 10 days during our summer growing season. By reducing the stocking densities this rate could be increased, however, this is the optimal stocking density for our system and its' location - the cost effectiveness has been proven.

Forced Upwell
The commonly used upwell system is based on the simple fact that water which flows into a container will also flow out - it's route can be manipulated. The inflowing water enters outside of the screened unit containing the clam seed, the water passes up through the seed mass enroute to the only exit which is situated within the seed unit. The stocking density for any upwell unit is based on food availability within the water and is directly related to flow rate.
The water tends to form channels as it flows upwards through the seed mass in a coke bottle upweller.

The small clams will climb the walls of the upwellers.

Manila Clams: Hatchery and Nursery Methods

The water tends to form channels as it flows upwards through the seed mass in a coke bottle upweller.

normally a flow rate of 20 to 25 litres per minute is optimal for each litre of seed. It is important to have clean screens and to maintain the most efficient flow rates to upwell units. This system is effective for all seed sizes, however, pumping costs may prove prohibitive for larger seed (as well as equipment costs for the larger volumes).

**Coke Bottle System**

This system is another type of upwell system designed to require less maintenance for small seed production. An inverted plastic coke bottle utilizes the bottle neck as the water inflow point, a marble as a check valve in the neck, and a plastic tube near the bottom (top when inverted) of the bottle as the water outflow. Seed is placed in the bottle on top of the marble and the water flow rate is adjusted to fluidize the clams, enough flow to move the seed away from the marble but not strong enough to keep the individual clams tumbling or constantly moving within the water column. The small animals will
Manila Clams: Hatchery and Nursery Methods

In the upwell unit some clams are always trying for the great escape. The water is pulled out of the central trough by the rotation of the paddle wheel. It is replaced by an upwelling current of water through the seed mass contained in the attached upwell units. It is a simple, efficient process.

create a large unified mass through the attachment of byssal threads to each other and to the sides of the container. This tends to create channeling of water flow (true for all upwell containers of clam seed) so that the animals next to the usual path of water flow through the clam mass receive more food, thus exhibit faster growth than other clams within the group. This phenomenon occurs with more frequency within the coke bottles due in part to the extended times between handling and because of the funnel shape of the upwell unit.

The stocking density for this system will be based on the water flow and the amount of food available in the water. We found that a reduced flow rate (5 to 10 litres per minute per litre of seed) was possible when cultured algae is added to the incoming water.

FLUPSYS - Floating Upwellers

A floating upweller can depend on tide or mechanical methods to force water to flow upward through a seed mass within containers (stacked trays or individual units) attached to the floating upwell unit. The most commonly used FLUPSYS are raft structures which

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support a series of individual containers (steel, plastic, wood, or fiberglass) along a central enclosed channel. The water is forced out of the channel, either by using a propeller or a paddlewheel, and is replaced by the upwelling flow of water through the attached clam seed containers.

We use a paddlewheel FLUPSYS which is powered by a 1/2 horse power electric motor. The use of a paddlewheel is the most efficient method to move water and has proven to be extremely effective in the production of clam seed (see diagram).

The floating system requires a protected site with warmer temperatures, and productive water (heavy blooms are neither necessary nor desirable as too much algae will clog the upwell unit's screens and restrict the water flow to the animals).
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Stocking Densities
All stocking densities are based on flow rate, so for animals larger than 2mm, the flow rate should be 20 to 25 litres per minute per litre of seed. This fluidizes the seed mass and provides optimal water flow to remove the metabolites and feces with the out-flowing water.

The following chart shows the number of individual upwell unit required to produce seed at 6-8mm outplanting size given a flow rate of 20l/min./seed.

<table>
<thead>
<tr>
<th>UPWELLER DIA.</th>
<th>NUMBER OF CLAMS</th>
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<tr>
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<td>22</td>
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Cages and Trays

Types:
Pearl Nets
Pearl nets are manufactured in different size meshes, however the smallest mesh, at 1.5 mm, is suitable for seed 2 mm. and larger. The nets are generally tied together to form a single line of nets and are weighted at the bottom to keep them vertical in the water column.

The nets should never be stocked at a density greater than 25% of the bottom surface area (single layer)
Manila Clams: Hatchery and Nursery Methods

Ted Kuiper of Kuiper Mariculture suggests hanging the Japanese onion bags with seed from long-lines or rafts.

Japanese onion bags have a small size and are suitable for use in most types of stacking trays. A quarter to a half litre of seed goes into each bag.

because of the tendency for seed to shift and pile up in a corner (see chart for numbers relative to volume). It should also be noted that if the clams are too small for the mesh, they will crawl through the holes.

Nestier Trays
Nestier trays can be lined with window screening for use with small seed (seed must be on the 1410 µ screen). An alternative for lining trays is use of Japanese onion bags. If the trays are being suspended, the stocking density should be 25% of the bottom surface area. The seed will also tend to pile up in the corners if they are maintained in high current areas. Trays which are maintained intertidally (either on racks or on the beach) should be stocked with substrate as well as the clam seed. The substrate will protect the animals from the extremes of temperature and of desiccation.

Other types of trays and nets are acceptable providing they have a compatible mesh size and stocking density. It should be noted that growth rates will be comparatively slower for seed which is grown
Manila Clams: Hatchery and Nursery Methods

...intertidally. Both methods depend upon the natural movement of the water to bring food to the animals so each site will have site specific variations in growth rates.

**Beach Nursery**

A beach nursery can be set up to increase the size of seed for out-planting. The beach area chosen for the nursery (under netting) should have a substrate of pea-gravel, be relatively flat so as not to have shifting or siltation problems, and be at the mid-tide height (too high means slow growth while too low gives more predators an opportunity). The area should be cleaned of all larger clams, and raked prior to planting the seed. The area must be covered with small mesh netting which should be buried on the edges. Planting densities can be quite high 2500 - 3500 per square meter since the animals will be removed from this nursery at a larger size for planting in other locations on the beach.

The mesh is buried on the edges to prevent infiltration by predators.
Planning Your Nursery

Site Selection
The type of nursery system that an individual grower will select will normally depend upon sites currently available, as it is usually not practical to consider obtaining a "new" lease site for this purpose. The nursery, will occupy only a small segment of a lease area, or can even be sited on land which doesn't necessarily front on saltwater. It is essential to look at the many nursery options available and weigh all the site specific characteristics against the cost of production at a given site, before making a final decision.

An important question to ask is, "do I need a nursery based on the scale of my operation, or is it more efficient to purchase the seed required for out-planting"?

In answering this question, it is important to recognize that a nursery can consist of buying 6-8 mm. seed, holding it in trays on the beach (protected from predators) until it is 12-15 mm., then out-planting the larger seed on the prepared beach area.
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The different types of nursery systems have been discussed in Chapter IV, so with reference to these systems, the site selection criteria will be discussed.

Oceanographic considerations
An important consideration when planning a nursery is the oceanographic conditions at the site, that is, factors affecting salinity, turbidity, temperature, food availability, wave action, and currents. Because these are not factors which can be easily controlled, it is crucial to site your nursery in a location which receives the least negative impact from these factors.

Siting is important to avoid storm damage to your nursery system.

Salinity
The overall freshwater influx within a nursery area can considerably change the salinity of the water. Water which is low in salts will freeze more rapidly during colder winter conditions and animals exposed to freezing will die. Clams also exhibit slower growth rates when subjected to salinities outside their optimal range. It is also important to realize that a seasonal stream which can cause the changing salinities, will often erode or cause movement of a beach substrate which is detrimental to any type of beach nursery.
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**Turbidity**
Large amounts of organic and inorganic suspended particles will affect overall growth rates and survival of the small clams. Turbid water is generally associated with a large influx of freshwater from a river in flood, or storm action where the bottom sediments have been stirred up. It is important to realize that clams, like other bivalves, will stop filtering if there are too many particles in the water, so growth rates are adversely affected. High turbidity associated with intense algal blooms can even prove to be deadly to small seed.

**Temperature**
Water temperature affects growth rate of clams significantly, so that a site which experiences depressed temperatures during the summer grow-out season will have reduced growth rates.

**Food Availability**
The productivity of the water is an important factor in the siting of the nursery facility. The amount of algae available as food for the clam seed has an affect not only on growth rates but also on carrying capacity of a system. Clams can die of starvation, particularly if the environmental conditions combine to give warmer water with less food available to individual animals.

**Wave action**
Any site, whether on the beach or floating, must be protected from wave action associated with storms or boat-wash. Not only are waves damaging to equipment, but water movement can cause movement of substrate or movement of clam seed within a cage system, thus causing density related problems as the clam seed is piled into a small area of the container.
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Currents
Tidal currents can be beneficial if the water movement is not too fast, however, it can cause a shifting of seed, similar to excessive wave action, or prove damaging to equipment if the currents are extreme.

Topographic
Topographic conditions affect both the siting of on-shore facilities and beach nurseries. It is important to site the beach nursery in an area which is of the appropriate tidal height (mid-tidal range) and which is not susceptible to either slope erosion or the accumulation of substrate deposits (small clams can be easily washed away or smothered).

The on-shore site must also consider the topography of the site. It should not be placed in an area susceptible to flooding (tidal or fresh), nor so far above the sea level that pumping costs are prohibitive. The access to water should be kept to a minimum distance, both because of the intake pipe/pumping costs, and because of the increased risk of damage to equipment. The best location for an on-shore nursery is one that is within one to several metres above the extreme high tide, and with very near-by access to deep water.

Nearby access to deep water reduces risks, costs and problems.
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Energy considerations
Each nursery system requires different levels of energy to make the system effective. The lowest energy requirements are for the beach nursery, or a floating nursery which utilizes the natural water movements to bring food to the animals and remove metabolic wastes from the system. These "low energy" nurseries consist of production systems that have restricted stocking densities. This does not make them ineffective but may make them the most cost effective alternative for many sites which have restricted access to the currently available energy sources. Even those sites which have easy access to electricity may not have a cost effective nursery.

Because we are located in a remote area without access to conventional sources of power, we have been forced to utilize efficient methods of moving water through a nursery system. For high volume production of larger seed, a floating nursery utilizing a paddle wheel has proven to be most effective and efficient.

Considerations of Scale
How much seed is required and how much space will that seed require? The following chart looks at space requirements for clam seed of various sizes based on the total volume of clam seed at differing sizes:

<table>
<thead>
<tr>
<th>Seed size mm</th>
<th>10000</th>
<th>20000</th>
<th>50000</th>
<th>100000</th>
<th>500000</th>
<th>1000000</th>
<th>5000000</th>
<th>10000000</th>
<th>50000000</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3</td>
<td>0.04</td>
<td>0.08</td>
<td>0.21</td>
<td>0.42</td>
<td>2.11</td>
<td>4.21</td>
<td>8.42</td>
<td>21.05</td>
<td>42.11</td>
</tr>
<tr>
<td>3-4</td>
<td>0.10</td>
<td>0.19</td>
<td>0.48</td>
<td>0.95</td>
<td>4.76</td>
<td>9.52</td>
<td>19.05</td>
<td>47.62</td>
<td>95.24</td>
</tr>
<tr>
<td>4-5</td>
<td>0.17</td>
<td>0.34</td>
<td>0.85</td>
<td>1.69</td>
<td>8.47</td>
<td>16.95</td>
<td>33.90</td>
<td>84.75</td>
<td>169.49</td>
</tr>
<tr>
<td>5-6</td>
<td>0.25</td>
<td>0.50</td>
<td>1.26</td>
<td>2.52</td>
<td>12.58</td>
<td>25.16</td>
<td>50.31</td>
<td>125.79</td>
<td>251.57</td>
</tr>
<tr>
<td>6-8</td>
<td>0.63</td>
<td>1.25</td>
<td>3.13</td>
<td>6.25</td>
<td>31.25</td>
<td>62.50</td>
<td>125.00</td>
<td>312.50</td>
<td>625.00</td>
</tr>
<tr>
<td>8-10</td>
<td>1.25</td>
<td>2.50</td>
<td>6.25</td>
<td>12.50</td>
<td>62.50</td>
<td>125.00</td>
<td>250.00</td>
<td>625.00</td>
<td>1250.00</td>
</tr>
<tr>
<td>10-12</td>
<td>3.03</td>
<td>6.05</td>
<td>15.14</td>
<td>30.27</td>
<td>151.35</td>
<td>302.71</td>
<td>605.42</td>
<td>1513.55</td>
<td>3027.09</td>
</tr>
</tbody>
</table>
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A nursery system, both size and type, will be based on total production needs for the year. For a grower who wants to produce sufficient seed clams to plant a relatively small beach area (requiring less than 1 million clams), a low-tech, low-energy system will probably suffice. For a larger scale farm operation, the energy costs, equipment costs, and labour costs must be analyzed to develop a nursery suitable for the site. The scale of the nursery can be minimized by planning to "plant" seed throughout the summer season as the animals reach the appropriate size. This "staggering" of planting times equates to much lower equipment costs and spreads out the associated labour required to produce the seed.

Size at Planting
There are conflicting opinions on the "correct" size at which to plant clam seed, both to ensure the optimum survival and to be cost effective when purchasing seed, but all agree that, "larger seed is better". To cost effectively produce larger seed (anything greater than 6 mm), the energy input must be minimal, equipment costs must be basic, and labour costs must be low. Therefore, it is important to consider a nursery system which may incorporate two or more systems to produce a larger clam for out-planting. The cost of seed is also a factor in the decision: "what size to plant", in order to put the costs into perspective and base a nursery decision on the "savings", it is important to consider the following:

<table>
<thead>
<tr>
<th>Cost</th>
<th>Number of Clams</th>
</tr>
</thead>
<tbody>
<tr>
<td>PER 1000</td>
<td>SEED SIZE</td>
</tr>
<tr>
<td>$1.50</td>
<td>1-2</td>
</tr>
<tr>
<td>$2.50</td>
<td>2-3</td>
</tr>
<tr>
<td>$3.50</td>
<td>3-4</td>
</tr>
<tr>
<td>$4.50</td>
<td>4-5</td>
</tr>
<tr>
<td>$5.50</td>
<td>5-6</td>
</tr>
<tr>
<td>$7.00</td>
<td>6-8</td>
</tr>
<tr>
<td>$9.00</td>
<td>8-10</td>
</tr>
<tr>
<td>$11.00</td>
<td>10-12</td>
</tr>
</tbody>
</table>
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European Clam Seed Production

Manila clam culture began in Europe in 1974 when the Satmar hatchery in France acquired broodstock from the Olympia Oyster Company of Washington State. Their annual clam seed production grew steadily during the next 15 years and peaked at 40 million in 1987. In most of Europe there is no natural recruitment of Manila clams so, except for Italy, all of their clam seed is hatchery produced.

The majority of the European clam seed producers are located in France. There are approximately fifteen producers in France, with Satmar being the leading and largest of the group. There are also two hatcheries in Spain, one on the Channel island of Guernsey and one in England, but the numbers often change as the industry changes.

Seasalter (UK)
The English facility, Seasalter, is operated by John Bayes, who pioneered many of the hatchery and nursery techniques that now have become standard practice. The three most notables are continuous-flow bag algal culture, continuous-flow larvae culture...
Manila Clams: Hatchery and Nursery Methods

and upweller technology. Guernsey Seafarms uses many of the same systems as Seasalter, as does the Tinamenor hatchery in Spain.

The Seasalter company is comprised of a hatchery, three nursery sites and two growout sites. The hatchery utilizes continuous-flow bag algae culture and continuous-flow larvae culture techniques.

Continuous-flow algae culture utilizes a system in which the incoming water is filtered then pasteurized at 90 °C, which kills all algae, ciliates, and bacteria. A heat exchange unit acts to cool the out-flowing water and preheat the incoming water. The nutrient media is injected into the in-flowing water before it is injected into the bags. As the enriched water flows into the bags, it forces the densely bloomed algae out. The flow into the bags is equivalent to about 1/4 to 1/3 harvest per day. On an average the bags can be continuously grown and harvested for about 3 months. Since the Seasalter Hatchery is situated on the Thames River estuary, it has always had larval survival problems associated with lower water quality during the summer months. For this reason the hatchery operates during the winter months. The larvae culture tanks are about 400 litres and the larvae densities begin at 200/ml and progress to 25 to 50/ml with flow rates in the tanks equaling 10 volume changes per day.

The clams are moved into the nursery as soon as possible. They metamorphose at 200 µ and are put into a modified recirculating system of upwellers at between 300-500 µ. When the clams are larger they are moved out into the upwell system outside of the hatchery which is fed from a large tank of bloomed algae kept in a separate building (greenhouse type roof).

The Guernsey site in a flooded rock quarry that is directly adjacent to the ocean and connected by a flood gate.
At Guernsey Sea Farms the larvae are grown in a continuous flow system that makes the most efficient use of available space. Mark and Penny Dravers operate Guernsey Sea Farms in a 4 acre flooded quarry on the Channel Island of Guernsey. Most of the hatchery methods are similar to Seasalter as that was where Mark got his shellfish training. After the seed comes out of the hatchery it is put into upwells on the docks. The dock upwells are supplied with (cultured) algae enriched water that is heated by the out-flow water from the hatchery. After the seed has reached the 1-2 mm size it is moved to the FLUPSY. The floating upweller system or FLUPSY consists of a paddle wheel set up at the end of a raft which contains individual units connected to the central outflow channel. As the wheel turns, it creates a current flowing out of the central corridor which is replaced by water flowing in through the seed units (upwell current). The speed of the wheel can be adjusted to allow for greater volumes of seed.

Coke bottle upwellers and clam setting rings at Guernsey Sea Farms
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Tinamenor’s hatchery with algae production greenhouse.

Small conical larvae tanks use the flow-through system for high production.

Recirculating downwelling system for setting

Tinamenor (Spain)

This hatchery mainly produces turbot and bream. The mollusc part of this multi-species facility was built in 1987. Most of the techniques used here were adapted from Seasalter. The two most impressive things about Tinamenor is the magnitude of their algal culture system and the size of their FLUPSYS.

The main algal production is in the greenhouse where approximately 10,000 litres are harvested per day from 80 semi-continuous culture bags. The bags are 400 l and continue to produce for an average of a month.

Their inside nursery is comprised of 28 downwell troughs. The outside nursery is contained within a large pond and consists of a system based on the Guernsey paddle wheel/raft. The clams are moved to the outside low flow system at 1 to 1.5 mm size and are transferred to the larger units at 2 mm or larger.
Satmar’s site incorporates greenhouse algae production and large numbers of upwell units for bivalve seed production.

Satmar began in 1972 with a contract between the Flat oyster growers in Brittany and Pacific Mariculture of California to produce Ostrea edulis oyster seed. Yves Le Borgne, the present general manager, has been with the company since the beginning. As the demand for Crassostrea gigas, the Pacific oyster and manila clam seed increased, so too did the size of their hatchery and nursery facility.

Satmar's hatchery methods are similar to those used on the west coast of North America as they grow their larvae in static water and use batch and semi-batch algae culture. The broodstock clams are conditioned for six to eight weeks at 20°C. The larvae are reared in 20,000 liter tanks that are changed every 2-3 days until the clams reach the setting size of 160µ. The clams are set on downwell screens then moved to upwells after they reach 500 µ. The clam seed is held at a depth of 2 inches and graded every month. After the seed reaches one millimeter it is moved into the outdoor upwell system that is supplied with bloomed water from their 2 HA pond.

The algae parent stocks are maintained in a separate room to avoid contamination.
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The clam seed they produce is generally sold at 2 to 4mm and the overlying protective mesh used is 5 mm. The general growing method used in France is that of modern agriculture. A field is prepared, planted, protected from perdition, allowed to grow and mature for the time necessary (2 years in France) and then harvested using mechanized equipment.

The French mechanical clam planting unit, spreads the seed, covers the area with netting, and buries the net edges, in ones smooth movement.
Getting Started

In planning and implementing a nursery suitable for your site, it is necessary to obtain the fundamental supplies for the system. Often the local plumbing supply and hardware stores will carry many of the items needed, however for more specialized equipment, you may have to build it yourself or look at specialized supply companies to fill these orders. The amount and type of equipment essential to each operation will depend upon the intensity and scale of the facility you are setting up. It pays to plan ahead and to shop around.

There are many companies serving the varied needs of the Shellfish industry. The following list of suppliers is not complete and any omissions are merely oversights on the part of the authors.

**Equipment Suppliers for Clam larvae and nursery production:**

**Fiberglass Tanks:**
Alpha Fiberglass Mfg. Co., Ltd.
10218 Bowerbank
Sidney, BC, V8L 3X4
phone: (206) 656-5121

Chemical Proof Corporation
19205 144th Avenue Northeast
Woodinville, WA 98072
phone: 1-800-521-0714 x 344

PRA Manufacturing Ltd.
P.O. Box 774, Stn. A
Nanaimo, BC, V9R 5M2
phone: (250) 754-4844
fax: (250) 754-9848
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Bags & Protective Netting:
Internet Inc.  
2730 Nevada Ave. N.  
Minneapolis, MN., 55427  
phone: toll free 1-800-328-8456, or (612) 541-9690

Norplex Inc.  
7048 S. 190 th Street  
Kent, WA., 89032  
phone: (206) 251-6050

Seed and or Clam Larvae:
Canadian Benthic, Ltd.  
P.O.Box 97  
Bamfield, BC., VOR 1BO  
phone: (250) 728-3274

Capestone Marine Resources Inc. (Lummi Hatchery)  
4232 Legoe Bay Road, Lummi Island  
WA. 98262  
phone: (360) 671-3806

Coast Oyster Co.  
P.O. Box 327, Quilcene  
WA. 98376  
phone: (360) 765-3474

Dahman Shellfish Co.  
393 S.E. Dahman Rd.  
Shelton, WA 98584  
phone: (360) 426-9880  
fax: (360) 426-9796

Innovative Aquaculture Products, Ltd.  
Skerry Bay, Lasqueti Island  
BC., VOR 2JO  
phone: (250) 248-8615 or  
Fax: (250) 755-9531
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Kuiper Mariculture, Ltd.
3025 Plunkett Road
Bayside, CA, 95524
phone: (707) 822-9057
fax: (707) 822-3652

Taylor United, Inc.
130 S.E. Lynch Rd.
Shelton WA 98584
phone: (360) 426-6178

Whiskey Creek Oyster Farm
2905 Bayshore Road
Tillamook, OR, 97141
phone: (503) 842-8365

Algae Paste:
Coast Oyster Co.
(see address under seed)
Innovative Aquaculture Products, Ltd.
(see address under seed)

Nytex screening:
B and Sh Thompson
8148 Devonshire Road
Mount Royal, Quebec H4P 2K3

Research Nets, Inc.
P.O. Box 249
Bothell, WA. 98041
phone: (206) 821-7345

Bag Filters, Cartridge filters, and Filter Housings:
Montgomery Brothers Inc.
14844 N.E. 31st. Circle
Redmond, WA 98052
phone: (206) 881-9393
fax: (206) 885-7999
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Peacock Incorporated
2325 Burrard St.
Vancouver, BC, V9J 3J2
phone: (604) 731-3185

Specialized technical equipment:
BDH Incorporated
60 East 4th Avenue
Vancouver, BC, V5T 1E8
phone: 1-800-663-3404

Canlab
7080 River Road #131
Richmond, BC, V6X 1X5
1-800-663-1891

Cole-Parmer
7425 North Park Avenue
Chicago, Illinois 60648
phone: 1-800-323-4340

Fisher Scientific Company
196 W Third Avenue
Vancouver, BC, V5Y 1E9
phone: (604) 872-7641

Sigma Chemical Company
P.O. Box 14508
St. Louis, Missouri 63178
phone: 1-800-325-3010

Western Scientific Services Ltd.
11620 Horseshoe Way
Richmond, BC, V7A 4V5
phone: (604) 274-4111