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Development of octopus aquaculture

Rearing, handling and systems design for *Octopus tetricus* commercial aquaculture

FRDC Project No 2009/206

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Contents

Introduction

The following document 'Development of octopus aquaculture, rearing, handling and systems designs for *Octopus tetricus* commercial aquaculture' contains protocols developed during the FRDC project 2009/206. These protocols encompass the most up-to-date rearing, handling and systems designs for *Octopus tetricus* commercial aquaculture.

These protocols are the result of extensive research and development work carried out over the past four years by the Department of Fisheries, Western Australia and summarised in the final project report.

The protocols represent the information needed for octopus aquaculture in a practical and hands-on description.

The document is divided to two sections:

1. Octopus ranching
2. Hatchery rearing

During the project period, the ranching of *O. tetricus* achieved commercial densities believed to be the highest reported in the world. Moreover, several system and rearing developments enabled the rearing of the octopus without any hides, which is a traditional method used in octopus grow out currently around the world.

This significant achievement improved the system efficiency and greatly improved the profitability by reducing manpower costs (significantly reducing cleaning and handling time), increasing biomass (kg harvest per unit volume), reducing mortality due to cannibalism and reducing capital costs (more biomass per volume means less tanks needed). While the system and protocols were developed for *O. tetricus*, it is the belief of the authors that these techniques will be suitable for grow-out of other octopus species such as the Mediterranean species *Octopus vulgaris*, which might present future opportunities for commercial octopus aquaculture elsewhere.

The hatchery protocols in this report present the current knowledge about *O. tetricus* broodstock and larvae rearing. During the project, different systems, handling and feeding protocols were developed to deal with some of the major issues affecting octopus larvae survival. While significant knowledge was gained during the project in this area, the protocols are yet to be developed to a commercial level. The hatchery protocols should be used as a base for future development.

1.0 *Octopus tetricus* ranching and grow out

1.1 Animal pick-up and transport

In most cases, octopus juveniles are caught by commercial fishing boats using commercial octopus pots. Transporting the octopus from the fishing harbour to the grow out location might take a few hours. To ensure good survival and optimal condition of the octopus arriving to a ranching facility, a simple transport system and handling methods were developed (Fig. 1). A more robust transport system, which includes water treatment and much larger containers, was developed for up to 48 hours holding time. The system is described in the report volume I and was used for transporting *Octopus berrima* from South Australia to Western Australia.

1.2 Equipment

A large esky (i.e. insulated cooler), with a volume greater than 500 l, is needed to be able to hold at least 2 oyster mesh baskets. Baskets facilitate splitting the animals into size groups, which prevents octopus escaping, as well as fighting and cannibalism that occurs between octopus with a large enough size differential. The baskets are made entirely of 5 mm oyster mesh with a square 15 mm PVC pipe frame at the top. The top half of the basket is covered with shade cloth, which the octopus are unable to adhere to and are thus unable to escape (Fig. 2). A piece of shade cloth is folded over the PVC frame. The interior side is sewn to the oyster mesh while the exterior side has a loop sewn in the end. A length of rope or elastic chord is threaded through this loop, which allows the shade cloth to be fitted tightly around the basket, and tied off (Fig. 3). At the base of the basket are 2 lengths of 40 mm PVC pipe, which gives the base of the basket some weight and stability when sitting in the esky (Fig. 4). These can be attached to the bottom of the basket with cable ties.

Figure 1. Equipment needed to transport juvenile octopus; (1) large esky (2) pure oxygen source or air pump (3) air stone (4) oyster mesh baskets (5) dissolved oxygen meter.

Figure 2. Basket profile (1) 5 mm oyster mesh (2) shade cloth covering (3) PVC frame at the top.

Figure 3. Basket profile. (1) Loop in exterior shade cloth and rope/elastic drawstring

Figure 4. Basket profile. (1) Positioning of the 40 mm PVC pipe lengths.

The octopus do not need to be electronically weighed before being split into the baskets, rather a visual grading of size is adequate. Electronically weighing octopus besides a wharf or pier before transport is time consuming and stressful to the octopus. A bigger esky increases the number of baskets able to used, meaning more efficient splitting of different sized octopus. The esky should be fitted with a dump valve at the base to allow easy removal of water after transport and a lid to stop water splashing out during transport as well as any escaping octopus (Fig. 5).

Figure 5. Esky profile during transport (1) dump valves (2) lid arrangement.1.2.1. Esky aeration & monitoring

A large density of juvenile octopus held in an esky during transport will consume high volumes of dissolved oxygen. Oxygen levels will fall due to;

1. Natural respiration. Octopus will uptake oxygen from the water to breathe
2. Octopus excreting. Faeces will use up oxygen to break down in the water.
3. Dissolved oxygen levels in a static body of water will naturally decrease if there is no water exchange.

Delivering pure oxygen or air via an air stone placed in the esky will help keep dissolved oxygen levels high during transport. An oxygen meter (Oxyguard, YSI etc.) will allow easy monitoring of dissolved oxygen. Levels in the esky should not fall below 4-4.5 mg lt^{-1} (60% saturation at 20°C T 35 ppt salinity) at any stage (Fig. 6).

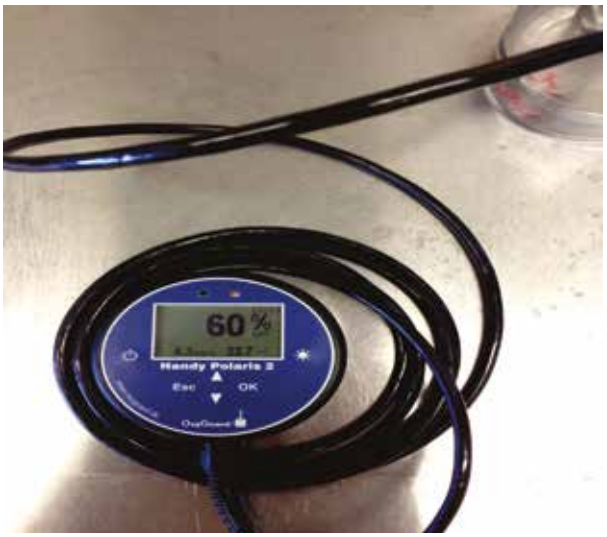


Figure 6. An Oxyguard meter reading 60% saturation (4.3 mg lt^{-1})

1.3 Holding (pre-stocking)

Upon arrival to a facility, juvenile octopus will need at least 24 hours to acclimatise after the stress of transport. Baskets containing octopus can be taken from the esky and once the temperature in the esky is matched to that of a holding tank, put straight into a tank that is large enough to fit multiple baskets. A 5000 lt tank is ideally used as a holding tank (Fig. 7).

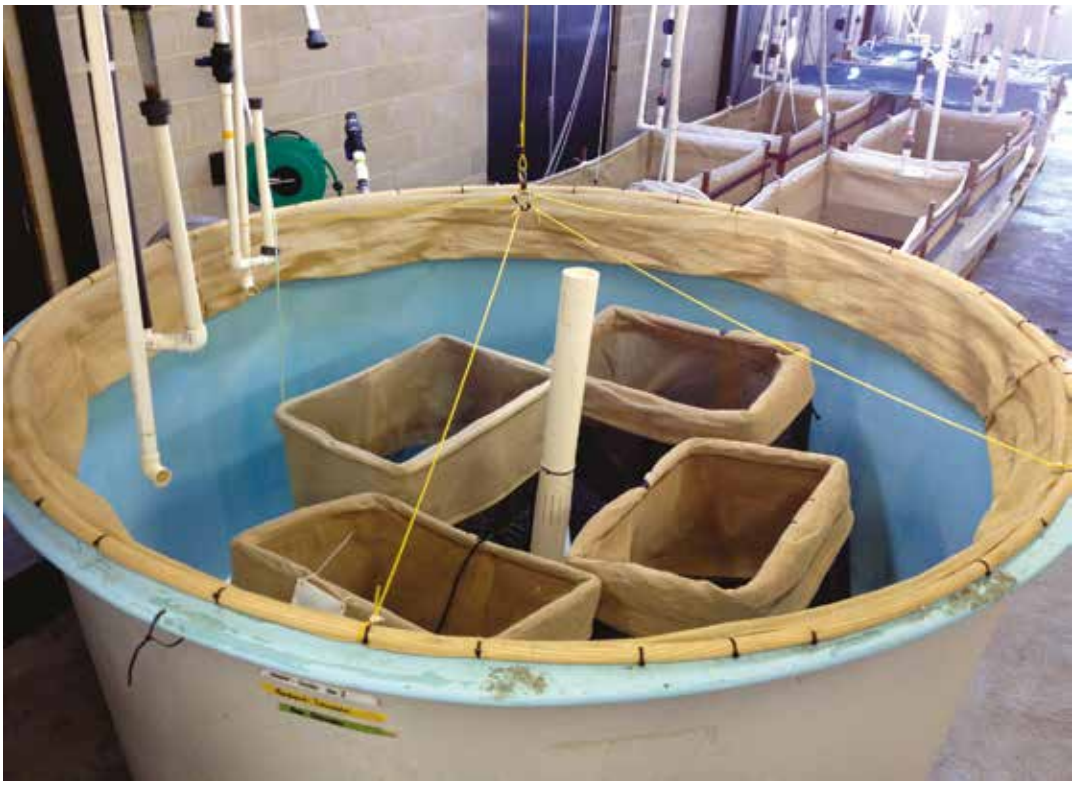


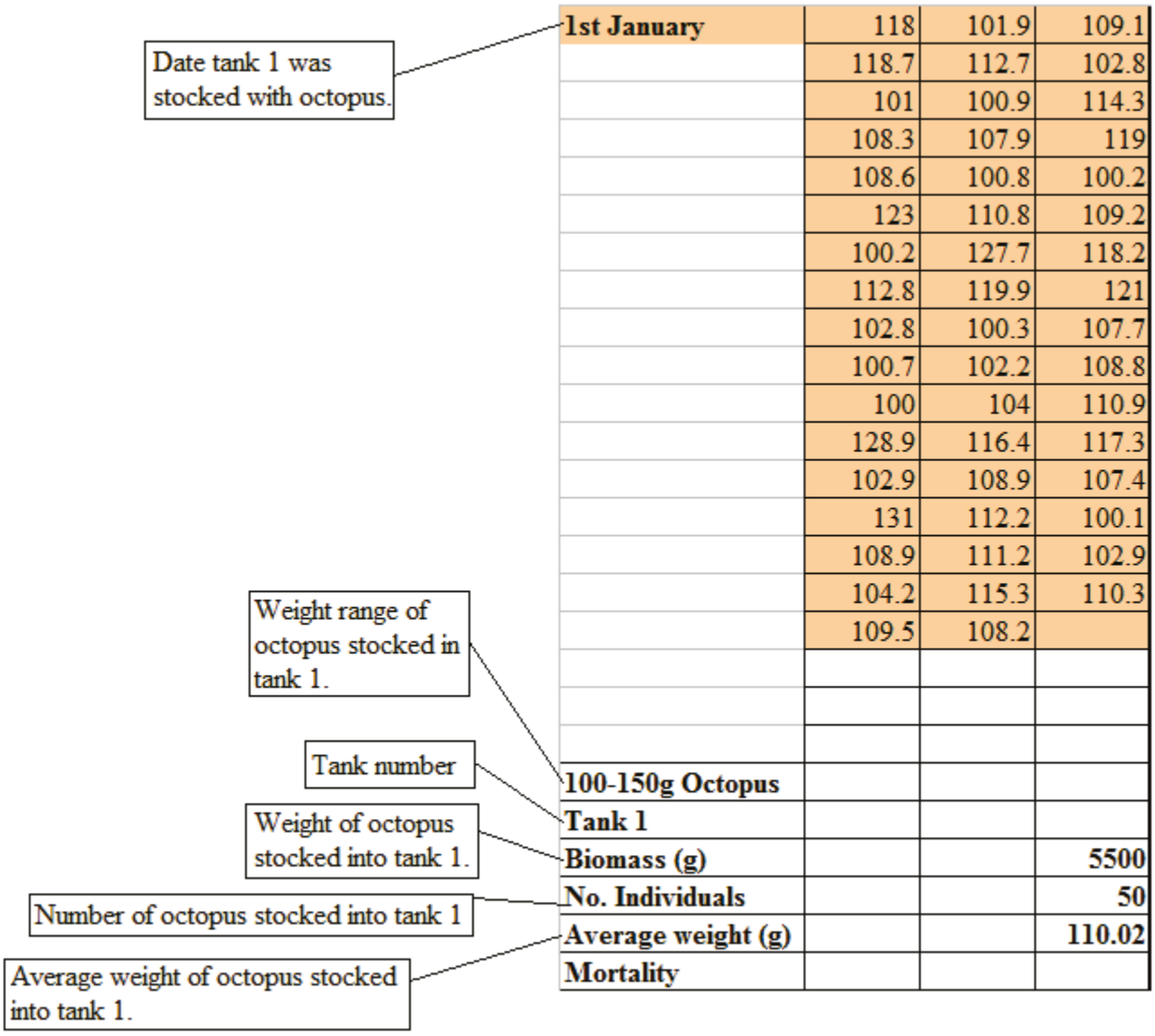
Figure 7. A 5 000 lt holding tank set-up containing multiple baskets.

Octopus that are held in baskets prior to stocking, only need to be fed once to satiation during the first 24 hours. Food can consist of any chopped, relatively cheap fresh feed such as Pilchards, Sardines or Prawns (Fig. 8). Incoming water should be open (flow-through) and set at a rate that keeps dissolved oxygen levels at 4-4.5 mg lt^{-1} (60% saturation at 20 °C at 35 ppt salinity) or greater. Dissolved oxygen should be measured 30 minutes after the octopus are fed, which is when levels are at their lowest. Water temperature between 16-23°C is ideal. Any octopus that are sick or have died during transport should be removed from the baskets prior to leaving the facility that afternoon. Uneaten food should be removed from the baskets the following morning prior to the octopus being weighed and stocked into grow-out tanks.

Figure 8. Fresh feed used to feed juvenile octopus. Prawns (left) and Pilchards (right).

1.4 Weighing and initial stocking

Once the newly arrived octopus have been acclimatised for 24 hours and the grow-out tanks are fitted and have running seawater, juvenile octopus are ready to be moved to the grow-out tanks at high densities. Octopus from the baskets need to be electronically weighed, and subsequent data recorded before they are stocked into the grow-out tanks (Fig. 9). This will allow the user to determine the initial weight range (Section 4.1) and initial biomass (Section 1.5) of the octopus in any tank. The octopus are best weighed individually for increased accuracy when calculating the initial biomass. A bucket containing seawater on a balance ensures reduced stress during this process (Fig.10).



1st January	118	101.9	109.1
	118.7	112.7	102.8
	101	100.9	114.3
	108.3	107.9	119
	108.6	100.8	100.2
	123	110.8	109.2
	100.2	127.7	118.2
	112.8	119.9	121
	102.8	100.3	107.7
	100.7	102.2	108.8
	100	104	110.9
	128.9	116.4	117.3
	102.9	108.9	107.4
	131	112.2	100.1
	108.9	111.2	102.9
	104.2	115.3	110.3
	109.5	108.2	
100-150g Octopus			
Tank 1			
Biomass (g)			**5500**
No. Individuals			**50**
Average weight (g)			**110.02**
Mortality			

Figure 9. Information on octopus stocked into a grow-out tank after acclimatisation

Figure 10. Equipment used when weighing and stocking octopus (1) Balance (2) Net (3) 10-20 lt bucket (4) Notepad and pencil.

1.4.1 Initial weight range

Octopus are highly cannibalistic, especially when the size differential between the largest and smallest octopus is high. If the largest octopus in a tank is double the weight of the smallest octopus (e.g. largest animal = 150 gr, smallest animal = 75 gr), the smallest octopus will be eaten by the larger octopus. As a result, the larger octopus will grow quicker than the rest of the octopus in the tank and hence will continue to predate on smaller octopus around it. Over time, the weight range of the octopus will increase meaning cannibalism of smaller octopus by larger octopus will increase. This means loss of stock and profit in a commercial facility. The largest octopus cannot be greater than 1.75 times the weight of the smallest octopus in a tank at the time of stocking.

Example: If the smallest octopus is 50 gr, than the largest octopus should be no greater than 87.5 gr. The weight range of octopus stocked into a tank will than be 50 – 87.5 gr.

** The weight range of octopus in a tank will increase slowly over time as feed rates will differ between individuals.*

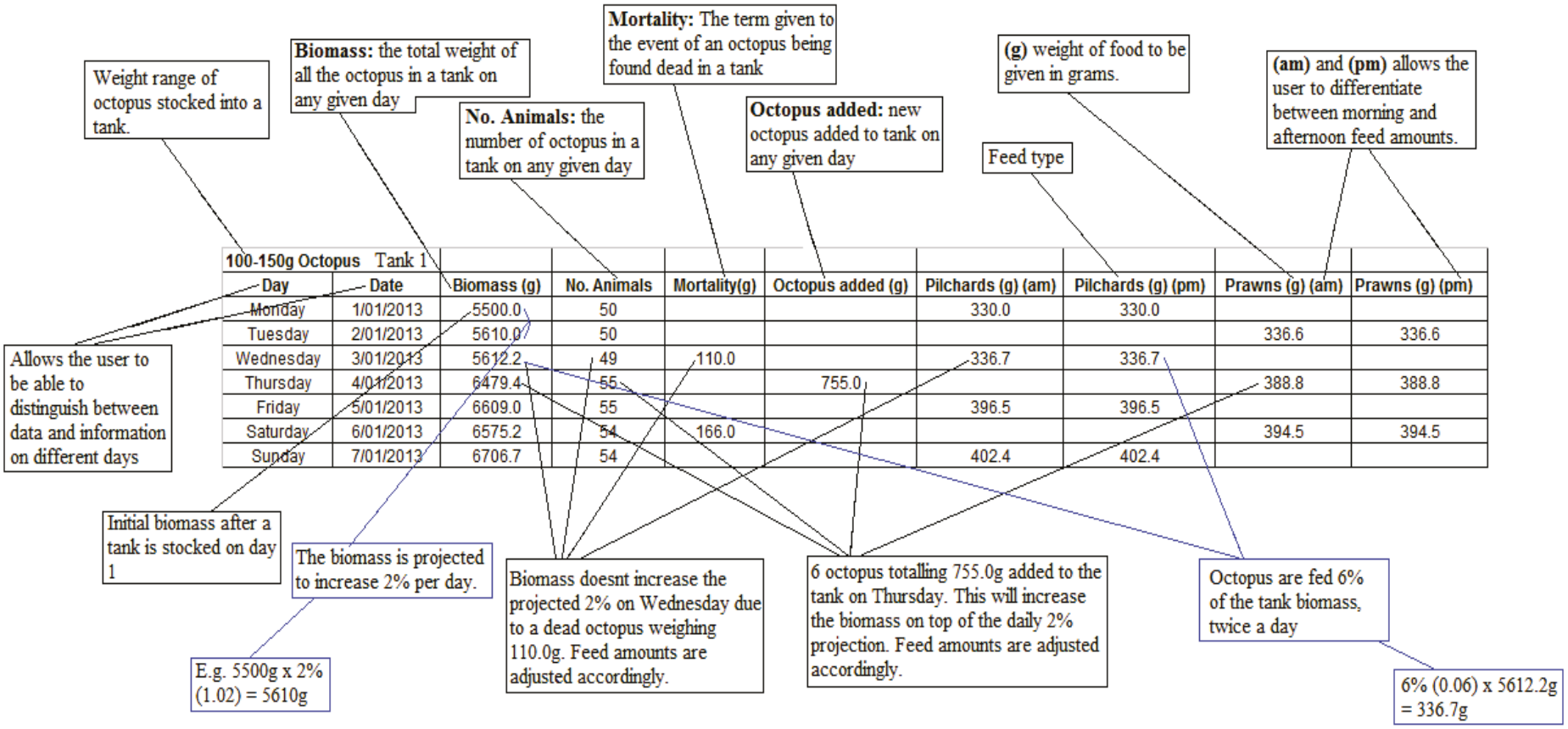
1.5 Initial biomass

The initial biomass will be the sum of the entire number of octopus stocked into a tank, once all the octopus in the baskets have been weighed and separated as described in Section 1.4. From here, daily feed amounts can be calculated and projected for the next 7 days, after which the octopus in that tank will need to be weighed and graded.

Following stocking of the grow-out tanks and the initial biomass being ascertained, a feed protocol (data sheet) can be created and followed for the next 7 days. A data sheet will give the

following details (Fig.11).

1. An accurate estimated biomass (total weight) of the octopus in a tank on any given day.
2. Feed type and amount that should be given each morning and afternoon.
3. Ability to enter the weight of any dead octopus (mortality), which will adjust the daily biomass and the amount of feed that needs to be given from that point forward.
4. Ability to enter the weight of new octopus added to the tank, which will adjust the daily biomass and the amount of feed that needs to be given from that point forward.
5. Detail of the number of animals in each tank on any given day.
6. Tank number and the weight range of octopus in that tank.



100-150g Octopus Tank 1									
Day	Date	Biomass (g)	No. Animals	Mortality(g)	Octopus added (g)	Pilchards (g) (am)	Pilchards (g) (pm)	Prawns (g) (am)	Prawns (g) (pm)
Monday	1/01/2013	5500.0	50			330.0	330.0		
Tuesday	2/01/2013	5610.0	50					336.6	336.6
Wednesday	3/01/2013	5612.2	49	110.0		336.7	336.7		
Thursday	4/01/2013	6479.4	55		755.0			388.8	388.8
Friday	5/01/2013	6609.0	55			396.5	396.5		
Saturday	6/01/2013	6575.2	54	166.0				394.5	394.5
Sunday	7/01/2013	6706.7	54			402.4	402.4		

Figure 11. A data sheet that could be used on a daily basis in a commercial octopus ranching facility

1.6 Daily feed and cleaning protocol

1.6.1 Tank checks

Upon arrival in the morning, the following things should be checked:

- Incoming water flow to all tanks should be checked and adjusted if they have fluctuated overnight.
- Aeration to the tanks.
- Look around the tanks for any dead or escaped octopus. If the octopus is dead, it needs to be weighed and that figure be entered into the data sheet for that tank.

1.6.2 Tank cleaning (morning)

Once all the appropriate checks have been carried out, the previous afternoons feed needs to be removed from the tank using the following procedure in order.

1. Drop the collapsible shade cloth ring to the tank by unhooking the lever from its anchor point
2. Remove the steel pin at the stop of the standpipe
3. Place bucket underneath the gate valve
4. Pull the handle on the gate valve ½ to ¾ of the way open so water is exiting the tank
5. Lift oyster mesh sleeve and vigorously move it up and down until all food is out of the tank.
6. Once all food is out of the tank, drop oyster mesh sleeve and insert the steel pin.
7. Close the gate valve and than raise the collapsible shade cloth ring.

Waste that is removed from any tank should be disposed of and should not be re-used to feed octopus under any circumstances. Any dead octopus that comes out with the food waste should be weighed and those figures entered to that tanks data sheet.

1.6.3 Morning feed (am)

Once the previous afternoons food has been removed all the tanks, the morning feed can be administered to all tanks. Octopus should be fed 6% of the tank biomass, twice a day. Feed type and amounts can be extracted from the data sheet for that tank (Fig. 20). A balance will need to be used to weigh out the feed, which can be chopped with scissors or a knife. Feed can be chopped into a bucket or bowl for ease of carrying feed to the tank (Fig.12). It is important to clean and disinfect (alcohol or other disinfectant) all food preparation tools and areas after each use.

Octopus in a grow-out tank will gather mostly on the tank wall rather down at the bottom (Fig.13). Because of this, food should evenly distribute along the edges of the tank ensuring the majority of the octopus receive some food.

Figure 12. Feed chopped and being weighed on a balance before feeding

Afternoon tank cleaning and feeding should follow the same procedure as that described for the morning cleaning (Section 1.6.2. and 1.6.3.).

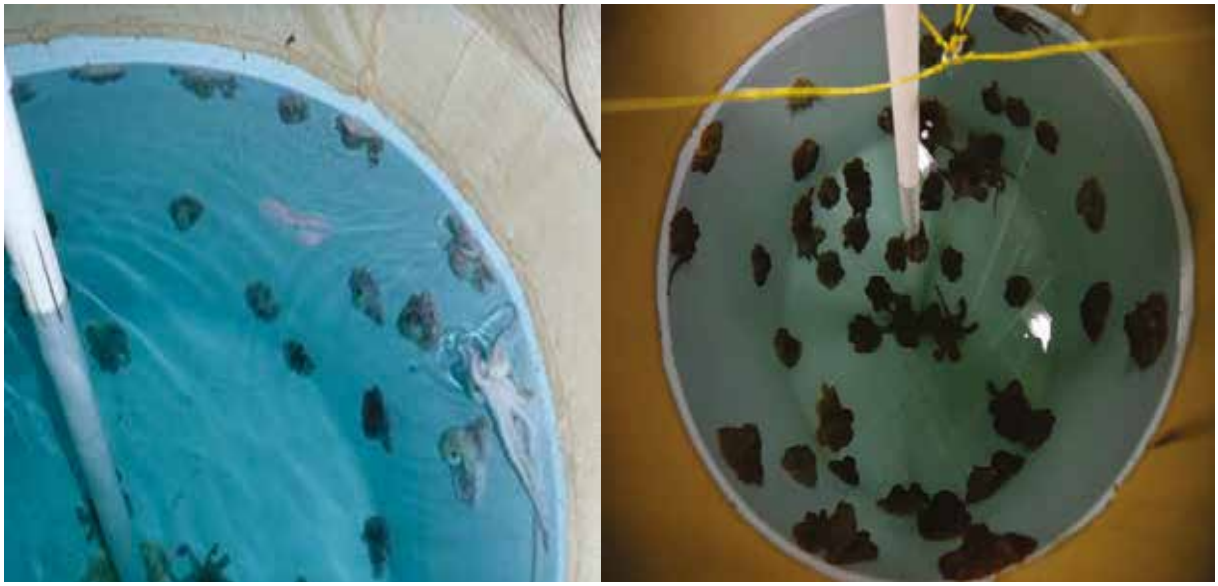


Figure 13. Octopus in a grow-out tank

1.6.4 Water quality parameters

For optimal growth while not affecting animal health, octopus should be grown-out at water temperatures between 16-23 °C. Incoming water flow rate should be ~ 100 lt kg octopus hr^{-1}.

For example; If there are 15 kg of octopus in a tank, the flow rate should be ~1500 lt hr^{-1}.

The flow rate can be adjusted dependent on dissolved oxygen levels in the tank, which should not fall below 4-4.5 mg lt^{-1} (60 % saturation at 20 °C at 35 ppt salinity) at any stage.

1.7 New animal arrival

1.7.1 Holding

New octopus will be transported regularly over the course of a week from the commercial fishermen to a ranching facility. The holding (pre-stocking) procedures are the same as those described in Section 1.3. Octopus that have been in the grow-out tanks for a period of time can become aggressive to newly added, wild caught octopus. It is important that acclimatisation for 24 hours takes place before new octopus are added to grow-out tanks.

1.7.2 Stocking

The stocking procedure for new octopus is the same as those described in section 1.4, however its important that new octopus that are added to grow-out tanks are stocked with octopus of similar size. Information on which tanks holds certain sized octopus can be found in the data sheet (Fig.11).

1.8 Weighing and grading

Weighing and grading of the octopus takes place on the 7th day after a tank is stocked. If a tank is left longer than 7 days without weighing and grading, the weight range of octopus in that tank will have increased enough for cannibalism to start occurring. This process involves weighing each individual octopus in all tanks so the following can be ascertained.

1. How many octopus have grown above the initial weight range stocked and have to be moved to a new tank (Fig.14).
2. If any octopus have reached market weight and therefore need to be culled (Section 8).

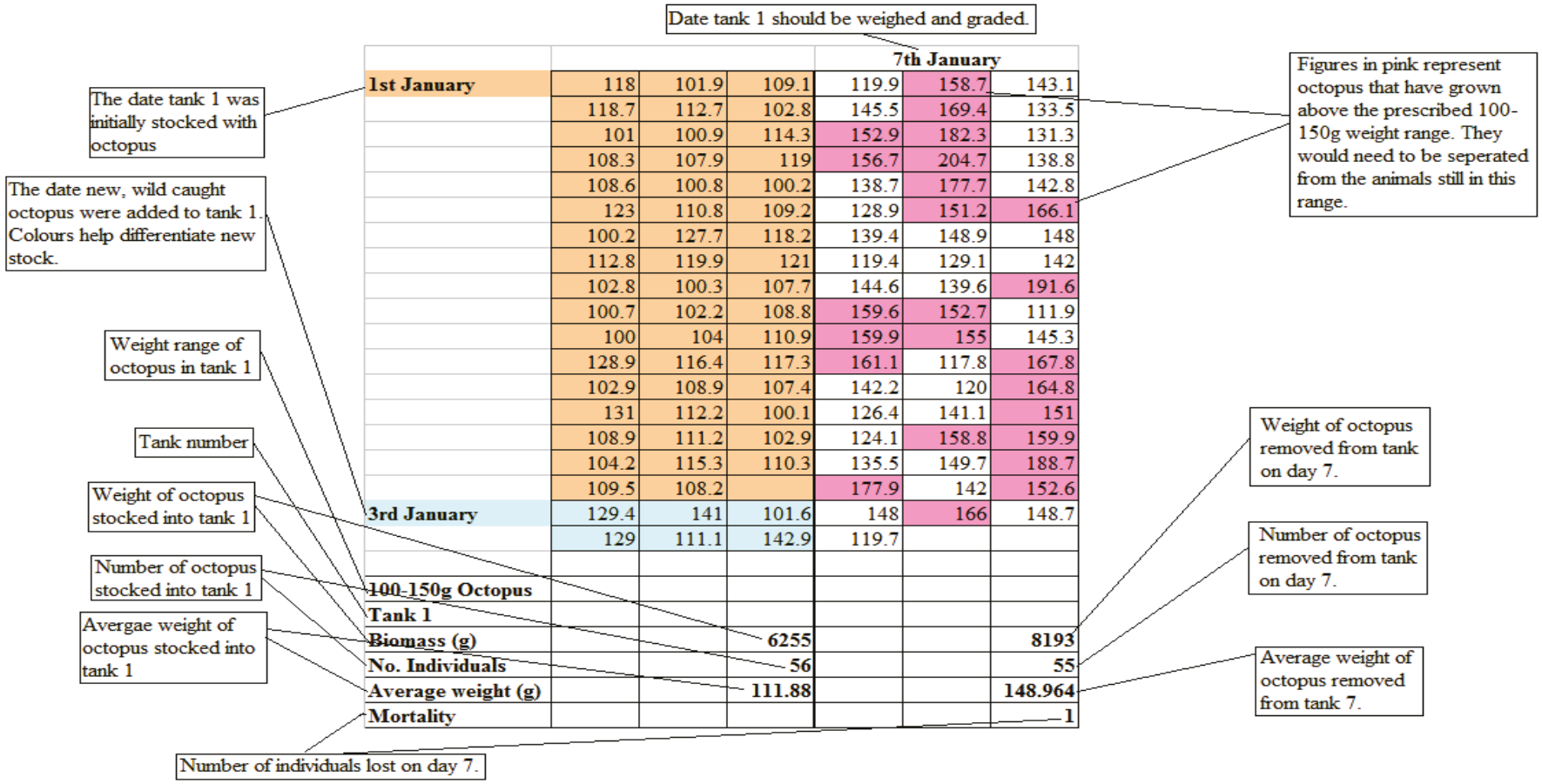


Figure 14. A table containing the weight and number of octopus from a tank stocked and harvested after 7 days.

1.8.1. Procedure

Due to the large number of tanks that will be running in a commercial facility, weighing and grading on a certain day will be labour intensive and time consuming. In a commercial facility, having numerous 5 m³ tanks that can fit multiple baskets (Fig.1) is ideal for the weighing and grading process. Having baskets labelled with pre-described weight ranges in 5 m³ tanks (Fig.15), will allow the user to weigh the octopus from every tank and put it in a basket already containing similar sized octopus.

Figure 15. Baskets labelled with pre-described weight ranges.

Once weighing and grading of the octopus is complete, all grow-out tanks will be empty and therefore need to be cleaned with an oxalic acid/freshwater mix and a scrubber. The tanks can be filled with seawater and flushed of any residual acid for 30 minutes. The tanks can then be designated a weight range, and then stocked with octopus from the baskets in the 5 m³ tanks. This can easily be done by tipping the basket allowing the octopus to fall out or transferring them into the tank by hand. Juvenile octopus will maintain good health if left out of the water for up to a minute during this process, however time out of the water should always be minimized.

1.9 Culling for market.

Market weight of an octopus will differ depending on the species, location and product it will be used for. In general, it will take up to 3-4 months in a commercial facility for *O. tetricus* to reach a market weight of 650 – 800 gr if initial average stocking weight was 50 gr. During the weighing and grading process (Section 1.7), octopus will be identified that are at market weight or over and, therefore, need to be culled. This can be done by placing the octopus against a hard flat surface. The tentacles of the octopus will adhere to the surface it is placed on, and can be held by the head and lifted slightly. Using a sharp knife, the head is cut off the octopus just under the eyes. Both the remaining ‘hands’ and head should be put into an ‘ice slurry’ (ice/seawater mix) to maintain product quality. The head can then be discarded.

1.10 Grow-out system

1.10.1 Tank design

Prior to weighing and initial stocking (Section 1.4), suitable grow-out tanks need to be fitted and installed ready to receive the octopus. A fibreglass, conical bottom tank of 2000 lt volume is ideal for grow-out (Fig.16). Its circular shape and conical base allows incoming water at the surface to undergo a circular motion before leaving the tank at the bottom. This circular motion means that any food or waste is directed to the base, centre of the tank. A 50 mm PVC external standpipe governs the height of the water in the tank (Fig.17,18).

Figure 16. A 2000 lt fibreglass, conical bottom tank.

Figure 17. Incoming water in the 2000 lt tank.

Figure 18. External Standpipe (1) attached to the base of the tank.

Figure 19. Gate valve with bucket underneath.

At the base of the tank, a 150 mm gate valve is attached. Its handle once pulled back (opened), allows easy, user-friendly removal of uneaten food and waste into a bucket (Fig. 19).

Attached to the inside of the tank at the top, is a collapsible shade cloth ring. As mentioned above, octopus are unable to adhere to shade cloth and hence this is fitted so octopus wont escape when the tanks unattended (Fig. 20).

Figure 20. (1) Erected, collapsible shade cloth ring.

The frame of the shade cloth ring is a length of 15 mm PVC pipe that is wrapped in a circle to form the same circumference as the top of the tank. A piece of shade cloth is than cut to that length (+ 100 mm) at a height of 500 mm. Shade cloth is attached to the PVC frame with cable ties and each end of the shade cloth is sewn together. The bottom of the shade cloth is adhered 100 mm under the inside lip of the tank with hot glue and marine grade silicone sealant. This acts as an anchor so that the ring can be raised and lowered (Fig. 21).

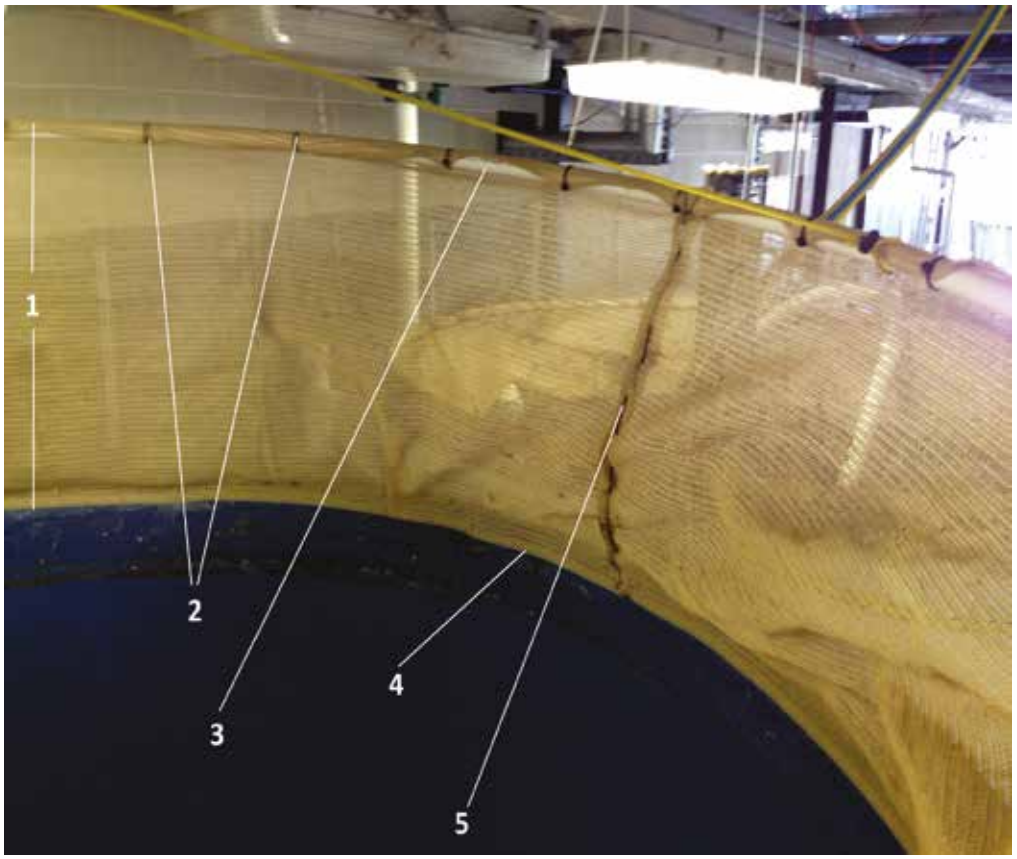


Figure 21. Shade cloth profile. (1) 500 mm height when erected (2) cable ties to attach shade cloth to the frame (3) 15 mm PVC frame (4) location of shade cloth attachment on tank (5) both ends of the shade cloth sewn together.

The shade cloth ring, once fixed to the inside of the tank, needs to facilitate being raised. Pieces of rope are connected to the PVC frame at 4 even points, which are long enough to meet in the middle of the circle that PVC frame creates. Ropes are attached to a steel ring or 2 large cable ties connected into a small circle. From here, a long piece of rope is attached to the circular cable tie arrangement, which is the lever for the user to be able to raise and lower the ring (Fig. 22). A clip is attached to the end of the lever, which facilitates it, being connected to an anchor point once the ring is raised (Fig. 23).

Figure 22. Shade cloth profile (1) lever attached to circular cable tie or steel ring (2) even spacing between 4 pieces of rope connected to PVC frame (3) location of circular cable tie arrangement.

Figure 23. (1) Clip attached to the end of the lever connected to an anchor point.

Inside the tank, a central standpipe and 5 mm oyster mesh sleeve stops octopus escaping and keeps food in the tank while allowing water pass through. The standpipe itself is 80 mm (PN12) PVC with large 30 mm holes cut from the base of the standpipe, 1500 mm upwards (Fig. 24). The oyster mesh sleeve is the same length of the standpipe.

Figure 24. (1) PVC standpipe and the location of the 30 mm holes (2) oyster mesh sleeve.

The large holes in the standpipe allow a high volume of seawater to pass through if a high flow rate is required. Octopus and uneaten food will also gather at the base of the tank over time covering the holes towards the base of the standpipe. The holes further up the standpipe allow water to pass out of the tank when this occurs.

Without an oyster mesh sleeve covering the standpipe, octopus and food would simply flow out of the tank. The sleeve stops this happening while allowing water to pass through. When it comes time to remove uneaten food from a tank, the gate valve (Fig. 19) is pulled open via the handle and the sleeve lifted to allow the waste out. Jiggling the oyster mesh sleeve up and down while the gate valve is open is effective in both removing stubborn waste around the base of the standpipe while forcing octopus towards the edge of the tank. A hole drilled through the top of the standpipe facilitates a short steel rod to be inserted which stops octopus lifting the sleeve (Fig. 25).

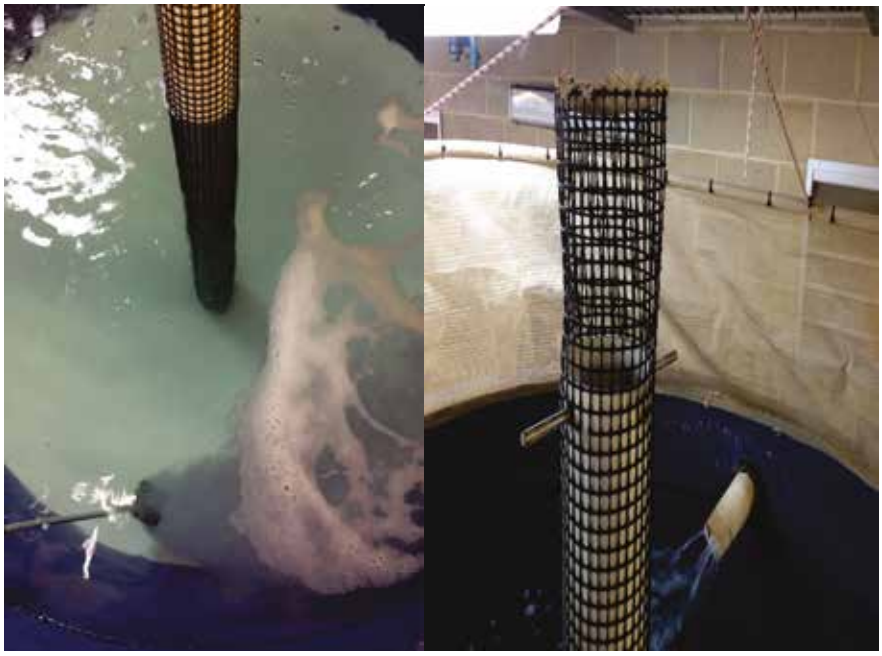


Figure 25. Standpipe profile in the tank and the location of the steel pin.

External air is delivered via an air stone at the base of the tank where the tank wall meets the conical base. It is important to locate the air stone here so as to aerate as much of the water column as possible, but not to stir up uneaten food and waste near the base of the standpipe (Fig. 25). An external air supply will keep octopus alive and in good health in case water supply stops for a prolonged period.

2.0 *Octopus tetricus* hatchery protocol

2.1 Broodstock

2.1.1 Transport & equipment

Broodstock of between 1.5 – 3 kg can be collected from commercial fishermen who are operating locally. An esky (i.e. insulated cooler) with volume of 500 lt or greater ensures there is enough space and water available when transporting multiple animals. Mash bags to individually separate the octopus can be used, however are not necessary as octopus of this size are quite docile during transport (Fig. 26). Air or pure oxygen delivery via an air stone is essential, as large octopus will consume a lot of oxygen in a static body of water during transport. Dissolved oxygen levels should be kept between 4 – 4.5 mg lt^{-1} (60 % saturation at 20 °C at salinity of 35 ppt). Dissolved oxygen and temperature can be monitored with an Oxygen meter.

Figure 26. Equipment needed for broodstock transport (right); (1) pure oxygen bottle and air stone, (2) mesh bag, (3) esky, (left) esky profile during transport.

2.1.2 Holding system

Upon arrival to a facility, there should be tanks with water running, ready to house the broodstock. Round fibreglass tanks of ~1000 lt are suitable as they can house up to 6 large octopus, which is necessary to trigger breeding and egg laying by females. The temperature in the transport esky should match that of the holding tanks before any octopus are stocked. Each tank should contain:

1. Internal standpipe; with small 10 mm holes at the bottom to allow incoming water to pass through, but to prevent octopus escaping.
2. Flow through seawater; incoming water located just above the surface of the tank so octopus will not attach to any plumbing and climb out.
3. Collapsible shade cloth ring; when erected, octopus are unable to escape when the tanks are not being tended to (See Section 1.9)

4. Screen Filter; to allow water to pass out of the tank, but to keep newly hatched larvae in the tank during spawning (Fig. 27).
5. Shelters; One shelter per octopus. Males and females will hide in them while females will also lay eggs in them after mating (Fig. 28).

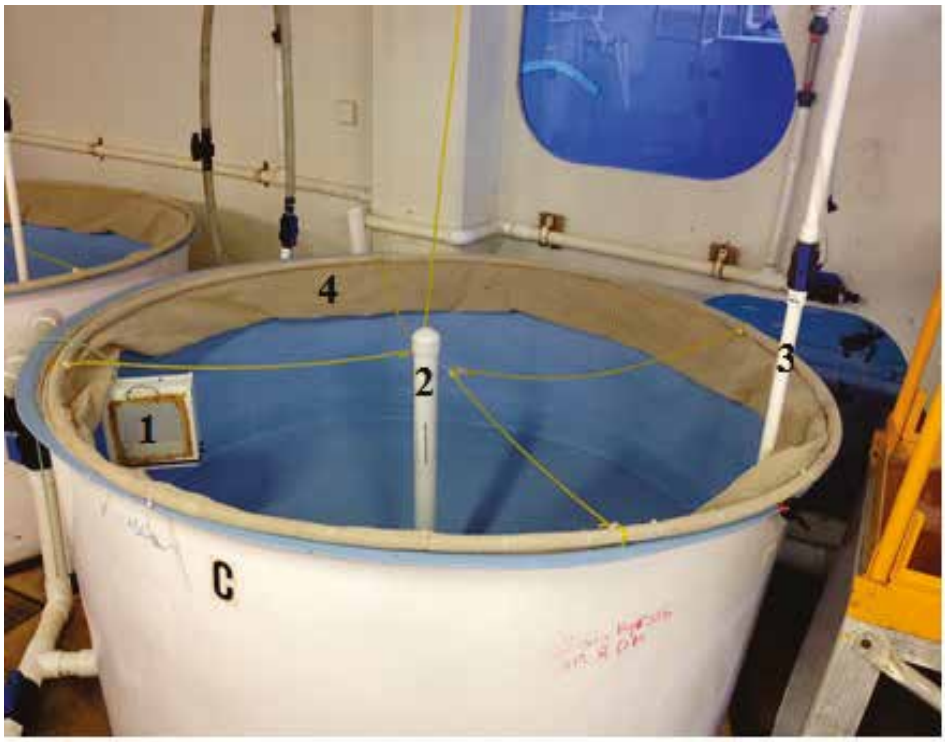


Figure 27. Broodstock tank profile (1) Screen filter with 250 µm interchangeable screens (2) internal standpipe (3) incoming water (4) collapsible shade cloth ring.

Figure 28. Shelter pots that are used to catch wild octopus make ideal broodstock tank shelters.

2.1.3 Feeding

Octopus in broodstock tanks should be fed once a day a fresh feed diet of Pilchards, Prawns, Lobster, Abalone or Crab. It is important to vary their diet as much as possible to match nutrition they would get in the wild. Feed should also be injected with a Nutrabrood broodstock additive (*Nutrakol Pty Ltd)* to enhance nutritional profile of the broodstock. Feed should be administered just prior to leaving the facility as octopus feed most actively at night.

2.1.4 Mating

Mating between broodstock in tanks can be observed by a male octopus extending the 3rd arm, clock wise from the right eye into the mantle cavity of the female. In most cases both animals will stay in their shelters during this process with only the arm of the male extending into the other shelter containing the female being observed.

Mating can occur instantly, but usually after 3-4 days from when broodstock are first stocked into tanks. If a period of weeks goes by without mating being observed, water temperatures can be raised or lowered 2-3°C over a period of 24 hours to induce mating. This is dependent on what the initial temperature is as O. tetricus have a tolerable temperature range of 16-23 °C.

Example; if the initial temperature is 23°C, then you would lower the temperature to 19-20°C. Raising the temperature 3-4°C to 26-27°C would stress and could subsequently kill the octopus.

Octopus in different tanks can also be mixed to change the ratio of females to males and also compatibility, as females are selective breeders where they choose the male. This practice can also trigger an increase in mating.

2.1.5 Egg laying and incubation.

If a female has laid eggs, they are usually attached to the roof or sides of the shelter. To observe if any eggs have been laid, the shelter containing the female can be lifted out of the water briefly, ensuring that water is still contained inside the pot, so egg clutches can easily be noticeable (Fig. 29). The eggs will be easily distinguishable as a clutch of very small white eggs hanging from the roof of the pot, usually hidden by the tentacles of a female (Fig. 30).

Figure 29. Female octopus in its shelter being checked for eggs

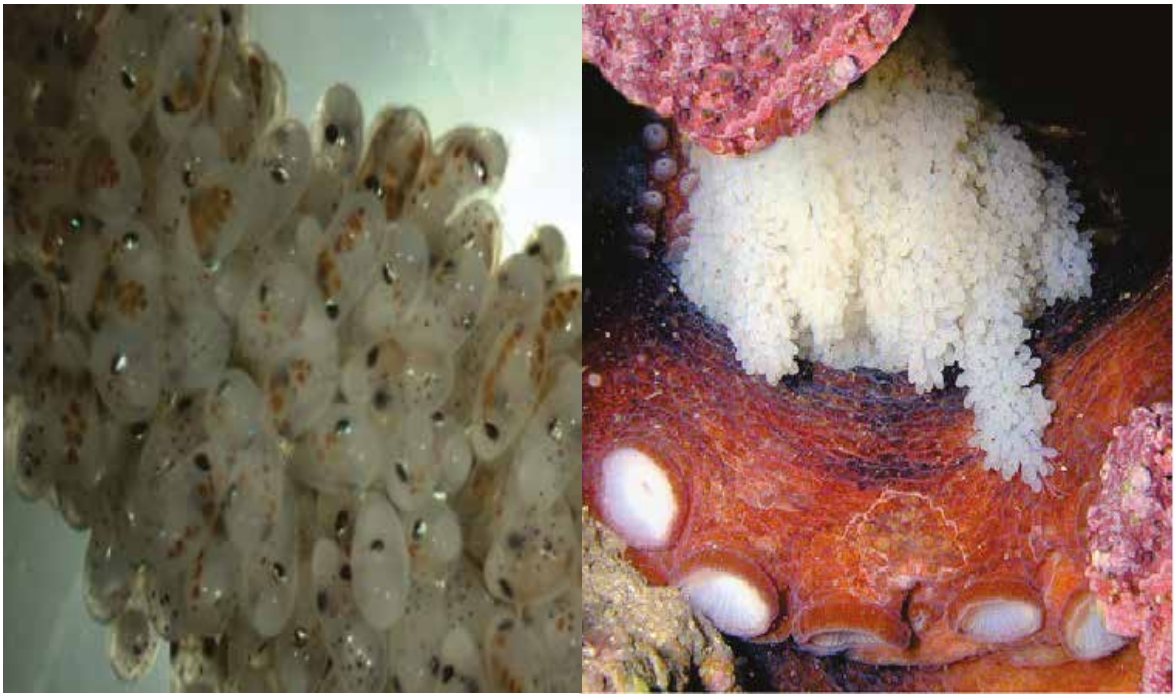


Figure 30. *O. tetricus* female guarding her egg clutch (Right), well developed egg clutch (Left).

If eggs are discovered, the shelter containing the female should be moved into separate individual tank to allow her to incubate her eggs undisturbed. A female will generally incubate her eggs for 35 – 40 days. However, this period can vary dependant on temperature. Higher temperatures (21-23 °C) can decrease incubation time to 25-30 days, while lower temperatures (16-18 °C)

can increase incubation time up to 45-50 days. Female octopus will eat small amounts for the first 2-3 weeks of their incubation period, but will cease feeding thereafter.

2.2 Larvae culture system

2.2.1 Seawater filtration and sterilisation

Seawater entering a facility needs to be filtered and sterilised prior to entering the larvae culture tanks. This will ensure that any harmful bacteria and foreign marine organisms that could potentially harm the larvae are removed. At the minimum, incoming seawater should pass through a 10 µm then a 5 µm filter before passing through an ultraviolet (UV) steriliser (Fig. 31).

Figure 31. Seawater filtration and sterilisation unit (1) incoming seawater (2) 10 µm filter + housing (3) 5 µm filter and housing (4) UV steriliser (5) outgoing seawater. Blue arrows represent water direction.

2.2.2 Larvae tank hydrodynamics

After filtration and sterilisation, water should enter the larvae tanks from the bottom, so it moves upwards and out of the top of the tank and into the external standpipe. This is known as an 'upwelling flow through' system where no water is recirculated back to the tank at any stage. A flow meter (rotameter) is helpful in letting the user know how much water is passing through the tank (Fig. 32).

Figure 32. A. rotameter (1), seawater inlet valve (2), B. water entering external standpipe, C. water entering at the tank bottom, D. water entering up through the base and exiting the top of the tank. Blue arrows represent water direction.

2.2.3 Larvae system description

The larvae culture system comprises of 6 x 1 m³ round, conical based, fibreglass tanks (Fig. 33). This volume ensures octopus larvae have enough room to swim and move around the tanks, while also giving them the ability to escape other aggressive larvae and limit exposure to external environmental factors. The conical base ensures water is distributed evenly throughout the tank and that organic matter will concentrate at the bottom of the cone around the standpipe (Fig. 34).

Figure 33. Larvae culture system

Figure 34. Larvae culture tank profile (left), inside view of the larvae culture tank (right)

2.3 *Artemia* hatching and enrichment system

Artemia hatching and enriching are processes that occur daily during intensive larvae culture, and as both processes require air, oxygen and heated seawater, they can be carried out using the same system (fig.35).

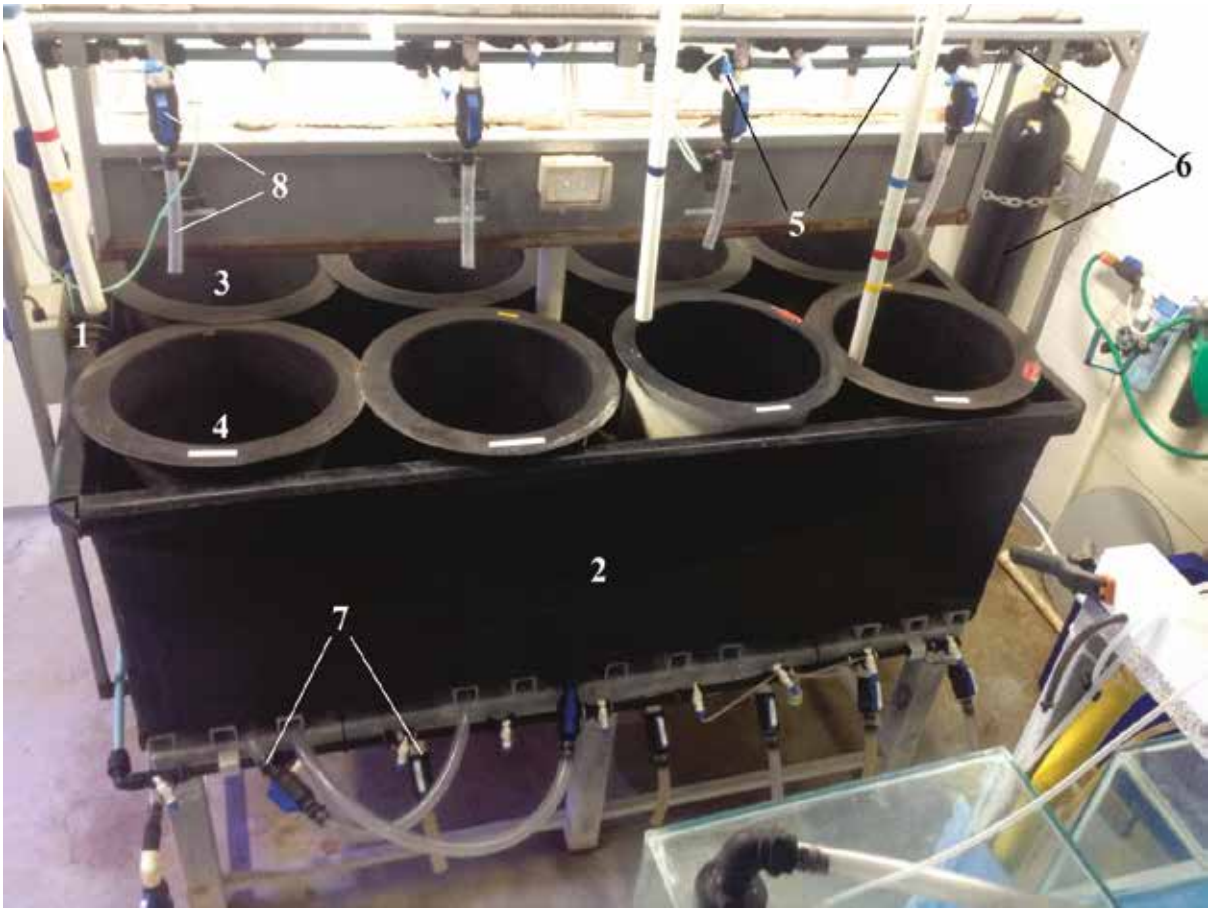


Figure 35. *Artemia* enrichment and hatch-out system. Heater (1), bath containing heated freshwater (2), hatching cone (3), enrichment cone (4), Air manifold (5), pure oxygen manifold and bottle (6), cone dump valve (7) and cone seawater inlet (8).

The system comprises of 8 x 50 lt fiberglass cone tanks that sit in a large 250 lt tub (Kolkovski et al., 2004). The tub contains freshwater that is heated to 29-30 °C, which evenly heats the water in the cones to between 27-30 °C. This is the optimal temperature for Artemia cyst hatching and enriching. Fitted to this system is a pure oxygen manifold which is connected to a size 'G' industrial oxygen bottle and a high volume, low pressure air manifold which is an extension of the current air delivery system at WAFMRL (Fig. 35).

The pure oxygen is needed for *Artemia* enrichment, which occurs in the front 4 tanks. Air is needed when both enriching and hatching *Artemia,* which occurs in all 8 cones. Hatching *Artemia* only occurs in 4 tanks separate to the enrichment cones. Air delivery is via a perforated standpipe while pure oxygen delivery is via an air stone.

A filtered seawater manifold is also fitted to this system, which is an extension of the filtered seawater manifold, which services the larvae tanks. Each 50 lt cone has its own seawater inlet and dump valve.

2.4 *Artemia* grow-out system.

2.4.1 Grow-out tanks

Once the *Artemia* have been hatched, harvested and rinsed, they can then be grown-out to larger *Artemia* in a separate system. *The Artemia* grow-out system is comprised of 6 x 1 m^3 round conical based, fibreglass tanks (Fig. 36). The tanks should contain an internal, central standpipe that delivers a high volume of air and a 100 µm screen filter (Fig. 37).

Each tank can receive temperature controlled or ambient seawater via a 25 mm PVC inlet valve depending on *Artemia* growth requirements. Temperatures higher than ambient will increase growth rates. Connected to the base of the tank is a 50 mm dump valve connected to an elbow containing a male quick release ('camlock') fitting. The camlock fitting is used when harvesting *Artemia.*

Figure 36. *Artemia* grow-out system

Figure 37. *Artemia* grow-out tank (left), (1) internal central standpipe (2) 100 µm screen filter (3) seawater inlet, *Artemia* grow-out tank profile (right)

2.5 Larvae tank components

2.5.1 Outlet filters

A 250 µm mesh filter allows for flow through of clean sterilized water into the tank while maintaining a constant water level and keeping larvae and live feeds inside the tank (Kolkovski et al., 2004). Mesh panels are interchangeable so that they can be replaced with clean panels if there is an accumulation of organic matter on the mesh. The filter is fitted with 6 mm airline that provides internal and/or external aeration to help prevent blockage and keep the screens clear of excess *Artemia* and organic matter (Fig. 37).

Figure 38. Interchangeable screens (1) fitted with 6 mm airline for internal and external aeration (2) Side profile of filter showing 250 µm mesh (3) positioning of filter inside tank (4)

2.5.2 Standpipe

A central standpipe at the apex of the tank bottom distributes water in an upwelling motion with a flow of 1000 lt hr^{-1}. Water enters through the base of the 40 mm standpipe and is distributed through 10 mm holes around the base of the standpipe, which are covered with 250 µm mesh to prevent larvae from escaping. A fitting connecting the base of the standpipe to the length of 400 mm pipe allows water only to flow into the base, leaving the rest of the standpipe airtight and dry. An end cap is fitted to the top of the standpipe to prevent larvae from escaping (fig.39).

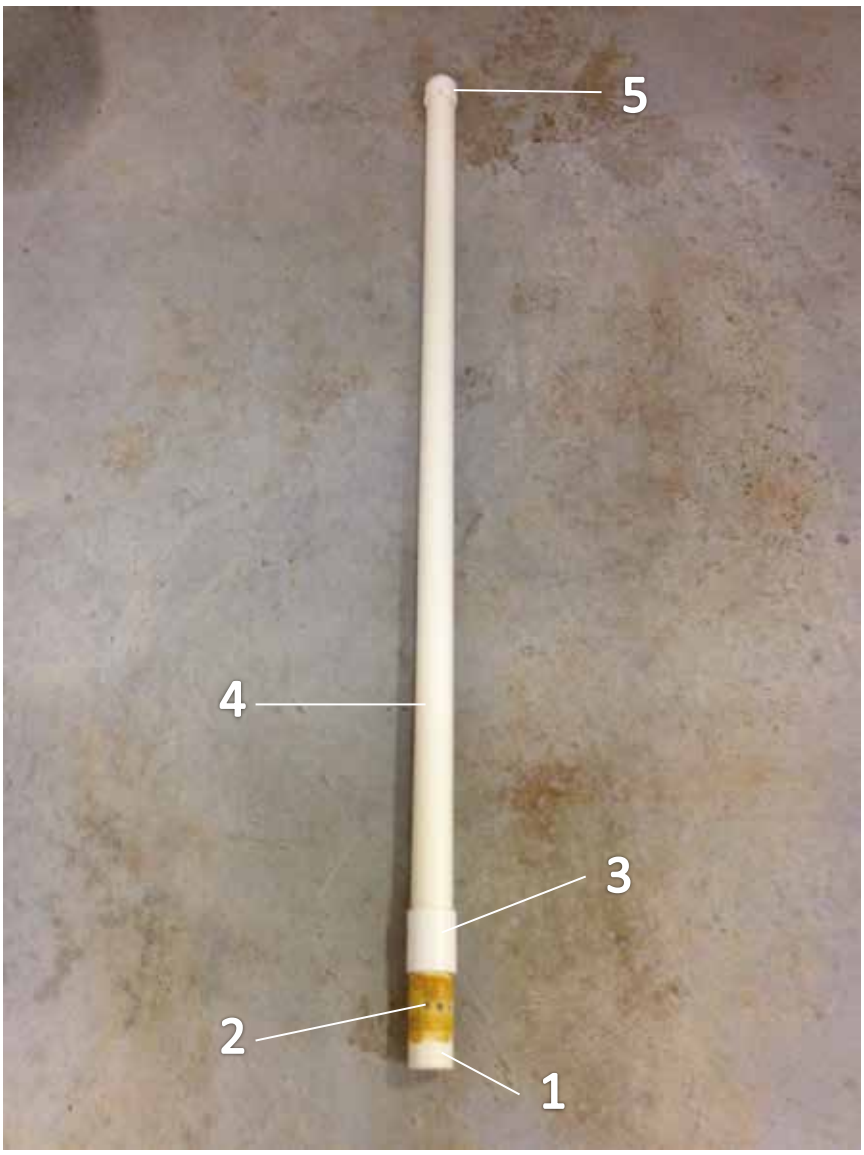


Figure 39. Base of standpipe (1) 10 mm holes covered in 250 µm mesh to distribute flow (2) fitting to restrict water flow to base of standpipe (3) 40 mm length of pipe (4) end cap fitting to prevent larvae escapes (5)

2.6 Double tank system

To keep tanks and larvae as clean as possible and to keep bacteria levels down, a method called 'passive transfer' is used to move larvae from an existing tank to a new sterile tank. A 'double tank system' is used to passively move larvae from one tank to another using only water flow with the aid of aeration (Fig. 40).

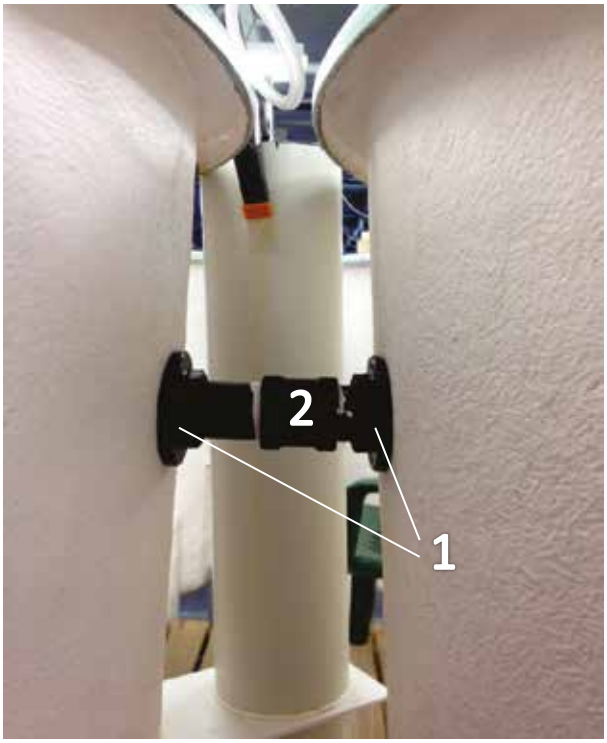


Figure 40. Two tanks connected using 40 mm tank adaptors (1) and a 40 mm threaded joiner (2)

Once the new tank is filled with clean sterile water, there are some steps that need to be taken before the passive transfer commences;

1. Flow adjustment: Water flow in the existing tank containing larvae is maintained at 1000 lt h^{-1}, while the adjacent clean sterile tank has a lower flow of 200 lt h^{-1} to create positive pressure in the existing tank.
2. Filter and standpipe into new tank: Placing a filter into the new tank ensures that larvae will not escape when they are moved across and allows for continuous flow of clean water into tank. The standpipe will prevent larvae from escaping.
3. Aeration: Along with the difference in water pressure, an aeration device directs water and larvae toward the new tank opening. The device consists of a frame of 20 mm PVC that sits on the conical base of the tank. Air supply is provided by 6 mm airline connected to 4 mm porous pipe, which is attached to the PVC frame. Porous pipe produces fine bubbles which creates an air curtain around half of the tank edge, directing larvae upward and in the direction of water flow (Fig. 41).
4. Removal of end cap: Removal of the end caps from tank adaptors in both the old and new tank allows the water from both tanks to combine. When there is positive pressure from the existing tank due to a higher flow, larvae are passively moved across to the new tank (Fig.42).

Adjustment of external standpipe: Attached to the outside of the tank is an external standpipe that acts as an overflow for water that passes through the filter mesh as it is replaced with new incoming water. Overflow can be regulated using a valve fitted to the external standpipe. In order to increase positive pressure in the existing tank with larvae, this valve can be closed so that the only place for water to escape is through the 'double tank' fitting and into the new tank (Fig.43).

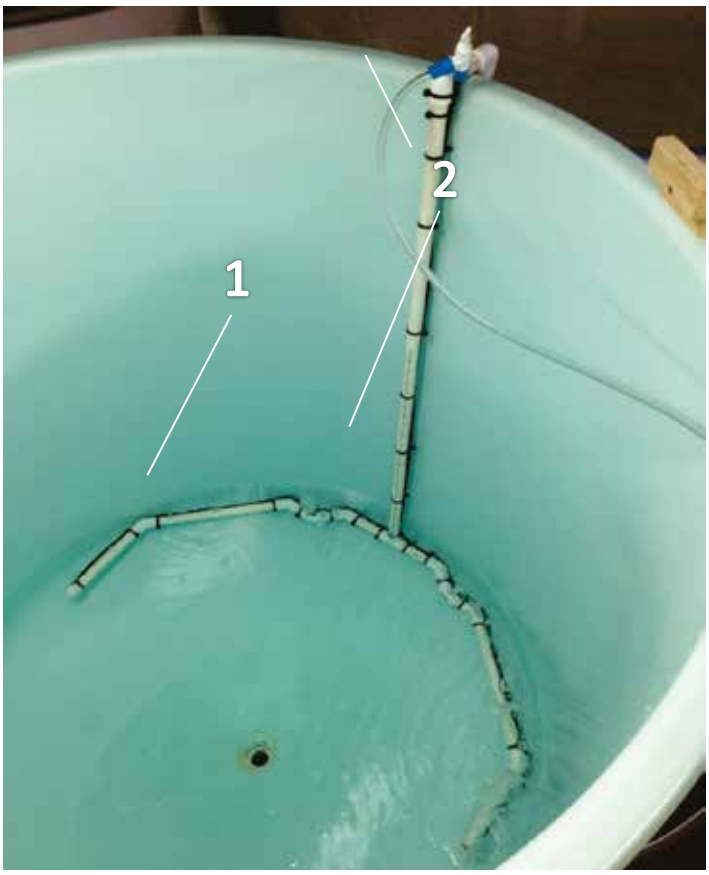


Figure 41. Aeration device showing PVC 20 mm frame (1) with 4 mm porous pipe attached (2)

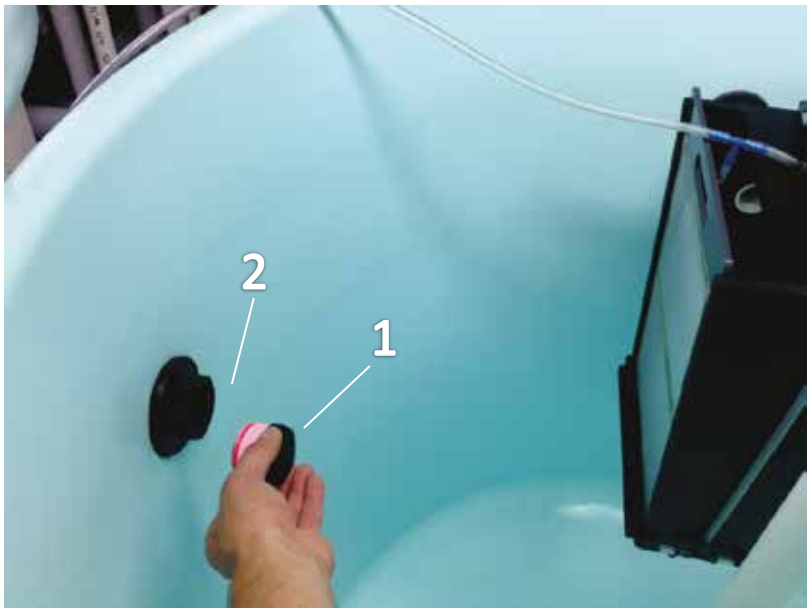


Figure 42. Removal of end cap (1) attached to tank from 40 mm tank adaptor (2) to allow water flow to new tank

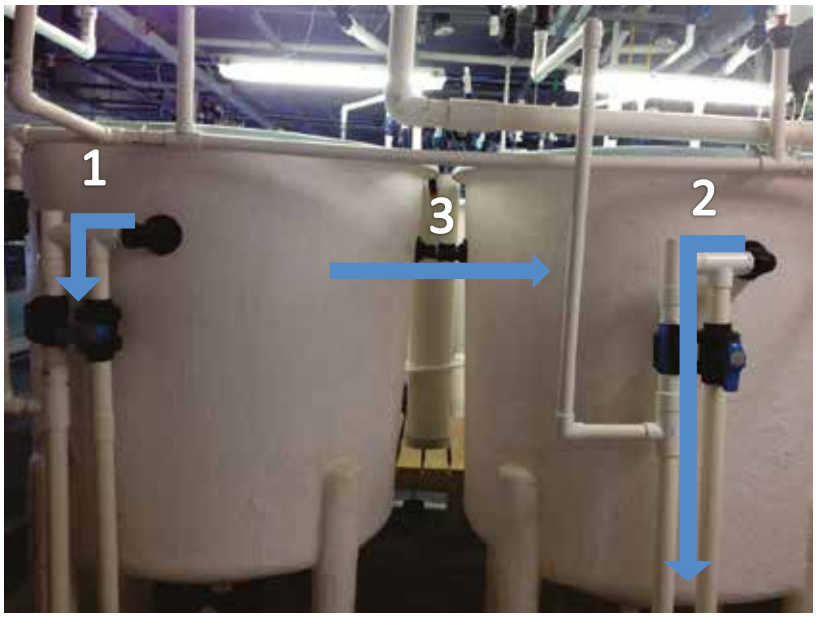


Figure 43. External standpipe with valve closed in existing tank during transfer (1) preventing water from overflowing and directing it to the tank connection (3) external standpipe with valve open, allowing normal overflow in new tank (2)

2.7 Automated feeding system

A semi-moist microdiet (Nutrakol Pty Ltd) was fed to larvae from 10 days post hatch via an automated feeding system (AMD, Department of Fisheries, Western Australia), comprised of a control box (Fig. 44) and automatic feeders that disperse microdiet (Fig. 45).

Figure 44. Front view of control box showing LCD display screen (left) and side view of cable (right)

Figure 45. AMD feeder attached to tank wall

2.8 Lighting

Suspended lighting above tanks was programmed on a photoperiod of 14 hours dark:10 hours light (Kolkovski et al., 2004). Fluorescent lighting ('day light' type) was between 550-600 lux and came on at 8.15 am and turned off at 6.15 pm. Spotlights were programmed to 'ramp up' and gradually turn on 15 minutes before fluorescent lighting came on, so as not to shock or cause stress to larvae and also to simulate a natural sunrise and sunset (Fig. 46).

Figure 46. Fluorescent light suspended above tank (1) and 'ramp up' spotlight (2)

2.9 Daily protocol

2.9.1 Stocking and stocking density

Upon hatching of octopus larvae by a female carrying eggs, larvae can be stocked into the culture tanks. It is important that (1) larvae from the first 10 days of spawning are used and (2) larvae are stocked from no more than 2 consecutive spawning days. Approximately 8,000 larvae can be counted into each 1 m³ tank in groups of 10 using a clicker counter (Fig. 47).

Figure 47. Clicker counter and 1 lt jug used for stocking (left) 1 m³ culture tank (right)

2.9.2 Hatching *Artemia*

Hatching of nauplii (newly hatched *Artemia* < 0.4 mm*)* is a process that occurs every 1-2 days during intensive larvae culture. These nauplii will be grown out to a size of 1.5 – 2.5 mm in a separate grow-out system prior to being enriched and fed to the octopus larvae.

Hatching should be carried out using the following steps:

1. Weigh ~40 g of unhatched dry cysts (*INVE sep-art*) into a beaker (Fig. 48).
2. Fill up a hatching cone in the *Artemia* system to 50 lt volume, place standpipe in the cone and turn on air so that aeration is vigorous.
3. Add 5 ml of hatch controller (*INVE Sanocare HC)* to the water and allow time for it to fully dissolve (Fig. 48).
4. Add cysts to the water once hatch controller has fully dissolved.

Figure 48. *Artemia* enrichment and hatch-out system components: hatch controller, cysts and balance (Left) 40 gr cysts weighed on balance (Right)

2.9.3 Post hatching harvest

After the cysts have been allowed to hatch for 24 hours in the cone, they are ready to be harvested. The harvester is an *INVE* product, which consists of a separator tube containing strong magnets (Fig. 49). The magnets attract all the unhatched and hatched shell, which are iron-coated, giving complete separation of the nauplii from the shells during harvesting. Decapsulation is not required when using these cysts.

Figure 49. INVE cyst separator set-up to harvest hatched cysts: cone dump valve (1) filtered freshwater hose (2) *INVE* cyst separator (3) 100 µm harvest bucket (4)

Post hatching harvesting should be completed using the following process:

1. Turn off air to the cone and remove the standpipe.
2. Fully open the cone dump valve to allow water from the cone to flow into the separator, slowing the flow to a trickle when water starts flowing into the 100 µm harvest bucket (Fig. 49).

3. Add a trickle from the filtered freshwater hose to assist nauplii and cysts through separator.
4. Allow time for the cone to completely empty and persist with the filtered freshwater rinse until all the nauplii have flushed through the separator.
5. Once all the nauplii have flushed through, persist with the filtered freshwater rinse until the water in the harvest bucket is 100% freshwater.
6. Switch to filtered seawater at this point and persist with this until water in the bucket is 100 % seawater.

2.9.4 Harvesting

Harvesting should be completed using the following process:

1. Once grow-out tanks containing *Artemia* are at a size suitable to feed octopus larvae (1.5-2.5 mm), they should be harvested. A 250 µm screen bucket connected to an air source and a 50 mm PVC hose with a female cam lock fitting at one end are required. The female cam lock fitting attaches to a male cam lock fitting at the base of the tank (Fig. 50).
2. Remove the standpipe from inside the tank and open the dump valve at the base of the tank to allow water to flow in to the harvest bucket, adjust the valve so that the screens in the bucket do not block causing the water to overflow. Take a sub sample from the bucket using a pipette and a counting cell to calculate how many *Artemia* you have harvested. Approximately 2 million *Artemia* should be harvested so that there are enough available across all feeding over the next 24 hours (Section 5).
3. Rinse the bucket containing *Artemia* with filtered fresh water until 100 % fresh water.
4. Following the freshwater rinse, rinse with filtered seawater until 100 % seawater.

Figure 50. Harvest method of grow-out *Artemia* tanks (Left) Hose attachment to tank set-up (Right)

2.9.5 Artemia pre–enrichment stocking

After the required amount of 1.5 – 2.5 mm *Artemia* has been harvested from the grow-out tanks, the *Artemia* should be enriched before feeding them to the octopus larvae.

Once rinsing is complete, the *Artemia* should be stocked into 3 enrichment cones filled with 20 lt seawater with vigorous aeration, a trickle of pure oxygen and 0.2 ml of 'Roti diet' paste (Reed Mariculture, USA). 600,000 *Artemia* should be stocked in the first two cones (8:00 am and 12:00 pm) with 800,000 *Artemia* being stocked in the third cone (3:00 pm) (Fig. 51). The extra 200,000 *Artemia* stocked into third cone should be cold stored for overnight dosing.

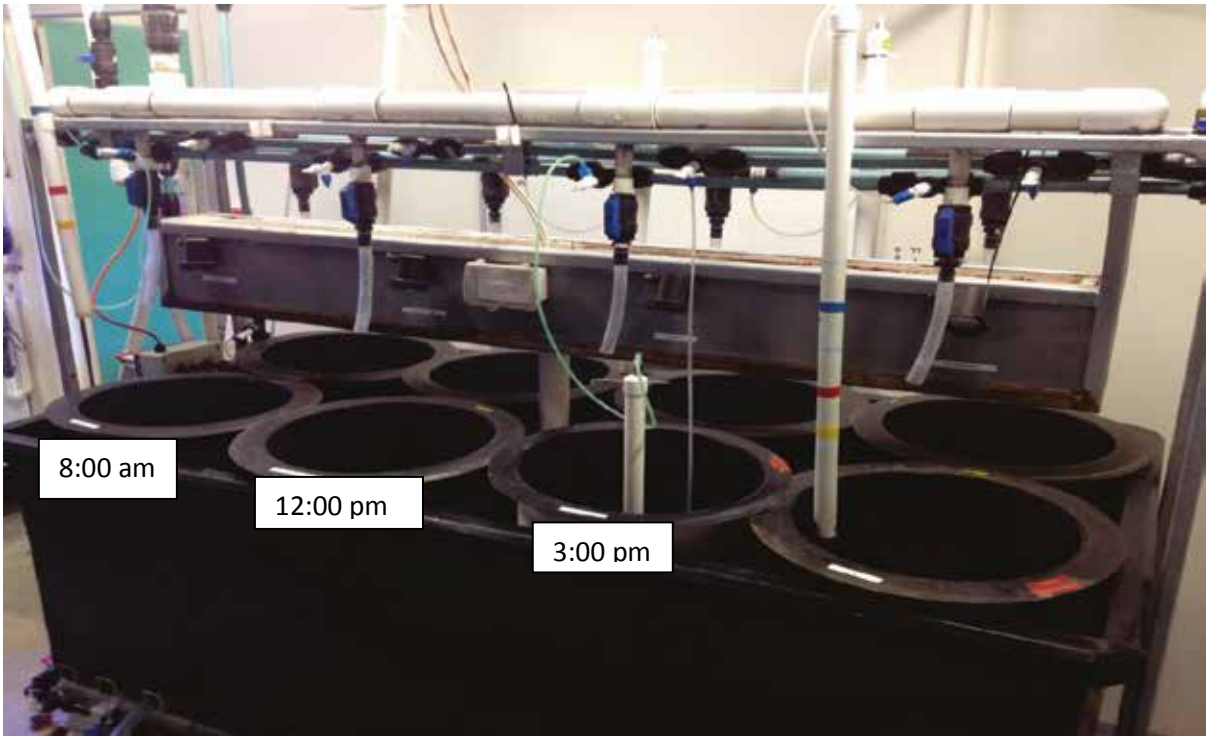


Figure 51. Cone designation for manual feed events during the day

2.10 *Artemia* enrichment

Enrichment involves feeding the *Artemia* a high quality feed, usually with a specific protein to lipid ratio with vitamins and minerals, to greatly enhance their nutritional quality, which subsequently will affect the octopus larvae. *Artemia* are suitable as live prey in larval culture because:

- They can be grown out at high densities to a variety of sizes.
- They will readily uptake any enrichment meaning there nutritional profile can be manipulated to suit any larvae dietary requirements.
- They are very hardy, tolerating saline, fresh and oxygen deficient water.
- They stimulate early hunting and feeding mechanisms in larvae.
- They are easy to obtain, store and hatch.

Artemia enrichment should be completed using the following process:

1. The amount of Artikol (Nutrakol Pty Ltd) enrichment required is 0.5 gr lt^{-1} seawater. Therefore, if the enrichment cone contains 20 lt of seawater, 10 gr of enrichment should be mixed with an amount of seawater (seawater is used to adequately blend the enrichment into a liquid prior to feeding to the Artemia). For three cones, 30 gr of enrichment is required.

2. In a jug, mix the 30 gr enrichment with 1.5 lt seawater for 30 seconds using a blender (Fig.52).
3. Separate the 1.5 lt mixture into 3 bottles: 2 bottles should be cold stored for a manual one hour enrichment at 11:00 am (12:00 pm feed) and 2:00 pm (3:00 pm feed) for feeding that day, and one bottle topped up with 500 ml seawater and cold stored to automatically enrich for 8:00 am cone at 2:00 am the following morning.
4. The overnight enrichment should be rigged to a peristaltic pump on a timer so that it automatically doses from the fridge at 2:00 am the following morning (Fig. 53).

Figure 52. Enrichment process; jug and balance used to weigh out the enrichment (Left) blender used to mix the enrichment with seawater prior to feeding to the *Artemia* (Right).

Figure 53. 2:00 am enrichment bottle connected to a peristaltic pump (1) and a feeding line (2)

2.11 Feeding

Larvae should be fed 6 times daily – 3 times manually during the day and 3 times via an automated system during the night, enriched *Artemia* that are between 1.5-2.5 mm.

Manual feeds to larvae during the day can be carried out using the following process:

1. Turn off air and oxygen and remove the standpipe from the cone.
2. Open the cone dump valve so that the contents of the cone fall into a 250 µm harvest bucket.
3. Rinse the bucket containing *Artemia* with filtered fresh water until water in the bucket is 100 % fresh.
4. Following the freshwater rinse, rinse with filtered seawater until water in the bucket is 100 % seawater
5. Count the *Artemia* in the bucket and use jugs to portion smaller amounts to feed to the octopus larvae (Fig. 54).

Figure 54. Post enrichment process; standpipe and *Artemia* from cone removed (1) filtered freshwater rinse (2) filtered seawater rinse (3) jugs to portion *Artemia* to feed to octopus larvae (4).

Night feeding: *Artemia* that are cold stored for overnight feeding should be programmed be fed via peristaltic pumps at 6:00 pm, 12:00 am and 06:00 am.

Night feeding should be completed using the following process:

1. Fill a 25 lt bucket with seawater and 200,000 *Artemia* from the 3:00 pm feed (50,000 *Artemia* for each tank).
2. The bucket should be inside an esky with ice as cold water slows down the metabolic process of the *Artemia* inside the bucket meaning they retain their enrichment.
3. Add aeration to the water.
4. 4 peristaltic pumps should be connected with 2 lines- the incoming line should be in the bucket containing *Artemia* while outgoing is in the larvae tank (Fig. 55).
5. Make sure there are 4 lines inside the bucket connected to 4 pumps. The pumps are set to work 3 times overnight at 18:00 pm, 24:00 am and 06:00 am.

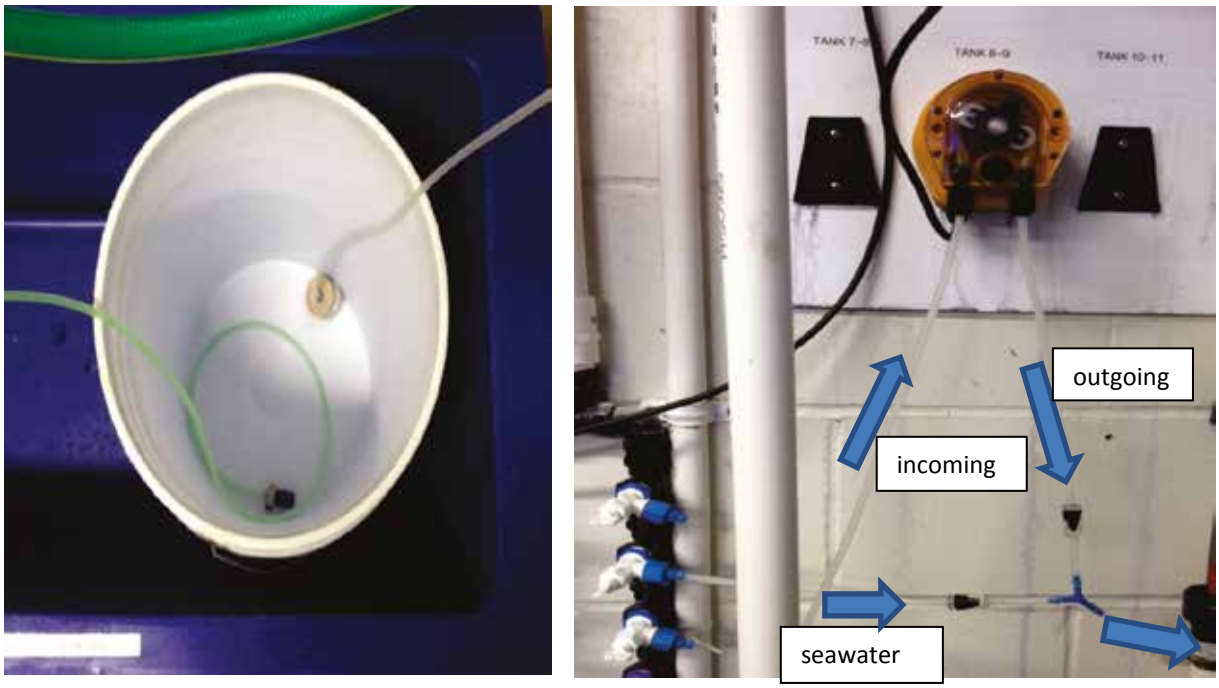


Figure 55. Cold storage esky containing a 25 lt bucket with air source and pump line (left) peristaltic pump profile with seawater addition, incoming and outgoing lines (right).

- Feed densities to be monitored as to not have excess of *Artemia*, but at the same time not to over feed larvae. Additional feeds between major feed events can be given if larvae are eating *Artemia* out quickly.
- Live copepods to be fed at 9 am each morning along with 25 g of frozen copepods. These should be mixed with 6 lt of seawater in a 10 lt bucket in an esky with ice, and dosed to treatment tanks at 11:00 am each morning.
- All tanks to be fed 500-800 µm micro diet from 10 dph via an automated feeding system.

2.12 Plankton collection

Live zooplankton can be collected from the ocean on a daily basis each morning. The collecting system is comprised of a floating 5 mm oyster mesh housing in which zooplankton were transported via a submersible pump to a 200 lt collector with 1000 µm filter. Plankton should be condensed using a 250 µm filter in a 100 lt collector. A spotlight to attract the plankton was attached to the mesh housing and was run from 6 pm to 6 am using a timer (fig.56). The majority of species that were collected were *Copepods* and some crab larvae. Smaller zooplankton can

be collected from this tank via a dump valve and hose directly into a 100 µm harvest bucket. Contents of the harvest bucket can be transferred to a bucket containing a lid for transport back to the facility.

Figure 56. Plankton collector containing the submersible and pond light directly above.

2.13 Transfers

To keep tanks and larvae as clean as possible and keep bacteria levels down, a method called 'passive transfer' was developed and is used to move larvae from an existing tank to a new sterile tank every 7 days, starting after 7 dph. A 'double tank system' is used to passively move larvae from one tank to another using only water flow with the aid of aeration (Fig. 57).

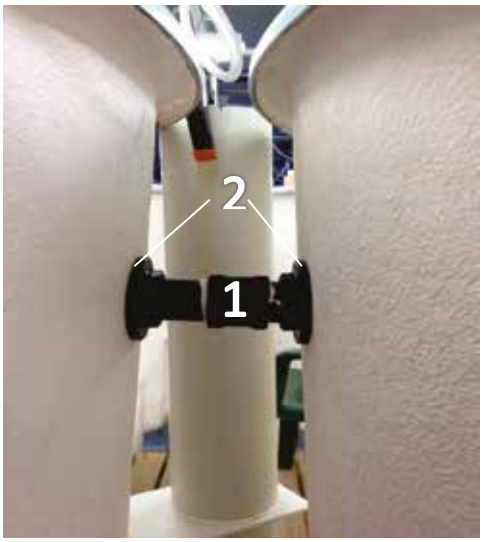


Figure 57. Two larvae tanks connected using 40 mm tank adaptors (1) and a 40 mm threaded joiner (2)

Once the new tank is filled with clean sterile water, there are some steps that need to be taken before the passive transfer commences;

1. Flow adjustment. Water flow in the existing tank containing larvae is maintained at 1000

lt h^{-1}, while the adjacent clean sterile tank has a lower flow of 200 lt h^{-1} to create positive pressure in the existing tank.

2. Filter and standpipe into new tank. Placing a filter and standpipe into the new tank ensures that larvae will not escape when they are moved across and allows for continuous flow of clean water into tank. The standpipe will direct water flow as upwelling while also preventing larvae escapes.
3. Aeration. Along with the difference in water pressure, an aeration device directs water and larvae toward the new tank opening. The device consists of a frame of 20 mm PVC that sits on the conical base of the tank. Air supply is provided by 6 mm airline connected to 4 mm porous pipe, which is attached to the PVC frame. Porous pipe produces fine bubbles, which create an air, curtain around half of the tank edge, directing larvae upward and in the direction of water flow (Fig. 58).
4. Removal of end cap. Removal of the end caps from the tank adaptors in both the old and new tank allows the water from both tanks to combine. When there is positive pressure from the existing tank due to a higher flow, larvae are passively moved across to the new tank.
5. Adjustment of external standpipe. Attached to the outside of the tank is an external standpipe that acts as an overflow in the larvae tank. Outgoing water can be regulated using a valve fitted to the external standpipe. In order to increase positive pressure in the existing tank with larvae, this valve can be closed so that the only place for water to escape is through the 'double tank' fitting and into the new tank (Fig. 59)

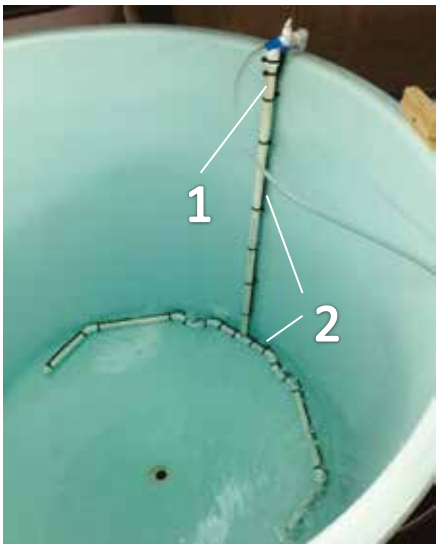


Figure 58. Aeration device showing PVC 20 mm frame (1) with 4 mm porous pipe attached (2)

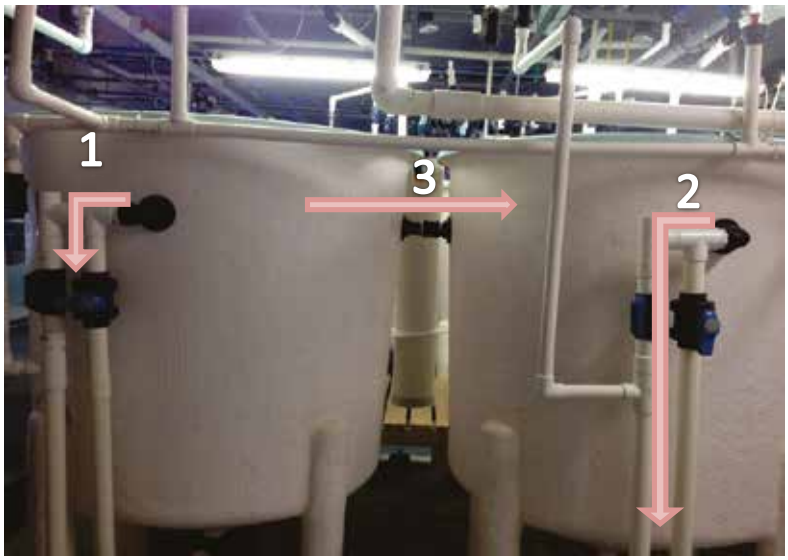


Figure 59. External standpipe with valve closed in existing tank during transfer (1), preventing water from overflowing and directing it to the tank connection (3), external standpipe with valve open, allowing normal overflow in new tank (2).

2.14 Photoperiod

The photoperiod should be 14 hr dark:10 hr light to mimic the natural photoperiod. Light intensity was 550-600 lux at the water surface via 'day light' fluorescent lights. It is advisable that the lights should ramp up and down each day to prevent stress to larvae with sudden lighting.

2.15 Water quality

Temperature and dissolved oxygen measurements should be taken daily using a dissolved oxygen meter. Water in all tanks should be maintained at 21 °C ± 1° C via a heater-chiller unit. A flow rate of 1000 lt hr^{-1} to be maintained at all times. The bottom of the larvae tanks and excess live feed should be siphoned daily into a 250 mm screen bucket. Any remaining live larvae that are siphoned into the bucket should be returned to the tank using a pipette (Fig. 60).

Figure 60. Siphoning method (1) acrylic rod used as the siphon tube (2) siphon hose placed in a 250 µm screen bucket