Development of octopus aquaculture
Final Report

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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

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1.0 Executive summary

During the past four years, the marine aquaculture group at the WA Fisheries and Marine Research Laboratories (WAFMRL) in collaboration with Occoculture Pty Ltd (subsidiary of Fremantle Octopus) and as a part of the FRDC project 'Octopus Aquaculture Development', investigated the potential of octopus aquaculture in Western Australia. Hatchery methods were developed in an attempt to close the life cycle. Advanced systems, rearing and feeding protocols were established for ranching *Octopus tetricus* juveniles.

The ever-increasing demand for octopus as a food source both nationally and worldwide, has placed higher importance for further developments into octopus aquaculture. And while research and progress into closing the life cycle of octopus in captivity received significant attention, it is the 'ranching' of octopus, which has increased in popularity and has become a potential solution to fill the shortfall in supply.

'Ranching' is the on-growing of wild caught octopus onshore or in cages and is gaining popularity in Europe and Latin America. Commonly, the grow-out systems involve the use of 'hides', usually PVC pipes that supply shelter to individual octopus.

Although positive growth has been achieved and marketable individuals obtained, these grow-out methods achieved relatively low biomass (up to 15 kgm^3), they are difficult to clean and harvest and promote territorialism and cannibalism amongst individuals.

During the FRDC project 'Octopus Aquaculture Development' new and innovative systems and protocols were developed for ranching the local octopus *Octopus tetricus*.

These systems and protocols have eliminated the need to use shelters in tanks, which has significantly reduced cannibalism and territorialism, subsequently making tanks extremely easy to clean and harvest. They have allowed the 'ranching' of octopus juveniles from as little as 50 gr at a biomass upwards of 54 kg m^3 in specifically designed tanks, which is the highest biomass reported achieved anywhere in the world to date.

Return on Investment (ROI) modeling based on these systems and methods, predicted a growth period of 18 weeks for a juvenile octopus reaching market weight (800 g). These predictions were confirmed in series of growout trials with different juvenile weights and biomass.

The growout systems and rearing protocols are commercial ready and are detailed in the *Octopus tetricus* commercial aquaculture manual as a product of this project.

In contrast to the promising results achieved in octopus juvenile ranching, octopus paralarvae rearing still constrains octopus industrial development. Commonly observed high mortalities and poor growth in early stages of larval development are thought to be associated with nutritional imbalances of live prey, feed additives and enrichments. However, the true reason (or reasons) for this bottleneck remain unconfirmed.

With limited studies on *O. tetricus* and no attempts at paralarvae rearing made in the past, this research was aimed at determining the most appropriate rearing conditions with regard to system design, nutrition and environmental parameters.

Over the past four years, focus has been on improving the health and survival of *O. tetricus* paralarvae and 'bridging the gap' between the free swimming planktonic stage and the stage at which metamorphosis is reached and paralarvae become benthic.

Trials over this period have tested various nutritional parameters, some of which include various live feeds to partially supplement feeding of *Artemia*, such as crabs *Portunus pelagicus* zoeae, *Copepod* sp. and lobster *Panulirus cygnus* phyllosoma. *Artemia* enrichment composition has been examined with regards to protein to lipid ratios, while testing various different proteins and lipids from both animal and plant sources. Various tank systems and designs were also considered with regards to hydrodynamic factors such as water flow and upwelling, tank volume, filtration and static or passive transfer of paralarvae to new tanks in order to manage bacteria levels. Environmental parameters such as water temperature, light intensity and photoperiod, as well as green water culture, were also considered integral to paralarvae success and were trialed over the duration of this project.

Strong emphasis was given to histological analysis of internal biological components of paralarvae under various treatment conditions, with particular focus on gut function and development.

The culmination of paralarvae development from a histological perspective, combined with the collection of qualitative data and observations while testing nutritional, environmental and system parameters has provided us with greater knowledge of requirements of *O. tetricus* paralarvae. However, further research is needed to 'close the gap' in order to achieve metamorphosis and completely close the life cycle of this species.

Keywords

Octopus tetricus, octopus culture, ranching.

2.0   Background

During the past decade, the octopus fishing industry in WA has developed from being virtually non-existent and catches being a by-product of other fishing activities, to a managed fishery that supports a thriving commercial manufacturing industry. Octopus is now supplied as raw fresh and/or frozen product for use in local restaurants and cafes and value added marinated varieties suitable for gourmet delicatessen outlets and supermarkets. The successful development of the industry has also resulted in its ability to sell product into eastern Australian markets.

Human demand for octopus has increased 5-fold during the past decade in parallel to a reduction in red and white meat consumption. This is partly due to the high Omega 3 content of octopus and an increased awareness of its health benefits.

This trend is reflected in an increase in market price from around \$4 to over \$12 kg^{-1} for raw product, and value added product fetching prices of around \$40 to \$50 kg^{-1} (at wholesale and retail levels respectively).

The same trend can be observed internationally, predominantly in the Mediterranean countries and Japan, where an increased demand from both local and export markets has inflated prices. Additionally, pressures on the octopus fishery in the Mediterranean Sea and off the North West Coast of Africa have resulted in, as much as, 40% decline in product supply, which further compounds the price issue. This trend is replicated worldwide and is likely to continue because of increasing restrictions on wild catch and growing demands for the product.

Fishing for octopus has encountered a number of commercial problems throughout the world in recent years, including low availability of stocks. However, the octopus fishers in WA have adopted sustainable and more effective fishing methods and equipment resulting in a substantial increase in catch in the last seven years. Nonetheless, this catch is still not sufficient to satisfy the demand in both the WA and Eastern States markets, which will require a substantial increase in production.

To date, 2 of every 3 octopus caught in WA have been returned to the ocean due to their small size, which was not suitable for commercial processing (although there is no minimum size for octopus fishing in WA). An opportunity to increase supply has been identified by ranching these smaller octopus in a commercially viable and environmentally sustainable manner. It was considered that this option will reduce the impact of boat and fishing activities on the environment and result in a higher volume of product. However, it was regarded as being a short-term solution, while in the longer-term the development of an octopus hatchery would substantially increase the production while vastly improving the sustainability of the industry.

The local Western Australian *Octopus tetricus* is an excellent candidate for aquaculture. Initial trials have indicated that *O. tetricus* is readily acclimated to captivity, maintains high growth rates (20% two-week^{-1}), will readily accept frozen/moist food and has a high reproductive rate. Furthermore, it fetches a high market price similar to wild catch and demand far outstrips supply and is not expected to reduce in the near future. These indicators ensure that capital investment will receive sustained strong returns.

Therefore, Occoculture Pty Ltd, a subsidiary of Fremantle Octopus (WA fishing company specialised in octopus fishing) teamed up with the marine aquaculture group at the Department of Fisheries, Western Australia (DoFWA) to develop the techniques and methods for octopus aquaculture. The project was funded through the Fisheries Research and Development Corporation (FRDC), DoFWA and Occoculture.

The project objectives were:

1. Develop the hatchery techniques for octopus larvae and juveniles; and

2. Optimising octopus ranching and grow out.

Ranching and growout of juvenile octopus was considered to be the priority in the project since the methods and techniques developed could be applied immediately for the commercial production of octopus. However, due to the seasonal fluctuation of octopus fishing and hence, juvenile octopus supply, prolonging the annual production period was considered important. Therefore, the long-term goal was to close the life cycle and shift the research focus towards hatchery protocols and growout techniques for octopus.

Intensive aquaculture of octopus suitable for human consumption does not yet exist anywhere in the world. There are several R&D centres looking at different aspects of the octopus life cycle and culture mainly in Spain (Vigo, Canary Islands) and Japan.

However, the ranching techniques used in these places were not considered to be commercially viable. Therefore, new ranching and growout techniques, systems, feeds and feeding protocols development were the initial priorities of the project.

Although octopus larvae are relatively robust compared to marine fish larvae, their nutritional requirements, physiology and environmental requirements were very much unknown. Therefore, the second objective of the project was to gain data and knowledge on *O. tetricus* larvae requirements and, if possible, to close the life cycle by investigating broodstock nutrition and husbandry, larvae nutrition and systems management.

Working on these two aspects in parallel was thought to ensure that the growout technology would be in place when the hatchery-reared juveniles are ready for stocking, thus preventing delays in the commercialization. An existing and increasing local, national and international demand for product adds greater certainty to the commercial viability of the aims of this project. Moreover, it was believed that if the development of technologies for growout and hatchery facilities prove to be successful, a market will also exist for the licensing and transfer of 'know how' to other stakeholders and locations.

The project answered all the criteria for the development of a new species of aquaculture, namely:

1. Market driven. There is strong and increasing demand for octopus products, both in Australia and overseas. Demand far outstrips the supply.

2. Being driven by industry with significant existing investment. Fremantle Octopus has invested over $2 million in R&D and is currently the biggest octopus fishing company in Australia. Occoculture has been specifically established to develop an octopus aquaculture facility and is in the process of raising private equity to assist in funding of the project. Both companies were an integral part of this project.

3. The cost of production was thought to be less than the farm gate price. In the Mediterranean, raw octopus has been selling at up to AU$70 kg^{-1} in the 2007/08 season. This compares with a current price of AU$11 kg^{-1} for local stocks. A conservative cost analysis of production was $9 kg^{-1}. On a value-added basis, wholesale sales in Australia can achieve $40 kg^{-1} (retailing at $50 to $55 kg^{-1}) with a 60% gross profit margin. In the European marketplaces, value-added products may achieve up to $100 kg^{-1}, giving a gross profit margin of more than 80%.

4. The species is endemic to Australia and builds on an established high value wild caught market with high export potential.

5. There exists the planning framework and access to resources to allow for the timely and orderly development of the project.

6. Octopus aquaculture is gaining strong interest in SA and Victoria. It is envisaged that the technology developed through this project will be transferable to other ventures in these states.

3.0   Objectives

- Develop the hatchery techniques for octopus larvae and juveniles; and

- Optimising octopus ranching and grow out.

4.0   Larvae rearing

4.1   Systems

4.1.1   40 lt system

4.1.1.1   Tanks

This system is comprised of 14 tanks of 40 lt volume which are cylindrical with a false bottom of 10 mm clear acrylic. The acrylic bottom has 1 mm holes drilled evenly across it which allows upwelling of flow through seawater. Water exits these tanks via a 32 mm outlet at the top. The inlet is a 20 mm valve socket plumbed into the tank wall underneath the acrylic bottom. The thread is located on the outside of the tank to facilitate attachment of a 20 mm female dovetail fitting on an inlet hose (Fig. 1) which allowed attachment via a 20 mm dovetail fitting connected to an inlet hose (Fig.1)
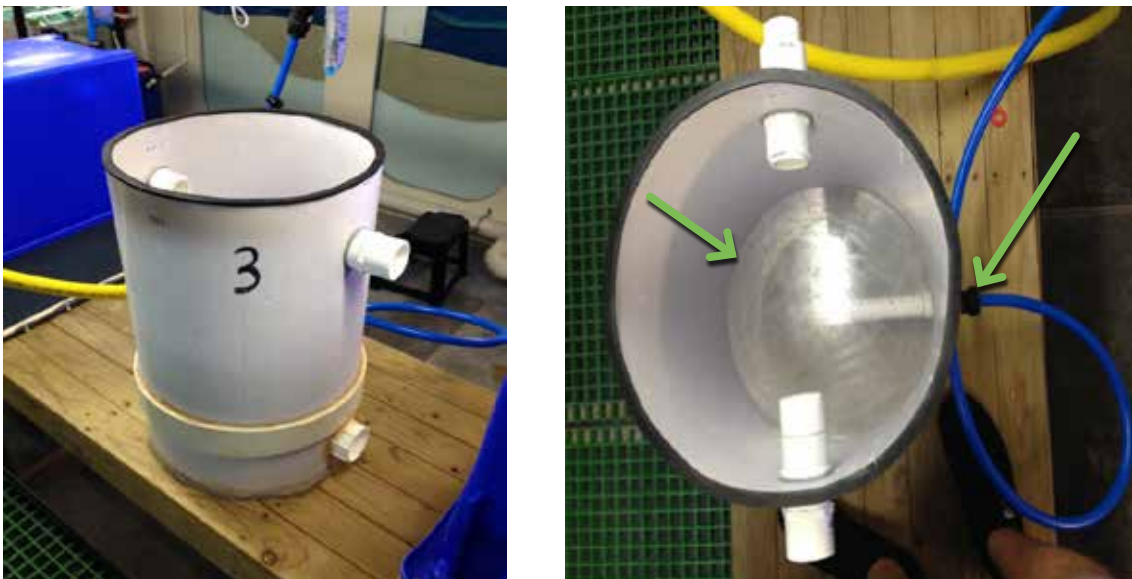
Figure 1.   40 lt tank profile (left) green arrows indicate acrylic bottom and attached seawater inlet (right).

4.1.1.2   Tank components

Each tank has a banjo filter screen connected to the 32 mm outlet at the top of the tank. Banjo screens are either 250 or 500 μm dependent on the larvae size during an experiment. Each banjo screen has a 4 mm poly valve and riser fitted at the top to facilitate internal air attachment (Fig. 2). Tanks are situated adjacent to each other to facilitate larvae transfer. Larvae are transferred from a dirty to a clean tank during experiments, which is done manually using pipettes (Fig. 3)

Figure 2.   Banjo screen profile (left) and its locality in the 40 lt tank, green arrow indicating poly valve and riser (right).

Figure 3.   Manual transfer of larvae during an experiment.

4.1.1.3   *Tank environment*

Flow rates to all tanks are set at 150 lt hr^{-1} (3.75 exchanges hr^{-1}). Water temperature is maintained at 21°C ± 0.5°C via a heater chiller unit. Photoperiod is 10 L 14 D with fluoro lights emitting 550 – 600 lux on the surface of the tanks during daylight hours.

4.1.2   270 lt system

4.1.2.1   Tanks

This system is comprised of 24 tanks of 270 lt volume which are fibreglass, cylindrical and have a conical base. Each tank is subject to up-welling flow through seawater by directly attaching a 20 mm flexible hose to the base of the tank, or flowing water through a de-gassing column that is fitted to the side of the tank beforehand. Water exits through a 32 mm outlet at the top of the tank (Fig. 4).

4.1.2.2   Tank components

Each tank is fitted with a box filter that facilitates interchangeable mesh screens. Screen size is 250 or 500 μm dependent on the larvae size during an experiment. Each screen has 4 mm airline and porous pipe fitted to allow an internal and/or external air source. Tanks also contain a removable 6 mm grey PVC plastic false bottom. The false bottom contains a handle to facilitate removal and 10 mm holes covered by 250 μm mesh to allow even water distribution (Fig. 5).

Tanks are lined up in 3 groups of 8 (24) which allows each tank to be paired up. Pairs of tanks are connected to allow transfer of larvae from a dirty to a clean tank. Connected to the 32 mm outlet on the outside of one tank is a 32 mm poly elbow and a 32 mm male cam lock fitting, while the tank next to it has a 32 mm barbed fitting attached to the elbow which can facilitate a clear PVC hose. On the end of the clear hose is a 32 mm female cam lock fitting which allows the hose to connect to the adjacent tank during transfer (Fig. 6).

Figure 4.   270 lt tank profile and 32 mm outlet (left) seawater inlet options; red arrow indicating degassing column and green arrows indicating 20 mm flexible hose.

Figure 5.   270 lt tank containing the false bottom (left) box filter with interchangeable screens (right).

Figure 6.   A pair of 270 lt tanks connected during larvae transfer

4.1.2.3   *Tank environment*

Flow rates to all tanks are set at 150 lt hr^{-1} (1.8 exchanges hr^{-1}). Water temperature is maintained at 20 – 21 °C ± 0.5 °C via a heater chiller unit. Photoperiod is 10 L 14 D with fluoro lights emitting 550 – 600 lux on the surface of the tanks during daylight hours.

4.1.3   1000 lt system

4.1.3.1   Tanks

This system comprises of 6 tanks of 1000 lt volume which are fibreglass, round and have a conical base. Each tank is subjected to upwelling flow through seawater via a 25 mm PVC manifold. Each set of 3 tanks are connected to each other with two 40 mm tank fittings and a threaded joiner to facilitate the transfer of larvae to a clean adjacent tank. Water exits the tank through a 40 mm outlet at the top of the tank (Fig. 7).

Figure 7.   1000 lt tank profile, green arrow indicating water inlet, red arrow indicating water outlet (left) two tanks connected with 40 mm tank fittings and joiner (right)

4.1.3.2   Tank components

Each tank has a large box filter with interchangeable mesh screens. Screen size is 250 or 500 µm dependent on the larvae size during an experiment. Each filter has 6 mm airline and porous pipe fitted to facilitate an internal and/or external air source. A 40 mm PVC standpipe is also located in each tank. The bottom of the standpipe is surrounded by 10 mm holes which are covered by 250 µm mesh. The holes allow water to pass through and the mesh stops larvae escaping. Each tank contains 2 automatic feeders that dispense a semi-moist 500 – 800 µm micro diet (Fig. 8).

During larvae transfer a 20 mm PVC frame with 4 mm porous pipe attached, is placed in the dirty tank. 40 mm threaded end caps on the inside of each tank, which normally cover the tank fitting holes, are removed. Removing the caps and closing the water outlet to the dirty tank in conjunction with the aeration from the frame, assist the larvae in shifting tanks (Fig. 9).

Figure 8. 1000 lt tank and the locality of the filter, standpipe and feeders (left) 40 mm standpipe profile and the locality of the 10 mm holes (right).
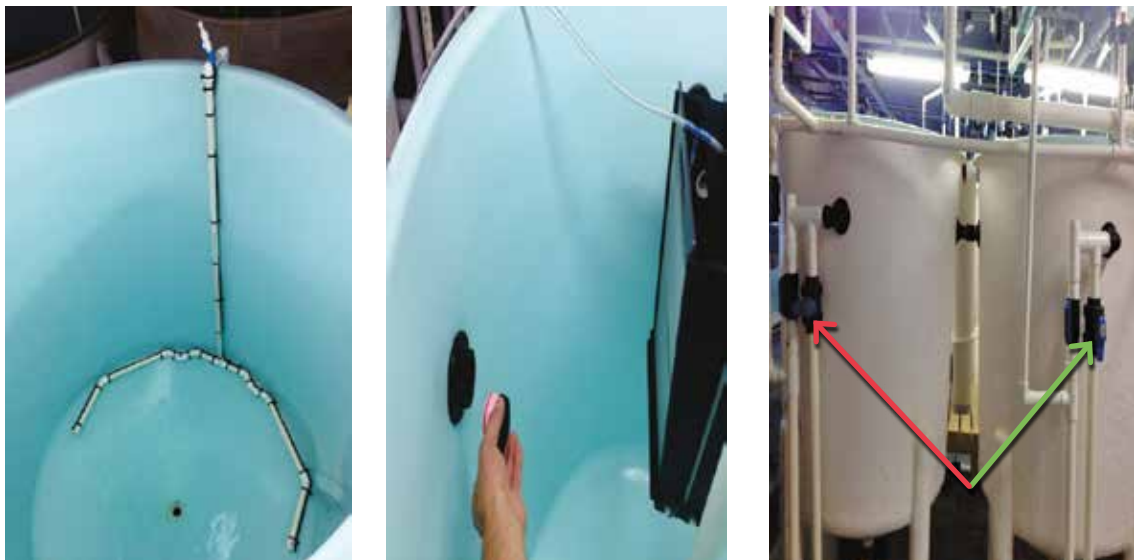
Figure 9. 20 mm PVC transfer frame with porous pipe (left) end cap removal prior to transfer (middle) external standpipe and valves. Red arrow indicating closed water outlet in dirty tank, green arrow indicating open water outlet in clean tank.

4.1.3.3 Tank environment

Flow rates to all tanks are set at 100 lt hr^{-1} (1 exchange per hr^{-1}). Water temperature is maintained at $21 - 23$ °C $\pm$ 0.5 °C via a heater chiller unit. Photoperiod was 10 L 14 D with fluoro lights emitting $550 - 600$ lux on the surface of the tanks during daylight hours.

4.1.4 Tank volume

4.1.4.1 Introduction

Previous studies on *Octopus vulgaris* have shown that tank volume significantly influences the growth of octopus larvae. Better results have been attained using larger tank volumes. It was thought that using 270 lt tanks will reduce stress that is generated in lower tank volumes as it

is suggested that fluctuations in physical conditions are less dramatic in larger volumes than in small ones (Allan and Burnell, 2013). The 40 lt tanks enable collection of data on survival and a better observation of individual larvae health during individual transfer.

4.1.4.2 Methods

System

The system is comprised of six pairs of transferrable tanks (270 lt) with flow through seawater, and three 40 lt tanks with flow through seawater. Two of these three tanks were 'transferred' and the other was 'stand alone'.

A wild caught female with eggs was sourced in March 2012 from a fisherman who fishes trigger pots off Rockingham (south of Fremantle). During this time another female produced eggs in captivity. This female was fed a mixed diet of fresh feed, consisting of Rock Lobster *Panulirus cygnus*, Blue Manna crabs *Portunus armatus*, mulies *Picton Herring*, and Prawns *Penaeus* sp. of which were injected with *Nutrabrood* (Nutrakol Pty Ltd) broodstock additive.

Table 1. Broodstock egg and larvae release information

Female	Date Eggs Found	Source of Eggs	Larvae Release (start date)	Incuba-tion Period	Water Temperature	Tanks Stocked	Comments
No.1	06/03/12	Wild	02/04/12	28 days	23°C	19/20, 23/24,K1, K2, K3, 17/18, 7/8, 5/6	Larvae released normally
No.2	17/02/12	Captive	20/03/12	33 days	23°C	9/10	Larvae released normally

* Tanks 5/6, 9/10 and 20 were originally stocked with captive larvae but crashed at ≤ 8 dph and were then restocked with wild larvae (omitted from table)

Table 2. Treatments and protocols at trial initiation

Tank	Larvae source	Stocking Density	Treatment	Algae Dosing	*Artemia* Feeds	Screen Size (μm)	Tem-perature (C°)	Flow Rate (L/hr)	Enrichment amount	Enrichment times and duration
5/6	Wild	1350	Clear water (no algae), no formalin, no transfer	None	3 x Daily	250	20	150	10g Nutrakol midday and afternoon, 20g Nutrakol Overnight	(1100 – 1200PM), (0900 – 1500PM), (0200 – 0800AM)
9/10	Captive	4000	algae, formalin, transfer	50ml in 20L SW	3 x Daily	250	20	150	10g Nutrakol midday and afternoon, 20g Nutrakol Overnight	(1100 – 1200PM), (0900 – 1500PM), (0200 – 0800AM)
17/18	Wild	4000	no formalin, algae, transfer	50ml in 20L SW	3 x Daily	250	20	150	10g Nutrakol midday and afternoon, 20g Nutrakol Overnight	(1100 – 1200PM), (0900 – 1500PM), (0200 – 0800AM)
19/20	Wild	4000	no formalin, transfer, algae	50ml in 20L SW	3 x Daily	250	20	150	10g Nutrakol midday and afternoon, 20g Nutrakol Overnight	(1100 – 1200PM), (0900 – 1500PM), (0200 – 0800AM)
23/24	Wild	4000	formalin, transfer, algae	50ml in 20L SW	3 x Daily	250	20	150	10g Nutrakol midday and afternoon, 20g Nutrakol Overnight	(1100 – 1200PM), (0900 – 1500PM), (0200 – 0800AM)
K1	Wild	500	No formalin, no transfer, no algae	None	3 x Daily	250	20	150	10g Nutrakol midday and afternoon, 20g Nutrakol Overnight	(1100 – 1200PM), (0900 – 1500PM), (0200 – 0800AM)
K2 & K3	Wild	500	No formalin, transfer	50ml in 20L SW	3 x Daily	250	20	150	10g Nutrakol midday and afternoon, 20g Nutrakol	(1100 – 1200PM), (0900 – 1500PM), (0200 – 0800AM)

* tanks 5/6, 9/10 and 20 were originally stocked with captive larvae but crashed at ≤ 8 dph and were then restocked with wild larvae
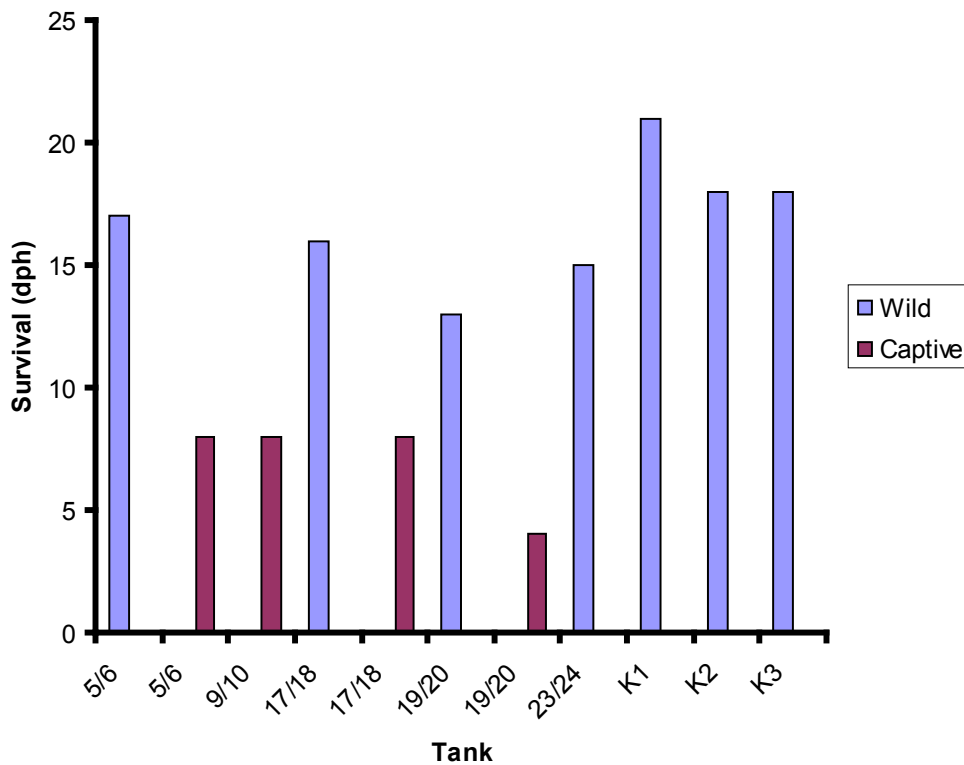
Figure 10.　　Survival dph of larvae sourced from both wild and captive eggs

Larvae hatched from eggs laid by a female in the wild yielded significantly higher survival than those larvae from eggs that were laid in captivity. The highest survival was achieved in tank K1, with larvae reaching 21 dph. All captive tanks showed lesser survival, with larvae reaching ≤ 8 dph (Fig. 10).
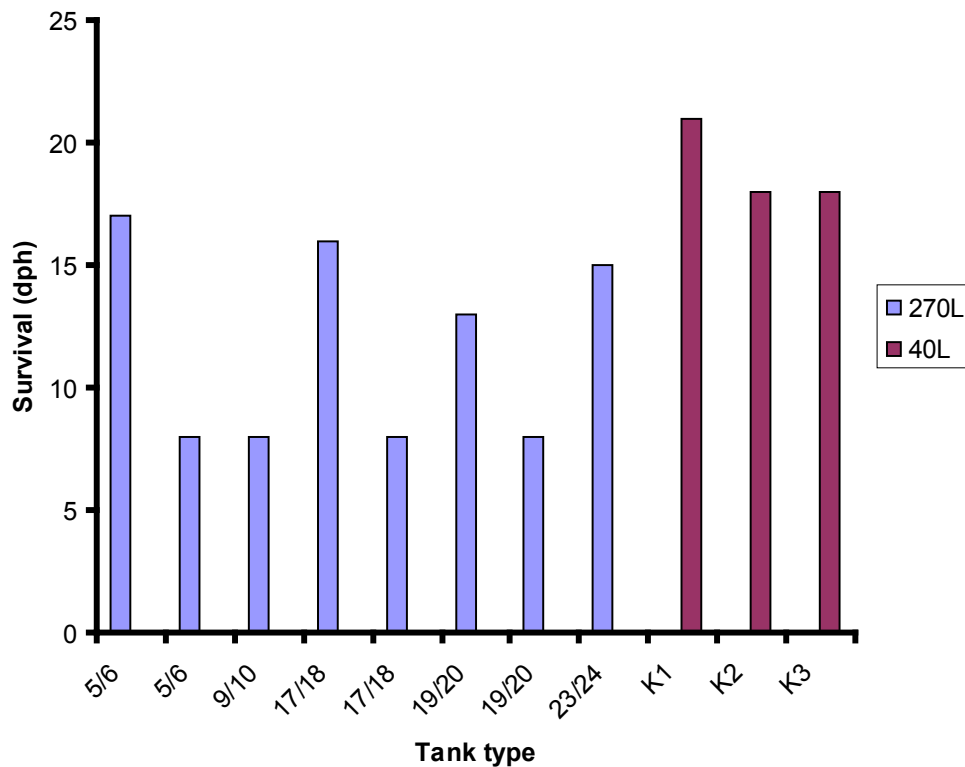
Figure 11.　　Survival dph of larvae reared in 270 lt and 40 lt (K) tanks

Larvae reared in 40 lt tanks had a greater survival than any other larvae in the 270 lt tanks, from both wild and captive broodstock. The greatest survival was achieved in K1 (40 lt), with larvae reaching up to 21 dph. These larvae were sourced from wild produced eggs. K2 and K3 achieved high survival overall with larvae reaching 18 dph. The highest survival of wild sourced larvae in the 270 lt tanks was in tank 5/6, with a survival of 17 dph (Fig. 11).
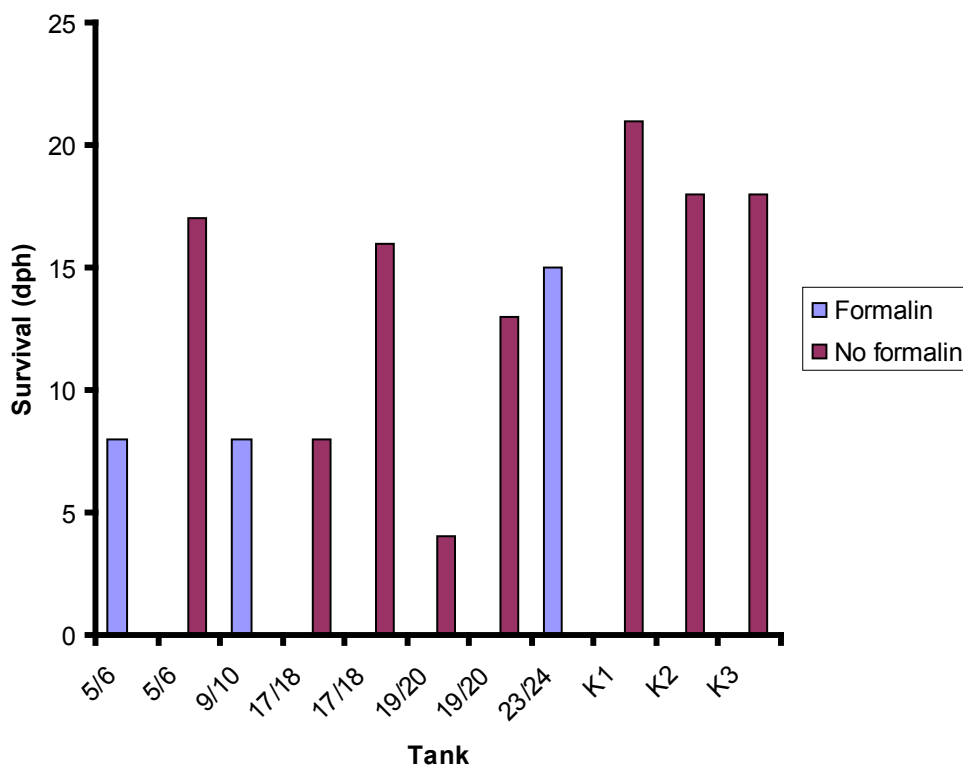
Figure 12.   Survival dph of larvae with formalin treatment and no formalin treatment

Larvae tanks that were not treated with formalin generally had a higher survival than those that were treated with formalin. Tank 17/18 and 19/20 were an exception to this trend. These tanks were operated as a recirculating system and had the lowest survival of tanks that were not treated with formalin, with a survival of 8 dph and 4 dph respectively. Tank 23/24 was the only tank treated with formalin that had larvae survives past 8 dph. The greatest survival was seen in tank K1, which was not treated with formalin, with larvae survival of 21 dph (Fig. 12).

4.1.4.3   Discussion

System

Over the duration of the run it was apparent that survival and growth of larvae was superior in the 40 lt (K) tanks. Larvae in these tanks survived longer and appeared visibly larger. A major factor, which may have facilitated this result, was a high water inflow of 150 lt hr^{-1} and an exchange rate (400%) that is eight times greater than in the 270 lt tanks (55%). This high water exchange greatly improves water quality, keeping the tank clean and free of any debris settling on the bottom, promotes better larval dispersal and improves dissolved oxygen levels in the water.

Larvae health and egg source

From our results it can be concluded that the hypotheses of wild sourced eggs producing better quality of larvae is supported, as almost all wild larvae survival was higher than that of captive larvae.

It can also be noted that formalin treatment of *Vibrio Spp.* in the tank did not appear to improve and may in fact be hindering larvae survival, with the five highest survival rates occurring in tanks that were not treated with formalin. Past experiments have shown that formalin treatments did aid in bringing levels of vibrios to a lower concentration in the tank, however it would appear that this strong chemical has adverse effects on larval survival. Transferring showed no consistent patterns of survival; although the greatest survival was achieved with no transfer, the second highest survival was achieved with transferring.

Larvae that were stocked in tank 5/6 with clear water and no addition of algae paste seemed to tolerate these conditions and survived to 17 dph. Omission of algae paste in this tank resulted in much better water quality and a cleaner tank, which may have contributed to these results. It can also be noted that tank 5/6 was not treated with formalin.

Observations (non-larvae related)

Toward the end of the trial it was discovered that contamination had occurred throughout our cultures, with the discovery of a large amount of *Copepod*s, ciliates and marine worms in the organic matter that had accumulated on the tank bottom. It is thought that these zooplankton are being transferred to the culture tank via the pot and eggs of the mothers which are being sourced from the ocean. There is a possibility that these zooplankton inhibited octopus larvae survival. The eradication of the contamination in the culture tanks needed to be addressed and a number of trials were performed to reduce bacteria levels in our cultures, and zooplankton contamination from females with eggs sourced from the ocean.

4.2   Nutrition

4.2.1   Amino acid treatment

4.2.1.1   Introduction

Nutrition is inhibiting larvae survival.

From previous survival and development results, it is apparent that larvae nutrition needs to be improved. New *Artemia* enrichments are to be developed and tested.

Survival can be improved by Amino Acid treatment. Based on literature research, it was established that survival of *O. vulgaris* larvae could be increased threefold with the addition of a daily amino acids solution (Villanueva *et al.*, 2004). Daily treatments of amino acids are to be incorporated into the tank in order to improve survival.

Treatments

1. *Artemia* with standard enrichment and 'Roti Diet'* (1 tank)

2. *Artemia* with crab enrichment (2 tanks)

3. *Artemia* with crab enrichment and mussel powder (2 tanks)

4. *Artemia* with crab enrichment, mussel powder and amino acid solution (2 tanks)

*Rotifer Diet 360 (Reed Aquaculture) is a marine microalgae concentrate of *Nannochlorpsis* sp and *Tetraselmis* sp that has a cell concentrate of ~57 billion per ml.

4.2.1.2 Methods

Cylindrical tanks in pairs of 7 (14 in total) with a volume of 40 lt were used for this experiment. Each tank had an interchangeable banjo screen, with temperature controlled flow through seawater. Tanks were operated as up welling with a flow of 150 lt hr^{-1}, where water flow was distributed through a clear acrylic diffuser with 1mm holes spread over the surface.

A 24 hr photoperiod was used with fluorescent lights positioned over tanks, emitting between 450-600 lux. Clear acrylic lids covered the top of each 40 lt tank.

Larvae were fed 6 times daily or as required, 1 to 6 hour enriched *Artemia* that were between 2-2.5 mm length (~ 14 days old). Overnight enrichment was over 3 hours (0600 – 0900 hr), lunch time enrichment over 1 hour (1100 –1200 hr) and afternoon enrichment over 1 hour (1400 – 1500 hr). *Artemia* were fed from cold storage via peristaltic pumps 3 times overnight from 4 buckets;

- 1 bucket: Enrichment w/Roti Diet (1 tanks)

- 1 bucket: Crab Enrichment (2 tanks)

- 1 bucket: Crab Enrichment w/mussel powder (2 tanks)

- 1 bucket: Crab Enrichment w/mussel powder + amino acids (2 tanks)

Feed densities were monitored to maintain required *Artemia* numbers, but at the same time not to over feed larvae. *Artemia* numbers were governed by size of mesh on screens and the jugging out excess when required.

Larvae were transferred individually into a new tank every Monday, Wednesday and Friday over the trial's duration. This was done with the use of a transfer pipette and a click counter to record survival.

Larvae for this trial were hatched from eggs that were spawned by a female held in captivity at Hillarys laboratories. The octopus was fed a fresh diet of prawns, mulies, abalone, rock lobster and crabs, all of which were injected with *Nutrabrood* (Nutrakol Pty Ltd) broodstock additive.

Table 3.　　　Amino acid composition (Villanueva, 1995)

AA	Mg lt^{-1}
Arginine	2.1
Histidine	2.1
Isoleucine	1.3
Leucine	1.3
Valine	1.2
Lysine	1.8
Phenylanine	1.7
Methionine	1.5
Threonine	1.2
Tyrosine	1.8

A treatment of Amino Acid solution (table. 3) was dosed to treatment tanks daily at a concentration of 600 mg lt^{-1}. Prior to treatment, flow in all tanks was stopped to allow for a static treatment for one hour. After one hour all flow was resumed to 150 lt hr^{-1}.
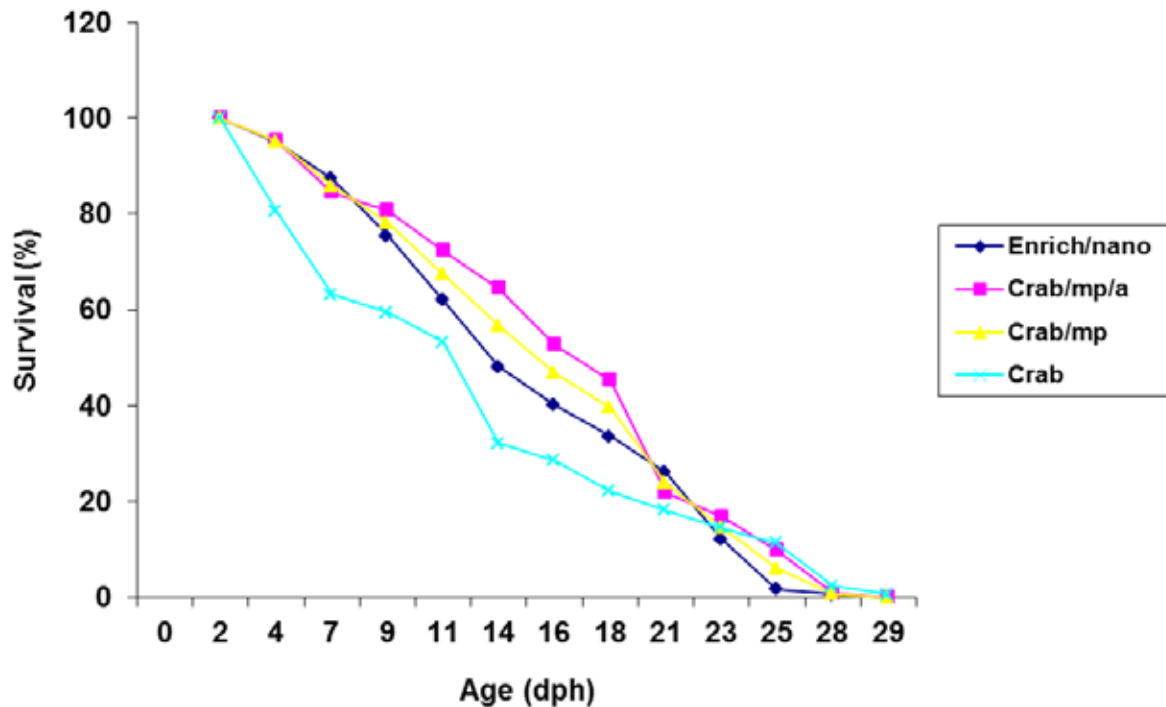
4.2.1.3   Results

Figure 13.   Average survival (%) of all treatments over trial duration.

It can be seen that all treatments ended with similar survival at 29 dph. The treatment which showed the greatest survival was the crab enrichment, with a survival of 0.6% when all other treatments were 0%. A decrease in survival can be observed for all treatments from 25 dph until trial completion at 29 dph (Fig. 13).

4.2.1.4   Discussion

Results suggest that *Artemia* with crab enrichment was the best form of nutrition for *O. tetricus* paralarvae in this trial. However, in order to reach a more conclusive result, more replicates of each treatment were needed.

Factors introduced in this trial which may have aided survival are the use of 40 lt tanks rather than 270 lt tanks, new transfer regime, the implementation of a 24 lt photoperiod and the addition of three automated feeds of enriched *Artemia* overnight. Use of the smaller 40 lt tanks to replace the 270 lt tanks was based on findings from a previous trial which showed superior survival in a tank with this volume. This was possibly due to a water inflow rate of 150 lt hr^{-1} causing an exchange rate that was eight times greater than what was experienced in the larger 270 lt tanks. A higher exchange rate in the 40 lt tanks looked to have greatly improved water quality and promoted better larvae dispersal and dissolved oxygen levels in the water. The change from passive transfer of larvae to a new tank, as was performed in previous trials, to individually transferring larvae was beneficial in a number of ways. It not only provided a more sterile environment for the larvae and minimised bacteria proliferation, but allowed us to accurately quantify our results in terms of larval survival. Transferring larvae three times per week provided a very clear overview of any major mortality events or high survival in a given tank at any stage of the trial. This also enabled us to observe each larvae individually and look at size, health, swimming behaviour, morphological changes or abnormalities.

4.2.2   Live feed treatment

4.2.2.1   Introduction

This experiment aimed to compare growth and survival of larvae fed solely *Artemia*, and a mix of live zooplankton and *Artemia* in an attempt improve larvae nutrition and therefore digestive health, growth and survival.

4.2.2.2   Methods

Two sets of 3 x 1000 lt fibreglass tanks with a conical base and 250 μm box filters. Tanks were operated as up welling, where water flow is distributed through 10 mm holes at the base of a 40 mm standpipe. The holes are covered in 250 μm mesh. 8500 larvae to each 1000 lt tank, counted using a click counter. Water in all tanks was maintained at 21°C via a heater chiller unit. A flow rate of 1000 lt hr^{-1} was maintained at all times. Temperature and Dissolved Oxygen measurements were taken daily using a dissolved oxygen meter.

Larvae tanks were transferred to adjacent clean tank every 7 days passively using the double tank system. A photoperiod of 10/14 light/dark at 550 - 600 lux was used.

Excess live feed from the tank bottom and filters was siphoned daily into screen bucket and remaining live larvae returned to tank using pipette.

Feeding

Larvae were fed 6 times a day, or as required, enriched *Artemia* that are between 1.5 - 3.0 mm. Overnight, 50,000 *Artemia* per tank were fed via cold storage 3 times at 1800 hrs, 0000 hrs and 0600 hrs. Each morning live *Copepod*s were fed together with 25 gr of frozen *Copepod*s mixed with 6 lt seawater. All tanks were fed 500-800 μm semi moist micro-diet from 10 dph via an automated feeding system.

Enrichment

Artemia were given 30 gr enrichment (Nutrakol Pty Ltd) poured into in 1.5 lt seawater, blended for 1 - 3 minutes and split into 3 bottles (500 ml each bottle). 1 bottle (overnight enrichment) was topped up with 500 ml seawater and put into a fridge for overnight dosing. *Artemia* were enriched for 6 hours overnight (0200 - 0800 hr), for 1 hour at 11:00 am, and 1 hour at 2:00 pm.

Live zooplankton was collected from the ocean at Hillarys boat harbour on a daily basis. The collecting system was 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 was then 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. The majority of species that were collected were *Copepods* and some crab larvae.
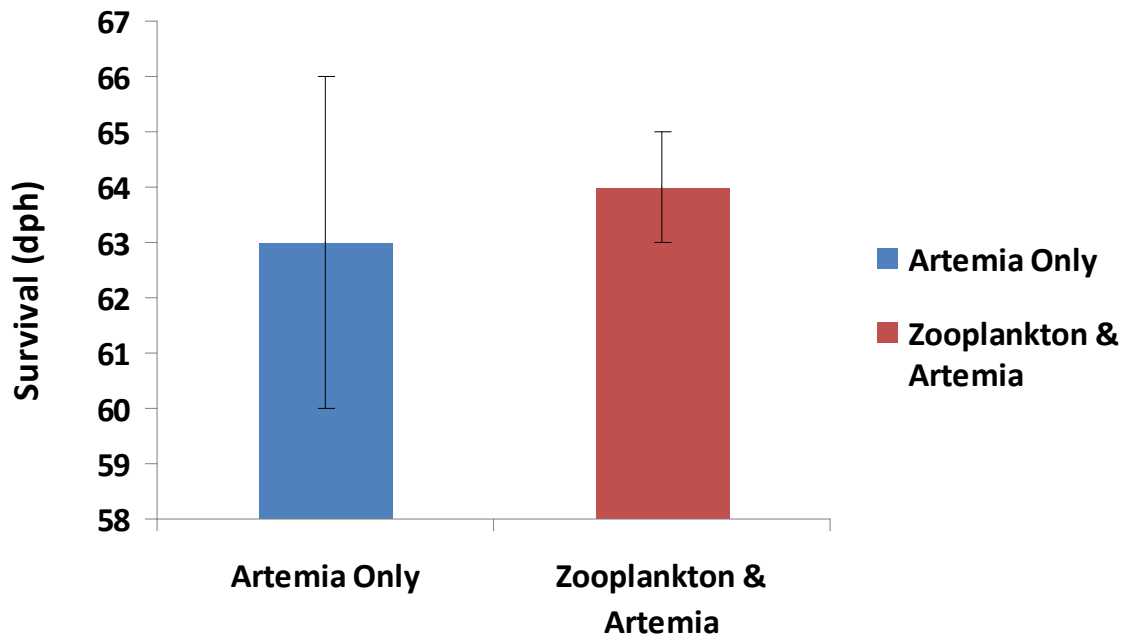
Figure 14.   Average survival (dph) at trial completion of two tanks fed solely *Artemia* and two tanks fed a mixture of live zooplankton and *Artemia*.

There was no significant difference in larvae survival between the two treatments. The two tanks fed solely *Artemia* finished with an average survival of 63 dph while the two tanks fed a mixture of live zooplankton and *Artemia* finished with an average survival of 64 dph (Fig. 14).
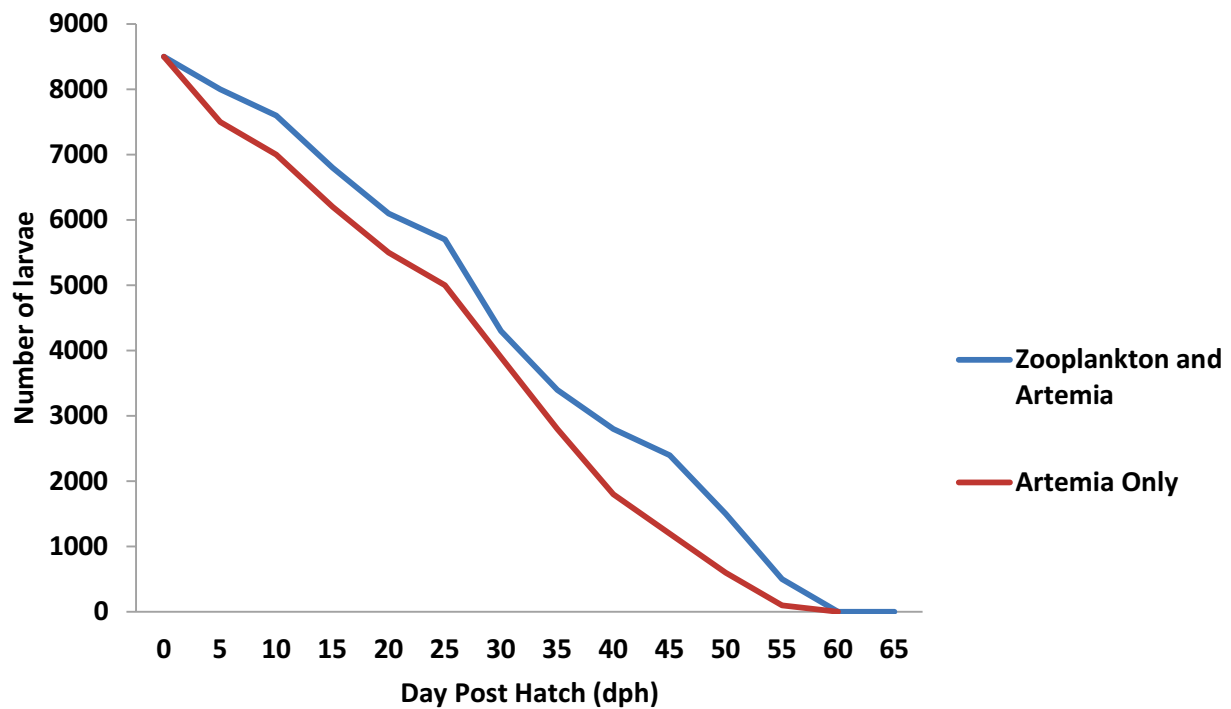
Figure 15.   The number of larvae recorded across both treatments over the trial duration.

The survival of larvae fed solely *Artemia* and a combination of zooplankton and *Artemia* did not vary greatly over the trial duration. The initial stocking density of 8500 larvae decreased steadily from day 0 to finish at an average of 1 remaining larvae at day 65 for the zooplankton and *Artemia* treatment and an average of 1 remaining larvae at day 60 for the *Artemia* only treatment. The greatest difference in larvae survival occurred on day 45 when 2400 larvae were recorded in the zooplankton and *Artemia* treatment and 1200 larvae recorded in *Artemia* only treatment meaning a difference of 1200 larvae (Fig. 15)
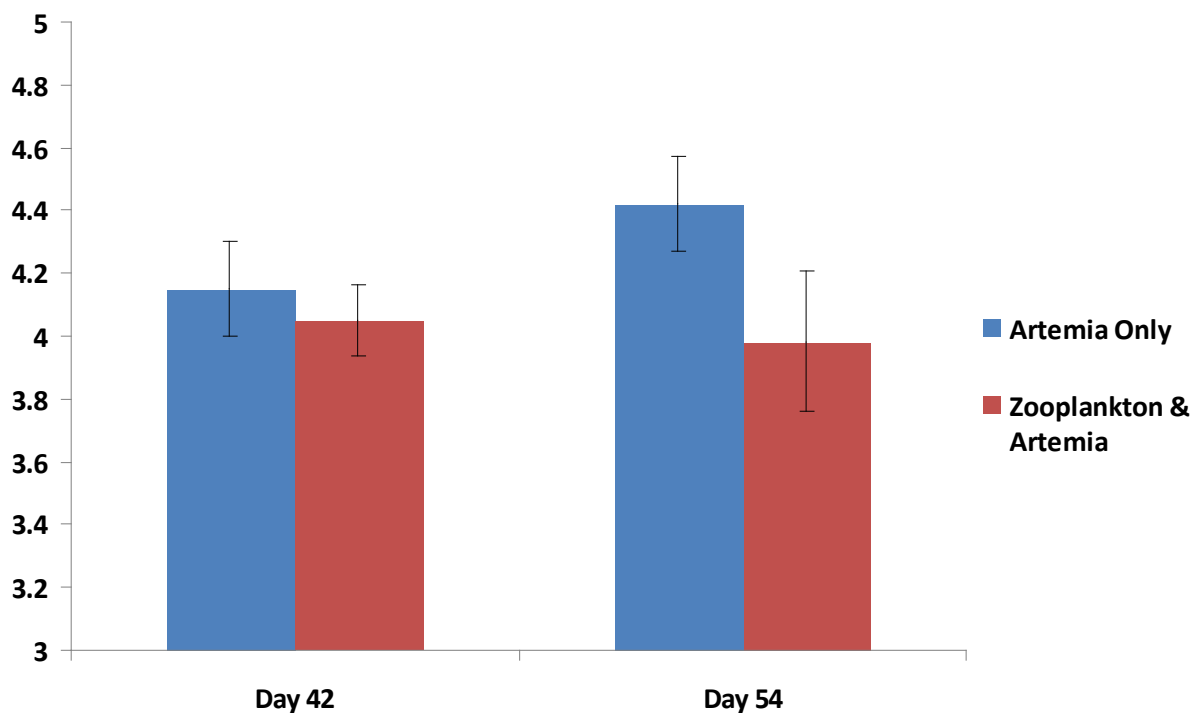
Figure 16.   Average total length (top of mantle to tentacle tip) of larvae from the two treatments at 42 dph and 54 dph.

The size of larvae did not increase across both treatments as the larvae got older. The larvae fed only *Artemia* increased in size over the 12 day period between 42 dph and 54 dph by 0.2675 mm, while the larvae fed a mixture of live zooplankton and *Artemia* decreased in size over the same period by 0.0663 mm. Larvae fed only *Artemia* measured 4.153 mm on day 42 and 4.42 mm on day 54 while larvae fed a mix of live zooplankton and *Artemia* measured 4.048 mm and 3.983 mm on day 42 and 54 respectively (Fig. 16).

4.2.2.4   Discussion

Larvae survival

The survival achieved in this trial was our highest since March 2011 when a 65 day old larvae was produced. It is thought that this was due to a combination of factors;

- Increasing tank volume from 40 lt to 1000 lt, which gave the larvae more space to feed, move around and escape other aggressive larvae.

- Persisting with a photoperiod (14Dark 10Light) for the whole trial which was more similar to that experienced in nature

- Feeding of micro-diet to the larvae from day 10 onwards giving them an alternate food

source to the *Artemia*. The micro-diet had a different protein-lipid ratio to the *Artemia* which may have given the larvae a better nutritional profile.

- Feeding of "wild" live and frozen zooplankton to two treatment tanks which provided an alternate food source to that of the *Artemia* and micro-diet.

- Daily siphoning of the bottom and screens in all tanks which eliminated the build-up of organic matter and bacteria.

- Passively transferring larvae from one tank to another every 7 days. This meant the larvae weren't over-handled like that of the 40 lt tank trials when larvae were individually counted 3 times a week.

Larvae behaviour and timeline of observations

Due to the longevity of this trial, a lot of visual observations were made across all treatment tanks. Observations made after 30 days old were especially noted, as larvae in our previous trials have had a tendency to die before then. Some observations of note were as follows:

- Day 1 – larvae observed eating frozen Copepods.

- Day 10 – larvae in all tanks observed eating 500-800 µm micro-diet.

- Day 24 – noticeable increase in larvae size and strength, numbers in tanks starting to taper off slowly.

- Day 34 – larvae in tanks observed attacking and wrestling with big Artemia (2.5 – 3mm).

- Day 36 – noticeable increase in feeding across all tanks with larvae getting through 100-150'000 Artemia in a couple of hours.

- Day 37 – size increase again in larvae although numbers seem to declining slowly again.

- Day 39 – larvae feeding actively at the surface on Artemia at an age where they thought to be on or around the bottom and sides of the tanks.

- Day 43 – decrease in larvae numbers across all tanks.

- Day 44 – feeding activity decreased across all tanks, a lot of Artemia remaining in tanks.

- Day 48 – larvae that are being fed 'wild zooplankton' look visibly bigger than those fed only Artemia.

- Day 50 – larvae still very active in the mid-surface part of the water column.

- Day 52 – decrease in larvae numbers across all tanks.

- Day 55 – Larvae moving near or on the bottom of most tanks, a few of them looking really well developed.

- Day 56 – 5 larvae moved to a 'pre settlement' tank with a volume of 50 lt and water exchange rate, light and feed regime to that experienced in the 1000L tanks. Hides made of 20 mm pipe put in that tank also.

- Day 57 – larvae numbers dropping all 1000 lt tanks, larvae in 'pre-settlement' tank not eating and mostly are on the bottom of the tank with tentacles attached and body vertically upwards.

- Day 58 – remaining larvae in 1000L tanks mostly on bottom or sitting on the wall of the tank under the feeders. One larvae in the "pre-settlement" tanks observed sitting in the hides provided.

- Day 61 – dramatic drop-off in larvae numbers across all 1000 lt tanks, < 10 in all tanks.
- Day 66 – All larvae dead.
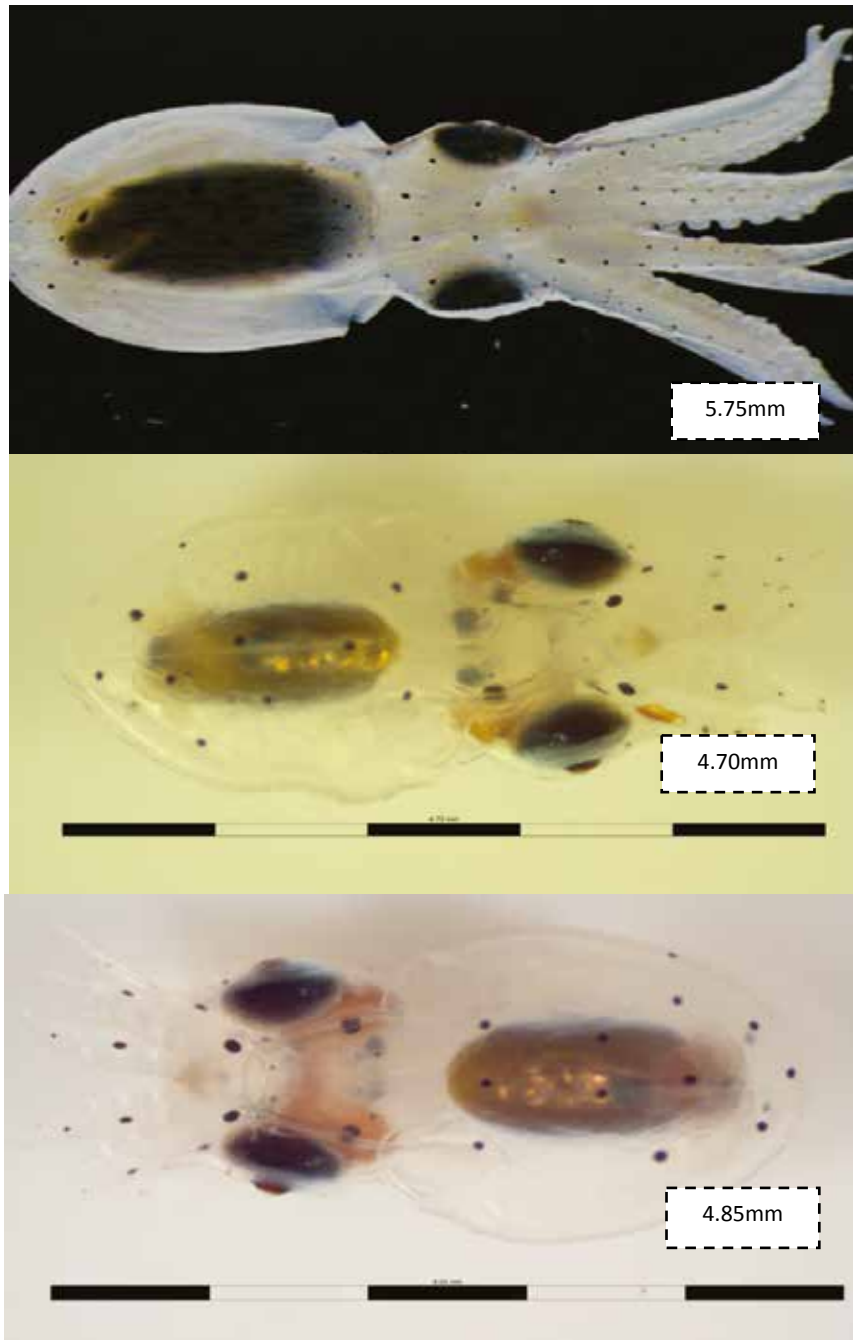
Figure 17.   A 54 day old larvae fed a combination of zooplankton and *Artemia* (top), a 54 day old larvae fed only *Artemia* (middle) and a 52 day old *Octopus vulgaris* larvae fed crustacean zoea (bottom, (Iglesias *et al.*, 2007, vol 266, p. 3))

Larvae measurements and photographs assisted our group in visually indicating that the larvae fed solely *Artemia* (top), were only slightly bigger than the larvae fed a mix of zooplankton and *Artemia* (middle) at day 54 (Fig. 17). The larvae from the two different treatments in this trial did not seem to differ greatly morphologically at this age with both larvae exhibiting tentacles and a mantle of similar length.

Available literature on the biology and morphology of *Octopus vulgaris* larvae suggest that there development during the planktonic phase closely mirrors that of *Octopus tetricus* larvae. This coupled with the fact that no literature exists on the larvae culture of *O. tetricus*, has forced our group to use techniques currently used in *O.vulgaris* larvae culture while implementing some of our own methods. Although the larvae survival in this trial was our best to date, the 52 day old *O.vulgaris* larvae at the bottom of figure 17 suggests that our larvae at day 54 were very much under developed. Our larvae exhibited much smaller tentacles and a less defined mantle, however their behaviour on day 55 and thereafter, is consistent with literature detailing behaviour during the pre-settlement of *O.vulgaris* larvae (Villanueva, R., 1995).

4.3   Environment

4.3.1   Live *Chlorella*

4.3.1.1   Introduction

Based on a series of results showing that bacterial treatments did not improve survival, along with literature research, it was decided that the focus needed to be shifted to larvae nutrition. Bacteria found on the larvae were possibly an indicator of poor health due to lack of adequate nutrition. Live *Chlorella* microalgae (Pacific Trading Pty Ltd) was used in this trial as greenwater. This concentrated *Chlorella* solution contains DHA and EPA required by many marine larvae in aquaculture. All tanks received enriched *Artemia* while half of the small tanks were also dosed with live *Chlorella* as a greenwater culture. One 1000 lt tank was run under the same culture conditions including greenwater. It is thought that larvae survival will improve with high protein/low lipid enrichment, and thatreenwater culture of *Chlorella* will improve larvae survival by providing DHA and EPA and vitamins to *Artemia*.

4.3.1.2   Methods

Three treatments were tested in this experiment; treatment 1: *Artemia* enrichment containing crab homogenate and *spirulina* (i.e. 'crab' enrichment), treatment 2: crab enrichment greenwater, and treatment 3: comparison of larvae survival between 40 lt and 1000 lt culture tanks.

The 40 lt tank system was comprised of 7 pairs (14) of cylindrical tanks with interchangeable 'banjo' screens. The 1000 lt system was comprised of 1 pair of fibreglass tanks with a conical base. The 40 lt tanks were operated as up welling, where water flow was distributed through a clear acrylic diffuser with 1 mm holes spread over the surface. The 1000 lt tanks were operated as up welling, where water flow was distributed through 10 mm holes at the flat base of the tank. Holes were covered in 250 μm mesh. Clear acrylic lids were used to cover tops of the 40 lt tanks to prevent larvae escaping and keep contaminants out.

550 larvae were stocked in the 40 lt tank, which were counted individually into tanks in groups of 10 using pipettes. The 1000 lt tank was stocked with 12,500 larvae counted using this same method.

Water in all tanks was maintained at 21°C. The flow rate for 40 lt tanks was 150 lt hr^{-1} while the flow rate for 1000 lt tanks was 1000 lt hr^{-1}. Temperature and Dissolved Oxygen measurements were taken daily using a dissolved oxygen meter (Oxyguard). The 1000 lt tank was siphoned once daily of excess *Artemia* and microdiet into screen bucket and remaining live larvae were returned to the tank using a pipette.

To prevent accumulation of debris and bacteria, larvae were passively transferred from culture tank to new clean tank, starting at 7 dph. Larvae were transferred to adjacent clean tank on an ad-hoc basis, judging tank cleanliness daily. These larvae were counted individually using a pipette in the 40 lt tank, while larvae were transferred passively when 1000 lt tanks were transferred.

Light intensity was 300-600 lux. 24 hour light was used initially, after which the photoperiod was changed to 10/14 light/dark when larvae were observed to have grown and shifted down the water column.

Larvae were fed 6 times a day or as required enriched *Artemia* that are between 1.5-2.5 mm. *Artemia* were enriched for 6 hours overnight and for 1 hour at 12:00 pm, and 3:00 pm. *Artemia* were fed to larvae via cold storage 3 times overnight at 1900 hrs, 0000 hrs and 0500 hrs. Feed densities were monitored as to not have excess of *Artemia*, but at the same time not to overfeed larvae.

The enrichment was specifically designed and made for octopus larvae (Nutrakol Pty Ltd). The enrichment includes fresh blue mana crab meat homogenate (filtered to 50 μm) as well as freeze dried *Spirulina*. These additives were included in the enrichment to increase the protein level (standard *Artemia* enrichment does not include any protein). From 7 dph, larvae were also fed semi-moist microdiet (250-500 μm, Nutrakol Pty Ltd).

Frozen and live blue mana crab *Portunus pelagicus* zoeae that were released from wild collected females were fed to octopus larvae on a daily basis.

The 40 lt tanks consisted of clear water culture for three tanks and greenwater culture for four tanks (*Chlorella*). Greenwater culture was used in the 1000 lt tank. Algae was dosed manually at 8:00 am (10 ml dosed to 40 lt tanks and 100 ml dosed to 1000 lt tanks) after which algae is dosed from a tub via peristaltic pumps. Greenwater culture was kept at 40-50 cm depth using secchi disk.

4.3.1.3   Results
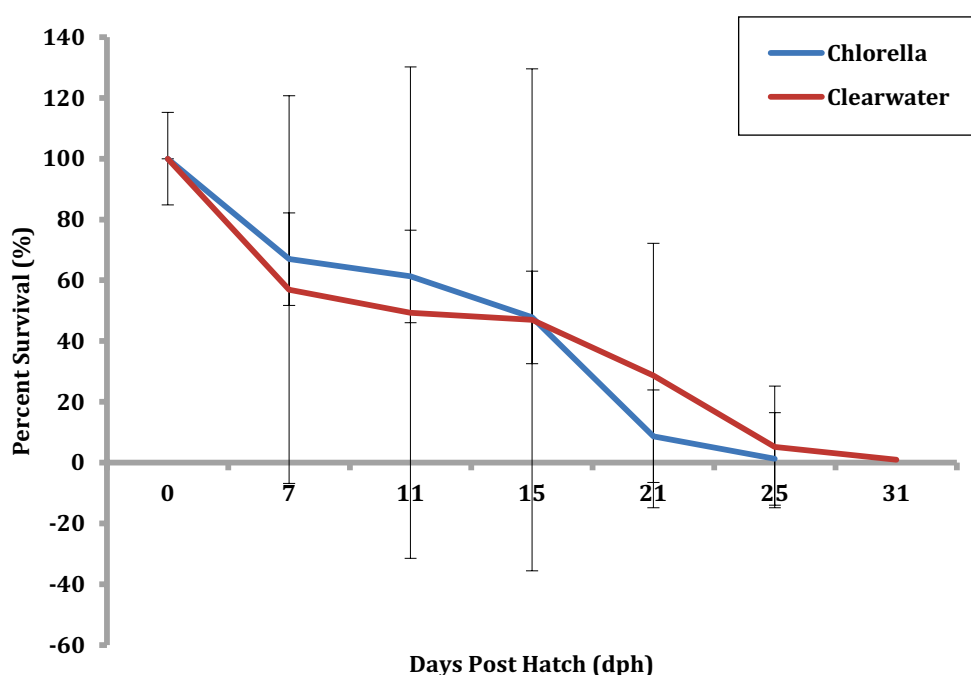
Figure 18.   Survival of larvae (%) showing both *Chlorella* and Clearwater treatments from hatching (0 dph) to 31 dph.

Larvae survival was not improved when greenwater culture was used. From 7 to 15 dph the *Chlorella* treatment had a higher survival with an average of 58.64% while the Clearwater treatment had an average survival of 51.03%. After 15 dph *Chlorella* treatment survival decreased and reached 1.2% at 25 dph while Clearwater continued until 31 dph reaching 0.9% survival. There was large variation in survival in both treatments throughout the trial duration (Fig. 18).

4.3.1.4   Discussion

From the data it would appear that addition of *Chlorella* as a greenwater treatment did not improve larvae survival in the 40 lt tanks and no trend was observed. However, according to histological analysis, there were a few differences between greenwater and clear water treatments and also the volume of the tank that larvae were cultured in. At 17-21 dph, larvae from the clear water treatment were reported to appear healthier than greenwater specimens at the same age. This was evident in the state of their digestive system. From 17-25 dph, larvae samples from the 1000 lt tank were observed to be more robust, much larger and in good condition with food in the digestive system. The fact that the 1000 lt tank was the only tank remaining with larvae at day 33 supports these findings. However, it was not possible to establish a survival percentage with this volume and high stocking density as passive transfer was used rather than individual transfer of larvae. Due to the fact that only one 1000 lt tank was used, it is difficult to establish whether higher survival was achieved as a result of the *Chlorella*, a larger tank volume with less fluctuation in physical conditions, or a combination of the two. Current experiments are now testing the use of large tanks.

4.3.2   Temperature

4.3.2.1   Introduction

This experiment was aimed at testing the effect of water temperature on growth and survival of *Octopus tetricus* larvae. It was decided to increase the water temperature to 23°C as studies on *Octopus vulgaris* have shown that increasing the water temperature can decrease the duration of the planktonic phase (Katsanevakis & Verriopoulos, 2006).

4.3.2.2   Methods

Two sets of 3 x 1000 lt fibreglass tanks with a conical base and 250 µm box filters were used. Tanks were operated as up welling, where water flow is distributed through 10 mm holes at the base of a 40 mm standpipe. The holes are covered in 250 µm mesh. Larvae were stocked at a concentration of 8000 to each 1000 lt tank, counted using a click counter.

Larvae were manually fed enriched *Artemia* 3 times a day at 0800 hrs, 1200 hrs and 1500 hrs and automatically via cold storage at 1800 hrs, 0000 hrs and 0600 hrs. All tanks were fed 500-800 µm micro-diet from 0 days dph via automated feeding system. Feed densities were monitored as to not have excess of *Artemia*, but at the same time not to overfeed larvae.

Artemia were fed 30 gr enrichment (Nutrakol Pty Ltd) poured into in 1.5 lt seawater and blended for 1-3 minutes and split into 3 bottles. Bottle 1 was cold stored for a 1 hour enrichment at 1100 hrs till 1200 hrs, bottle 2 was for a 1 hour enrichment at 1400 hrs till 1500 hrs, while bottle 3 was topped up with 500 mls seawater for an automatic 6 hour enrichment at 0200 hrs till 0800 hrs.

Water in all tanks was maintained at 23°C via a heater chiller unit. A flow rate of 1000 lt hr^{-1} was maintained at all times. Temperature and Dissolved Oxygen measurements were taken daily using a dissolved oxygen meter.

Larvae tanks were transferred to adjacent clean tank every 7 days passively.

A photoperiod of 10 hrs light and 14 hrs dark at 550-600 lux was used. Tank bottom and excess live feed was siphoned daily into screen bucket and remaining live larvae returned to tank using pipette. Larvae hatched from eggs laid by captive brood stock were used in this trial.

4.3.2.3   Results

Survival
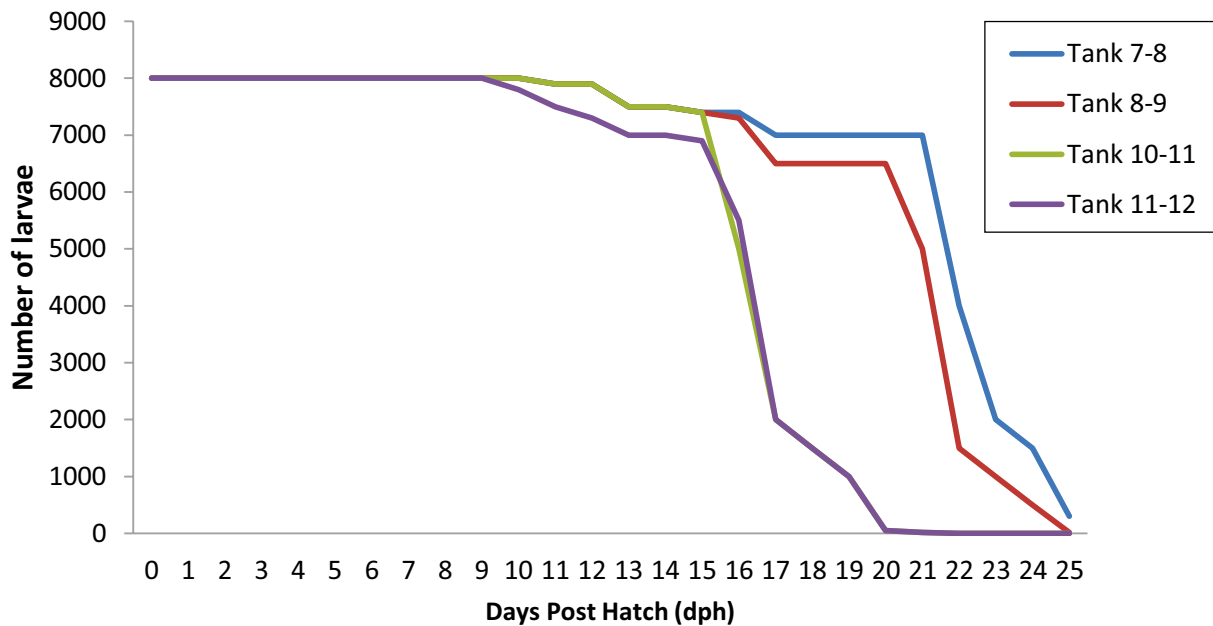
Figure 19.   Larvae survival across all 4 treatment tanks over the trial duration.

Larvae survival was poor across all tanks with the trial ending at day 25 (Fig. 19). Tank 7-8 had the highest survival at the end of the trial with an approximate survival of 3.75%, while tank 8-9 had the second highest at approximately 0.25%. The tanks with the lowest survival were tanks 10/11 and 11/12 with 0% survival at 22 dph.
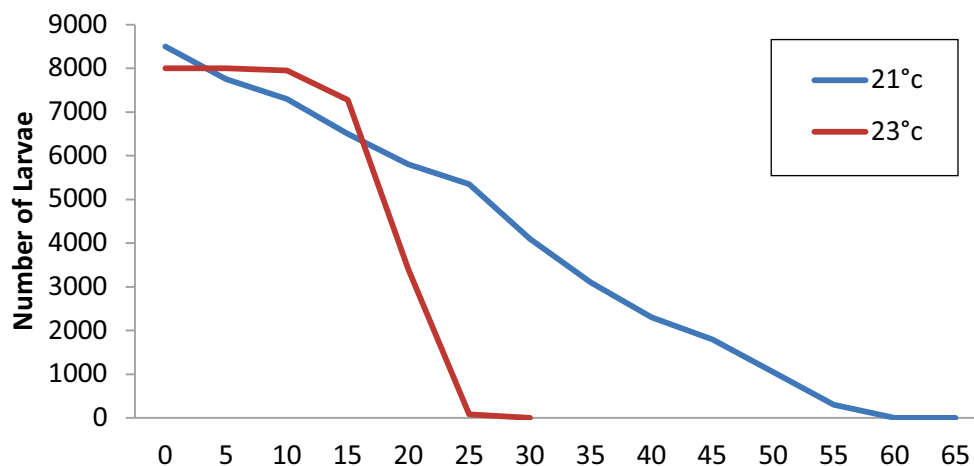
Figure 20.   Comparison of larvae survival when culture at 23°C and 21°C

* Larvae cultured at 23°C were hatched from captive brood stock

Larvae survival was much less when the temperature was increased to 23°C from 21°C (Fig. 20). On average, larvae cultured at 21°C survived 65 days, compared to 23°C in which larvae survived to 30 dph. A pronounced drop off at day 15 at 23°C was recorded in which survival dropped from an average of 7275 to 80 larvae over 10 days, while survival at 21°C decreased steadily over the duration of the trial.

Growth and Morphology
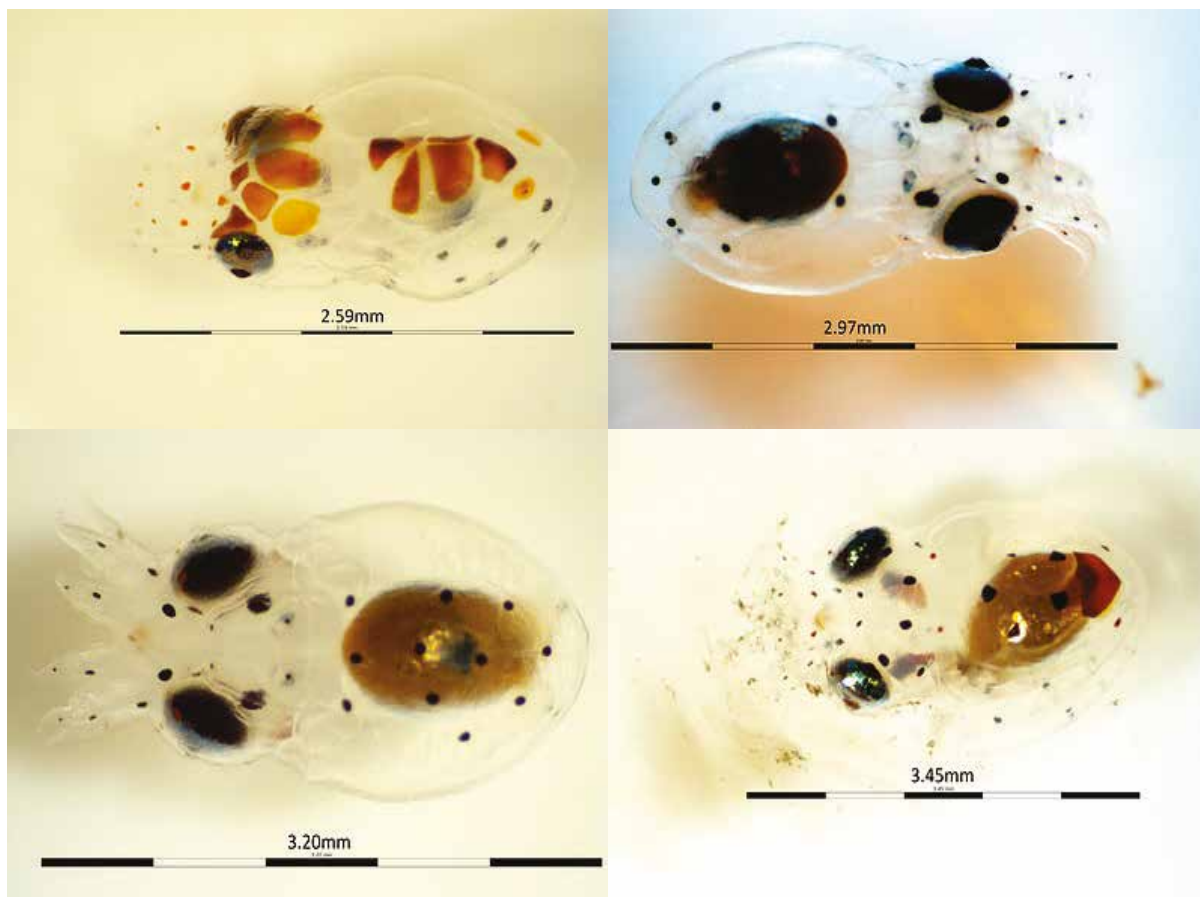
Figure 21.   Larvae profile at 0 dph (top left), 7 dph (top right), 14 dph (bottom left) and 22 dph (bottom right)

Octopus larvae did increase in size over the duration of the trial; however morphological changes were minimal over this period (Fig. 21). Newly hatched octopus larvae (day 0) measured ~ 2.59 mm, and increased to ~ 3.45 mm 22 dph, meaning overall growth over this period was ~ 0.86 mm. No measurements were taken prior to 37 dph in the previous trial at 21°C.

4.3.2.4   *Discussion*

It was evident during this trial that raising the water temperature by 2°C from 21-23°C not only did not accelerate larvae development during the planktonic phase, but also caused ill health with subsequent mortalities not long after. Mortality events were recorded as early as 10 dph, which is uncommon at 21°C, due to the larvae being very much still in their planktonic phase. During this phase they are predominantly near surface waters away from the bottom of the tank (Villanueva & Norman 2008) where decomposing organic matter and bacteria can harm them. At 10 dph in this trial, they were observed to be resting or skipping along the bottom and also mid water clinging to the side of the tanks. Larvae observed exhibiting these behaviours were usually found dead the next day. Mortality was observed almost daily after 10 dph until 25 dph when the trial was stopped.

Larvae that were used in this trial were hatched from eggs laid by captive broodstock. Although broodstock held at WAFMRL are fed high quality fresh feeds with nutritional additives, the quality of larvae they produce does not compare with that from females carrying eggs sourced from the wild. This may have been a factor that contributed to low survival in this trial.

Due to the higher temperatures in this trial, tanks became dirtier than normally observed at 21°C. This was seen in the form of protein build-up on the surface of all the culture tanks. Micro-diet being dosed in to the tanks tended to break down and leach protein quicker at high temperatures; it is not sure how this affected larvae health and well-being.

A higher occurrence of pink bacteria was also observed in this trial on the bottom of the culture tanks. This was thought to result from organic matter breaking down quicker on the tank floor at higher temperature. Occurrence of these bacteria on the bottom of the tanks where larvae were feeding and inhabiting was detrimental to their health and caused mortalities.

4.4   Bacteria treatment

4.4.1   Formalin treatment

4.4.1.1   Introduction

Based on the theory that bacteria is inhibiting larvae survival, formalin treatment was implemented to prevent bacterial proliferation and therefore improve larvae health and survival.

4.4.1.2   Methods

Tanks were operated as up welling, with a 6 mm PVC plate placed at the apex of the cone. The plate contained 2mm holes spread across drilled at the corner of 20 mm squares (Fig. 23). The temperature was kept constant at 20°C with the use of a heater chiller. The inflow to each larvae tank was 150 lt hr^{-1}.

Figure 22.   Double tank system for passive transfer of larvae

Figure 23.   The plate placed in the bottom of the larvae tank

A wild octopus female in a shelter pot with her eggs was sourced from Cockburn Sound with the help of Fremantle Octopus fishermen. The octopus was put into a flow-through tank on the boat and transported to The Department of Fisheries Watermans Bay Research Centre. The larvae hatched after 40 days at 20ºC.

Larvae were stocked at a rate of 10,000 larvae per 27 lt hr^{-1} tank (~40 larvae lt^{-1}) using jugs. Due to limited larvae numbers (octopus larvae hatched over 7-10 days and therefore daily hatching numbers are limited), three double-tanks and two standard 'stand-alone' tanks were stocked.

Artemia were hatched and grown for 5-10 days to reach a length of 2-2.5 mm. The *Artemia* were fed with *Dunaliella salina* microalgae paste (Cognis Australia). Prior to feeding the larvae, the *Artemia* were harvested from the growout tanks and enriched with 'tailor-made' enrichment (Artikol, Nutrakol Pty Ltd) and 'Roti Diet' (concentrated green algae, Reed Aquaculture) for 3 hours. Larvae were fed 3 times a day at a rate of 20,000-30,000 *Artemia* per feeding event. *Artemia* densities were monitored to prevent an excess of feed, but at the same time, not to under feed the larvae. Excess *Artemia* were jugged out prior to each addition of freshly enriched *Artemia*.

To create 'green water' in the larvae tanks, concentrated algae (Reed Aquaculture) dosed via peristaltic pumps from tub at a concentration of 85 ml/larval tank/day^{-1}. The algae tank was cleaned and sterilised daily before being refilled.

Temperature, Dissolved Oxygen and ammonia measurements were monitored daily. Water samples for bacterial analysis were taken from each tank during the run.

In the double-tank setup, the larvae were transferred overnight from the 'dirty' tank to the adjacent clean tank every 4 days. The flow in existing larvae tank was set at 150 lt hr^{-1} while the adjacent (clean) tank was set at 50 lt hr^{-1}. Light aeration in existing larvae tank was used to assist in the lateral movement of larvae. Since the outlet of the existing tank was connected to the inlet of the adjacent tank, the water flow to the clean tank with larvae creating passive

and gentle transfer of larvae overnight. A volume of 10 ml of algae concentrate was manually dosed to the new clean tank before transfer was started. The algae dosing line was left in the original tank during transfer. The following morning, the larvae were all in the 'clean' tank and the previous tank was drained cleaned and disinfected ready for the next transfer.

Every tank was treated with formalin at a concentration of 10 ppm (2.7 ml Formalin/tank) every 4 days. Formalin was diluted in seawater and the solution was dosed via peristaltic pumps for 15 minutes. After the formalin dosing, the tanks water flow was turned off for 1 hour. Water flow was restored to 150 lt hr^{-1} after the 1 hour treatment was completed.

The purpose of bacteria sampling was to analyse the effects of both formalin treatments and vacuuming as a method of reducing bacteria levels in larvae tanks.

Sampling was conducted on the 6th and the 11th of April. The bacteria counts were the result of water samples that were taken from the tanks before and after the tested treatment. The sampling procedure was the same before and after the treatment was carried out. The procedure was as follows:

- A number of sterile 50 ml sample vials were labeled with a number that corresponded to a tank number and treatment in an electronic data sheet.

- The vial was then filled with water from each tank that was to be tested and the lid screwed on. Gloves were worn during this time to prevent contamination.

- Samples were then placed on ice and kept below 4°C until transportation to the Department of Agriculture.

Microbiological analysis was carried out by the Animal Health Unit at the Agriculture Department as follows: water sample from larvae tanks were serial diluted (Quinn *et al.*, 1994) and a 100 µl sample was spread onto MSA-B (Marine Salt Agar-Blood) using TSBA (Trypticase Soya Broth Agar). Samples were plated at each of the dilutions produced. All agar plating was carried out in a laminar flow cabinet in sterile conditions. All equipment was sterilized using flame, ethanol spray or an autoclave. Dilution media was tested for microbial activity to guarantee sterility of media and methods.

The plates were left at room temperature (~25ºC) for 24 h before counting colonies formed per plate. Whole plates were counted when possible or two quarter plate areas were counted, averaged and multiplied by four, when colony numbers were high (Quinn *et al.*, 1994). Complete bacterial identification was completed according to Quinn *et al.*, (1994). Identification focused on Vibrio sp., as this genus of bacteria are known marine finfish pathogens and are commonly found associated with *Artemia* (Olafsen, 2001; Verschuere *et al.*, 2000).

Tank #	Group	Stocking date	# of larvae	Artemia size (mm)	Initial temp	Temp after 5 dph	Average DO (mg lt^{-1})	Formalin	Cleaning	Enrichment	Lighting (lux)	Survival (dph)	Mother
7/8	A	26/03/11	5000	~2-2.5	20	20	7.5	Every 4th day 10ppm/1hr	Transfer every 4th day	5 ml rot diet 2XAM, 10 ml PM	320	32	Wild with eggs
17/18	B	26/03/11	5000	~2-2.5	20	20	7.5	Every 4th day 10ppm/1hr	Transfer every 4th day	5 ml rot diet 2XAM, 10 ml PM	320	62	Wild with eggs
19	C	01/04/11	10000	~2-2.5	20	20	7.5	Every 4th day 10ppm/1hr	Vacuum every 2-3 days	5 ml rot diet 2XAM, 10 ml PM	320	45	Wild with eggs
20	B	28/03/11	4000	~2-2.5	20	20	7.5	Every 4th day 10ppm/1hr	Vacuum every 2-3 days	5 ml rot diet 2XAM, 10 ml PM	320	65	Wild with eggs
23/24	C	04/04/11	10000	~2-2.5	20	20	7.5	Every 4th day 10ppm/1hr	Transfer every 4th day	5 ml rot diet 2XAM, 10 ml PM	320	37	Wild with eggs

Table 4.   Larvae trial summary

4.4.1.3 Results

Although no post metamorphosis larvae resulted from the experiment it was the most successful larval run to date. In both treatments some larvae reached metamorphosis around 60 dph.

The last larvae was found dead at 65 dph on the edge of the false bottom (Fig. 24a, b). It is thought that this was an attempt to find shelter. This is considered typical behaviour for larvae during and after the metamorphosis stage when they become benthic.

The factors and/or combination of factors that contributed to the success of the experiment could have been the following;

1. Better tank hydrodynamics facilitated by implementation of false bottoms and creating evenly spaced upwelling throughout the base of the tank.

2. Lower bacterial load as a result of formalin treatment and/or tank exchange.

3. Better nutritional profile due to a combination of 'tailor-made' enrichment and concentrated algae paste.

4. Good quality eggs from a wild caught mother.

There were no significant differences between the two treatments, double-tanks and stand alone tanks (Fig. 25). There was a significant decrease in vibrio strains and total colony forming units (cfu) of bacteria after treating the tanks with formalin (Fig. 26 & 27). Likewise there was a significant reduction in vibrio and total cfu of bacteria after vacuuming the larval tanks see (Fig. 28 & 29).

Figure 24a.   Larvae at 65 dph and larvae at 0 dph respectively
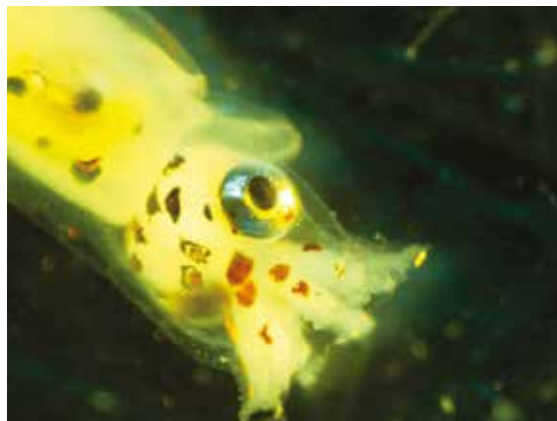
Figure 24b.   Side view of larvae at 65 dph
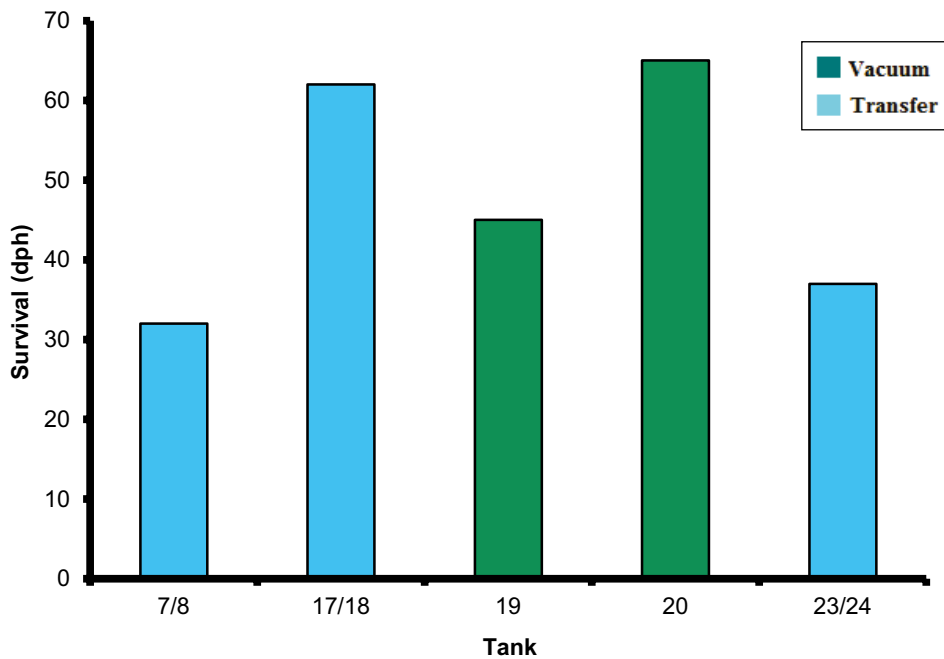
Figure 25.   Comparison of octopus larvae survival rates from vacuum and transfer treatments
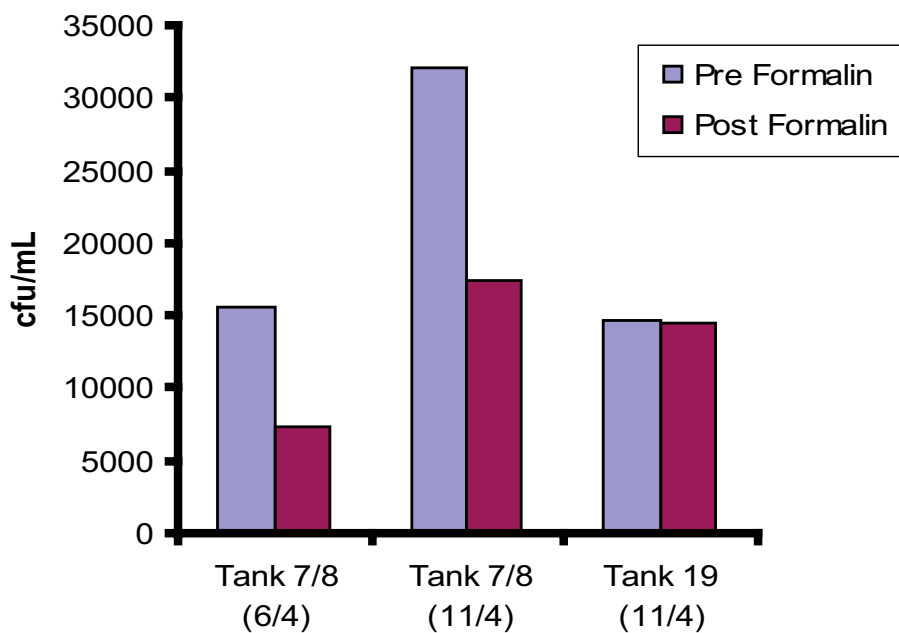
Figure 26.   Results from water samples analysed for Vibrio species of bacteria before and after formalin treatments of tanks

It is evident that the Vibrio counts were reduced with the addition of Formalin (Fig. 26). Samples were taken randomly over two dates; 6th and 11th of April 2011. The greatest decline of Vibrio sp. was in tank 7/8 on the 11th of April, with a reduction of 14800 cfu ml^{-1}. The smallest decline was observed in tank 19 on the 11th of April 2011, with a reduction of 50 cfu ml^{-1}. The average decline in Vibrio across all tanks was 7700 cfu ml^{-1}. Different methods of cleaning were applied to each of the tanks; tank 7/8 was transferred and tank 19 was vacuumed.
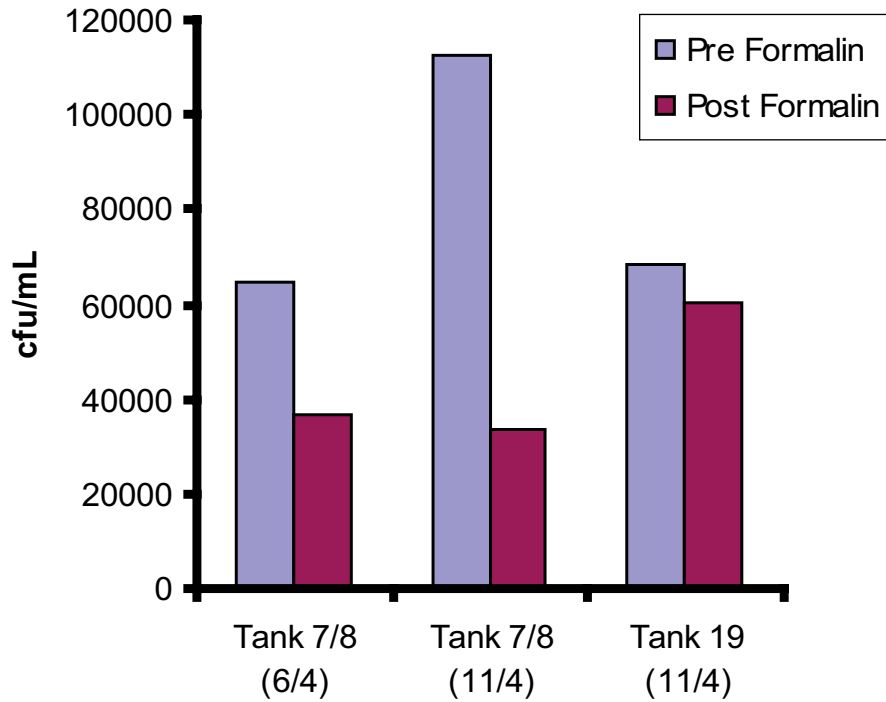
Figure 27. Results from water samples analysed for total cfu of bacteria before and after formalin treatments of tanks

Total bacteria counts from the same samples also showed a decline post Formalin treatment (Fig. 27). It can be seen from the graph that trends are similar to those of the Vibrio samples. The greatest decline of total bacteria was in tank 7/8 on the 11th of April 2011, with a reduction of 79000 cfu ml^{-1}. The smallest decline was observed in tank 19 on the 11th of April 2011, with a reduction of 8200 cfu ml^{-1}. On average the decline in total bacteria across all tanks was 38266 cfu ml^{-1}.
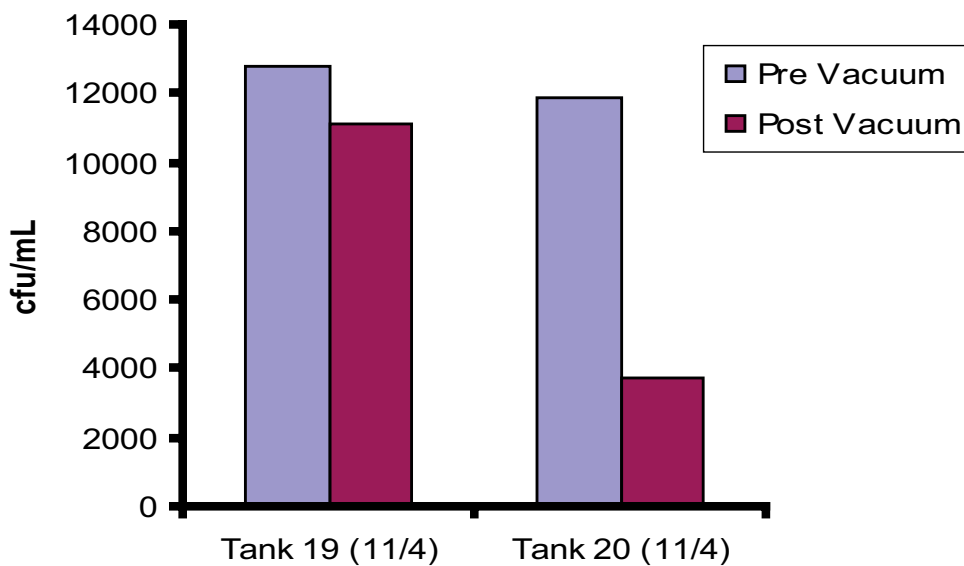
Figure 28. Results from water samples analysed for Vibrio species of bacteria before and after vacuuming tanks

A decline in Vibrio counts can be observed post vacuum (Fig. 28). Tank 20 showed the greatest decline with a reduction of 8200 cfu ml^{-1} while tank 19 was reduced by 1700 cfu ml^{-1}. Both samples were taken on the 11th of April 2011. On average the reduction in Vibrio post vacuum was 4950 cfu ml^{-1}.
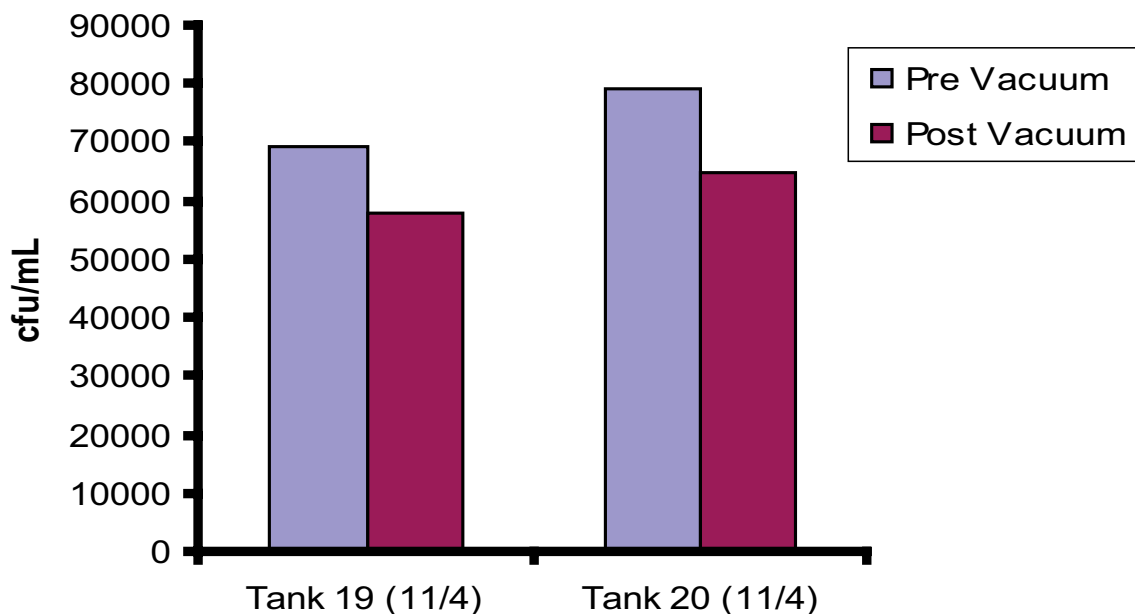
Figure 29. Results from water samples analysed for total cfu of bacteria before and after vacuuming tanks

Total bacteria counts also declined post vacuum. The greatest decline was seen in tank 20 with a reduction of 14600 cfu ml^{-1}, while tank 19 was reduced by 11200 cfu ml^{-1}. On average vacuuming reduced total bacteria by 12900 cfu ml^{-1}.

4.4.2 Formalin treatment 2

4.4.2.1 Introduction

Eight wild caught adult octopus females and males were grown at 20ºC in a 10000 lt tank. After 15 days eggs were detected in one of the pots used as shelters. The pot with the female and eggs was transferred to a separate tank with a continuous supply of seawater at 20ºC. Following hatching after 45 days, the captive bred larvae were stocked into 6 tanks in order to test the impact of formalin treatments on the survival of the larvae. Although in the previous larvae run all tanks were also treated with formalin, the specific effect of formalin was not determined.

4.4.2.2 Methods

Six x 270 lt double-tanks as described previously were used. All tanks had false bottoms to create even upwelling and to allow the same passive larvae transfer system as previously described.

Table 5 describes the experiment parameters. Larvae stocking was up to 10,000 larvae tank^{-1}, (27 larvae lt^{-1}). Larvae were stocked using jugs. Tanks were stocked in 3 blocks according to hatching days (as described previously, octopus larvae hatched during several days from the same egg batch) marked as group A, B and C.

Initial flow was kept at 100 lt hr^{-1} until 7 dph at which flow was increased to 150 lt hr^{-1}.

To create 'green water' in the larvae tanks, concentrated algae (Reed Aquaculture) dosed via peristaltic pumps from tub at a concentration of 85 ml/larval tank/day^{-1}. The algae tank was cleaned and sterilised daily before being refilled.

Artemia were hatched and grown for 5-10 days to reach a length of $1.5-2.5$ mm. The *Artemia* were fed with *Dunaliella salina* microalgae paste (Cognis Australia). Prior to feeding the larvae, the *Artemia* were harvested from the growout tanks and enriched with 'tailor-made' enrichment (Artikol, Nutrakol Pty Ltd) and *Spirulina* and *Chlorella* powder for 3 hrs. Larvae were fed 3 times a day at a rate of 10000-20000 *Artemia* per feed. *Artemia* densities were monitored to prevent an excess of feed and feed was given according to the survival rate and number of larvae in tanks. Excess *Artemia* were jugged out prior to each addition of freshly enriched *Artemia*.

Artemia were fed 10 gr of enrichment (Artikol) in 1000 ml seawater was blended with 0.5 gr of *Spirulina* and 0.5 gr of *Chlorella* powder for three minutes. The enrichment was added to the *Artemia* in 20 lt of seawater, for 9 am and 12 am feeds. 0.5 gr of Probiotic (Inve, Belgium), was given to the *Artemia* 3 hours before the designated feed. 20 gr enrichment in 50 ml seawater was blended with 1 gr of *Spirulina* and 1 gr of *Chlorella* for overnight enrichment. *Artemia* was enriched in 40 lt of seawater. The overnight enrichment was dosed at 6 am with the use of a time controlled peristaltic pump. 5 ml of rotifer diet was also added to the overnight enrichment tank at 3 pm daily.

Temperature was kept at 20ºC initially and increased to 21ºC after 5 dph.

From each hatching group (blocks A, B and C), one tank received formalin treatments as described in the previous experiment at a 10 ppm concentration every 2 days. The formalin administering protocol was as follows; once the formalin was administered by the peristaltic pump to the larvae tank, the water flow was reduced to 50 lt hr^{-1} (rather than stopped) for one hour to increase the circulation of the formalin. Following the treatment, water flow was increased back to the regular flow of 150 lt hr^{-1}.

Table 4.   Larvae trial summary

Tank #	Group	Stocking date	# of larvae	*Artemia* size (mm)	Initial temp	Temp after 5 dph	Average DO (mg l-1)	Formalin	Cleaning	Enrichment	Lighting (lux)	Survival (dph)	Mother
5/6	B	03/06/11	10000	~1.5-2	20	21	7.5	No	Transfer every 7th day	0.5gr Chlorella 0.5gr Spirulina 2xAM/1gr of each PM.	320	19	captive
7/8	A	26/05/11	9000	~1.5-2	20	21	7.5	Every 2th day 10ppm/1 hr	As above	As above	320	27	captive
17/18	C	03/06/11	10000	~1.5-2	20	21	7.5	Every 2th day 10ppm/1 hr	As above	As above	320	17	captive
19/20	C	03/06/11	10000	~1.5-2	20	21	7.5	No	As above	As above	320	17	captive
21/22	B	03/06/11	10000	~1.5-2	20	21	7.5	Every 2th day 10ppm/1 hr	As above	As above	320	20	captive
23/24	A	26/05/11	9000	~1.5-2	20	21	7.5	No	As above	As above	320	28	captive

4.4.2.3   Results

It can be seen that group A; tank 7/8 and 23/24 had the greatest survival with 27 and 28 dph, respectively (Fig. 30). Both tanks were stocked on 26/05/11 at a stocking density of 9000 larvae (Table 5). Tank 7/8 was treated with formalin at a concentration of 10 ppm for one hour on every second day while tank 23/24 was not treated with formalin. Group C; tank 17/18 and 19/20 had the lowest survival rate with 17 dph. Both tanks were stocked on 03/06/11 at a stocking density of 10000 larvae (Table 5.). All other variables were kept constant with an initial temperature of 20ºC which was then increased to 21ºC at 5 dph, an average DO reading of 7.5 mg lt^{-1} and an average *Artemia* size of 1.4 mm. Survival within each group had similar or the same survival rate; Group A; Tank 7/8 had the highest survival rate at 27 dph.
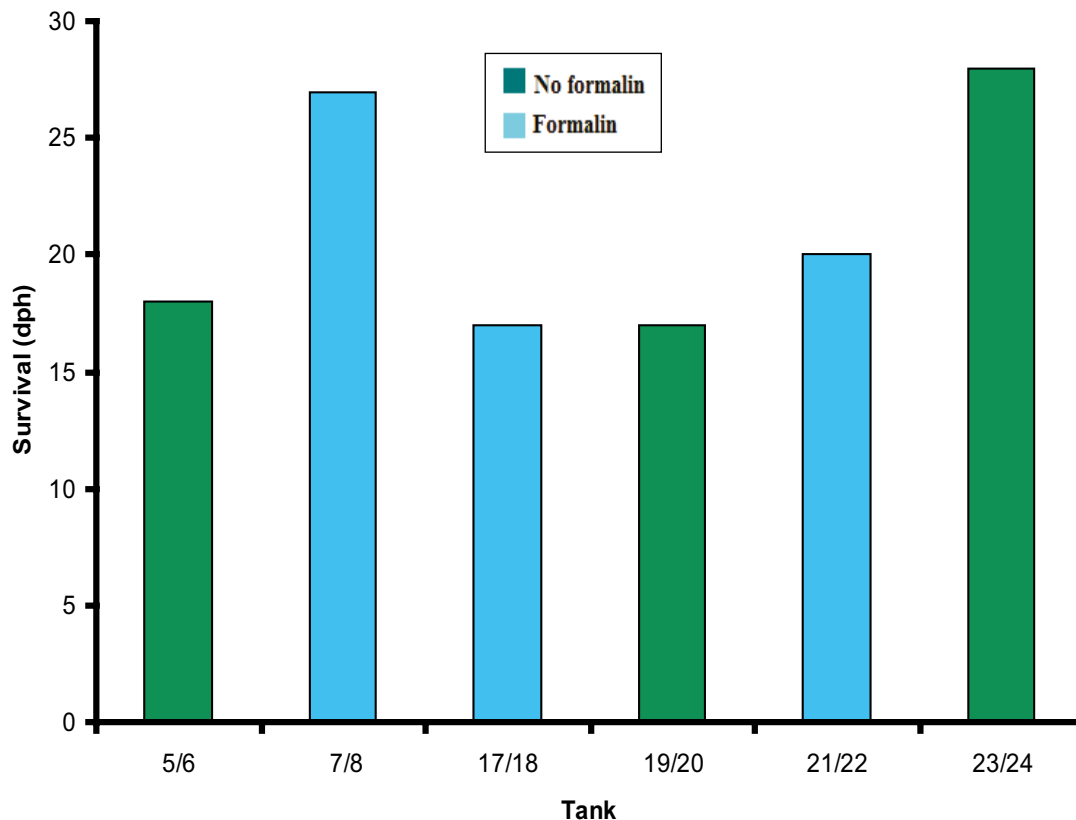
Figure 30.   Octopus larvae survival from the two treatments tested

Formalin at the concentration of 10 ppm did not show any major benefit to the larvae, there was no significant differences in survival between treatments with or without formalin. The general low survival of larvae in this experiment may be related to the source of the eggs i.e. captive bred female compared to wild caught female. Another factor was the different nutritional profile of the *Artemia* resulting from using *Spirulina* and *Chlorella* powder rather than the 'Roti Diet'.

It is interesting to point out that significant differences between blocks were detected (Table 5). These differences related to the hatching date from the same batch. It seems that the first larvae to hatch were also the best and survived 27-28 days compared to the later hatching larvae that survived 17-20 days.

4.4.3 Formalin treatment 3

4.4.3.1 Introduction

A Wild caught female with eggs in a trigger pot was sourced from a commercial octopus fisherman from Two Rocks (50 km north of Perth). The females and eggs (with the pot) were kept at 20ºC with flow though seawater. The larvae from the female were stocked into six tanks to test the use of formalin at a higher concentration than had previously been used on wild larvae.

4.4.3.2 Methods

System

Six double-tank systems with false bottoms, as described previously, were used. The light intensity was reduced from 320 lux to 106 lux with the use of shade cloth covers over the day light fluorescent lights.

Initial flow was kept at 100 lt hr^{-1}. At 7 dph flows were increased to 150 lt hr^{-1}.

To create 'green water' in the larvae tanks, concentrated algae (Reed Aquaculture) dosed via peristaltic pumps from tub at a concentration of 85 ml/larval tank/day^{-1}. The algae tank was cleaned and sterilised daily before being refilled.

Table 6 describes the experimental parameters. Larvae stocking was 10,000 larvae tank^{-1}. Larvae were stocked using jugs. Larvae stocking was done in 3 blocks according to hatching days, groups A, B and C.

Artemia were hatched and grown for 5-10 days to reach length of 1.5-2.5 mm. The *Artemia* were fed with *Dunaliella salina* microalgae paste (Cognis Australia). Prior to feeding the larvae, the *Artemia* were harvested from the growout tanks and enriched with 'tailor-made' enrichment (Artikol, Nutrakol Pty Ltd) and *Spirulina* and *Chlorella* powder for 3 hrs. Larvae were fed 3 times a day at a rate of 10000-20000 *Artemia* per feed. *Artemia* densities were monitored to prevent an excess of feed and according to the survival rate and number of larvae in the tanks. Excess *Artemia* were jugged out prior to each addition of freshly enriched *Artemia*.

Artemia were fed 10 gr enrichment (Artikol) in 1000 ml seawater was blended with 0.5 gr of *Spirulina* and 0.5 gr of *Chlorella* powder for 3 minutes. The enrichment was added to the *Artemia* in 20 lt of seawater, for 9 am and 12 am feeds. 0.5 gr Probiotic (Inve, Belgium), was given to the *Artemia* 3 hrs before the designated feed. 20 gr enrichment in 50 ml seawater was blended with 1 gr of *Spirulina* and 1 gr of *Chlorella* for overnight enrichment. *Artemia* was enriched in 40 lt of seawater. Enrichment was dosed at 6 am with the use of a time controlled peristaltic pump. A volume of 5 ml of 'Roti Diet' was also added to the overnight enrichment tank at 3 pm daily.

Temperature was kept at 20ºC initially and increased to 21ºC after 5 dph. Due to problems with temperature control, temperature fluctuated from 19 to 21ºC from 5 dph onwards.

Tank #	Group	Stocking date	# of larvae	Artemia size (mm)	Initial temp	Temp after 5 dph	Ave DO (mg/lt)	Formalin	Cleaning	Enrichment	Lighting (lux)	Survival (dph)	Mother
5/6	A	30/07/11	10000	~0.9-1.5	18	increased to 22 over 10 days	7.5	No	Transfer every 7th day	0.5gr Chlorella 0.5gr Spirulina 2xAM/1gr of each PM.	106 lux	19	captive
7/8	A	30/07/11	10000	~0.9-1.5	18	As above	7.5	Every 4th day. 100ppm/1 hr	As above	As above	106 lux	19	captive
17/18	B	28/07/11	7000	~0.9-1.5	18	As above	7.5	Every 4th day. 100ppm/1 hr	As above	As above	106 lux	21	captive
19/20	B	28/07/11	7000	~0.9-1.5	18	As above	7.5	No	As above	As above	106 lux	21	captive
21/22	C	29/07/11	7000	~0.9-1.5	18	As above	7.5	Every 4th day. 100ppm/1 hr	As above	As above	106 lux	21	captive
23/24	C	31/07/11	7500	~0.9-1.5	18	As above	7.5	No	As above	As above	106 lux	21	captive

Table 6. Larvae trial summary

4.4.3.3   Results

For the first time, larvae were found on the outside walls of the tanks at ~ 15 dph. This phenomenon was observed in all tanks (Fig. 31). The dried larvae were cleaned off daily and found again on all tanks the following day. To prevent the 'crawling' larvae, a spray bath was installed on one of the tanks to see if keeping water flowing down the tank walls would stop the larvae from climbing up (Fig. 32). The following morning there was a large number of dead larvae in the bottom of this tank. It was thought that the spray intensity damaged and killed the larvae. If this phenomenon was to occur again, a gentle water flow over the tank walls might be a better solution, however this would need to be tested.

Figure 31.   Circled area displaying larvae escaped from tank
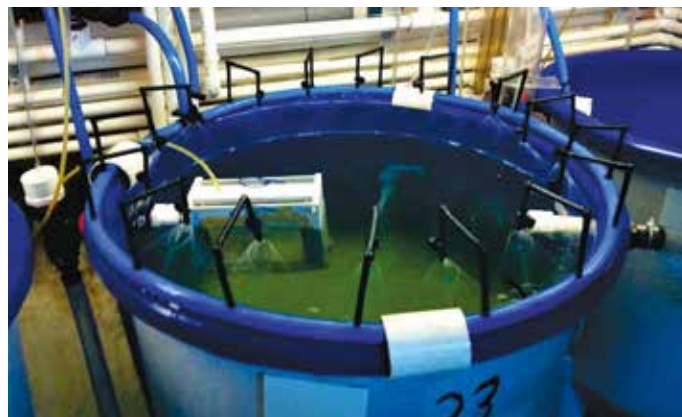
Figure 32.   Sprayers set up to keep water flowing down the tank freeboard to prevent larvae from escaping
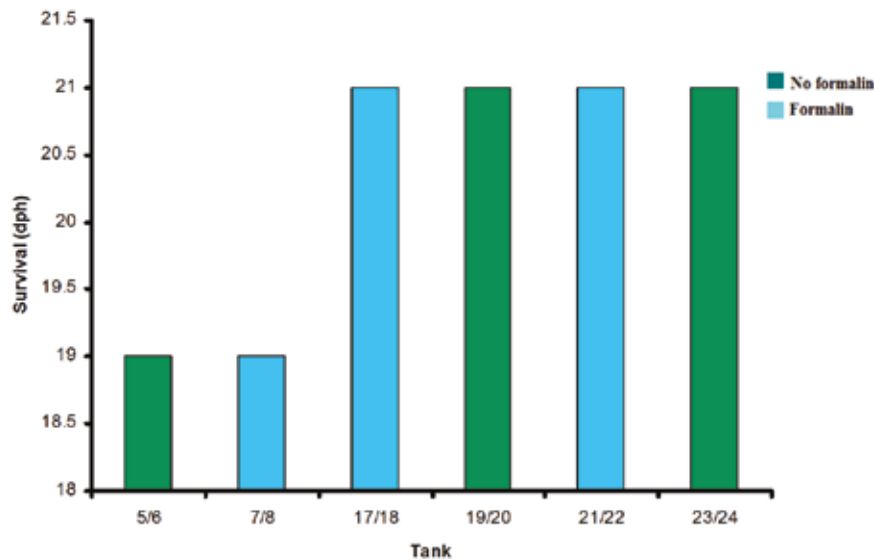
Figure 33. Larvae survival in different tanks with different treatments

The larvae survival across all tanks and treatments was relatively low at 20 dph (Fig. 33) compared to previous runs. It was believed that the change in the light intensity may have affected the larvae in this run. The increased dosage of formalin from 10 ppm to 100 ppm may have adversely affected the larvae. On the other hand, no formalin treatment may have also led to bacterial problems for the larvae. The *Chlorella* and the *Spirulina* may not have been a suitable nutritional supplement in comparison to the 'Roti Diet'. The fluctuating water temperatures which resulted from heater chiller malfunction may have also caused stress to the larvae resulting in their low survival rate.

4.4.4 Antibiotics treatment

4.1.4.4.1 Introduction

Antibiotic treatment will reduce bacteria and hence improve larvae survival.

Previously, a number of water quality and larvae samples were sent to the Department of Fisheries Fish Health unit for analysis (Section 4.4.6). It was discovered that the octopus larvae were becoming encased in a mat of filamentous bacteria. After consultation with members of Fish Health unit, a decision was made to treat some tanks with a ten day course of antibiotic (Oxytetracyline) bath in an attempt to eradicate this problem.

4.4.4.2 Methods

Treatments

1. *Artemia* with crab enrichment (3 tanks)

2. *Artemia* with crab enrichment and antibiotic treatment (4 tanks)

System

Cylindrical tanks in 7 pairs (14) of 40 lt with interchangeable 250 μm banjo screens and temperature controlled flow through seawater were used in this experiment. Tanks were operated as upwelling, where water flow is distributed through a clear acrylic diffuser with

1 mm holes spread over the surface. A 24 hour photoperiod with fluorescent lights positioned over tanks was implemented, emitting between 450-600 lux. Clear acrylic lids to cover top of 40 lt tank.

Larvae were fed 6 times a day or as required, with 1-3 hour enriched *Artemia* that are between 1.5-2.5 mm total length. Overnight enrichment over 6 hours (0300 – 0900), lunchtime enrichment over 1 hour (1100 – 1200) and afternoon enrichment over 1 hour (1400 – 1500).

- Artemia fed via cold storage at 3 times overnight from 2 buckets.
- 1 bucket: Crab Enrichment (3 tanks)
- 1 bucket: Crab Enrichment (4 tanks)

Feed densities were monitored as to not have low numbers of *Artemia*, but at the same time not to over feed larvae. Additional feeds between major feeds if larvae are eating *Artemia* out quickly. *Artemia* numbers were governed by size of mesh on screens and the jugging out excess animals when required. Screens were 250 µm. A semi moist microdiet 220-500 µm to be fed from 7 dph onwards before each *Artemia* feeding event (was later increased to 500-800 µm as larvae showed preference to larger pieces of diet). Live Porntunus pelagicus larvae were fed when available before each *Artemia* feeding event 3 times daily most days throughout the trial there were newly hatched crab zoeae available and fed to tanks.

Larvae for this trial were hatched from eggs that were spawned in the wild; the octopus with eggs was caught south of Rottnest Island at a depth of 15-25 m. The octopus incubated the eggs for 35 days prior to spawning. Tanks were not stocked with larvae until 12 days after the octopus started releasing larvae due to only a small number being released gradually until this time.

4.1.4.4.3 Results
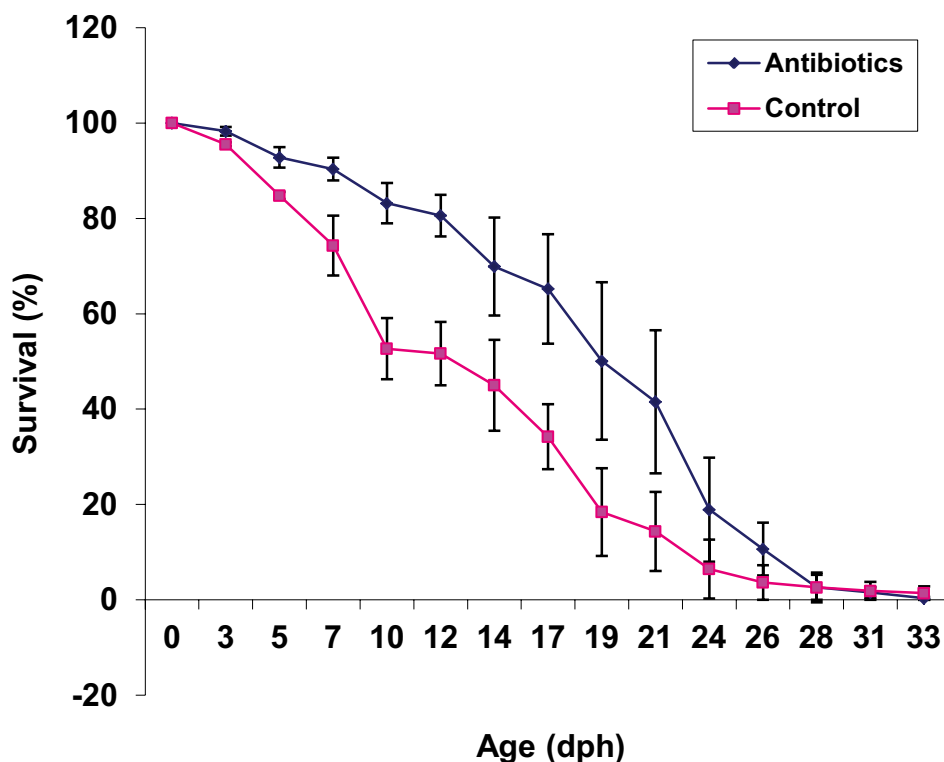
Figure 34. Percent survival (%) of larvae in tanks with or without antibiotic treatment across trial duration.

Percentage survival in tanks with the antibiotic treatment did not result in higher larvae survival at trial completion, with an average of 1.41% survival in tanks not treated and 0.3% in tanks that were treated. A significant difference can be seen from 5 dph to 21 dph, but was not seen again thereafter. There is a steep decrease in survival across both treatments from 21 dph onward until trial completion at 33 dph (Fig. 34).

Larvae Health and Analysis

Throughout the trial, samples of larvae without antibiotic treatment (Treatment 1) and with antibiotic treatment (Treatment 2) were submitted to the Department of Fisheries, Fish Health unit for histological analysis and routine weekly health testing. It was found that some larvae from Treatment 2 had filamentous bacteria present in the connective tissue and the skin (Fig. 35). The development of haemocytes was also found in Treatment 2; cells that play a role in the immune system of invertebrates. The presence of haemocytes suggests that larvae are developing some immune response to bacteria. The digestive gland of larvae from samples of Treatment 1 appeared improved in comparison to Treatment 2, where the cells appear 'washed out', irregular in size and unstructured within the gut. Vacuoles within a number of larvae were found to have accumulated undigested material within lysosomes, indicating that the digestive system is not functioning properly and food is not being efficiently broken down.
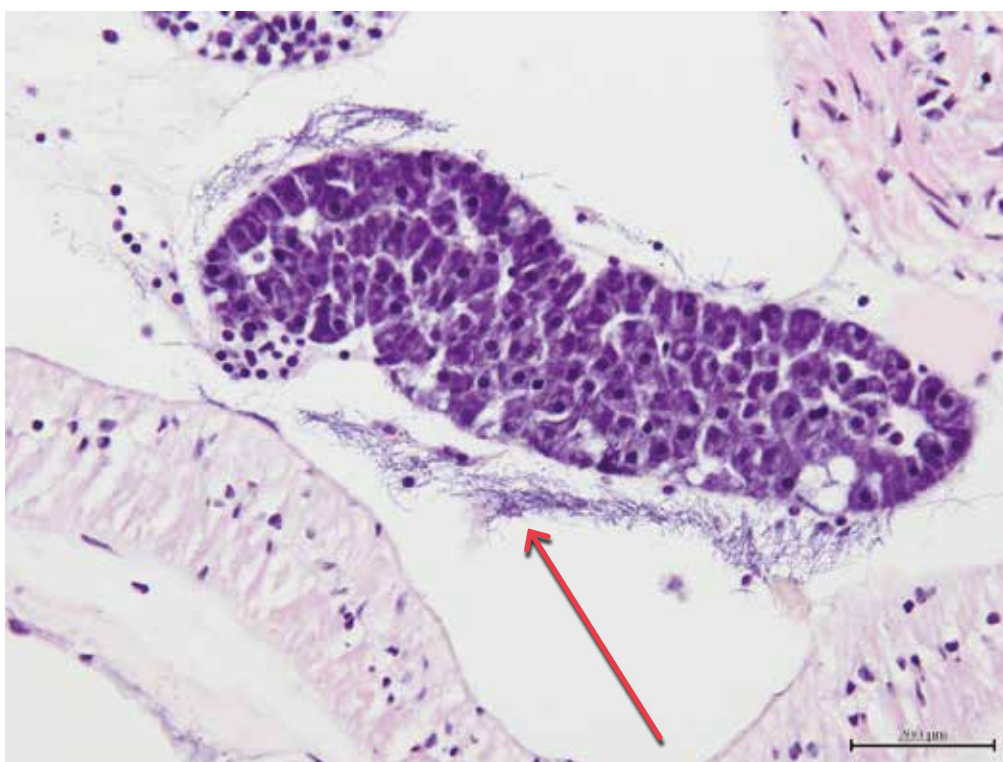
Figure 35. Mat of filamentous bacteria in connective tissue and dermis of 19 dph larvae (see arrow).

Toward the end of this trial, several larvae were found that were 'swollen' and filled with fluid between the muscle tissue and the skin (Fig. 36). This fluid is most likely haemolymph that has leaked out due to breakdown of the gut and caused the body to dilate, where a healthy gut would normally absorb fluid.
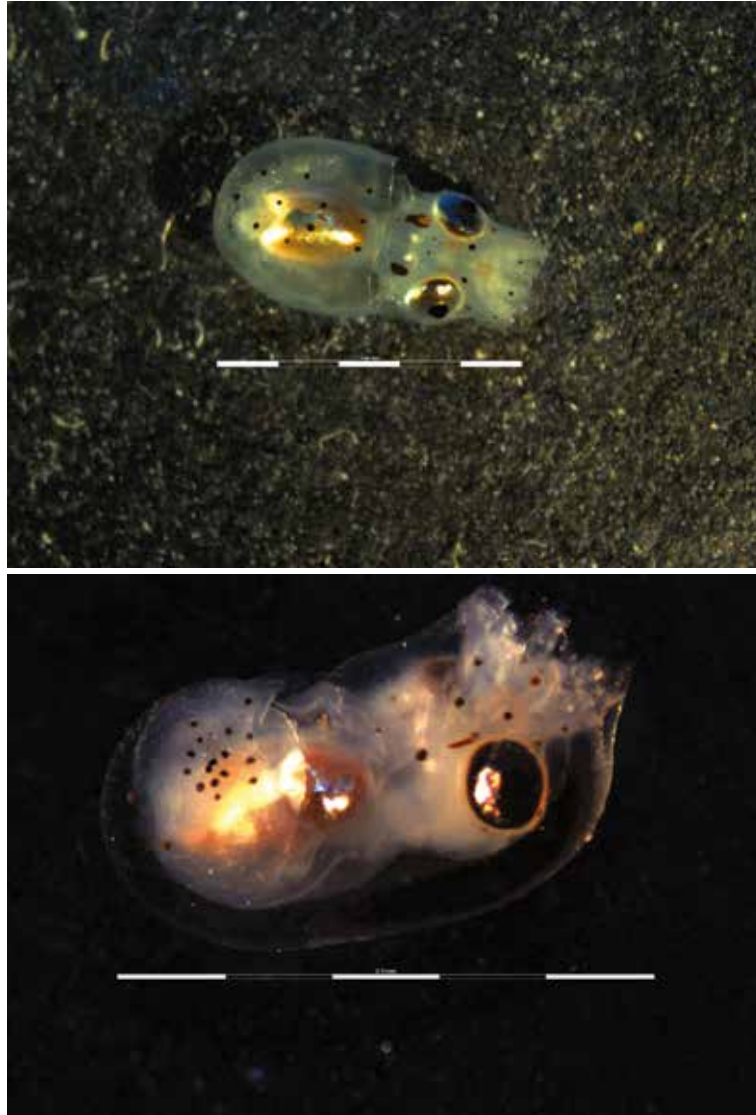
Figure 36.   Normal larvae at 33 dph (top) and larvae with dilated haemolymph channel at 23 dph (bottom)

4.4.4.4   *Discussion*

Although the average survival of larvae was greater in the antibiotic treatment tanks for the majority of the trial, survival was actually slightly greater in tanks not treated with antibiotics at trial completion (33 dph). Antibiotic treatments did not aid in reducing bacteria. This was concluded based on a number of larvae being found to have filamentous bacteria present in the connective tissue and the skin, while also causing poorer gut condition. This suggests that these animals were stressed due to conditions in their surrounding environment or the nutrition they were receiving was not adequate to promote healthy immune function. The improved gut condition of larvae in Treatment 1 suggests that additional feeds of *P. pelagicus* zoeae and micro-diet may be improving larvae health; however it is unknown at this stage whether gut improvement is a developmental change brought on by larvae age, while condition of the gut and digestive system still does not appear optimal. Based on literature detailing negative phototaxic behaviour when *O. vulgaris* larvae become benthic and display reclusive behaviour (Villanueva, 1995), illumination was reduced when larvae reached 33 dph. Within a short time, almost all larvae were observed to move from the tank bottom to the mid and surface waters of

the tank, suggesting that they were most likely showing negative phototaxic behaviour. To date, this has been the most successful trial since June 2011 in terms of larvae health and survival and has provided scope for improved trials in the future of this project.

4.4.5 *Octopus tetricus* larvae health analysis

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Specimen preparation for histological examination

All live specimens were euthanased on ice slurry (for approximately 20 minutes) and placed into 10% neutral buffered formalin for fixation. After 24 hours, the specimens were set in agarose gel and embedded into paraffin wax for histological sectioning and staining with H&E (haematoxylin and eosin).

4.4.5.1 Amino acid treatment (see 4.2.1)

Submission 1.

- 18 dph

- Marked mortalities occurring around 8-10 dph

- Recently dead larvae submitted.

4.4.5.1.1 Histopathological description

There was severe diffuse growth of bacteria throughout the larval tissue.

4.4.5.1.2 Comments

Bacterial culture of dead larvae revealed a heavy growth of swarming bacteria including *Vibrio alginolyticus*, *Vibrio rotiferianus* and *Vibrio pomeroyi*-like bacteria. These and other bacteria are ubiquitous and expected to be present in culture water. Vibrio species of bacteria are implicated in numerous aquatic animal diseases and are commonly isolated from aquatic species including molluscs. In particular, *Vibrio alginolyticus* is known to cause systemic disease, ulcerative disease, necrosis and wound infection in molluscs (Buller, 2004). Some predisposing risk factors for contracting vibriosis include high environmental temperatures, overcrowding, organic pollution and other stressors (Buller, 2004). Gram stain revealed the presence of gram negative bacteria adhered to the connective tissue and dermis (Fig. 37). Antibiotic therapy (ten day immersion treatment 100 mg Oxytetracycline) was recommended to control the systemic gram negative bacterial infection.
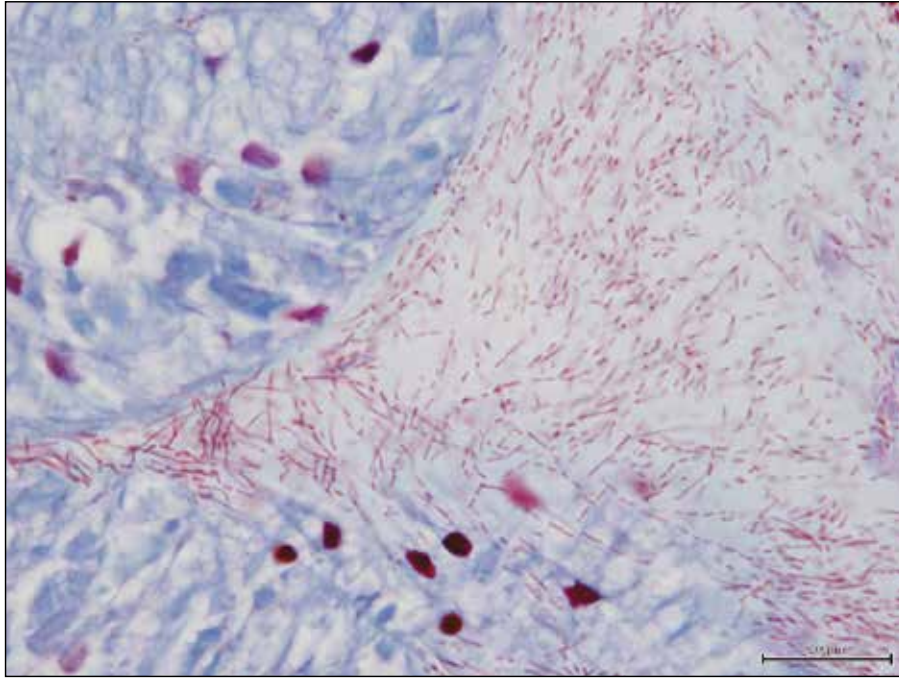
Figure 37.   Gram negative bacteria (pink rods) adhered to the connective tissue and embedded in the dermis of 18 dph captive bred larvae. (100X oil immersion).

4.4.5.1.3 Diagnosis

Bacterial septicaemia.

Submission 2

- 19 dph
- Increasing mortalities as animals age
- Larvae submitted in three different conditions:
  - *Captive broodstock larvae:* healthy, sick and dead from tanks 1,2,3,4,6 and 7
  - *Wild broodstock larvae:* 0 dph submitted for histological comparison.

4.4.5.1.4 Histopathological description

Wild larvae:

0 dph: There were no significant lesions. The digestive gland appeared to resemble normal mollusc structure but the cytoplasm was amorphous and eosinophilic in appearance and contained no digestive inclusions (Fig. 38).
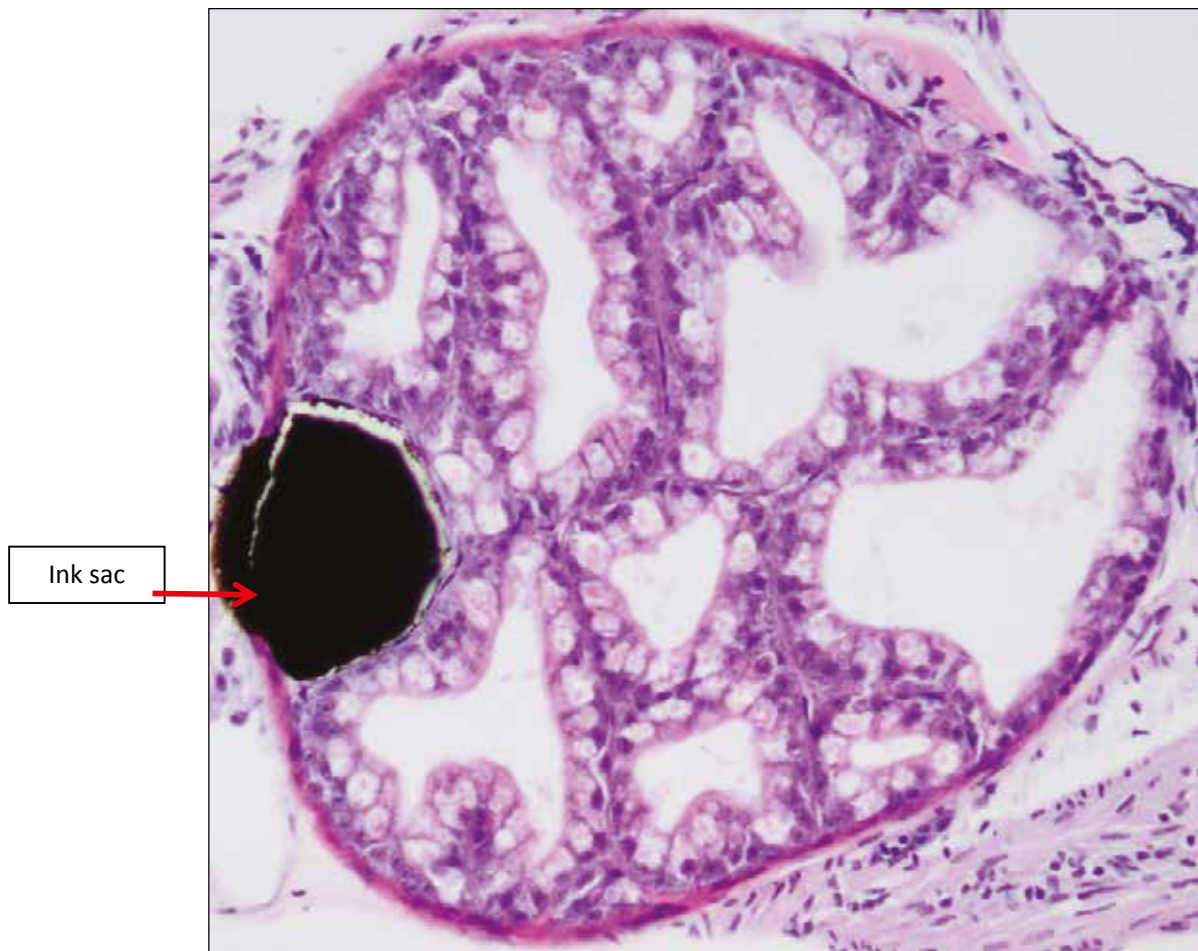
Figure 38. Digestive gland of 0dph wild hatched larvae with an intact luminal brush border and regular digestive gland vacuole and epithelial cell structure. The ink sac can be identified in this section (red arrow) (20X).

4.4.5.1.5 Captive broodstock larvae

Tank 1: There was degradation of enterocytes in the digestive gland in larvae submitted in all three conditions (good, sick and dead) with cell debris in the gut lumen (Fig. 39). Filamentous bacteria were identified on the skin and dermis in specimens submitted in sick and dead condition (Fig. 40).
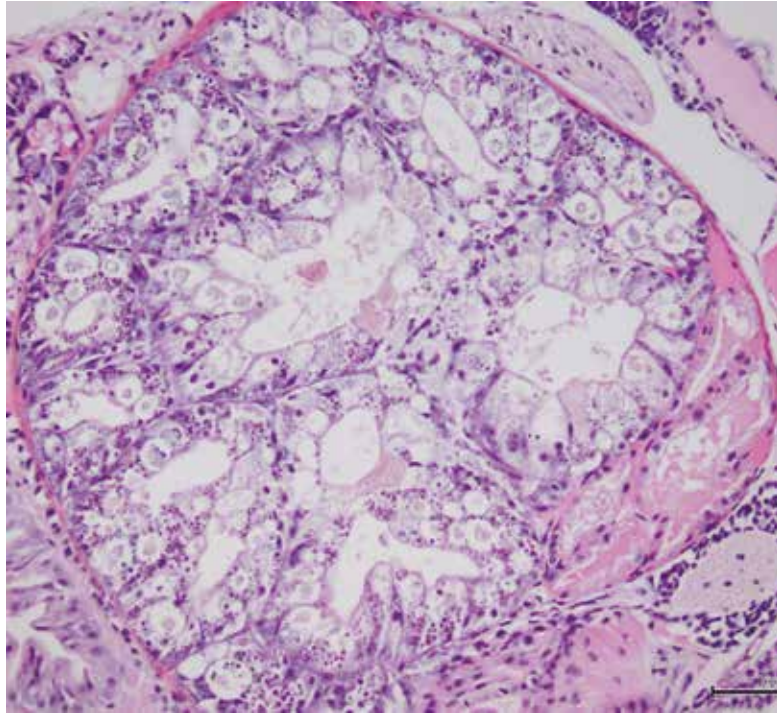
Figure 39.　　Digestive gland of 'good' captive bred larvae showing degradation of enterocytes in the digestive gland with cell debris in the gut lumen (20X).
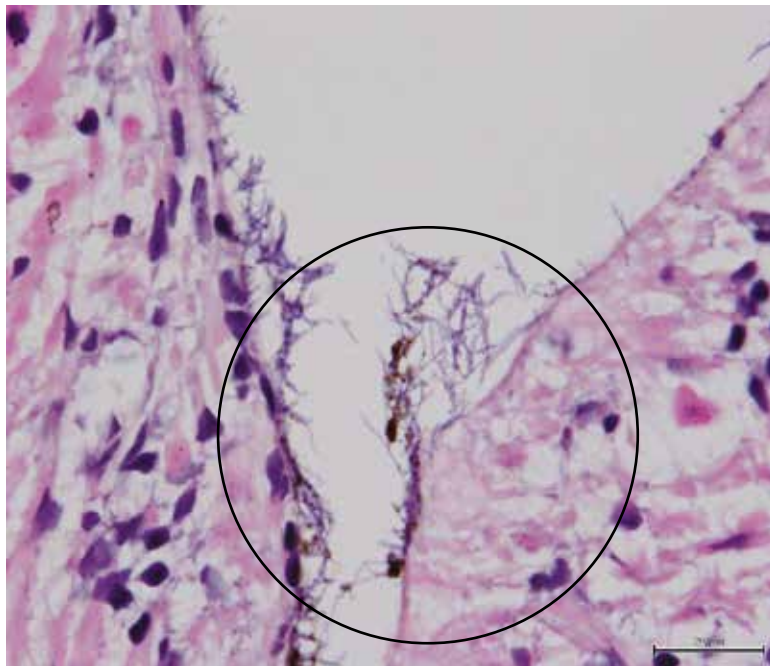
Figure 40.　　Filamentous bacteria (within circle) adhered to the connective tissue of a 'sick' captive bred larvae in tank 1 (100X oil immersion).

Tank 2: Rod-shaped bacteria were identified in the digestive gland and connective tissue in the specimen submitted in dead condition. Small basophilic inclusions (suspected to be residual bodies after lysosome activation has occurred to remove cellular debris) within enterocytes were identified in the digestive gland of one specimen submitted in good condition (Fig. 41). No lesions were identified in the specimen submitted in sick condition.
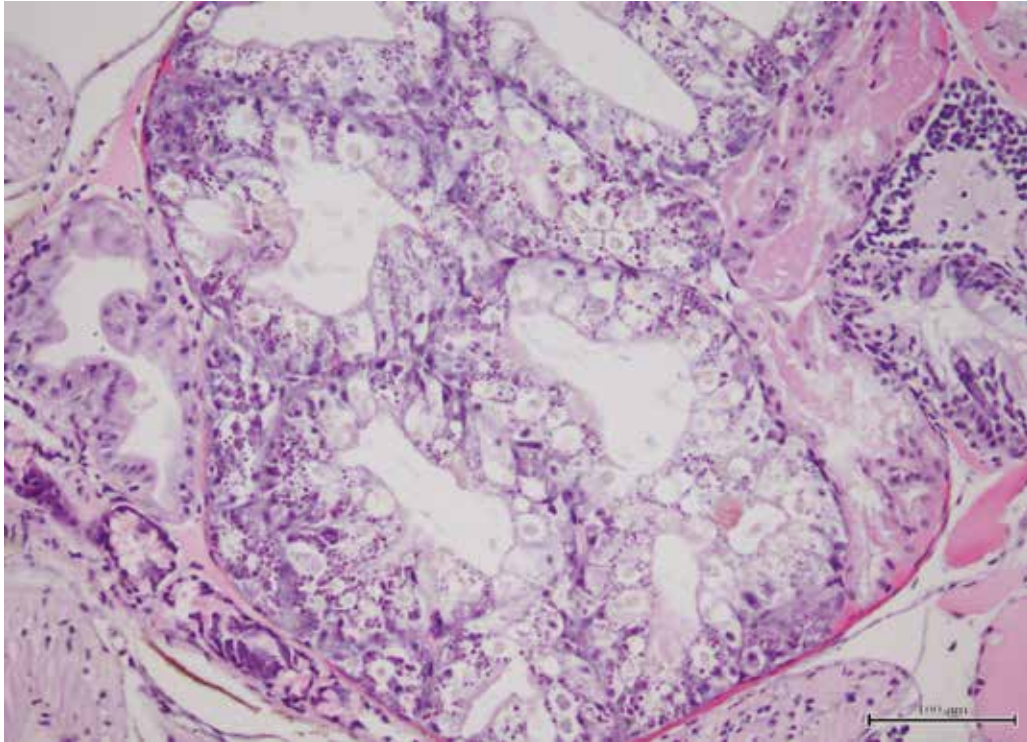
Figure 41.   Digestive gland of larvae from tank 2 in 'good' condition showing basophilic inclusions (suspect residual bodies) within enterocytes (20X).

Tank 3: All specimens submitted in good condition had small basophilic inclusions in enterocytes in the digestive gland but no bacteria could be identified. All specimens submitted in sick condition had mild degradation of digestive gland enterocytes and no bacteria could be seen. One specimen submitted in dead condition had atrophy of digestive gland enterocytes and bacteria were identified throughout all tissues in the other specimen.

Tank 4: Two out of three specimens submitted in good condition had small basophilic inclusions in enterocytes in the digestive gland but no bacteria could be identified. Small basophilic inclusions within enterocytes were identified in the digestive gland in the specimen submitted in sick condition, but no bacteria could be identified. The digestive gland of the specimen submitted in dead condition appeared to resemble normal mollusc structure and there was a cluster of bacteria near the gills.

Tank 6: External fouling and necrosis of the epithelium of the skin was observed in both specimens submitted in good condition and small basophilic inclusions were present within enterocytes. One out of two specimens submitted in sick condition had small basophilic inclusions within enterocytes in the digestive gland and filamentous bacteria were present in the connective tissue. One out of two specimens submitted in dead condition had a severe infection of the skin and connective tissue with filamentous and rod-shaped bacteria present. One of these specimens also had small basophilic inclusions within enterocytes in the digestive gland and the other had filamentous and rod-shaped bacteria in the skin and connective tissue.

Tank 7: Both specimens submitted in good condition and one of two submitted in sick condition had small basophilic inclusions within enterocytes in the digestive gland but no bacteria could be seen. There were no significant lesions observed in the one specimen submitted in dead condition.

4.4.5.1.6 Comments

Vibrio alginolyticus and other bacteria were cultured from the tank water and histologically, rod-shaped and filamentous bacteria were present within the connective tissue and dermis in most specimens throughout this submission. The current tank environment is most likely favouring the growth of these pathogens and as a result, the specimens are being stressed causing failure to thrive. Only one specimen had digestive gland structure similar to the normal structure found in other molluscs (in tank 4) and the rest had varying degrees of enterocyte degradation and basophilic inclusions within the gland. It appears in most specimens that the digestive gland is breaking down so the functional capacity of the organ is being compromised and the animal cannot grow or develop further. The presence of many lysosomes/residual bodies within the digestive glands suggests that there is some dysfunction with the digestive process occurring in these specimens. Lysosomes bodies usually store and collect waste material in animals. It must be questioned why there are so many of these residual bodies in these specimens who due to their young age, should be healthy and efficiently able to process waste material.

It is difficult to compare the structure of the digestive gland of the wild caught 0 dph larvae to the captive bred specimens in this submission as the wild larvae have not yet fed; the digestive gland in Figure 38 is therefore not typical of a feeding mollusc. Figure 33 below shows the digestive gland structure of a wild caught adult Octopus submitted to the Fish Health Laboratory in 2009. The digestive gland of this specimen contains amorphous red-brown particles within digestive vacuoles (*) and does not contain any of the small round basophilic particles that were identified in this submission in 2012.
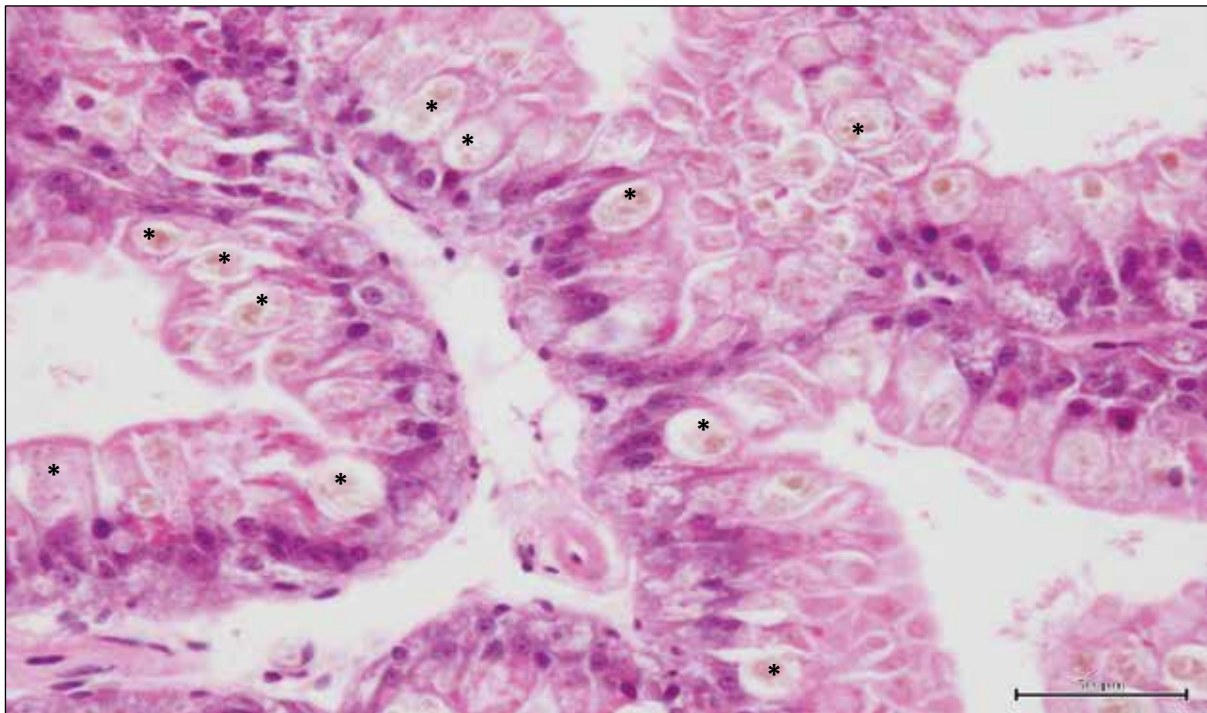
Figure 42.   AS-09-3398. Digestive gland of wild adult Octopus submitted to the Fish Health Laboratory in 2009. Digestive vacuoles contain amorphous red-brown material (*) and the overall structure of the gland appears to be intact (40X).

4.4.5.1.7 Diagnosis: Bacterial infection and enteropathy.

4.4.5.1.8 Conclusions from amino acid treatment trial

It is likely that larvae mortality in this trial is due to nutritional deficiencies in the current diet on offer which has facilitated a secondary bacterial infection. The immune system of these specimens may have not yet developed, so the larvae are susceptible to secondary bacterial infection from pathogens in the surrounding environment.

4.4.5.2 Antibiotics treatment (see 4.4.5)

Submission 1

- 10 dph

- 4/7 tanks treated with 100mg/lt Oxytetracycline for 10 days: 12/11/12 is the 10th and final day of antibiotic therapy

- Mortalities occurring;

  - Treatment 2 (tanks treated with antibiotics) has greater survival then Treatment 1.

4.4.5.2.1 Histopathological description

At 10 dph, all larvae including those not treated (treatment 1) and those treated with antibiotics (treatment 2) had digestive glands lined by hypertrophied epithelial cells with a villous border that contained large membrane bound cytoplasmic vacuoles (up to 10 μm diameter) containing yellow-brown material (suspect food material) and numerous variably sized round (3-5 μm) basophilic structures (suspect residual bodies) (Fig. 43, 44 and 45). Both cytoplasmic structures are interpreted to be accumulated material within digestive vacuoles.
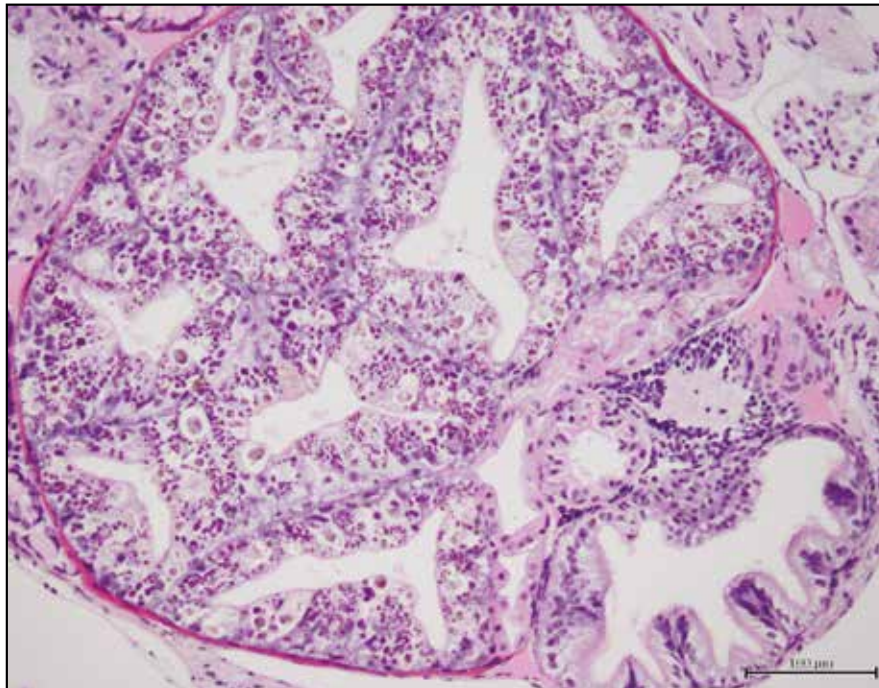
Figure 43.   Digestive gland of larvae from tank 4 (treated with antibiotic) showing round basophilic structures (suspect residual bodies from lysosome activation to remove waste products) (20X).
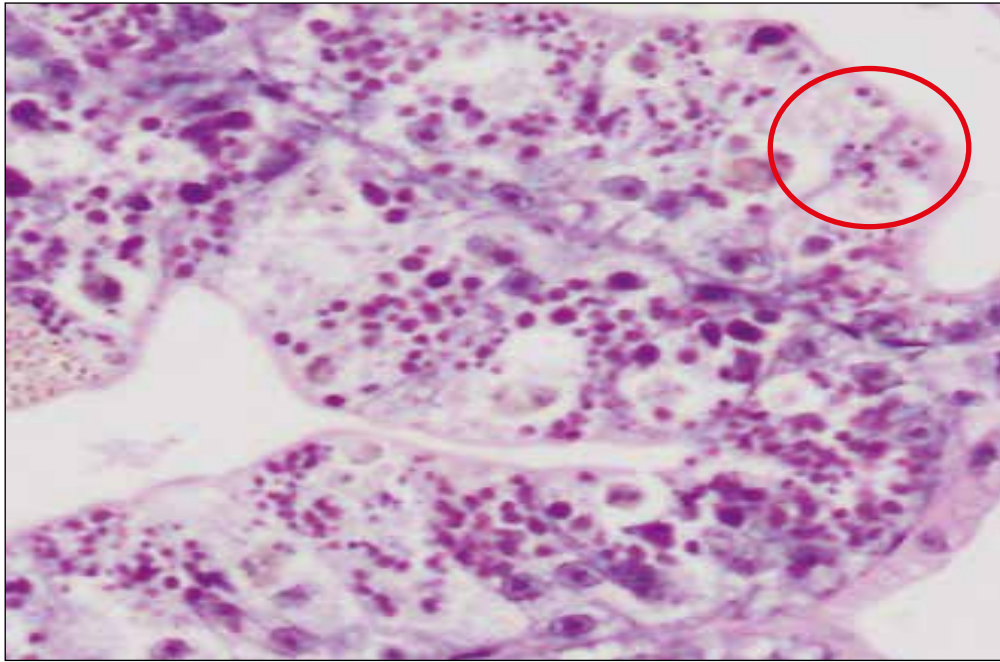
Figure 44.   Amplification of Figure 7 showing basophilic structures (suspect residual bodies) in red circle (20X).
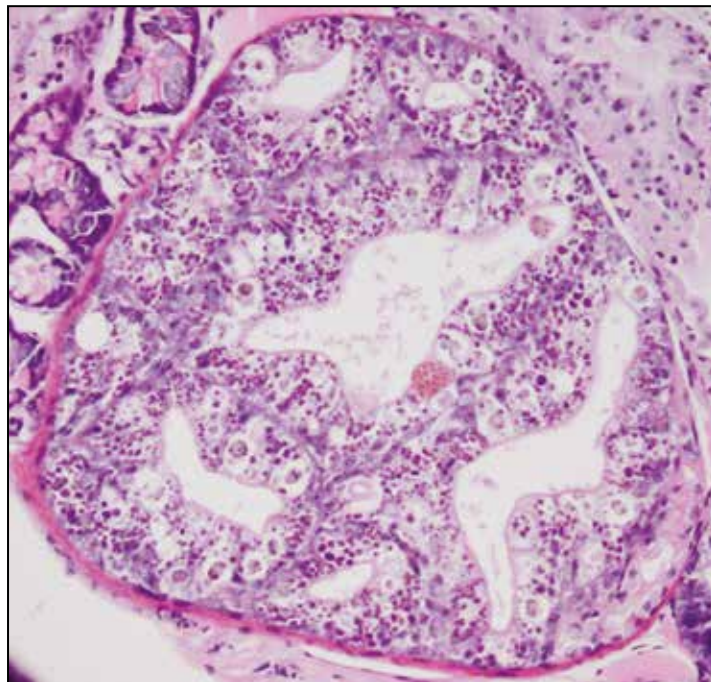
Figure 45.   Digestive gland of larvae from tank 3 (not treated with antibiotic) showing round basophilic structures similar to larvae in tanks treated with antibiotic (Fig. 43) (20X).

4.4.5.2.2 Comments

There was no histological difference in the structure of the digestive gland between specimens in treatment 1 and 2. The histological appearance of the digestive gland suggests that food is accumulating in gut epithelial cells, but is not breaking down into digestible components. It appears that the specimens are 'starving' as they are not able to absorb nutrients and energy from the feed they are consuming to grow and develop.

4.4.5.2.3 Diagnosis

Digestive gland maldigestion.

Submission 2

- 17 dph

- 4/7 tanks treated with 100 mg lt^{-1} Oxytetracycline for 10 days: 19/11/12 is day one of the second ten day antibiotic treatment course

- Mortalities still occurring;

  - Treatment 2 still has greater survival then Treatment 1

- Larvae have started to exhibit 'bottom dwelling' behaviour.

4.4.5.2.4 Histopathological description
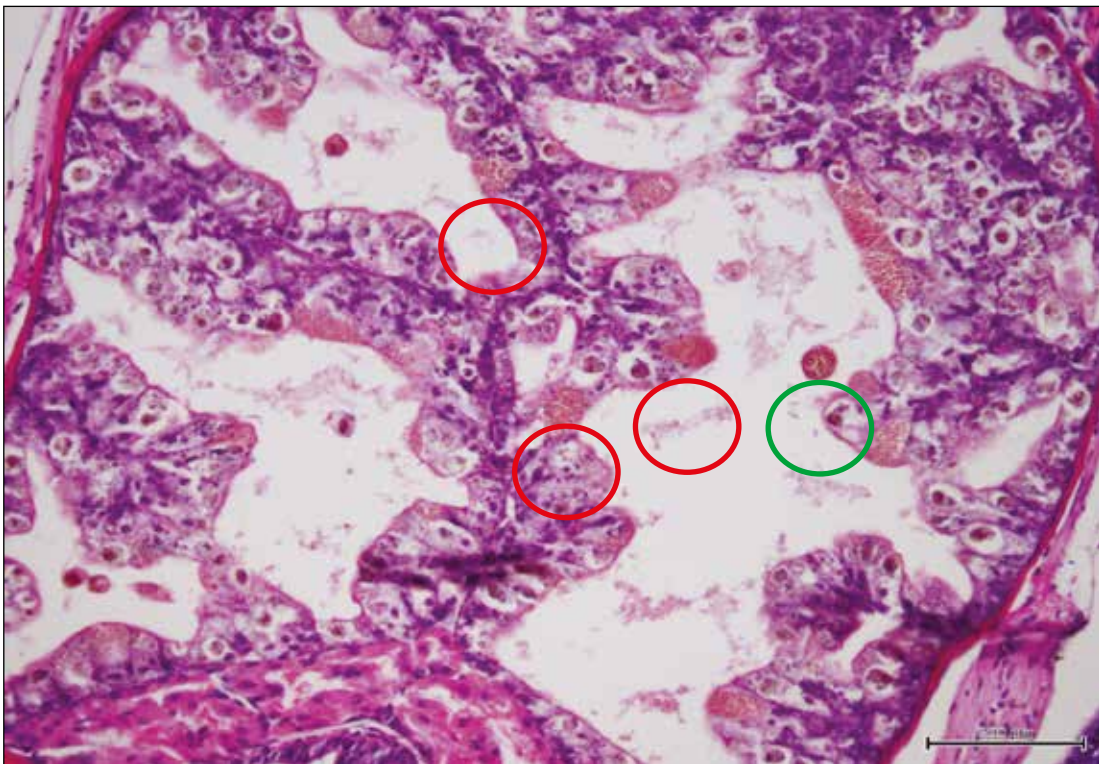
Figure 46. Digestive gland of larvae from tank 4 (treated with antibiotic) showing yellow-brown material (red circles) within vacuoles. This yellow-brown material is also sloughing off into the lumen (green circle). The digestive gland tubules are also irregular in shape (10X).

At 17 dph, the cytoplasmic vacuoles containing the yellow-brown material are larger and more prominent than in larvae from the previous submission (Fig. 46). In some specimens, these vacuoles are sloughing into the lumen and the digestive gland epithelial cells are enlarged and irregular in shape. Some specimens also have dilated haemolymph channels and no bacteria can be identified.

Figure 47.   Dilated haemolymph channel (circled) in larvae from tank 2 (10X).

4.4.5.2.5 Comments

The digestive gland pathology is worse than in the previous submission (12/11/12) and the digestive gland tubules are becoming irregular in shape. One specimen from a tank not treated with antibiotics has a shrunken (atrophied) digestive gland. Both specimens collected from the bottom of the tank and those collected higher in the water column have dilated haemolymph channels.

4.4.5.2.6 Diagnosis

Digestive gland maldigestion and haemolymph channel dilation.

Submission 3

- 24 dph

- 4/7 tanks treated with 100 mg lt^{-1} Oxytetracycline for 10 days: 26/11/12 is day eight of the second ten day antibiotic treatment course

- Mortalities still continuing;

  - Treatment 2 still has greater survival than treatment 1

- Some larvae have a 'balloon' appearance and most are moribund

- Three classes of larvae were submitted:

  - Dead

  - Moribund but alive

  - Alive

4.4.5.2.7 Histopathological description

There was a variable appearance in the digestive gland morphology across the submission. In larvae submitted dead, there was complete breakdown of digestive gland structure. In moribund but alive larvae, the digestive gland tubule structure was still present but there is more sloughing of vacuoles into the gut lumen and in some specimens, the gut epithelial cells are degenerating. There was also severe oedema of the sub-epithelial tissues and skin in these moribund specimens. In larvae submitted alive, the digestive gland morphology was also variable in appearance. Basophilic cells were still present within vacuoles and these are larger and more numerous and there are other vacuoles containing dark brown (suspect pinocytic vesicles that draw material into the cell) and yellow-brown staining particles (Fig. 48). In some specimens the brush border lining the digestive tubules was discontinuous and cell contents have sloughed into the lumen.
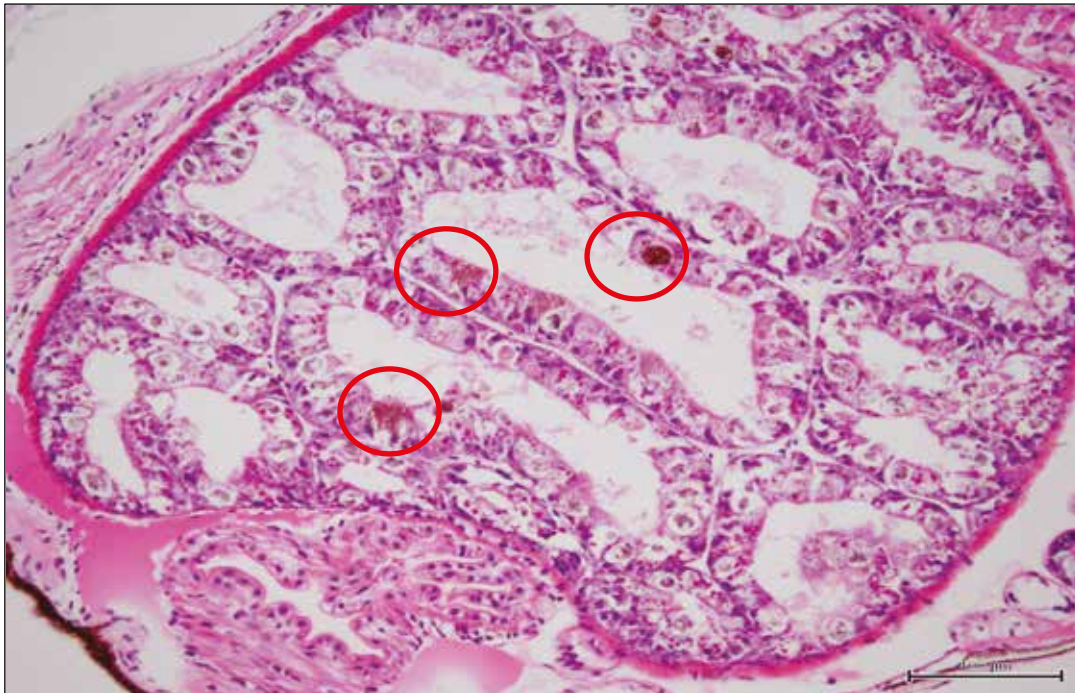
Figure 48. Digestive gland of 'live' larvae from tank 4 (treated with antibiotics) showing the presence of basophilic particles (suspect residual bodies) and dark brown particles (suspect pinocytic vesicles in red circle) within vacuoles (20X).

4.4.5.2.8 Comments

Across this submission, the condition of the digestive gland is generally worse than in the previous submission. There is more sloughing of cell contents into the gut lumen and the epithelial cells are severely irregular in shape containing larger basophilic bodies. The 'balloon' appearance of the mantle of the moribund larvae is most likely caused by oedema from the loss of control of osmoregulation.

4.4.5.2.9 Diagnosis

Digestive gland maldigestion and oedema of the mantle.

Submission 4

• 33 dph: furthest days post hatching any batch has been grown out to

- Antibiotic treatment completed
- Mortalities still continuing;
  - Approximately 30 remaining larvae remaining in tanks;
    - Tank 1: Antibiotic treated: Two larvae submitted
    - Tank 2: Not treated with antibiotic: Two larvae submitted
    - Tank 4: Antibiotic treated: Three larvae submitted
- Larvae are noticeably larger in size.

4.4.5.2.10　Histopathological description

Tank 1: Filamentous bacteria and haemocytes present in the connective tissue as well as in the skin (Fig. 49). No digestive gland was visible in this plane of section.
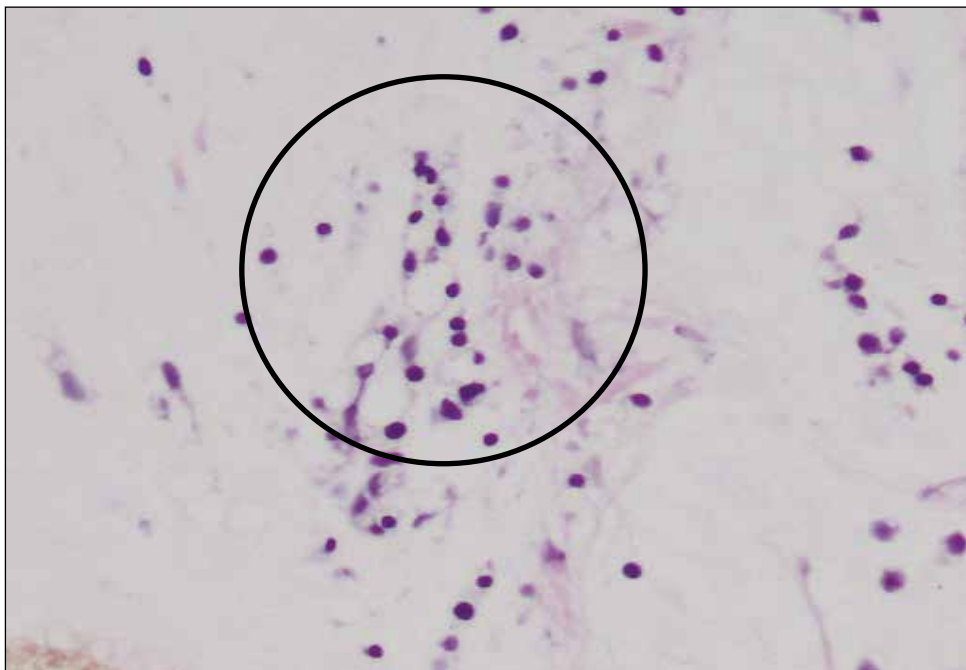
Figure 49.　　Haemocytes (in circle) within the connective tissue near the beak in larvae from tank 1 (20X).

Tank 2: The digestive gland vacuoles contained many brown-yellow particles and material sloughed off into the lumen (Fig. 50).
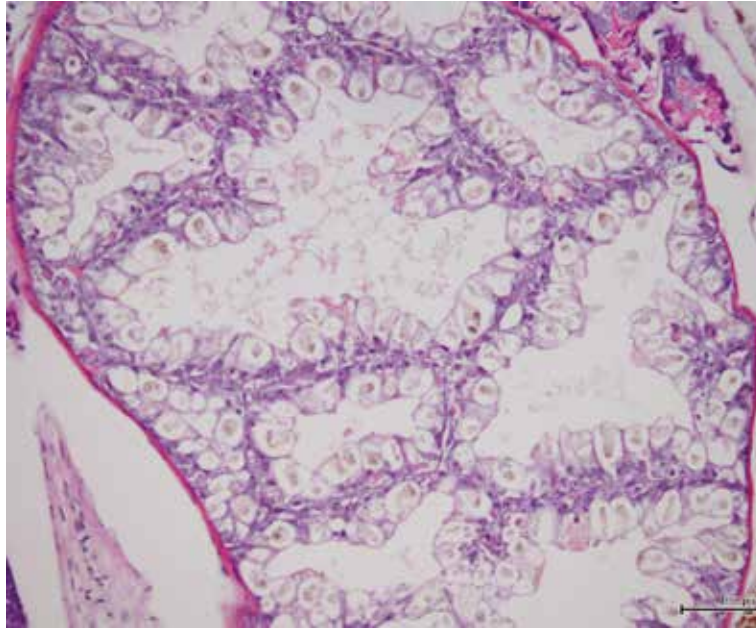
Figure 50.   Digestive gland from larvae in tank 2 (<u>not treated</u> with antibiotics) with yellow-brown particles within vacuoles (20X).

Tank 4: Two out of the three specimens had digestive glands containing many brown-yellow particles within vacuoles (Fig. 51). No basophilic particles were seen in these specimens. The third specimen had large numbers of small homogenous red-brown vesicles and brown-yellow amorphous vacuoles similar to the other specimens and a few basophilic inclusions within the digestive gland tubules. The villous border was also irregular in this third specimen.
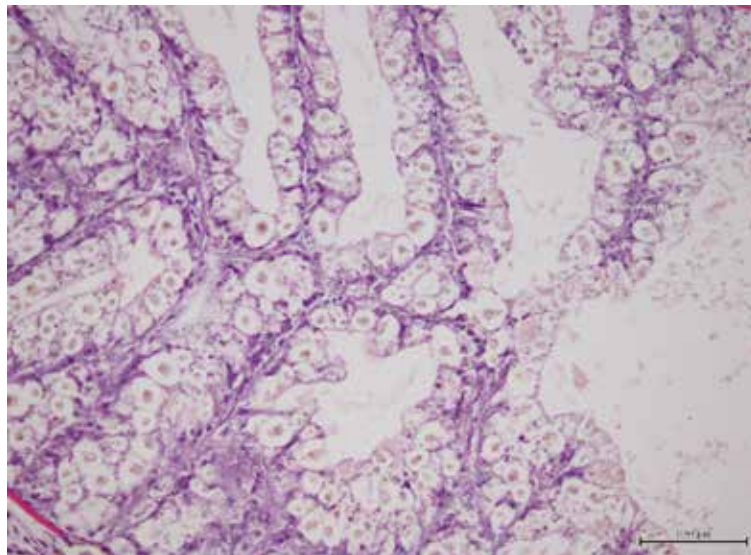
Figure 51.   Digestive gland from larvae in tank 4 (<u>treated</u> with antibiotics) with yellow-brown particles within vacuoles (20X).

4.4.5.2.11   Comments

The structure of the digestive glands of specimens in Tanks 2 and 4 appear to contain less evidence of gut degradation compared to those in the earlier submissions. It appears that the gut lesions in these specimens are less severe or improved; however as the understanding and

recognition of 'normal' digestive gland structure in these cephalopod creatures is currently limited, it is difficult to ascertain whether the digestive gland degradation is resolving. The presence of haemocytes in specimens from Tank 1 suggests that the animal is able to mount an immune response to the invading pathogens (filamentous bacteria).

4.4.5.2.12   Diagnosis

Tank 1: Bacterial infection.

Tanks 2 and 4: No diagnosis.

4.4.5.2.13   Conclusions from antibiotics treatment

Digestive gland morphology

There is no significant histological difference in the digestive gland structure between specimens in treatment groups 1 and 2. Specimens treated and not treated with antibiotics both showed digestive gland degradation throughout submissions in this trial. It is possible that the current diet on offer is not suitable or nourishing enough to support digestive function in these creatures and this may predispose them to internal and external bacterial infections that were identified on histological examination throughout the submission.

It appears that the digestive gland is not able to process the food material on offer and this ingesta is being trapped within digestive vacuoles in the gut. The presence of numerous lysosomes/ residual bodies suggests that there may be a problem with waste processing in these specimens. The specimens may be starving as they are unable to assimilate nutrients they need for growth and development from the diet they are consuming.

In other species of molluscs the digestive tubules undergo a sequence of cytological changes during a digestive cycle. For example, in bivalves a holding, absorptive and disintegration phase within the digestive gland have all been described and recognised. Each phase consists of distinct characteristics that are displayed when the bivalve is undergoing a specific digestive phase; *'in the fasting animal, the digestive cells are flat and the lumen wide; this is known as the holding phase. Absorption and intracellular digestion begin with food arrival. The digestive cells become taller and filled with digestive vacuoles. The lumen is narrow and the crypt cells develop cilia. At the final phase, the apical part of the digestive cells swell and protrudes into the lumen releasing numerous free spherules subsequently discharged into the stomach. The disintegration phase is followed by a reconstituting phase during which crypt cells regenerate new digestive cells'* (Grizel, 2003). It is possible that a 'digestive cycle' is being observed as the dph increases.

Further investigation is required to identify the composition of the lysosome material. Samples have been stored in glutaldehyde fixative for TEM (transmission electron microscopy) to examine the structure of the residual bodies.

Bacterial infection

Despite two 10 day treatments with Oxytetracycline therapy, bacteria were still present histologically in the connective tissue in some specimens in the antibiotic treated tanks. This suggests that these animals were stressed due to conditions in their surrounding environment or the nutrition they were receiving was not adequate to promote healthy immune function. The presence of haemocytes in a specimen from submission four (trial 3) suggests that at 33 dph the larvae are able to mount an immune response to invading pathogens. This is a significant

finding in this trial and subsequent trials will confirm this phenomenon.

References

Grizel, E.H. (2003). An Atlas of histology and cytology of marine bivalve molluscs. Ifremer. France.

Buller, N.B. (2004). Bacteria from Fish and other Aquatic Animals; A Practical Identification Manual. CABI Publishing, Oxfordshire, UK.

4.4.6   Static vs transfer treatment

4.4.6.1   Introduction

Hypotheses aimed to determine whether already established methods of passive transfer of larvae reduced total cfu of bacteria in culture tanks and hence prolonged survival of larvae. Static tanks with no passive transfer were used as control treatments. Through establishing protocols with the Department of Agriculture to determine total cfu using bacteria plating techniques, bacteria levels in tanks from both treatments were monitored weekly. Fresh mussel and crab meat were used as the protein components in the *Artemia* enrichment

4.4.6.2   Methods and protocol

Tanks were operated as upwelling, where water flow was distributed through 10 mm holes at the base of 40 mm standpipe. Holes were covered in 250 μm mesh. System was comprised of two 'double tank' fibreglass tanks and two 'stand alone' static tanks all 1000 lt volume with conical base and 250 μm box filters.

Larvae were counted into tanks in groups of 10 using click counter. Approximately 8000 larvae to 1000 lt tanks counted using this same method.

Larvae were fed 6 times a day or as required, 1-6 hour enriched *Artemia* that are between 1.5-2.5 mm. 6am enrichment over 6 hours (0200 – 0800 hrs), 12 pm enrichment over 1 hour (1100 – 1200 hrs) and 3pm enrichment over 1 hour (1400 – 1500 hrs). *Artemia* were fed via cold storage 3 times overnight at 1800 hrs, 0000 hrs and 0600 hrs. Feed densities were monitored as to not have excess of *Artemia*, but at the same time not to over feed larvae. *Artemia* numbers were governed by size of mesh on screens and the jugging out excess animals when required. 50 000 *Artemia* per tank were fed overnight to all tanks. All tanks were fed 500-800 μm semi moist micro diet from 10 dph via automated feeding system. Enrichment was made using a mussel homogenate.

Artemia were fed 30 gr of enrichment mixed with 1.5 lt seawater, blended for 30 seconds. Blended enrichment was split into 3 bottles (500 ml each bottle). The overnight enrichment in fridge was topped up with 500 ml seawater. Enrichment cones were filled to 20 lt with a trickle of pure oxygen.

A water temperature of 21°C was maintained via heater chiller unit with a flow rate of 1000 lt hr^{-1} for 1000 lt tanks. Temperature and Dissolved Oxygen measurements were taken daily. Clear water culture was used for all tanks.

One pair of larvae tanks were transferred to adjacent clean tank after 7 dph on an ad-hoc basis, judging tank cleanliness daily (approximately every 4th day). Two remaining tanks were left static with no transfer.

A 14 hours dark/10 hours light photoperiod was used with 550-600 lux.

250 μm screen that was siphoned in 1000 lt tank once daily of *Artemia* and copepods, returning larvae back to tank. Tank bottom was siphoned daily into screen bucket and remaining live larvae returned to tank using pipette.

Two bacteria samples from each tank were plated and counted at regular intervals according to protocol provided by Department of Agriculture. Measurements of 4 larvae from each tank to be taken every 7th day and weekly samples of larvae submitted for histological analysis of digestive system.

4.4.6.3   Results

Bacteria
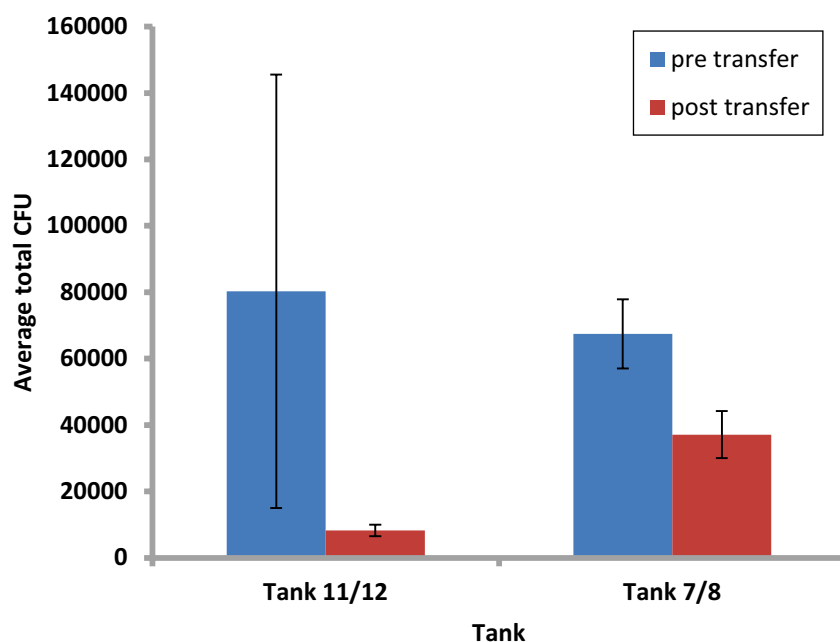
Figure 52.   Average total cfu of transfer tanks pre and post transfer at -2 dilution

The average total cfu in both tanks decreases significantly post transfer. Tank 11/12 initially shows average total cfu of 80250 and is reduced to 8250 average total cfu post transfer. Tank 7/8 shows average total cfu of 67500 and is reduced to 37125 cfu post transfer (Fig. 52).
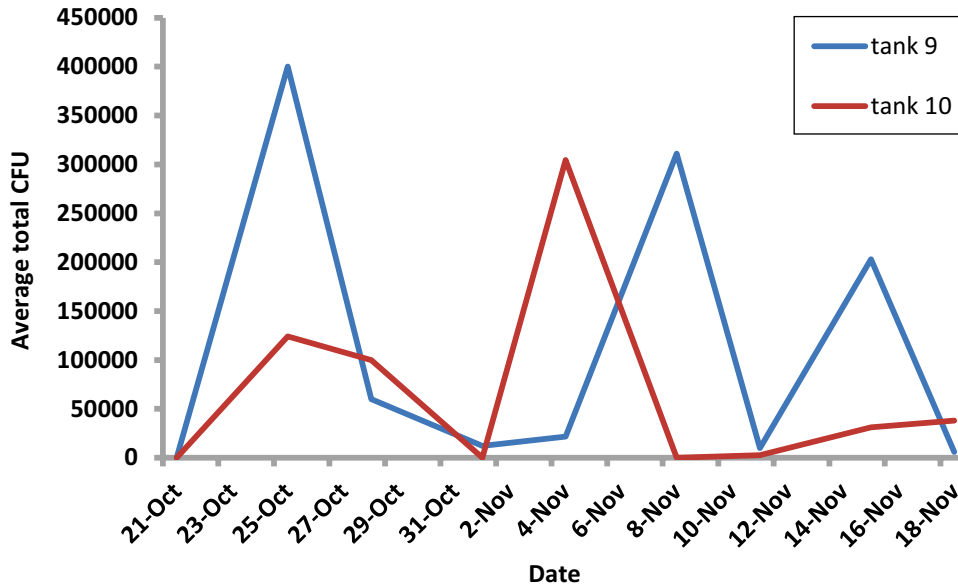
Figure 53.   Average total cfu of static tanks 9 and 10 at -2 dilution from 25th October - 18th November

Static tanks 9 and 10 show a series of sharp increases, followed by sharp decrease in average total cfu. This pattern is mirrored in both tanks with a lag period of 3–4 days in tank 9. Tank 10 reaches a peak average total cfu of 304500 on 4th November while tank 9 reaches a peak average total cfu of 311000 on 8th November. On 18th November at trial completion average total cfu is 6000 in tank 9 and 38000 in tank 10 (Fig. 53).
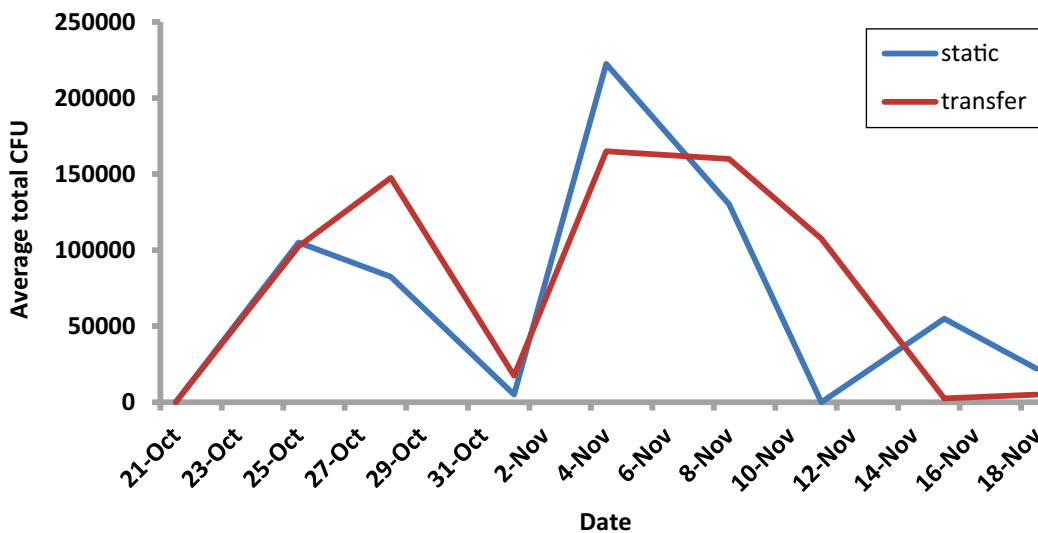
Figure 54.   Average total cfu of static and transfer tanks at -3 dilution from 21st October – 18th November

Static and transfer treatment tanks both show similar increase and decrease patterns in average total cfu (Fig. 53). Both static and transfer treatments show a significant decrease in average total cfu on 1st November, where static tanks are 5000 cfu and transfer tanks are 17500 cfu. Both treatments reach their peak on 4th November where static tanks are 222500 cfu and transfer tanks are 165000 cfu. The increases and decreases in total cfu are greater in static tanks (Fig. 54).

Growth
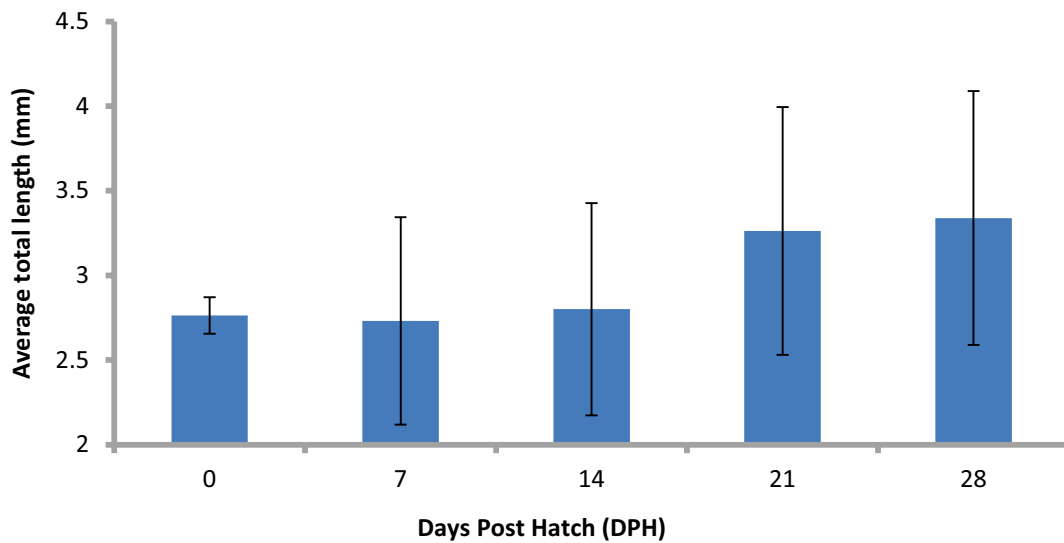
Figure 55.   Average total length of larvae in all tanks from 0 to 28 DPH

The average length of larvae at 0 dph is 2.76 mm, while the average length of larvae at 28 dph is 3.33 mm, showing an increase in length of 0.57 mm. there was a decrease in average total length from 0 dph to 7 dph of 0.03 mm. The greatest increase in average total length is between 14 dph to 21 dph of 0.46 mm (Fig. 55)
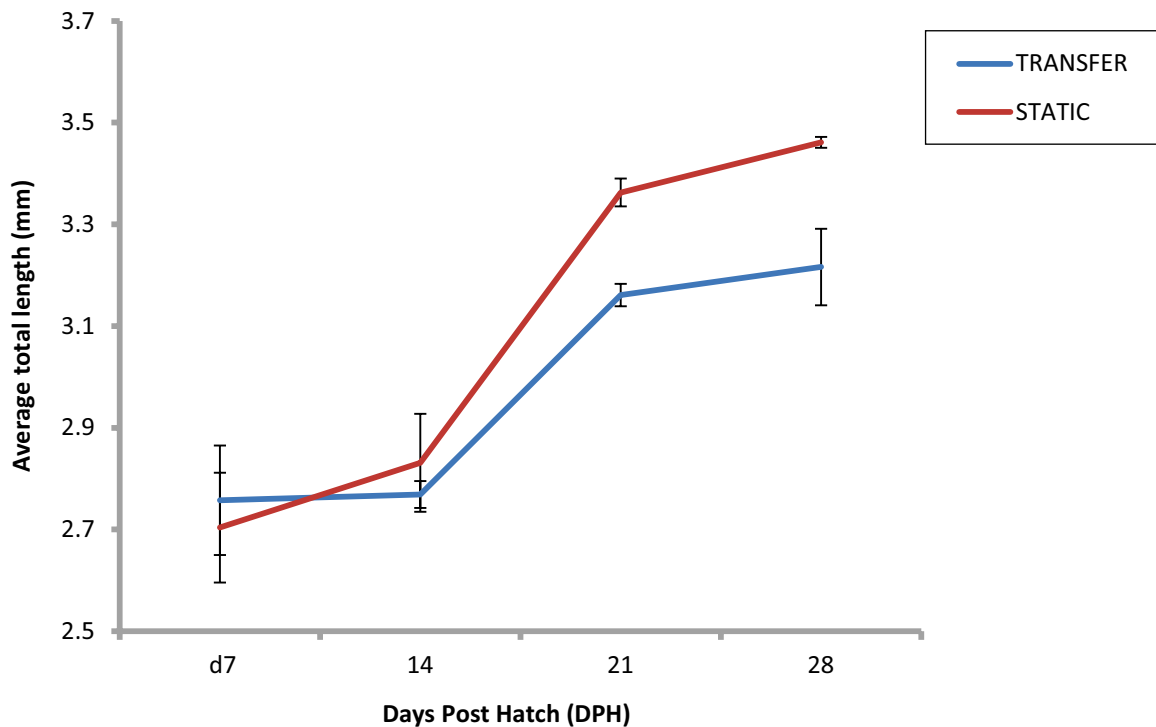
Figure 56.   Average total length of larvae showing transfer and static treatments from 0 to 28 dph

The average length of larvae at 0 dph is 2.76 mm. The average length of larvae is greater in the static treatment with an average total length of 3.46 mm at 28 dph and an average length in the transfer treatment of 3.21 mm at 28 dph, showing a difference in average length of 0.25 mm.

There is a decrease of average total length at 7 dph in static treatment. The average length of larvae in the static treatment at 7 dph is 2.7 mm while the average length of larvae in the transfer treatment at 7 dph is 2.75 mm (Fig. 56).

4.4.6.4   *Discussion*

From the results it is evident that although bacteria is reduced in transfer tanks from pre to post transfer, the overall total cfu is relatively similar for both static and transfer treatments. There appears to be a trend in cfu levels in both transfer and static treatments which shows significant increase and decrease patterns. These results suggest that it would be optimal to leave tanks static as this is likely to prevent any ill health of larvae caused by stress of being moved from one tank to another. Static treatment produced a greater total length of larvae on average compared to transfer treatment at trial completion. It is possible those aspects of the passive transfer process are inhibiting larval growth or that the larvae are generally under stress when being passively transferred and as a result their growth is less than those larvae in static tanks.

It is noteworthy that the larvae used in this experiment were sourced from eggs produced in captivity and potentially not as resilient or healthy as wild produced larvae, therefore survival may have been influenced by this factor. Counts of bacteria colonies were compromised in some cases where an agar plate was covered in swarming vibrios bacteria, causing other colonies that may have formed to be outcompeted and hence altering total cfu. From daily observations of larvae in comparison to spikes and drops in total cfu, it remains unknown as to whether bacteria has any significant effect on larvae health and survival.

5.0 Ranching (*Octopus tetricus*)

5.1 Initial grow-out and commercial system install.

Several systems were installed and/or modified for octopus grow-out throughout the duration of the project. These include

- 15 x 1000 lt conical tanks (grow-out)
- 18 x 400 lt rectangular tanks (broodstock)
- 3 x 2300 lt (grow-out)
- 1 x 10,000 lt (broodstock)
- 1 x 5000 lt (quarantine, broodstock)

Several different tanks (fibreglass, plastic moulded) were tested as potential tanks for commercial farming. Based on initial small scale experiments using existing aquaculture tanks at WAFMRL, two different tanks were designed and constructed. The tanks were tested and one was found to be ideal for commercial farming combining ease of use and being relatively inexpensive to manufacture. See ranching protocol for details on prototype tank developed for commercial grow-out.

5.1.1 System 1

Tanks

This system comprised of 15 x 1000 lt conical based, fiberglass tanks. Each tank had incoming, flow through sea water at the surface, with drainage at the base of the tank.

Tank Components

Standpipe

Each tank contained a 40 mm (PN9) PVC internal standpipe which had 10 mm holes extending 300 mm up from the base. The holes allow water to flow out the base while keeping octopus and food in the tank.

Surface skimmer

Each tank contained a 40 mm (PN9) PVC surface skimmer which also contained 10 mm holes. These holes allow protein and faeces to be skimmed off the tank surface, but keep octopus inside the tank

Shadecloth ring

Each tank contained a 500 mm collapseable shadecloth ring. Octopus cannot adhere there tentacles to the shadecloth and hence could not climb out of the tanks when the ring was erected. The ring could be easily raised and lowered at the user's discretion (Fig.57). See the ranching protocol for the shadecloth ring's components and dimension.

Figure 57.   1000 lt system and tank components.

5.1.2   System 2 (commercial prototype)

Tanks

This system comprised of 3 x 2300 lt conical based, fiberglass tanks. Each tank had incoming, flow through sea water at the surface, with drainage at the base of the tank. Incoming seawater is hard plumbed into the wall of the tank to prevent octopus using it as a form of escape (Fig. 58).

Tank Components

Figure 58.   2300 lt system and tank components. Red arrow indicates hard plumbed seawater inlet.

Standpipe

Each tank contained an 80 mm (PN12) PVC standpipe with large 40 mm holes extending 1500 mm up from the base. The 40 mm holes ensures adequate water drainage as these tanks were at times, subjected to high water exchange coupled with high biomass. The standpipe is tightly covered by an oyster mesh sleeve which keeps octopus and food in the tank. The sleeve was held in place by a steel pin at the top. Removal of the pin allows the user to lift the sleeve and remove un-eaten food and waste from the tank in conjunction with opening the 150 mm gate valve at the base of the tank (Fig. 59).

Figure 59.   2300 lt tank components.

Shadecloth Ring

See (section 5.1.1)

Gate Valve

A 150 mm gate valve was fitted to the base of all tanks. This allows for a user friendly and easy method of discharging uneaten food and waste from the tanks into a screen bucket (Fig. 60).

Air Stone

An external air source in the form of an airstone is hard plumbed into the wall of all tanks. A valve on the outside of the tank allows volume control. Hard plumbing is essential as octopus can use the line as form of escape (Fig. 60).
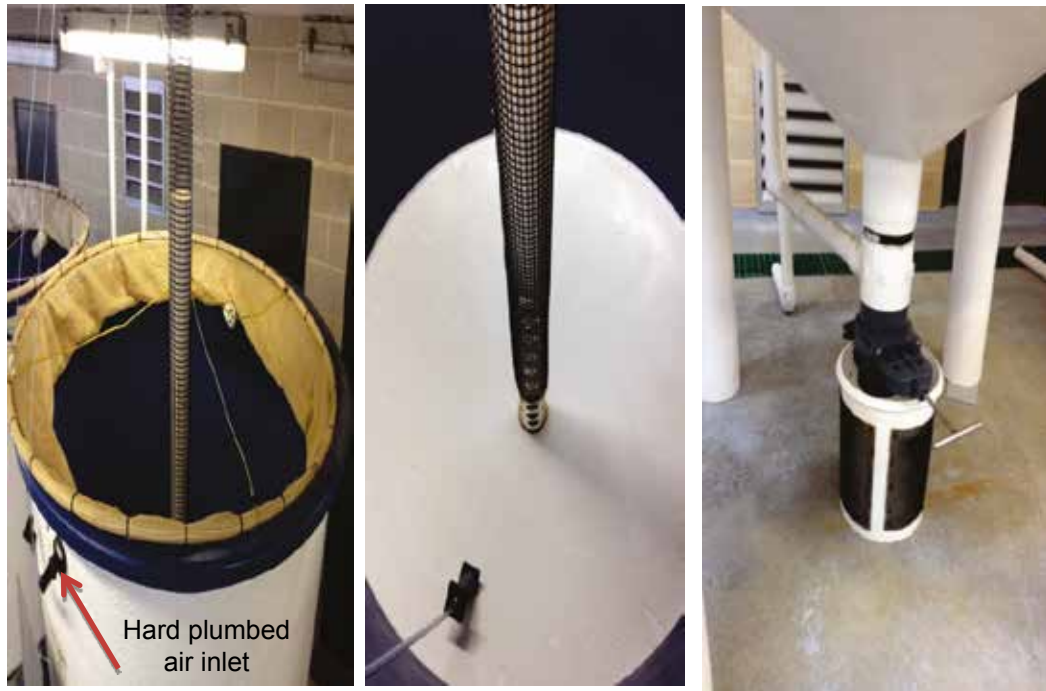
Figure 60.   2300 lt tank components. Red arrow indicates external air valve.

5.2   *Feed and feeding (including fresh, frozen and formulated)*

Growth and food intake of juvenile *O. cf. tetricus*

Vincent Boer[1], Justin King[2] and Sagiv Kolkovski[2]

[1]Wageningen University, Netherland, [2]Department of Fisheries Western Australia

5.2.1   Introduction

This study, within the bigger study of closing the lifecycle of *O. cf. tetricus* for aquaculture, focused on the growth of *O. cf. tetricus* juveniles. Just as with studies on *O. vulgaris*, it is important to find the right nutritional food. To understand the nutritional requirement of *O. cf. tetricus*, it is important to look at their natural diet. Unfortunately, not much is known so far, although observations show that it consists of crustaceans and molluscs (Joll, 1977). Diet studies on *O. vulgaris* (Smith, 2003) showed similar results, with diets consisting of 63,6% crustaceans, 37,6% molluscs, 11.2% teleosts and 10.8% polychaetes. Most probably, diet is highly dependent on the food sources available. Because both crustaceans and molluscs are expensive products, it is important to investigate the growth of *O. tetricus* on commercial, non-expensive foods that are available locally.

Garcia and Valverde (2005) showed that with part of the diet consisting of crab (*Carcius mediterranus*) combined with fish (*Boops boops*), *O. vulgaris* had higher growth rates than with diets consisting only (non-expensive) fish. A study done by Rodriquez *et al.* (2006) looked at the on-growing of *O. vulgaris* in cages, which proved successful with a mixed diet of fish, crab and mussel. Pham and Isidro (2009) found that also with a mono-diet consisting of fish (Mackerel, *Scombercolias*), octopus can reach commercial size quickly, but with high aggressiveness and mortality, probably due to the limited nutritional value of the food. Eventually, these studies must lead to an understanding of the nutritional demands of *O. vulgaris*, which in the future

will lead to the development of a commercial, artificial diet (Quintana 2008, Rosas 2007, CerezoValverde and Dominquez 2007, Aguila 2007).

In this study, we focused on the growth and food intake of octopus fed on three different mono-diets; Krill (*Euphausia sp.*, Hikari Bio Pure ©), Whitebait (low value juveniles of several fish) and Mulie (Australian Pilchards, *Sardinops neopilchardus*). These diets were tested on small locally caught *O. cf. tetricus* juveniles that were kept separately under experimental conditions. As a continuation of the experiment after a significant data collection period, half of the octopuses on each diet were switched to one of the others. This gave an insight in any long lasting effect of the nutritional composition of the previous diet and the response of a new diet on growth and food intake.

5.2.2   Methods

Animals

The octopus used in this study were caught with shelter pots off the coast of Fremantle, Western Australia, by fisherman operating under the exemption of Fremantle Octopus Pty Ltd. A total of 24 octopus were caught and used in this study, ranging from 13.05 to 307.47 gr in weight at the start of the experiment. The animals were kept together in a 500 lt holding tank before the experiment, without shelters.

Set-up

The tanks used in this experiment were 24 small glass aquariums (60 x 30 x 40 cm, 60 lt), which were separately connected to an open system of sand-filtered, cleaned seawater (32 ppt salinity). Seawater flowed into the tanks at a rate of approximately 30 lt hr^{-1} resulting in water exchanged every two hours. Temperature, oxygen and pH were checked twice daily at the start (morning, before cleaning, afternoon after feeding) and daily afterwards (first day morning, next day afternoon etc.) The octopus were put separately into each tank five days before the start of the experiment. Each octopus received a piece of PVC-pipe (diameter 40 to 100 mm corresponding to body size) as a shelter.

To prevent escaping, each tank was covered with a plastic lid that was partly glued on, with two removable hatches. An airline with air stone and seawater-inlet were provided through the lid to the bottom of the tank, while the seawater-outlet was positioned on the opposite sides of the tanks, extracting water from the surface.

Both the seawater inlet and outlet were covered with 20 mm PVC tube with 2 mm holes, to prevent escaping of octopus.

To minimize any possible effect of the set-up, the treatments were randomized. Octopus were caught one by one from the holding tank and placed randomly in one of the tanks.

Three different feeds (treatments) were supplied to the animals. The feeds were chosen based on their commercial and local availability and the fact that they can be obtained at low costs in large quantities. Krill (*Euphausia sp.*, Hikari Bio Pure ©) is a commercial caught small shrimp-like invertebrate marine animal found in the Antarctic region. It is used as a feed for ornamental marine fish. Whitebait is a locally caught species that is used as bait in the recreational fisheries and as well as for human consumption. Mulie (Australian Pilchards, *Sardinops neopilchardus*) is a locally caught species used as bait.

Daily protocol

The octopus were fed to satiation once a day, in the end of the afternoon. The amount of food in WW (Wet-Weight) that was given to the octopus was weighed and put in an aluminium tray. All diets consisted of complete pieces of food, without removal of guts, heads etc. Because mulies were too big to be fed whole to the small animals, the octopus on the mulie diet received only half a fish, first day a head part, the next day a tail part. The amount of food supplied daily ranged from 7–10% of the total bodyweight of the octopus. After preparing all the food, the octopus were fed at the same time. In the morning, all the leftovers were collected per tank with a small net (maze 1.0 mm) and put in a pre-weighed aluminium tray. These were dried in an oven at 100 °C for 48 hours, and weighed afterwards to measure the DW (Dry-Weight) content of the food left.

Because food was measured in WW when feeding the octopus and in DW when measuring the leftovers, a DW-WW ratio was calculated for the different diets. To do so, 16 mulie parts (8 head, 8 tail) and 12 pieces of krill and whitebait were weighted and dried as described above. The WW/DW ratio was then calculated.

The octopus were weighed every two days from the start of the experiment. To minimize stress and to simplify this task, all the shelters were weighed before the experiment, to allow octopus to be weighed together with their shelter. An octopus with it's shelter was taken out of a tank and placed into a small 5 lt bucket on a scale and weighed. The octopus was then quickly put back in its tank, while the remaining water in the bucket was weighed. The total weight minus the shelter and the remaining water gave the weight of the octopus in WW.

Observations that were made daily included inking of octopus while weighing or cleaning and the reaction of octopus when receiving food.

Protein, lipid, ash and moisture content was determined for all feeds by the Chemistry Centre, WA.

Analyses

Absolute growth rate (AGR) and specific growth rate (SGR) were calculated per octopus over each two day period:

1) $AGR = ((BW_{T-2} - BW_T) / t)*100$

2) $SGR = ((BW_{T-2} - BW_T) / BW_{MID} / t)*100$

Where BW_T and BW_{T-2} are the weight of the individual octopus on a weighing day and two days before, BW_{MID} is the mean weight of an octopus over a two day period and t is the time in days.

The weight gain (WG) over the initial bodyweight over the whole experiment was calculated with the following:

3) $WG = ((BW_{FIN} - BW_{INI}) / BW_{INI})*100$

Where, BW_{FIN} and BW_{INI} are the weight of the individual octopus at the end and the beginning of the experiment, BW_{MID} is the mean weight of an octopus over a two day period and t is the time in days.

Food intake (FI) and food conversation ratio (FCR) were calculated with the following:

4) $FI = ((F_{DW} / BW_{MID}) / t)$

5) FE = WG / F_{WW})*100

6) FCR = F_{WW} / WG

Where F_{DW} is the amount of food eaten in DW over a two day period, BW_{MID} is the mean weight of an octopus over a two day period, t is the time in days, WG is the weight gain of an octopus over the total experiment and F_{WW} is the total amount of food eaten in WW over the total experiment.

The DW/WW ratio (DWR) of each diet was calculated with this formula:

7) DWR = F_{DW} / F_{WW}

Where F_{DW} is the amount of diet left after drying and F_{WW} is the amount of diet measured before drying.

Statistical analyses was done with Statistica (version 7.1, Statsoft Inc ©). WG, SGR, FCR and FI were compared between diets with an independent sample T-test. Correlations were made over BW_{INI}, BW_{MID} and WG, SGR, FCR and FI with a linear fit.

5.2.3   Results

Experiment 1

All octopus seemed to eat well at the start of the experiment, all the food that was given was taken instantly. After a couple of days, at least visually, some changes were noticed. The mulie that was given to several octopus was eaten irregularly, while the krill and whitebait were accepted with enthusiasm. The experiment was stopped at day 20, after a significant difference occurred between the growth of the whitebait group and the mulie and krill group respectively.

Out of the 24 octopus used during this experiment, only one octopus died (not from natural causes). The lid of the tank where the octopus was kept wasn't closed properly resulting in the octopus escaping.

Mean water temperature 17.45 °C (± 0.40°C). pH ranged from 8.1 to 8.4.

Growth

The group of octopus that were fed whitebait increased almost 36% in weight during the 20 days of the experiment, while the krill and mulie group stayed behind with an increase of 4.92% and 7.98% respectively (Table 7). SGR was also much higher in the whitebait group (1.49% BW per day^{-1}) than in the krill and mulie group (0.23% and 0.36% BW per day^{-1} respectively) (Fig. 61).

Table 7.   Summary of weights, growth rates and feeding of the octopus on three different diets

Diet	Krill	Mulie	Whitebait	Significance
N	8	6	8	
BW_{INI} (g)	178,4±44,3	220,2±53,0	177,4±68,0	n.s.
BW_{FIN} (g)	187,1±46,4	240,3±73,8	234,1±78,4	n.s.
WG (%BW_{INI})	4,92a±5,75	7,98a±11,74	35,59b±16,41	p<0,005
SGR (%BW/day)	0,233a±0,282	0,359a±0,550	1,490b±0,602	p<0,005
FE (%WG/F_{WW})	7,72a±9,88	12,85a±22,61	37,80b±11,03	p<0,05
FI (%DW/BW/day)	0,46a±0,04	0,62b±0,09	0,80c±0,10	p<0,005
FCR (F_{WW}/WG)	9,27±8,72	0,15±6,74	2,87±0,91	n.s.

N = Number of octopus, BW_{INI} = Initial Bodyweight, BW_{FIN} = Final Bodyweight, WG = Weight gain, SGR = Specific Growth Rate, FCR = Food Conversion Ratio, FI = Food Intake.
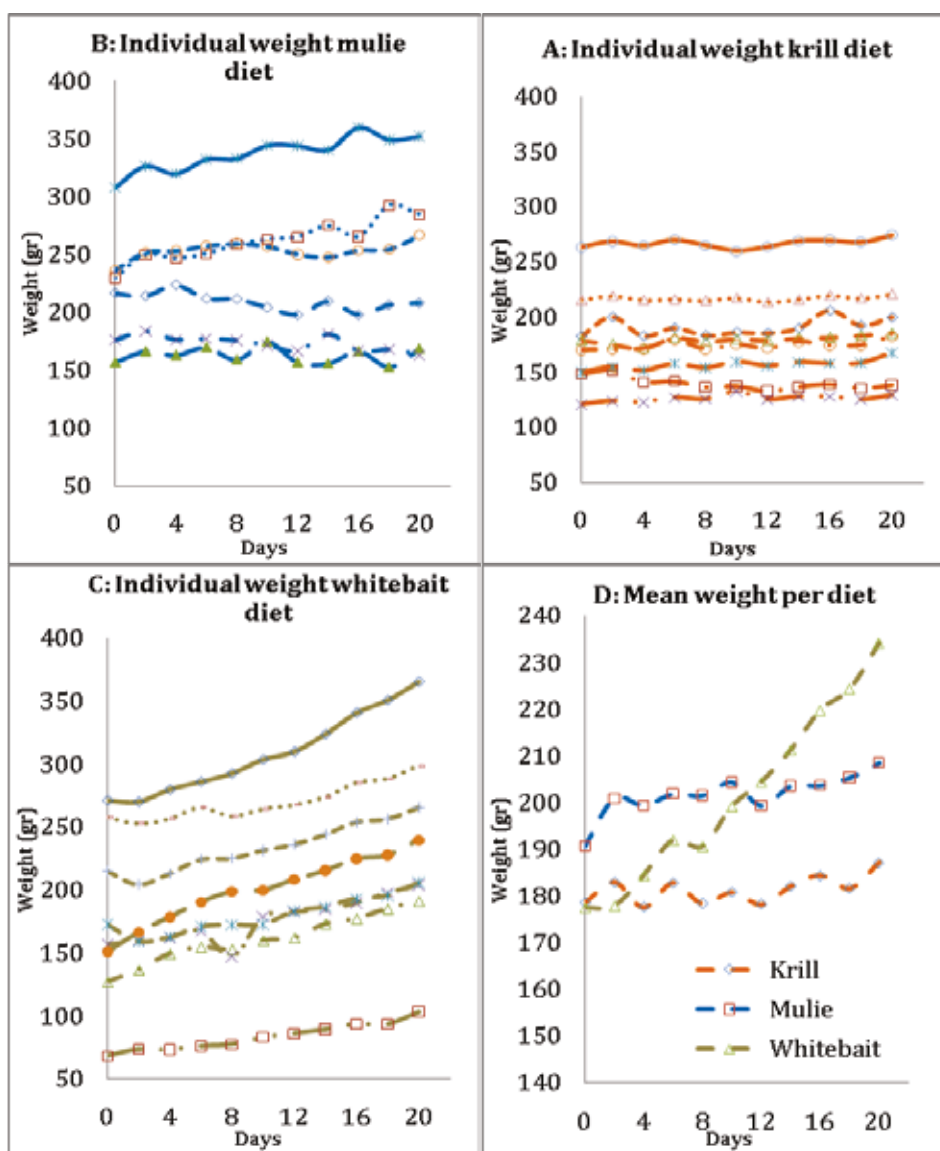
Figure 61.   Growth of individual octopus on the three different diets (A: krill, B: mulie, C: whitebait) and the mean growth of the three diets as a total (D).

No correlation were found between BW_{INI}, BW_{MID} and WG, SGR, FCR and FI in the krill and mulie octopus groups, and only between BW_{MID} and FI in the whitebait group, with a decreasing FI with bigger octopus (Table 7)

Experiment 2

Set up

Whitebait was shown to be the best diet from the previous experiment. Following that, it was interesting to see how the octopus that have been fed on mulie and krill, that somehow have a lower nutritional value, will react in growth and food intake on receiving the best diet out of the first experiment; whitebait. Therefore four out of each eight octopus of the mulie and krill diet were randomly selected for the whitebait diet, while the other four stayed on the previous diet.

It was interesting to see if a mixed diet would give any nutritional benefits to the octopus. This was carried out by giving four octopus from the whitebait a mixed diet consisting of krill and whitebait. Krill was chosen above mulie due to the added vitamins in the Hikari Bio Pure product. Since octopus had to eat enough of both of the feeds in the mixed diets, each diet was given on alternate days.

Treatment:

KK= krill (no change)l, KW=switch to whitebait (from krill), MM= mulie (no change), MW= switch to whitebait (from mulie), WW=whitebait (no change), WKW=switch to mixed diet krill/ whitebait (from whitebait).

This set-up gave the opportunity to compare within a group that was previously on the same diet. Therefore the octopus of group 'KK' can be compared with 'KW', group 'MM' with 'MW' and group 'WW' with 'WKW' with a independent t-test. Furthermore, all groups can be tested against the 'background' values out of the previous experiment. This means that KK/KW, MM/MW and WW/WKW can be compared (dependent t-test) with the original growth rates and food intake found in the krill, whitebait and mulie diets respectively.

Experiment 2

All octopus seemed to eat well during the whole experiment.

No mortalities occurred during the experimental period. One octopus (Mulie diet) was left out of the results because it laid eggs after 10 days of experiment.

Mean water temperature was 19.3°C (± 0.38 °C).pH ranged from 8.1 to 8.4.

The octopus that switched to whitebait after an initial period on krill started growing from the start, while the octopus that stayed on krill remained at approximately the same weight during the experiment (Fig. 61A and 61B). A more varying trend was observed in the octopus that received whitebait after an initial period on mulie. Three out of four octopus started growing from the start, while two out of three octopus on a mono diet of mulie only lost weight (Fig. 61C and 61D).All of the octopus in the groups that remained on whitebait and the one that received a mixed diet of krill and whitebait after an initial period on whitebait grew during the experiment (Fig. 61E and F)
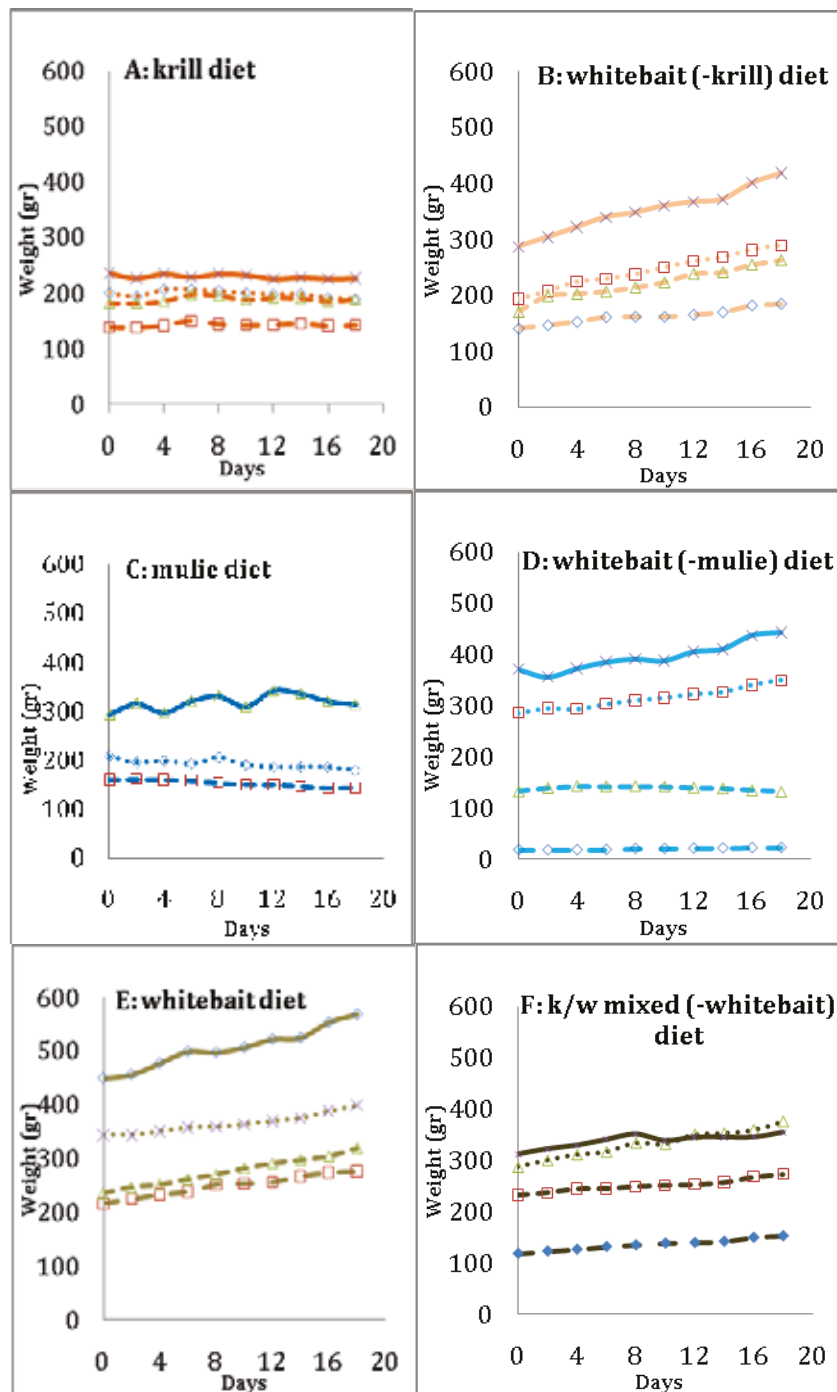
Figure 62. Growth of individual octopus on the six different diets (A: krill, B: whitebait (initial krill), C: mulie, D: whitebait (initial mulie), E: whitebait, F: krill/whitebait mixed diet (initial whitebait).

The increase in weight was more than 45% after 18 days of trial in the group that was fed whitebait after it had krill in the first experiment. A significantly lower increase in weight was observed in the three other groups that contained whitebait. The octopus that kept receiving a mono diet of krill and whitebait showed a negative growth rate (Table 8)

Table 8.   Summary of weights, growth rates and feeding of the octopus on six different diets

Diet	Krill (K)	Whitebait (K)	Mulie (M)	Whitebait (M)	Whitebait (W)	K/W (W)	Sign.
N	4	4	3	4	4	4	
BW_{INI} (g)	188,84 ±41,21	197,85 ±63,24	220,30 ±66,76	202,39 ±157,49	311,19 ±107,75	237,21 ±86,56	n.s.
BW_{FIN} (g)	186,57[a] ±34,79	288,64[ab] ±97,40	211,96[ab] ±89,42	236,65[ab] ±193,05	390,77[b] ±128,53	289,21[ab] ±101,60	p<0,05
WG (%BW_{INI})	-0,61[a] ±4,78	45,13[b] ±10,18	-5,75[ac] ±11,40	15,71[cd] ±10,95	26,52[d] ±8,15	23,01[d] ±8,46	p<0,05
SGR (%BW/day)	-0,039[a] ±0,267	2,058[b] ±0,399	-0,379[a] ±0,536	0,791[c] ±0,552	1,298[c] ±0,361	1,140[c] ±0,384	p<0,05
FE (%WG/F_{WW})	2,35[a] ±14,37	39,01[b] ±4,25	-16,22[a] ±27,66	20,95[ab] ±14,90	31,48[b] ±7,35	26,90[b] ±9,82	p<0,05
FI (% DW/BW/day)	0,38[a] ±0,17	1,07[b] ±0,09	0,64[c] ±0,05	0,71[cd] ±0,18	0,85[d] ±0,10	0,67[c] ±0,03	p<0,05
FCR (F_{WW}/WG)							

N = Number of octopus, BW_{INI} = Initial Bodyweight, BW_{FIN} = Final Bodyweight, WG = Weight gain, SGR = Specific Growth Rate, FCR = Food Conversion Ratio, FI = Food Intake.

The octopus that were fed a mixed diet of krill and whitebait received both food types on consecutive days. Besides a total food intake, this allows a separate calculation of food intake of both krill and whitebait which can be compared with the food intake of octopus fed solely one of the diets (Table 9). The mixed diet group ate significantly more whitebait than octopus that received whitebait every day (p<0,01), while krill was not eaten less (p<0,1).

Table 9.   Food intake between octopus that were fed mono-specifically on krill and whitebait and octopus that were fed a mixed diet of both.

Diet	Krill (K)	Krill (KW diet)	Significance
FI	0,38a±0,17	0,19b±0,07	p<0,1 (n.s.)
Diet	Whitebait (W)	Whitebait (KW diet)	significance
FI	0,85a±0,10	1,15b±0,01	p<0,01

A longer lasting effect of being fed on a mono diet during the experimental period was examined by comparing the results of the first and second experiment. No significant changes were observed in the specific growth rate (SGR, table 10), feeding efficiency (FE, table 11)and food intake (FI, table 12). The krill and mulie group both had a lower SGR in the second experiment, but only on a lower level of significance (p<0.10 and p<0.13 respectively).

Table 10.   Growth of the octopus fed mono-specifically on krill, mulie and whitebait during experiment 1 and 2.

Mean growth (%BW/day)

Diet	Experiment 1	Experiment 2	Significance
Krill (n=4)	0,322±0,140	-0,039±0,267	p<0,10 (n.s.)
Mulie (n=3)	0,015±0,531	-0,379±0,536	p<0,13 (n.s.)
Whitebait (n=4)	1,286±0,606	1,298±0,361	n.s.

Table 11. FCR of the octopus fed mono-specifically on krill, mulie and whitebait during experiment 1 and 2.

Mean FE (%WG/FWW)

Diet	Experiment 1	Experiment 2	Significance
Krill (n=4)	10,58±4,46	2,35±14,37	n.s.
Mulie (n=3)	-0,44±24,33	-16,22±27,66	n.s.
Whitebait (n=4)	34,14±11,56	31,48±7,35	p<0,10 (n.s.)

Table 12. Food intake of the octopus fed mono-specifically on krill, mulie and whitebait during experiment 1 and 2.

Mean Food Intake (%DW/BW/day)

Diet	Experiment 1	Experiment 2	Significance
Krill (n=4)	0,46±0,04	0,38±0,17	n.s.
Mulie (n=3)	0,55±0,05	0,62±0,03	n.s.
Whitebait (n=4)	0,77±0,13	0,85±0,10	n.s.

5.2.4   Discussion

From the start of the experiment, all of the different diets were taken almost immediately by all octopus. Normally, an octopus would collect all the pieces of food with its arms, to keep it underneath its head, even when the total amount of food was too much to eat that day. Although they were kept separate, this is probably caused by the need to keep it safe from other octopus that can possibly eat it (Pham and Isidro, 2008), enhanced by the fact that they could see each other in the glass aquaria. A couple of days after the start of the experiments, it was observed that mulie was taken straight away after feeding, but discarded within a couple of seconds. This was not observed in the octopus feeding on krill and whitebait which started feeding immediately. Sometimes the mulie was eaten later on during the day, on other occasions it stayed untouched until removed the next day.

No mortalities due to natural causes happened during the total period of the two experiments. This suggests that all of the diets were sufficient to keep the small octopus alive during the 7 weeks of experiments. Because some octopus from the krill and mulie diet were losing weight, it is certainly possible that mortalities would have occurred if the experiments were stretched over a longer period of time.

The mean water temperature was significantly different between the two experiments (E1: 17.45 °C ± 0.40 °C; E2: 19.3 °C ± 0.38 °C). This of course could have affected the comparison of growth between the experiments (Table 10).

Growth figures show that the growth of the octopus occurred with small bumps. This could be partly caused by the measuring method of live octopus, because they can keep a certain amount of water in their head which can vary between weighing days. Nevertheless, trends in the growing pattern can be seen quite clearly between the diets (Fig. 62). In the first experiment, all of the octopus on the whitebait diet grew almost constantly, while the krill diet octopus hovered around the same weight. However, the growth of the mulie-fed octopus is more varied, with octopus decreasing in weight and/or remaining the same weight.

The first experiment shows that in all aspects whitebait were the best diet, with a higher SGR,

FCR and FI than krill and mulie. Compared with other studies that used isolated octopus to calculate growth, the SGR in this study of whitebait fed octopus (1.49% BW per day^{-1}) is higher than the SGR of other fish fed *O. vulgaris*, like with Boops boops (1.11% BW per day-1 Garcia Garcia and Cerezo Valverde, 2007) and Engraulis encrasicolus (0.43 – 0.96% BW per day^{-1}); Petza *et al.*, 2006) comparable with frozen squid (1.60% BW per day) Quintana *et al.*, 2008) and lower than crab (Carcinus maenas) fed octopus (2.04% BW per day^{-1}) (Garcia Garcia and Cerezo Valverde, 2007). It has to be taken into account that there is a large variety in factors of other studies (temperature, initial weight, housing, time of experiment) and the fact that there could be differences between species. Nevertheless, the results of the whitebait diet are promising. Mulie had a higher FI than krill, but showed no difference in the other variables, which could suggest that it has a lower nutritional value. At the time of writing this report, analytical results for all the feeds were not available and therefore linking the current results to the nutritional profile of the feeds can only be based on literature. Mulie is known for its high levels of fat that can range from 10–20% during different seasons. Studies on *O. vulgaris* indicate that octopus require high levels of protein as their energy source (O'dor *et al.* 1984) and, as seen in the cuttlefish, small amounts of lipids that are used for synthesis of cell membranes, cholesterol and steroid hormones (Lee, 1994). Higher lipids content fat could not only lower the nutritional value directly, but also obstruct or deplete the intake of necessary nutrient like amino acids (Garcia Garcia & Cerezo Valverde, 2007), further decreasing the value of food with a high lipid content. A higher lipid content could therefore cause a higher food intake to compensate for the low digestibility (Garcia Garcia & Agaudo Gimenez, 2002), which could explain the higher food intake of mulie in comparison with krill. Krill has a high protein level and less lipids (11.0% crude protein en 1.4% crude lipids, Hikari Biopure ©), moreover, a range of added vitamins and minerals. However, the total intake of krill stayed behind that of mulie and whitebait, although the octopus seemed to like it when fed. A cause of the lower intake of krill could be its size. Roughly, the krill size varied from 10 to 30 mm, which could be quite small to handle for an octopus, also when considering the size of edible part of the krill. Furthermore, eating small food particles like krill with only a relatively small edible portion has a low energetic benefit per eaten piece of krill, which is less efficient than bigger food pieces with a higher flesh content.

No correlations were found between specific growth rate and weight gain and the midpoint weight of the octopus. Studies with *O. tetricus* and *O. vulgaris* showed that bigger octopus tend to have a lower growth rate than smaller octopus. FI shows a decreasing trend in bigger octopus of the whitebait group, supporting results found by Joll (1978) with octopus fed on a crab diet. Although not significant, the mulie group almost showed an increasing FI with bigger octopus. This indicates that certain food types like mulie can become nutritionally sufficient for bigger octopus. It is possible that this is different when using other food sources, since the nutritional demand can change with growing individuals in different life stages.

The growth trends that were seen for the octopus that were fed the same mono diet in the second experiment are comparable with those of the first experiment. The octopus that switched to a new diet in the second experiment (W(K), W(M), K/W) had constant growth on their new diet. This shows that octopus can improve their growth after a period on a less suitable diet. These results are important when considering the on-growing of wild caught octopus, since food supply in nature fluctuates, with periods of more and less food or even starvation. This study shows that after a period of a couple of weeks with a less than optimal diet, octopus growth can be improved. It has to be seen if this would lead to octopus reaching similar weights as octopus that were fed ad libitum on a suitable diet the whole time.

No differences between mono diets in experiment 1 and 2, but lower growth in octopus that were fed krill and mulie suggest a possible depletion of nutrients by a mono diet, which could become a significant effect if the experimental period was of a longer duration. Similar growth rates can be seen in the mono specifically fed whitebait group, which shows that it still has a sufficient nutritional value. The difference in temperature between the experiments (17.45 °C and 19.3 °C) was not taken into account here. Although a higher growth would be expected with a higher temperature, no relation between growth and temperature in other studies with *O. tetricus* was found in the past (Joll, 1977). In this study, it could be that growth rates are approximately the same at both temperatures or in a certain range of temperature. Studies with *O. vulgaris* showed a peak in growth at 17.5 °C (Aquado Gimenez and Garcia Garcia, 2005), even with weight loss at temperatures above 23 °C. This shows that it is important to discover an optimum in temperature and growth rates for *O. tetricus*. Furthermore, the midpoint weights of the octopus in both experiments showed that whitebait fed animals were significantly bigger in the second experiment. The weight of octopus has an influence on growth (Joll, 1977), but no models are available yet to predict the combination of weight and temperature on growth. It can be concluded that in the range of 17.5 °C – 19.3 °C and mean group weights of 236 – 351 gr juvenile *O. tetricus* on a whitebait diet showed growth rates of 1.49% BW day^{-1}.

5.2.5 Conclusion

Whitebait showed to be the best diet in this study, showing higher growth and feeding rates than mulie and krill. Furthermore, compared with other octopus growth studies, whitebait seems to be a suitable fresh food source for octopus aquaculture, as a mono diet, or possibly as a base for a mixed diet or for studies with formulated feeds. Juvenile octopus in this study demonstrated ability to 'catch-up' on growth when switched from less suitable diet to a better one. The use of specific feed such as whitebait or combination of several feeds is yet to be determined on commercial scale and cost benefit analysis.

5.2.6 References

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5.3 Feed and feeding I

5.3.1 Introduction

Grow-out trials on juvenile *O.tetricus* in tanks with increased biomass, were undertaken after initial feed trials were completed (Section 5.2). Even though octopus showed a preference for whitebait and krill during these experiments, obtaining these feeds with any regularity and at a cost that was commercially viable was not possible. It was decided to persist with mulies as they were relatively cheap at ~$1.80 per kg^{-1} and were easily obtained. The mulies would be complemented with Prawn heads. Occoculture Pty Ltd had completed some feed trials at Fremantle Maritime TAFE prior to the beginning of the project, from which they provided our group with data detailing positive growth from octopus fed this product. They were of no cost to our group and were easily obtained as they were a waste product from a prawn processing facility.

The data collected during initial feed trials (Section 5.2) was during the cooler, winter months in Perth when ambient water temperatures are between 16–19 °C. Cool water temperatures result in a slow-down in the growth and feed rate of *O. tetricus*, especially when grown in captivity.

These grow-out trials would be conducted during the summer months and would provide a direct comparison from the data (growth, feed rate, mortality) collected during winter. The aim of these growth trials was to increase stocking densities of all tanks to observe maximum capacity while optimizing the flow of water and varying feed rates.

5.3.2 Methods

The system used in experiments 1 – 4 is described in 5.1.1, while the experiments 5 – 7 were carried out using the system described in Section 5.1.2 . Maximum flow rates to the tanks were 400 lt hr^{-1} (10 exchanges of water per day) while minimum flow rates were 150 lt hr^{-1} (3.6 exchanges of water per day). Octopus in all tanks were fed 6% of the tank biomass daily (3% am, 3% pm) with uneaten feed and waste siphoned out of the tanks before every feed. The maximum stocking density to any tank was 15 kg m^3 with weight ranges at the time of stocking of 0 – 50 gr ('x' individuals), 50 – 100 gr (< 50 individuals), 100 – 200 gr (< 40 individuals), 200 – 400 gr (< 30 individuals), 400 – 600 gr (< 20 individuals) and > 600 gr (< 10 individuals). Octopus were weighed every 7 – 10 days with dissolved oxygen and temperature measurements taken daily. The biomass of each tank was projected to increase 2% per day for the purpose of projecting daily feed amounts.

Table 13.   Experimental treatments

Experiment	Treatment	Treatment Description
1	Water Flow	Reduced from 24 exchanges per day (1000 lt hr^{-1}) across all tanks, to 3.6 – 21.5 exchanges per day (150 – 900 lt hr^{-1}). Adjusted flow rates dependent on biomass at the time of stocking. Lower flow rates for low biomass.
2	Feed Rate	Some tanks randomly fed 4% of the tank biomass per day rather than the normal 6%.
3	Aeration/Feed Rate	External aeration via an airstone added to all tanks. All tanks returned to a 6% a day feed regime from a 4% a day regime
4	Aeration/Feed Rate	All tanks fed a 6% a day feed regime. Airstone removed from all tanks.
5	Stock Type	Comparison of growth between new wild caught stock and compromised captive stock
6	Feed Rate	New wild caught stock fed 8% tank biomass once a day
7	Feed Rate	New wild caught stock fed twice a day. 10% am and 8% pm

5.3.3 Results

Table 14. Experiment 1 (week 1)

Tank No.	Biomass (Day 1/ gr)	Weight Range (gr)	St Dev (Day 1)	No. Individuals (Day 1)	Avg Weight (Day 1)	Biomass (Day 10) (gr)	Biomass Increase/ Decrease (gr)	St Dev (Day 10)	No Individuals (Day 10)	Mean Water Temp (Day 1 – 10)	Avg Weight (Day 10)	Flow Rate (lt hr⁻¹)	Feed Rate (% per day⁻¹)	Biomass Increase (% per day⁻¹)
1	9644.3	400 - 600	54	20	482.2	9779.1	+134.8	80.2	19	19.8	514.7	400	6	0.14
2	683.5	0 - 100	16.3	9	75.9	820.2	+136.7	18.5	8	19.8	102.5	150	6	2.00
3	9153.0	400 - 600	61.4	19	481.7	10248.9	+1095.9	74.7	19	19.8	539.4	350	6	1.20
4	10002.6	600 <	87.8	14	714.5	10665.1	+662.5	103.3	14	19.8	761.8	400	6	0.66
5	8425.8	400 - 600	41.6	17	495.6	9251.8	+826	54.2	17	19.8	544.2	300	6	0.98
6	4463.0	200 - 400	53.8	15	297.5	4851.2	+388.2	55.5	15	19.8	323.4	200	6	0.87
7	2315.1	100 - 200	29.7	17	136.2	2698.0	+382.9	34.6	16	19.8	168.6	150	6	1.65
8	8430.4	200 - 400	55.1	28	301.1	9663.1	+1232.7	65.4	27	19.8	357.9	300	6	1.46
9	9514.2	200 - 400	56.7	30	317.1	11042.2	+1528	59.5	30	19.8	356.2	400	6	1.61

Table 15. Experiment 1 (week 2)

Tank No.	Biomass (Day 1) (g)	Weight Range (gr)	St Dev (Day 1)	No. Individuals (Day 1)	Avg Weight (Day 1)	Biomass (Day 7) (gr)	Biomass Increase/ Decrease (gr)	St Dev (Day 7)	No Individuals (Day 7)	Mean Water Temp (Day 1–7)	Avg Weight (Day 7)	Flow Rate (lt hr⁻¹)	Feed Rate (% per day⁻¹)	Biomass Increase (% per day⁻¹)
1	7992.0	400 - 600	73	17	470.1	8405.1	+413.1	64.7	17	20.3	494.4	300	6	0.7
2	10048.9	400 - 600	48.4	21	478.5	8987.1	-1061.8	48	19	20.3	473.0	400	6	- 1.5
3	11179.7	200 - 400	52.2	35	319.4	9276.5	-1903.2	48.7	29	20.3	319.9	350	6	- 2.4
4	13355.7	400 - 600	64.6	27	494.7	12572.0	-783.7	60.3	23	20.4	546.6	500	6	- 0.8
5	13260.5	600 <	57.9	20	663.0	13495.5	+235	57.9	20	20.4	674.8	550	6	0.18
6	2680.2	100 - 200	24.1	18	148.9	2933.7	+253.5	24.4	17	20.3	172.6	150	6	0.95
7	7559.8	200 - 400	43.1	23	328.6	8065.7	+505.9	45.1	23	20.3	350.7	300	6	0.9
8	350.9	0 - 100	12.6	4	87.5	390.8	+39.9	13.9	4	20.4	390.8	150	6	1.14

An increase in biomass was recorded across almost all tanks during week 1 of the experiment, with only tank 1, 2 and 3 not recording an increase in week 2 (Table 15). The highest growth was recorded in tank 2 in week 1 with an increase of 2.00% per day⁻¹. The lowest growth was recorded in tank 1 on week 1 with an increase of 0.14% per day⁻¹ (Table 14). Tanks that produced negative growth across both weeks were those that were initially stocked with 10 kg of octopus or greater. Positive growth was recorded on only one occasion when the initial stocking density was > 10 kg. This was 0.18% per day⁻¹ in tank 5 on week 2. Mortalities were low during week 1 with only 2 octopus lost across all tanks; however they increased after week 2 with 13 animals lost.

Table 16.   Experiment 2

Tank No.	Biomass (Day 1) (gr)	Weight Range (gr)	St Dev (Day 1)	No. Individuals (Day 1)	Avg Weight (Day 1)	Biomass (Day 8) (gr)	Biomass Increase/Decrease (gr)	St Dev (Day 8)	No Individuals (Day 8)	Avg Weight (Day 8)	Mean Water Temp (Day 1–8)	Flow Rate (lt hr^{-1})	Feed Rate (% per day^{-1})	Biomass Increase (% per day^{-1})
1	10871.0	600 <	56.2	16	679.4	11365.1	+494.1	68.1	16	710.3	20.9	600	4	0.5
2	14146.4	400 - 600	72.9	28	505.2	13181.2	-965.2	67.9	25	527.2	20.9	900	4	- 0.8
3	6408.8	200 - 400	37	19	354.6	6737.9	+329.1	48.9	19	354.6	20.8	250	6	0.6
4	2693.8	100 - 200	33.2	18	149.7	3012.4	+318.6	30.4	15	200.8	20.9	150	6	1.47
5	9417.9	400 - 600	47.3	20	470.9	8276.0	-1141.9	57.6	17	486.8	20.9	600	6	- 1.5
6	9430.1	400 - 600	44.9	18	523.9	9872.6	+442.5	48.3	18	548.5	20.8	600	6	0.5
7	9477.0	200 - 400	53.2	31	305.7	9934.8	+457.8	53.1	31	320.5	20.9	400	4	0.6

Positive growth was recorded in tanks fed both 4% and 6% per day^{-1}. The highest growth was recorded with the 6% feed rate which was 1.47% per day^{-1} in tank 4. The lowest increase was recorded in tanks 1 and 6 which was 0.5% per day^{-1} (Table 16). They were fed 4% and 6% respectively. Negative growth of 0.8 and 1.5% per day^{-1} was recorded in tanks 2 and 5 which were also fed 4 and 6% respectively. Mortalities were recorded across both feed rates with the loss of 3 animals in tanks 4 and 5 (6%) and tank 2 (4%).

Table 17. Experiment 3

Tank No.	Biomass (Day 1) (gr)	Weight Range (gr)	St Dev (Day 1)	No. Individuals (Day 1)	Avg Weight (Day 1)	Biomass (Day 7) (gr)	Biomass Increase/Decrease (gr)	St Dev (Day 7)	No Individuals (Day 7)	Avg Weight Day (7)	Mean Water Temp (Day 1–7)	Flow Rate (lt hr^{-1})	Feed Rate (% per day^{-1})	Aeration	Biomass Increase (% per day^{-1})
1	10155.1	400 - 600	62.5	21	483.6	8133.1	-2022.0	60.6	16	508.3	21.3	600	6	Yes	- 2.8
2	12251.6	600 <	60.9	18	680.6	12773.5	+521.9	81.5	18	709.6	21.3	750	6	Yes	0.5
3	9123.5	200 - 400	49.4	29	314.6	8489.1	-634.4	55.4	26	326.5	21.4	400	6	Yes	- 0.9
4	10892.2	600 <	54.6	21	518.7	10783.8	-108.4	58.7	20	539.2	21.3	600	6	Yes	- 0.1
5	1022.2	100 - 200	17.8	6	170.4	1128.4	+106.2	24.2	6	188.1	21.4	150	6	Yes	1.29
6	8267.4	200 - 400	71.2	29	306.2	9765.4	+1498	64.9	29	336.7	21.3	300	6	Yes	2.26
7	7092.9	400 - 600	47.7	13	545.6	7237.2	+144.3	55.5	13	556.7	21.4	250	6	Yes	0.25

Positive growth was only in recorded in 4 tanks during this experiment. The highest growth was 2.26% per day^{-1} in tank 6 while the lowest was 0.25% per day^{-1} in tank 7 (Table 17). Negative growth was recorded in the remaining 3 tanks with the highest decrease being – 2.8% per day^{-1} in tank 1. 9 octopus were lost across all tanks.

Table 18. Experiment 4

Tank No.	Biomass (Day 1) (rg)	Weight Range (gr)	St Dev (Day 1)	No. Individuals (Day 1)	Avg Weight (Day 1)	Biomass (Day 9) (gr)	Biomass Increase/ Decrease (gr)	St Dev (Day 9)	No Individuals (Day 9)	Avg Weight (Day 9)	Mean Water Temperature (Day 1 – 9)	Feed Rate (% per day^{-1})	Biomass Increase (% per day^{-1})
1	6256.8	200 - 400	55.4	20	312.8	6346.0	+90	50.9	19	334.0	22.1	6	0.15
2	8293.5	400 - 600	67.5	15	552.9	8680.5	+387	84.1	15	578.7	22.1	6	0.5
3	8879.4	200 - 400	64.4	30	296	8980.1	+100.7	63.4	29	309.7	22.1	6	0.12
4	10738.9	600 <	53.8	16	671.2	9574.5	-1164.4	72.3	14	683.9	22.1	6	- 1.2
5	10038.1	400 - 600	55.6	20	501.9	8621.9	-1416.2	68.4	18	524.3	22.1	6	- 1.5
6	9922.3	400 - 600	64.2	20	496.1	8621.1	-1301.2	77.1	17	507.1	22.1	6	-1.45

Positive growth was recorded in 3 tanks during this experiment. The highest growth was 0.5% per day^{-1} in tank 2, while the lowest was 0.12% per day^{-1} in tank 3 (Table 18). Negative growth occurred in the remaining 3 tanks, with highest decrease being – 1.5% per day^{-1} in tank 5. Total negative growth of - 4.15% per day^{-1} across tanks 4, 5 and 6 outweighed total positive growth of 0.77% per day^{-1} in tanks 1, 2 and 3.

Table 19.   Summary of experimental data (experiments 1-4)

Weight Range (gr)	Biomass (Day 1/gr)	Biomass (Day 10) (gr)	Biomass Increase/ Decrease (gr)	Biomass Increase (% per day⁻¹)	No. Individuals (Day 1)	No. Individuals (Day 10)	Water Temperature (C°)
0 – 100	517.2	605.5	+88.3	1.7	7	7	20.1
100 – 200	2177.8	2443.1	+265.3	1.21	15	14	20.6
200 – 400	8148.8	8468.3	+319.5	0.39	26	25	20.8
400 - 600	9373.9	9419.2	+45.3	0.05	20	18	20.9
600 <	11336.2	11442.7	+106.5	0.09	18	17	21.0
AVERAGE	6310.8	6475.8	+164.9	0.26	17	16	20.7

Data averaged from experiments 1 – 4 indicates that positive growth was recorded across all weight ranges (Table 19). Octopus between 0 – 100 gr recorded the highest growth of 1.7% per day⁻¹. The lowest growth of 0.05% per day⁻¹ was recorded in 400 – 600 gr octopus.

Table 20. Experiment 5

Tank No.	Stock	Biomass (Day 1) (gr)	Weight Range (gr)	St Dev (Day 1)	No. Individuals (Day 1)	Avg Weight (Day 1)	Biomass (Day 12) (gr)	Biomass Increase/ Decrease (gr)	St Dev (Day 12)	No Individuals (Day 12)	Avg Weight Day (12)	Mean Water Temp (Day 1 – 12)	Feed Rate (% per day^{-1})	Biomass Increase (% per day^{-1})
1	New	1868.0	33.8 – 163.3	35.2	19	98.3	1592.5	-275.5	42.3	9	176.9	22.6	6	- 1.2
2	New	2443.5	113.4 – 212.8	26.8	16	152.7	2774.6	+331.1	35.1	16	173.4	22.6	6	1.12
3	Leftover	7144.2	406.4 – 596.6	52.7	15	476.3	6146.6	-997.6	65.4	12	512.2	22.6	6	- 1.1
4	Leftover	6070.8	209.5 – 506	66.5	20	303.5	4957.4	-1113.4	84.4	15	330.5	22.6	6	- 1.5

Positive growth was only recorded in one tank during this experiment. The new stock outperformed the octopus that were leftover from previous trials. Tank 2 recorded an increase of 1.12% per day^{-1}, while the remaining tanks recorded negative growth. The highest decrease was – 1.5% per day^{-1} in tank 4 which contained leftover compromised stock (Table 20).

Table 21.   Experiment 6

Tank No.	Biomass (Day 1) (gr)	Weight Range (gr)	St Dev (Day 1)	No. Individuals (Day 1)	Avg Weight (Day 1)	Biomass (Day 7) (gr)	Biomass Increase/ Decrease (gr)	St Dev (Day 7)	No Individuals (Day 7)	Avg Weight (Day 7)	Mean Water Temperature (Day 1 – 7)	Feed Rate (% per day⁻¹)	Biomass Increase (% per day⁻¹)
1	2868.0	142.5 – 199.8	19.6	17	168.7	3134.4	+266.4	33.1	16	195.9	24.3	8	1.30
2	2821.9	150.3 – 243.8	31.4	14	201.6	2779.7	-42.2	36.4	12	231.6	24.9	8	- 0.21
3	2571.9	175.2 – 280.4	37.3	11	233.8	2972.4	+401.5	46.2	11	270.2	24.7	8	2.23
4	2972.4	198.3 – 336.5	46.2	11	270.2	3068.0	+95.6	83.2	9	340.9	24.5	8	0.45
5	2297.5	61.6 – 132.7	26.2	27	85.1	2220.0	-77.5	36.4	17	130.6	24.8	8	- 0.48
6	6664.0	255.3 – 423.2	78.9	18	339.6	2928.4	-3735.2	83.3	11	418.4	25.4	8	- 4.6
7	4384.3	312.6 – 578	107.1	10	438.4	4678.1	+294.8	139.2	9	519.8	24.8	8	0.96
													0.78

Positive growth was not recorded across all tanks when using completely new stock. Positive growth was recorded in only 4 tanks with the highest growth being 2.23% day⁻¹ in tank 3 (Table 21). The lowest increase was 0. 45% day⁻¹ in tank 4. Mortality was high in tanks which recorded negative growth, with the loss of 10 octopus in tank 5 out of 17 octopus overall. Tank 5 subsequently recorded the highest decrease in biomass with -4.6% day⁻¹.

Table 22. Experiment 7.

Tank No.	Biomass (Day 1) (gr)	Weight Range (gr)	St Dev (Day 1)	No. Individuals (Day 1)	Avg Weight (Day 1)	Biomass (Day 7) (gr)	Biomass Increase/ Decrease (gr)	St Dev (Day 7)	No Individuals (Day 7)	Avg Weight (Day 7)	Mean Water Temperature (Day 1 – 7)	Feed Rate (% per day⁻¹)	Biomass Increase (% per day⁻¹)
1	6964.0	72.1 – 249.8	29.39	54	129.0	8790.4	+1826.4	40.4	53	165.9	25.3	10 & 8	3.74
2	4361.6	26.7 – 101.5	19.4	63	69.2	5786.1	+1425.1	29.5	58	99.8	25.8	10 & 8	4.66
3	3639.1	100.2 – 195.5	23.9	30	121.3	4459.9	+820.3	33.7	28	159.3	26.1	10 & 8	3.22
4	9376.0	101.9 – 198.5	24.5	66	142.1	11679.0	+2303.0	41.6	58	201.4	26.1	10 & 8	3.50
													3.78

An increase from once a day to twice a day feeding resulted in positive growth across all tanks. The highest growth was 4.66% per day⁻¹ in tank 2 while the lowest increase was 3.22% per day⁻¹ in tank 3 (Table 22). Mortalities occurred in all tanks with the highest loss recorded in tank 4 where 8 animals were lost.

Table 23. Food Inputs & Consumption: Experiment 7

Tank No.	Biomass (Day 1) (gr)	Biomass (Day 7) (gr)	Growth Increase/ Decrease (gr)	Total Input: Mulies (Wet Weight) (gr)	Total Input: Prawn Heads (Wet Weight) (gr)	Dry Weight of Total Input: Mulies (gr)	Dry Weight of Total Input: Prawn Heads (gr)	Dry Weight of Uneaten Feed: Mulies (gr)	Dry Weight of Uneaten Feed: Prawn Heads (gr)	Dry Weight of Consumed Feed: Mulies (gr)	Dry Weight of Consumed Feed: Prawn Heads (gr)	FCR (Feed: Growth (gr)
7	4383.3	4678.1	+294.8	1645.7	2569.5	421.3	555.0	132.6	270.3	288.7	284.7	2.00: 1
11	9376.0	11679.0	+2303.0	7381.8	10368.1	1889.7	2239.5	425.2	832.7	1464.5	1406.8	1.24: 1

5.3.4   Discussion

Experiment 1

The attempted optimization of water flow to tanks containing juvenile octopus wasn't an overall success. Positive growth occurred in week 1 across all tanks which was initially encouraging; however after a 2nd week of lower water flows, feeding decreased and some negative growth occurred. An increase in mortality after week 2 further indicated that low water flow affected octopus health.

The negative growth was due to low dissolved oxygen levels caused by the low flow rates. Juvenile octopus use a lot of oxygen when held in tanks, especially when feeding. Two weeks of lower than usual dissolved oxygen levels made a large percentage of the octopus sick. They reduced, or in some cases stopped feeding which lead to deaths by the end of the second week. Analysis of some sick juvenile octopus by the DoF Fish Health section indicated bacterial infection (*Vibrio harveyii*) as a cause of death during this period.

Experiment 2

The continued declining health of the octopus after experiment 1, was a large factor in the variable growth that resulted from lowering the daily feed rate from $6 - 4\%$. It was assumed that sick octopus would naturally recover over time and so they were mixed with healthy octopus in this experiment. Healthy octopus eventually became sick too which affected results in relation to the two different feed rates.

Positive growth of 0.5 and 0.6% day^{-1} from two tanks fed 4% indicated that the lower feed rate was somewhat effective. However similar growth in two tanks fed 6% and even higher growth of 1.47% day^{-1} in tank 4 meant that the 6% feed rate was more effective. Overall daily growth across all tanks during this experiments, although positive, was lower than normal due to declining octopus health.

Experiment 3

Attempts to assist in the recovery of sick octopus in this experiment by adding an airstone to the tanks was partially successful. Positive growth did outweigh negative

growth across all tanks, however a higher decrease of $- 2.8\%$ day^{-1} (tank 1) compared to an increase of 2.23% day^{-1} (tank 6) indicated that recovery powers were poor in juvenile octopus once they had become sick. Continued mortalities during the experiment indicated this was the case (Table 10)

Experiment 4

Removal of the airstone from all tanks in this experiment coupled with a return to a 6% daily feed rate was the last attempt to bring sick octopus back to good health and was not successful. Negative growth far outweighed positive growth which reinforced that once juvenile octopus are sick in captivity, they cannot recover. All sick octopus were culled after this experiment.

The averaged data (Table 12) indicated that smaller octopus, namely $0 - 100$ gr animals, tolerated the low oxygen conditions experienced in experiments $1 - 4$ a lot better than the larger animals. Although smaller octopus were stocked at a lot lower biomass than that of larger octopus, visual observations of health and body condition as well as the size of the remaining healthy animals suggest this was the case.

Experiment 5

Growth comparisons of the new wild stock compared to that of the leftover compromised stock was not accurate due to the large size difference at the time of stocking. Negative growth of 1.2% per day^{-1} from new stock in tank 1 (Table 14) was the result of cannibalism as the largest octopus was 5 times greater than the smallest octopus. A tighter initial weight range in tank 2 resulted in positive growth. Negative growth in tanks containing leftover stock was due to them falling sick and dying. All remaining stock from experiments 1 – 4 were culled after experiment 5.

Experiment 6

An influx of new stock after experiment 5 meant initial weight ranges were able to be a lot tighter in this experiment. Negative growth was due to the once a day feed regime. Increasing the daily feed amount to 8% still wasn't adequate with large amounts of cannibalism observed. High mean water temperatures of between 24 °C and 25.5 °C contributed to the increased mortality, however it was evident after this experiment that feeding octopus in tanks once a day wasn't suitable. It meant that octopus would go ~ 20 hours with out food over the course of a day.

Experiment 7

Increasing the feed regime to twice a day in this experiment proved to be successful. A feed of 10% first thing in the morning and 8% last thing in the afternoon, meant octopus were fed to satiation. Positive growth of 4.66% per day^{-1} in tank 2 (Table 15) was the highest recorded since the start of the project. Cannibalism was still observed in all tanks especially tank 2 and 4 losing 5 and 8 animals respectively, however due to the large amount of uneaten feed remaining after siphoning, cannibalism was attributed to size difference between the octopus at the time of stocking and the high water temperatures.

Food conversion ratio's (FCR) obtained from tanks 7 and 11 during experiment 7, indicated that octopus are very efficient at converting food eaten into weight gained. An FCR of 1.24 : 1 indicates that octopus need 1.24 gr of feed for 1.00 gr of weight gain in those tank conditions (Table 16).

Tank Dynamics

A few adjustments needed to be made to the new fiberglass tanks following previous experiments

1. At low stocking densities, water would flow easily from the top, exiting out the bottom of the tank through large holes 40 mm holes in the 100 mm internal PVC standpipe. As stocking densities increased, more food would collect at the base of the standpipe, which would block water flow. This caused the tanks water level to increase above the level that was initially set. Holes had to be drilled further up the PVC standpipe to allow for the increased water flow.

2. Octopus were able to lift the oyster mesh cover sleeve and escape via the external standpipe, or sit above the 100 mm gate valve at the base of the tank. The problem was two-fold as animals that escaped would die, while animals that would sit above the gate valve would not have access to food and block water flow in the process. To solve this problem a hole was drilled either side at the top of the standpipe and a metal rod pushed through it.

5.3.5   Conclusions

- Recovery by juvenile octopus who become sick in captivity is not possible

- An external air source via an airstone does not aid in octopus recovery, but is beneficial in increasing dissolved oxygen levels at times of high biomass.

- Flow rates must be at 100 lt hr^{-1} per kg^{-1} of octopus at all times to prevent this

- Water temperatures must not rise above 26 °C at any stage. The optimal temperature range is 18°C – 23 °C.

- A twice a day feed regime is absolutely necessary for maximum growth, with feed rate dependent on water temperature.

- A tight weight range at the time of stocking is important in lowering potential cannibalism, although further experimental work is needed in this area.

5.4   Feed and feeding II

5.4.1   Introduction

Growth trials using fresh feeds were continued on juvenile *O. tetricus* during this period. Our main aim over this was to conclude trials using fresh feeds and compile results from all trials that were conducted from May 2010 to present, as well as to start to develop an artificial diet and begin trials to compare growth rates on fresh and formulated feeds.

This period of time was also used to finalize a suitable tank design which would, on a commercial scale, reduce the amount of labour required to grow octopus while at the same time being affordable and easy to construct.

The following data is separated into seasonal growth. Two data sets are showing the growth of octopus during the summer and winter months in Perth, while combined graphs following this, merged food conversion ratio (FCR) and mortality data from both seasons.

5.4.2   Methods

The indoor aquarium system is described in Section 5.1.1 and the outdoor aquarium is described in Section 5.1.2. Flow rates were set at 100 lt hr^{-1} seawater for every 1 kg of octopus biomass. A minimum flow rate of 250 lt hr^{-1} for < 2.5 kg octopus

Table 24.   Experimental treatments

Experiment	Treatment	Treatment Description
1	Feed Rate	Summer Feed Rate – 6% of the tank biomass twice a day to all tanks
2	Feed Rate	Winter Feed Rate – 3% of the tank biomass twice a day to all tanks

Feeds were alternated between mulies and prawn heads. Stocking densities in the indoor aquarium were set at a maximum of 10 kg of octopus per 1 m^3 of seawater in the indoor aquarium and 15 kg of octopus per 2 m3 of seawater in the outdoor aquarium. Weight ranges at the time of stocking were 0 – 50 gr, 50 – 100 gr, 100 – 200 gr, 200 – 400 gr and 400 – 600 gr. octopus were weighed every 7 days when fed a summer feed rate and every 8 days when fed

a winter feed rate. Dissolved oxygen and temperature measurements were taken daily. The biomass of each tank was projected to increase 2% day^{-1} for the purpose of projecting daily feed amounts.
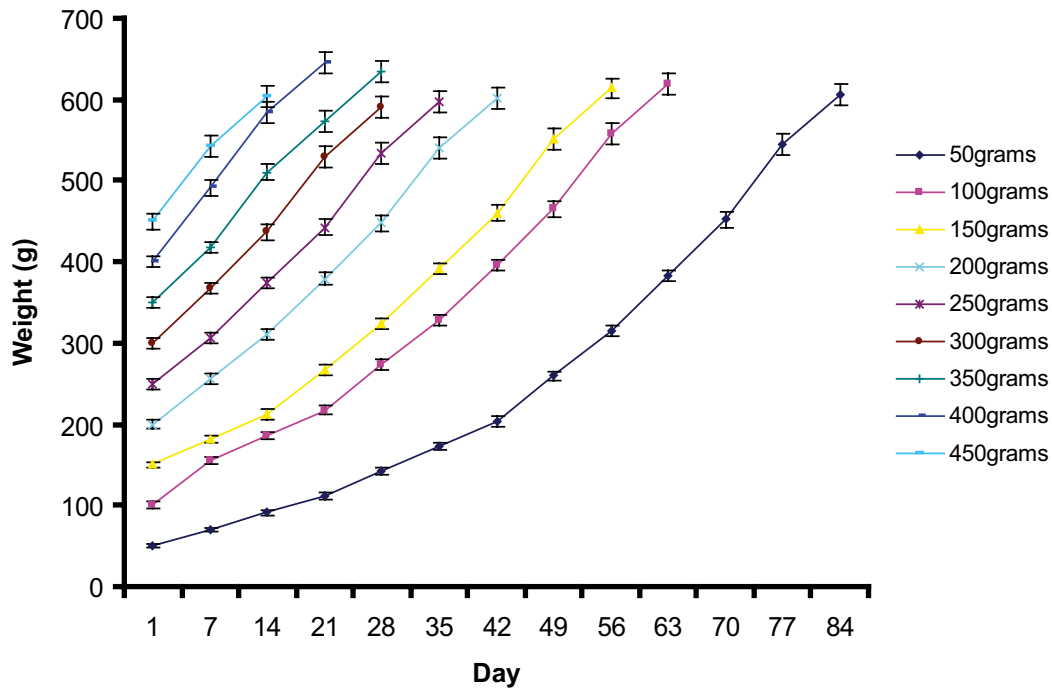
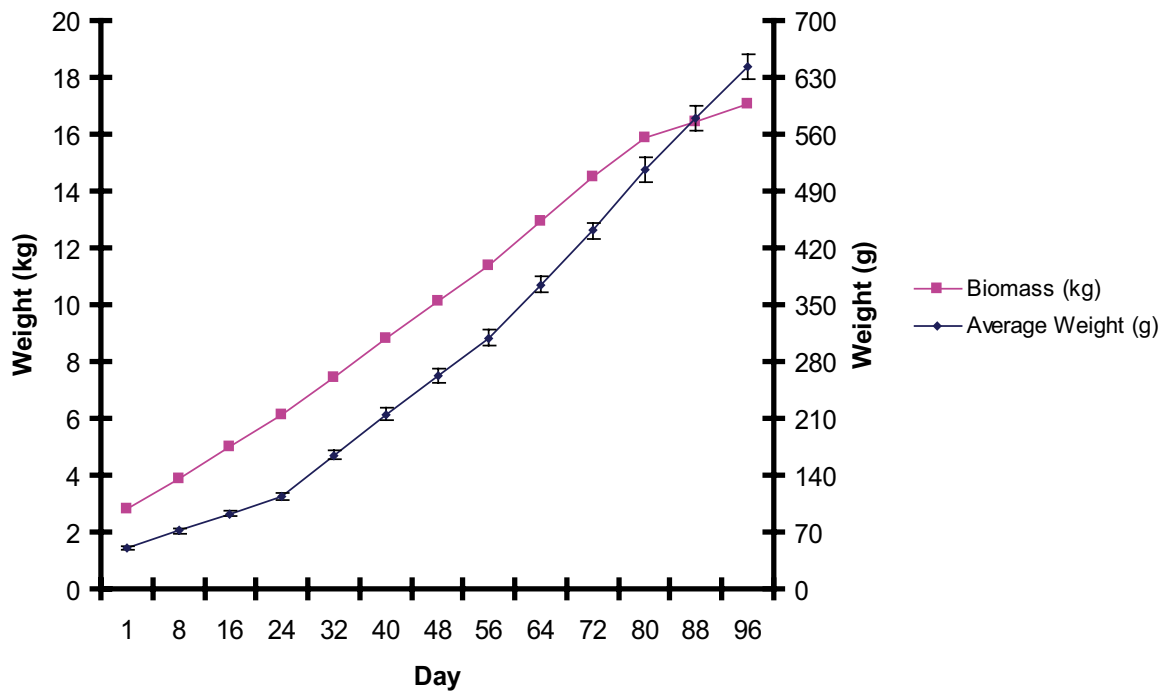### 5.4.3   Results
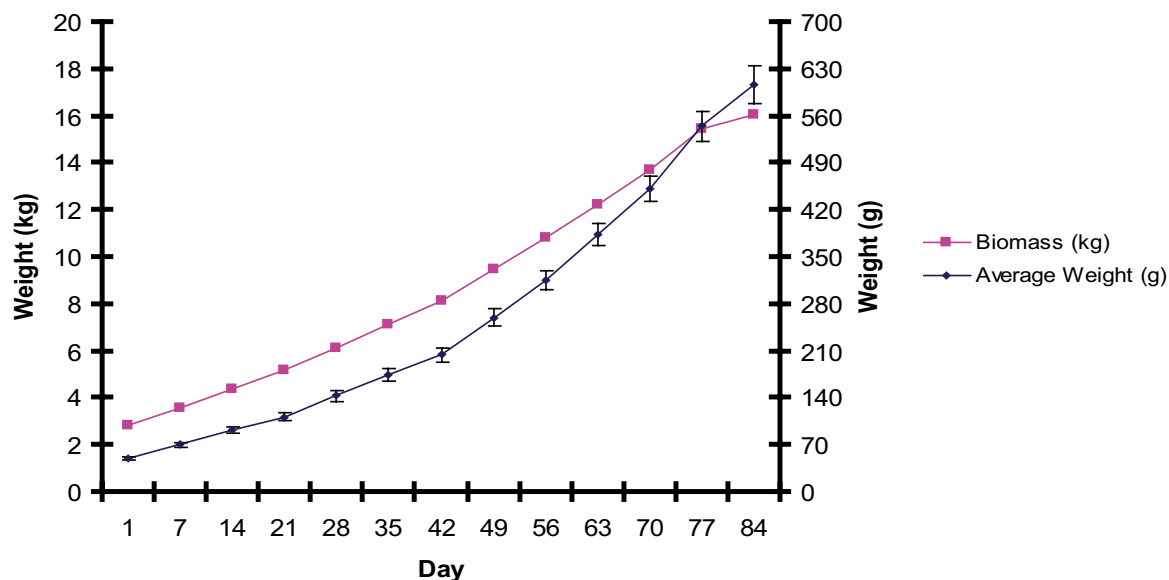
Summer (21°C – 23 °C).

Figure 63.   Average weight and projected biomass increase of ~ 50.0 gr octopus at initial biomass of 2.8 kg over a 7 day period. Achieved under a summer feed regime.

50.0 gr octopus stocked at 2.8 kg biomass during summer, achieved growth to harvest weight (585.0 gr) in approximately 80 days with a harvested biomass of ~ 15.7 kg (Fig. 63). On average, the biomass after a 7 day period increased 1.17 kg (0.167 gr day^{-1}) with an increase in average weight of 48.9 gr (6.98 gr day^{-1}). There was a tendency for biomass increase to be greatest from animals stocked between 200 – 550 gr which was 1.48 kg (0.211 gr day^{-1}) with the average weight increasing 67.96 gr (9.70 gr day^{-1}). The biomass from animals stocked between 50 – 100 gr increased 0.86 kg (0.12 gr day^{-1}) with the average weight increasing 20.4 gr (2.91 gr day^{-1}).

Figure 64. The average increase in weight of different sized octopus over a 7 day period. Achieved under a summer feed regime.

50.0 g octopus take approximately 84 days to reach market weight under a summer feed regime (Fig. 64). Growth is slow when octopus are growing from 50 to 100 gr taking approximately 20 days. From then on, growth is faster in octopus greater than 100 gr. Increasing the initial average weight of octopus by 50 gr can decrease time in captivity by 7–10 days while achieving similar final weights.


Figure 65. Average weight and projected biomass increase of ~ 50.0 gr octopus at 2.8 kg initial biomass over an 8 day period. Achieved under a winter feed regime.

50.0 g octopus stocked at a initial biomass of 2.8 kg in winter, achieved growth to harvest weight in approximately 90 days with final biomass of ~ 16.8 kg (Fig. 65)

On average, after an 8 day period the biomass increased to 1.18 kg (0.147 gr day^{-1}), with the average weight increase being slightly greater during summer at 49.42 gr (6.17 gr day^{-1}). Biomass increase tended to be highest in tanks stocked with octopus between 100–500 gr at 1.397 kg (0.175 gr day^{-1}) with an average weight increase of 57.33 gr (7.14 gr day^{-1}), however tanks stocked with octopus between 50 – 100 gr had higher biomass increase than those during summer at 1.1 kg (0.138 gr day^{-1}) and greater average weight increase of 21.3 gr (2.66 gr day^{-1}).
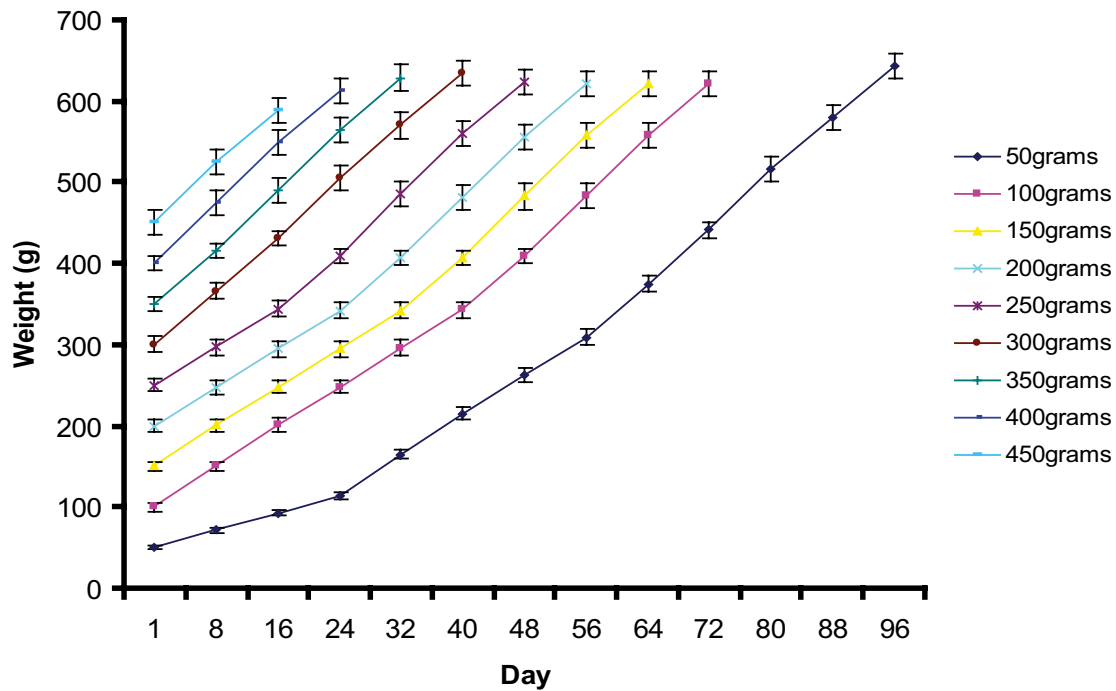

Figure 66.   The average increase in weight of different sized octopus over an 8 day periods. Achieved under a winter feed regime.

50.0 gr octopus take approximately 96 days to reach market weight under a winter feed regime (Fig. 66). Like summer, growth of octopus between 50 gr to 100 gr was slow taking approximately 20 days. If the average weight of octopus stocked is increased by 50 gr, it can be seen that time until harvest can be decreased by 7 – 10 days.
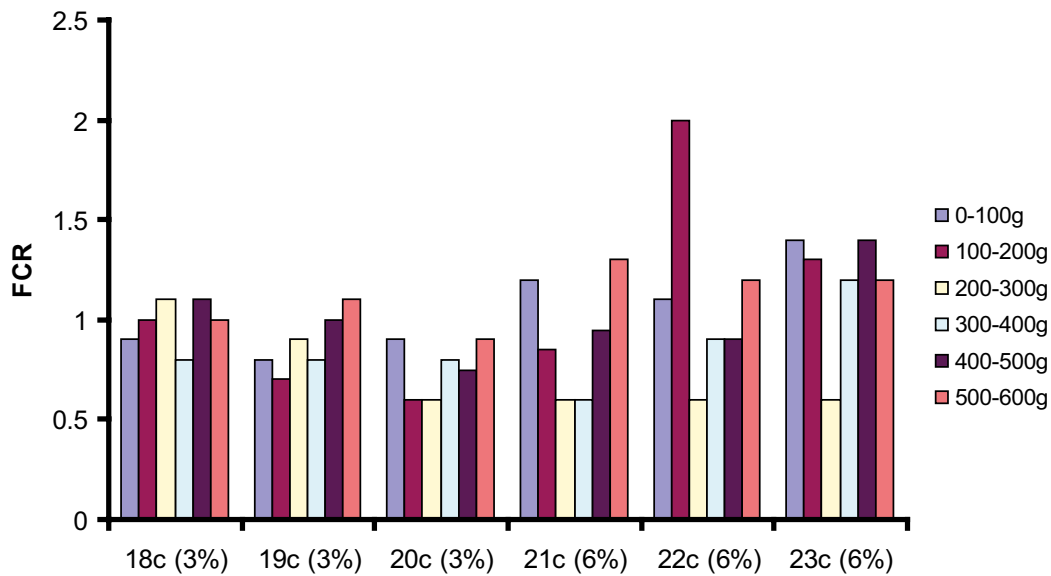
Figure 67. The food conversion ratio (FCR) of different sized octopus during summer and winter. The percentage (%) represents feed rate.

An average FCR of 0.88 was calculated across all temperatures during winter while an average FCR of 1.07 was calculated across all temperatures during summer (Fig. 67). The optimal temperature for growth was 20 °C with an FCR of 0.76, while the least optimal temperature was 23 °C with an average FCR of 1.18. The weight range of octopus that exhibited most efficient growth in winter was the 100-200 gr animals with an FCR of 0.76, while in summer the 200-300 gr animals showed the most efficient growth with an FCR of 0.6. The best performed weight range across both seasons was the 200-300 gr animals with an FCR of 0.73. These FCR rates are considered to be very efficient compared to most other cultured marine organisms.
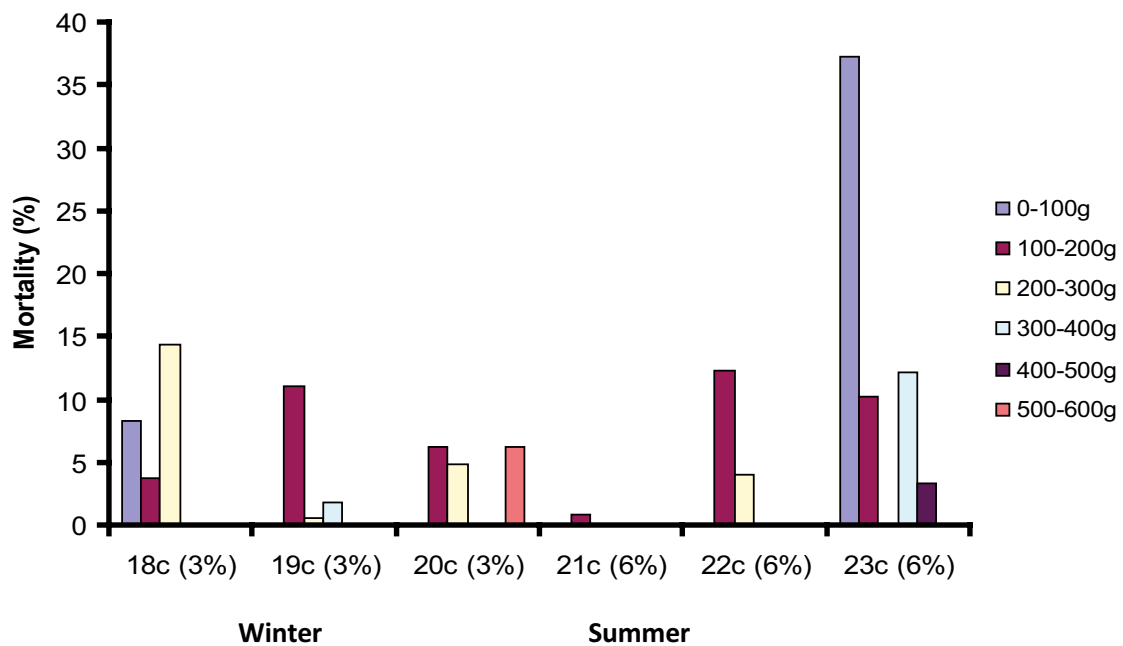
Figure 68. The percentage mortality of different sized octopus during summer and winter. The percentage (%) indicates feed rate.

Increasing water temperature led to an increase in mortality (Fig. 68). Across all water temperatures during summer, an average mortality of 4.43% was recorded while during winter, an average mortality of 3.16% was recorded. The water temperature which rendered the highest mortality rate was 23 °C at 10.46%, while the lowest mortality rate was observed at 21 °C at 0.13%. The best performed weight range was the 400-500 gr animals with an average mortality rate across both seasons of 0.55%, while the weight range that showed the highest mortality rate were the 0-100 gr animals at 7.57%.

5.4.4 Discussion

Water Temperature and Mortality

An increase in temperature up to 23 °C resulted in a large spike in mortality. Reasons for high mortalities at this temperature were:

1. Cannibalism. High water temperatures increased octopus metabolism causing a greater demand for food. As it was imperative to keep a balance between feeding and maintaining good water quality, there were times when the octopus would have been under-fed and therefore the largest animals in the tanks would predate on the smallest or weakest animals. On most occasions, the entire octopus would be eaten leaving no evidence that this had occurred.

2. Disease. Bacterial infection in octopus usually arose from over-feeding of the tanks. Excess food left in the tanks for long periods resulting in water quality deterioration, namely high ammonia and low oxygen. Octopus would then become sick and leave themselves prone to bacterial infection (*Vibrio harveyii*). Octopus affected by this would cease feeding, which would leave more feed in the tanks. Death wouldn't necessarily occur straight away, with animals sometimes staying alive for weeks. Signs animals were affected by bacteria were skinny tentacles, lesions on the mantle and tentacles, constant darkness in colour and a complete disinterest in feeding.

The results were variable between water temperatures. The largest animals, the 400-500 gr and 500-600 gr octopus, were the least susceptible to changes in water temperature with mortality rates of 0.55% and 1.04% respectively, across both seasons. Greater strength and a well-developed central nervous system provided greater protection against bacterial infection. Cannibalism was low in octopus greater than 400 gr. It was observed that octopus would predate on other octopus if they were twice the size. No animals were held at 800 gr or above.

Mortality was highest in animals between 0-100 gr because at this weight range, gaps between different sized animals is greatly exaggerated as an animal of 60 gr is four times the size of an animal at 15 gr. Animals of this size are constantly hungry and competitive for food. In most instances animals were under-fed and a case can be made for animals of this size to be fed 8-10% twice a day to avoid cannibalism.

Water Temperature and Growth

The defined feed rates for winter (3%) and summer (6%) were reached after several trials using different feed rates. It was clear, quite early on in most cases, that octopus being fed above or below the now designated rates were being over or under fed. These observational trials, usually lasting only a few days, weren't electronically captured and were done solely to give us a clear understanding of how much to feed the octopus.

It was interesting to note that overall, FCR's were lower in winter compared to summer. This was due to the higher mortality rate in summer and the fact that animals are hungrier and more

competitive during this time with bigger animals assuming dominance in tanks. Food isn't as evenly distributed and therefore bigger animals become even bigger, while smaller animals can miss out on food. This results in greater weight variance, which leads to a lower total biomass increase and a higher individual average weight increase.

Octopus seemed more docile in winter with food more evenly distributed meaning less weight variance and a greater increase in total biomass. Lower water temperature in winter saw greater tank stability with higher solubility of oxygen in the water, meaning octopus were generally healthier and less prone to bacterial infection. Cannibalism still occurred in winter but we found that this could never be fully eradicated.

Feeding

All octopus during the fresh feed trials were fed a diet of mulies and prawn heads which alternated on a daily basis. Prawns and mulies were cut in halves or even thirds for animals under 200 gr to ensure evenness in feed distribution. Feeds were always administered manually, spreading the food evenly throughout the tank once in the morning before 10 am and once in the afternoon after 4 pm.

Cleaning & Drying of Feed

Tanks that were stocked in system 1 (Section 5.1.1) were cleaned of uneaten food and waste by a large siphon twice a day prior to feeding. These tanks, although suitable in size, did not facilitate easy removal of waste via an exit valve and appropriate standpipe. Uneaten food was siphoned into a 20 lt bucket with 1000 μm mesh and dried in an oven on foil trays for 24 hr at 100 °C to obtain dry weights and subsequent FCR's.

Octopus that were stocked in system 2 (Section 5.1.2) were cleaned of uneaten food and waste via a large 150 mm gate valve located at the bottom of the tanks. The 20 lt bucket with 1000 μm mesh was placed underneath the half open valve while at the same time climbing up an adjacent ladder to lift the oyster mesh on the standpipe. The feed was removed while the act of lifting the standpipe would force the octopus away from the outlet. The sleeve was then dropped and the valve closed. Uneaten feed was dried in the same way as stated above.

Weighing & Grading Stock

During growth trials all tanks that contained octopus were weighed and graded every 7 days during summer and 8 days during winter. Grading octopus into designated wight ranges helped reduce cannibalism, allowed tanks to be cleaned thoroughly of residual waste and allowed time to analyze the data and make any necessary changes.

Octopus were weighed individually by scooping them out with nets and transferring them into a bucket that had been placed on a set of scales. Weights were recorded and the octopus were either placed into a temporary holding tank or to another tank already allocated for the next trial period.

It is interesting to note that this system of grow-out tanks without any hides for the octopus is unique and currently the only one in the world. All other R&D centers and/or companies working with octopus (Mexico, Chile, Greece, Spain, Italy) are using individual hides (usually PVC pipes). Avoiding hides is thought to be much more commercially viable; saves cleaning time, catching the animals during weighing (time and stress), reduce fouling of the tank, and reduce territorial behaviour leading to higher cannibalism.

5.5   Feed and feeding III

5.5.1   Introduction

Upon completion of grow-out trials at the end of 2011, it was realized that grow-out trials at a biomass of 10 kg m^3 were not commercially viable. Tank biomass would need to exceed 20 kg m^3 to make commercial ranching of *O. tetricus* profitable when factoring in utility, transport, equipment, material and value-adding costs.

Attempts made previously to stock juvenile *O. tetricus* at densities greater than 10 kg m^3 resulted in increased mortality with the rearing protocol that was in place. The average mortality rate in 2011 was 3.79%. However, a rate anywhere from 10-25% was experienced especially when water temperatures were $\geq$ 24 C°. To achieve growth at high densities while minimizing mortality, an adjusted protocol was developed and evaluated.

The use of the custom-made octopus culture tanks (Section 5.1.2) was put on hold while a maximum stocking density per cubic metre was ascertained in smaller tanks. Juvenile *O. tetricus* were obtained on numerous occasions from fisherman operating off Fremantle, transferred to the Western Australia Fisheries Marine Research Laboratories (WAFMRL) and stocked the same day. The experiments commenced the next day after grading.

5.5.2   Methods

The system used is described in Section 5.1.2. Flow rates were set at 100 lt hr^{-1} per kg octopus. Tanks were operated at half the original volume (500 lt) and had added aeration via an airstone and pure oxygen source. Feed rate was set at 6% of the tank biomass twice a day. Tank biomass projected to increase 2.5% per day^{-1} for purposes of projecting daily feed amounts. Uneaten feed was removed before fresh feed given. Prawn heads and mulies were alternated daily. Weight range at the time of stocking were 150 – 250 gr, 250 – 450 gr and 300 – 500 gr. Initial biomass would start at 20 kg and increase until a limit is reached (i.e. reduced growth and/or higher mortality). Water temperatures would be controlled between 19–21 °C. Octopus were weighed after 7 days.

5.5.3 Results
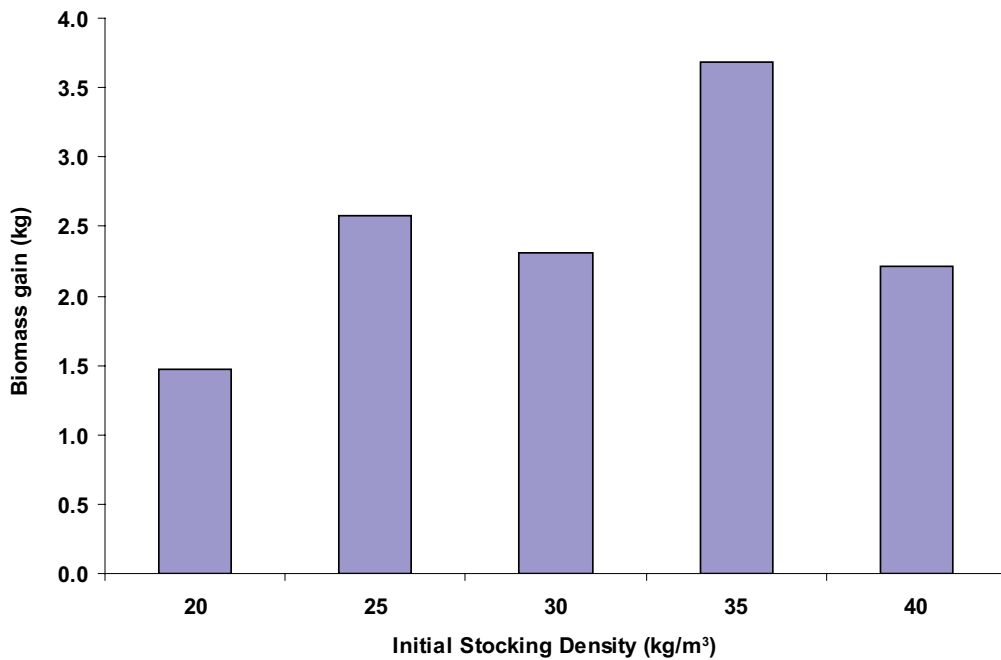
**Figure 69.** Average biomass (kg m³) increase over a 7-day period

Positive growth was detected over all stocking densities greater than 20 kg with biomass gain range between 1.46 kg (7.3% increase) and 3.68 kg (10.5% increase) (Fig. 69). The highest growth was recorded over 7 days when 35 kg was stocked; the lowest was 1.46 kg when 20 kg was stocked. The average growth gain across all stocking densities was 2.45 kg (8.3%) over a 7-day period.

Pure oxygen was injected continuously into the rearing tank water at 1 lt min^{-1}. This practice kept dissolved oxygen levels at > 5.4 mg lt^{-1}. Approximately 8.9 m³ of water was used every 7 days.
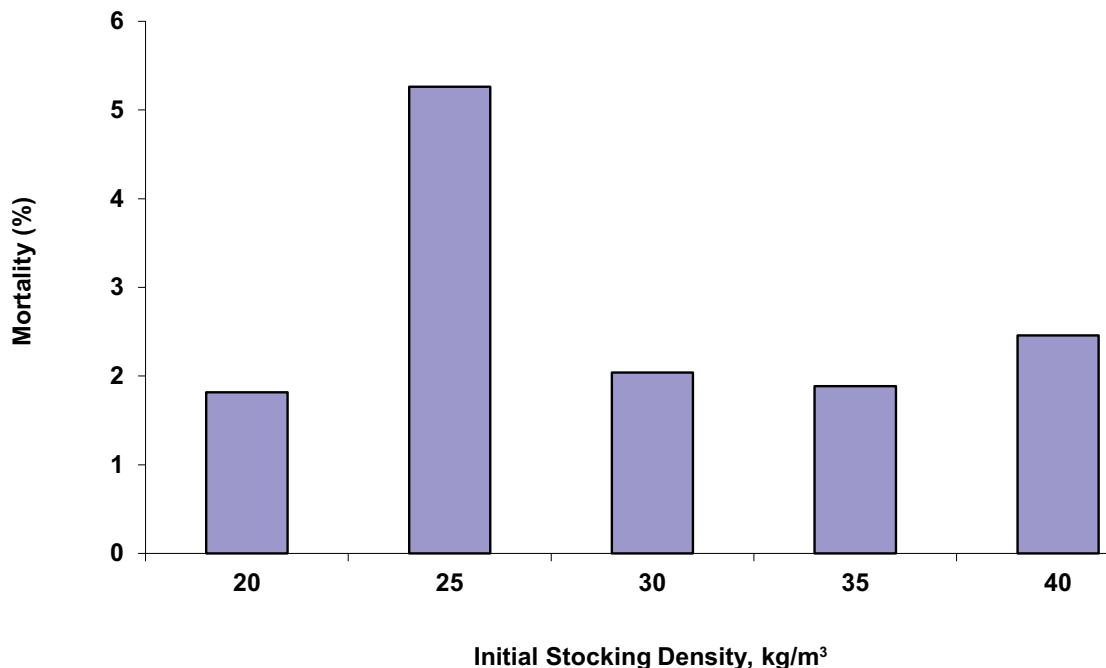

Figure 70. Mortality rate (%) over a 7-day period

A mortality rate of 5.26% was highest at 25 kg initial biomass and the lowest when initial biomass was 20 kg, at 1.81% (Fig. 70). The average mortality rate was 2.69% over a 7-day period. It is interesting to point out that there was no correlation between initial biomass stocking densities and mortalities. The mortality rate at 20, 30, 35 and 40 kg m^3 were not significantly different.
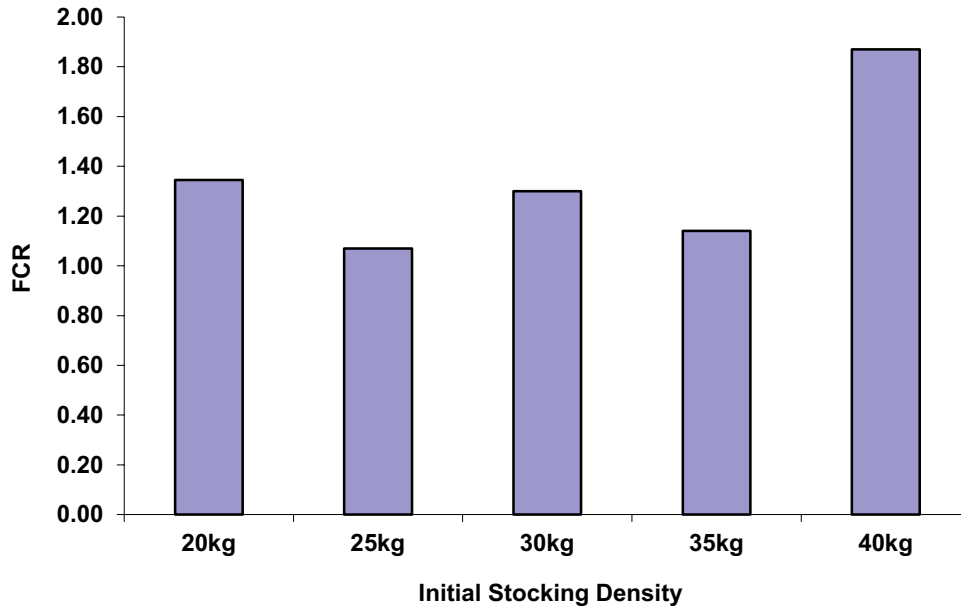
Figure 71. FCR (Food conversion ratio) over a 7-day period.

Stocking densities upwards of 20.0 kg recorded FCR's of between 1 and 2 (Fig. 71). The most efficient FCR was recorded at an initial stocking biomass of 25 kg at 1.07, while the least efficient FCR was recorded at 40 kg initial biomass, at 1.87. The average FCR recorded was 1.35 over a 7-day period.
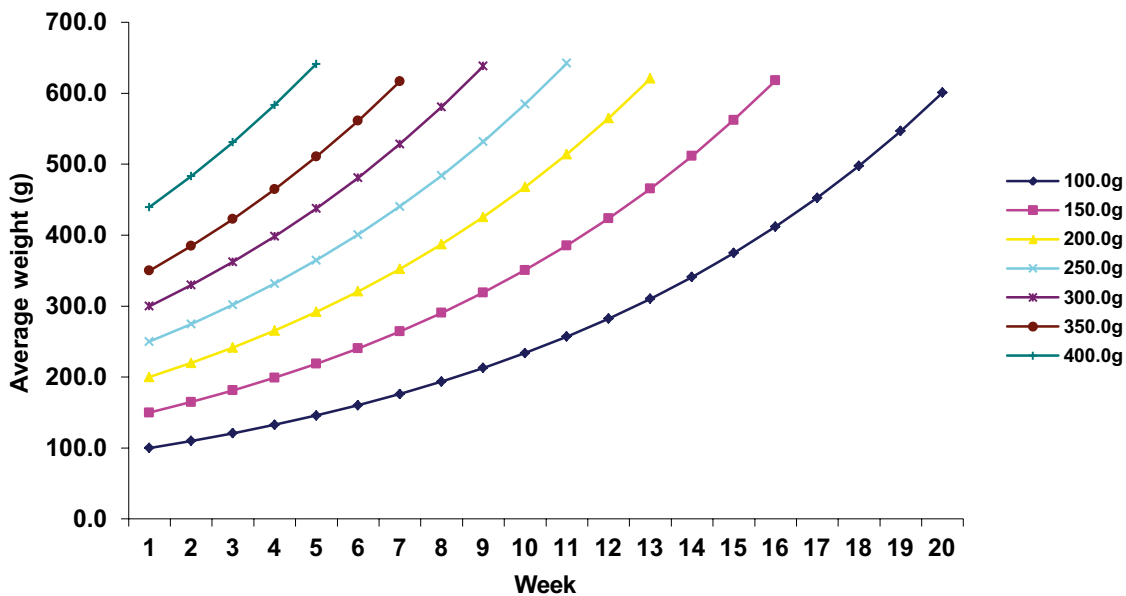
Figure 72. Projected time taken for *O. tetricus* to grow to market weight (600 gr).
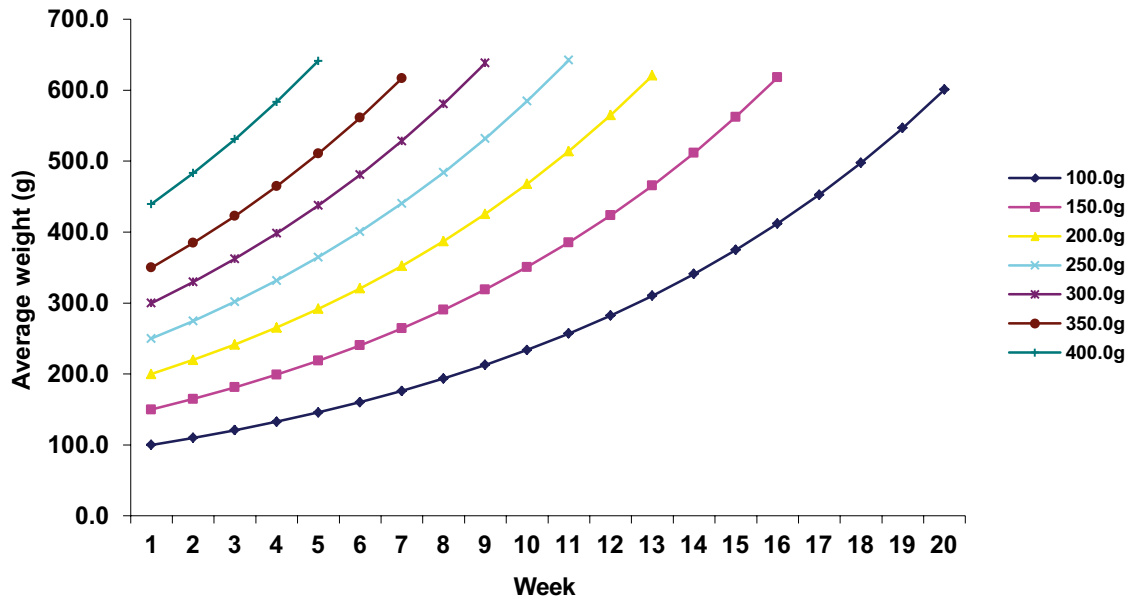
Figure 73.   Projected time taken for *O.tetricus* to grow to market weight (800 gr).

It takes ~ 20 weeks to ranch 100 gr individuals to 600 gr (Fig. 72) while it would take ~ 24 weeks to ranch 100 gr individuals to 800 gr (Fig. 73) with time decreasing thereafter as the initial average weight is increased. Increasing the initial stocking weight by 50 gr reduces the time to reach market size to 16 and 19 weeks for 600 gr and 800 gr, respectively.

5.5.4   Discussion

Developments of ranching protocol

Biomass & water temperature

Previous experiments where octopus were stocked at biomass > 10 kg m^{-3} (Section 5.3.3), were problematic due to ambient water temperatures of 24 C° and higher. Temperatures this high cause an increase in feeding in octopus due to increased metabolism. This results in more organic matter in the tanks which decreases water quality. Dissolved oxygen levels fall and ammonia levels rise which makes octopus sick and leaves them prone to bacterial infection. Controlling water temperature in this experiment via a heater/chiller unit at 21 C° had an immediate improvement in octopus health and dissolved oxygen solubility. Cannibalism in *O. tetricus* fed twice daily reduced significantly while mortality from sickness was completely eliminated.

Biomass & weight range stocked

Another factor which contributed to positive growth at high biomass was the review and adjustment of the initial weight range of octopus stocked into the tanks. Previously, the weight ranges stocked were 50 − 100 gr, 100 − 200 gr, 200 − 400 gr and 300 − 600 gr (Section 5.3).These ranges allowed, on some occasions, the largest animal to be twice the weight of the smallest animals in tanks. Growth was un-even with the biggest animals growing larger and quicker by either cannibalism on smaller animals, or by out-competing smaller animals for food. The adjustments to the weight ranges (Section 5.3.11) eliminated these problems, allowing even growth between individuals and significantly reducing cannibalism from 3.79% to 2.69% in this experiment.

Biomass & average weight increase

Stocking 100 - 400 gr octopus at a biomass ≥ 20 kg m^3 has increased the time taken for octopus to grow to market weights of 600 and 800 gr. Results from the previous experiment (Section 5.3.8) illustrate that it takes ~ 12 weeks in summer and ~ 14 weeks in winter for 50.0 gr octopus to grow to a market weight of 600.0 gr. However these experiments were conducted at biomass < 20 kg per m^3 and controlled water temperatures of 21 °C. The overcrowding of tanks meant that food was not evenly spread amongst individuals while cooler water temperatures would have slowed metabolism. Subsequently octopus of 100.0 gr would now take ~ 20 weeks to reach a market weight of 600.0 gr, allowing longer for octopus of 50.0 gr.

5.5.5   Conclusion

Grow-out trials on juvenile octopus were concluded after this experiment and a commercial protocol developed (see ranching protocol). Final biomass achieved was 54 kg m^{-3} resulting in positive growth, however supply of new wild juveniles was unavailable after this point which meant no further experimental work could be done. It is believed there is room for further increase from 54 kg m^3 based on visual observations, mortality and water quality data.

5.6   Formulated diet I

5.6.1   Introduction

Formulated semi-moist diet trials were conducted on remaining stock after grow-out trials had finished. The initial diet which was composed of a certain percentage of fish meal and blended fresh feeds (mulies and prawn heads). Being able to eventually ranch octopus solely on a formulated diet would cut down the labour required to feed and clean tanks significantly, while also not having to rely on a constant supply of fresh feed.

5.6.2   Methods

The system used is described in Section 5.1.2. Flow rates are described in Section 5.3.2.

The formulated diet consisted of a 1:1 ratio of prawn heads and mulies which were mashed to a paste using a commercial food processor. The paste was then mixed with fish meal, squid meal, vitamin mix and mineral mix to create heavy dough. The meal percentage was 35% (DW basis) while the mash was 48% (WW basis). Gelatin was dissolved in hot (80 ℃) water and was added to the mix. After a few hours setting, the mix was pushed through a commercial meat mincer with sausage making nozzle, into a cellulose sausage skin (Fig. 74). The sausage was then put into a fridge for setting. After 24 hours, the sausage could be cut into small pieces for feeding or kept frozen for later use (Fig. 75).

Figure 74.   Octopus diet in cellulose sausage skin.

Figure 75.   Octopus diet ready to be fed.

2 tanks were fed a winter fresh feed regime (Table 17) while 2 tanks were fed the formulated sausage diet twice a day at 1.75% the tank biomass. Weight ranges at the time of stocking and the time at which octopus are graded are also described in Section 5.3.7.

5.6.3   Results

Table 25.   Results of the trial comparing formulated and fresh feed.

Tank/Treatment	1/Fresh (200-400 gr)	2/Fresh (400-600 gr)	5/Formulated (400-600 gr)	6/Formulated (200-400 gr)
Initial Biomass (kg)	4.62	5.72	5.86	4.58
Final Biomass (kg)	4.31	6.13	5.54	4.41
Growth/Loss (kg)	- 0.31	+ 0.41	- 0.32	- 0.17
Initial Average Wt (g)	330.1	476.3	489.1	327.3
Final Average Wt (g)	331.8	510.8	503.7	315.2
Growth Loss (g)	+ 1.7	+ 24.5	+ 14.6	- 12.1

The first formulated feed that was made was not effective with negative growth of 0.32 kg and 0.17 kg occurring in tanks 5 and 6 respectively (Table 25). An average weight increase in tank 5 of 14.6 gr indicates that some octopus ate and grew off the diet.

5.6.4   Discussion

Future larger scale trials will be conducted using formulated diets on new wild caught octopus. Over this time we hope to get useful data on growth while learning more about a suitable size, shape and texture of diet that will appeal to octopus in captivity.

5.7   Formulated diet II

5.7.1   Introduction

New wild caught octopus were obtained for the next formulated diet trial. It was thought that new healthy wild stock would give our group a better indication whether this formulation was suitable as a food for octopus grow-out. The previous experiment was conducted on octopus that may have been compromised following grow-out trials at high biomass. They were fed fresh feed for an extended period and could have perhaps had trouble adjusting to a semi-moist diet.

5.7.2   Methods

The system used was the same as is described in Section 5.1.2. Flow rates used were the same as described in Section 5.3.2. Feed rates were set at 6 and 4% of the tank biomass twice a day. Each tank was allocated a different feed rate. Uneaten feed and waste was removed before each feed was given. Water temperature controlled at 21 °C. Octopus were weighed after 7 days.

Table 26.   Experimental treatments and stocking information

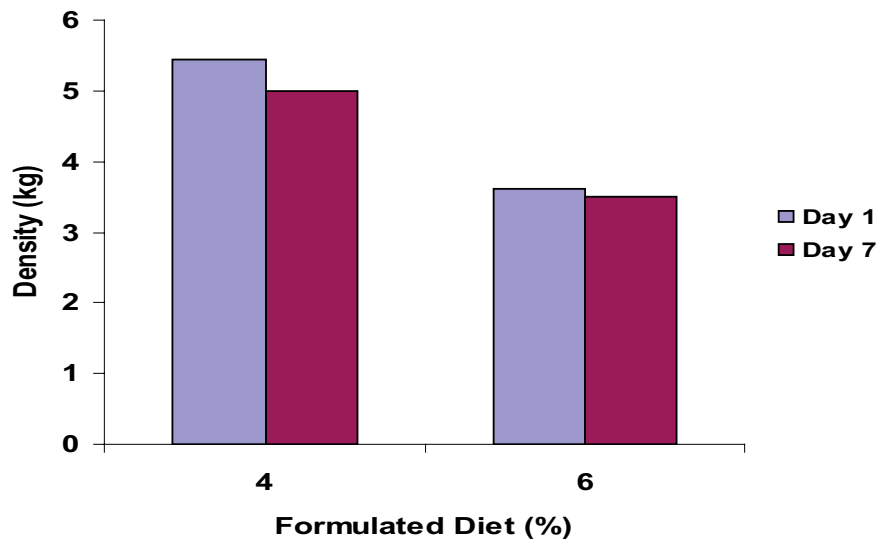| Tank	Feed Rate	Average weight	No. Individuals
1	4%	202 ± 25.16 g	27
2	6%	212 ± 24.8 g	17

5.7.3   Results

Figure 76.   Initial and final biomass of two tanks fed 4% and 6% tank biomass twice daily.
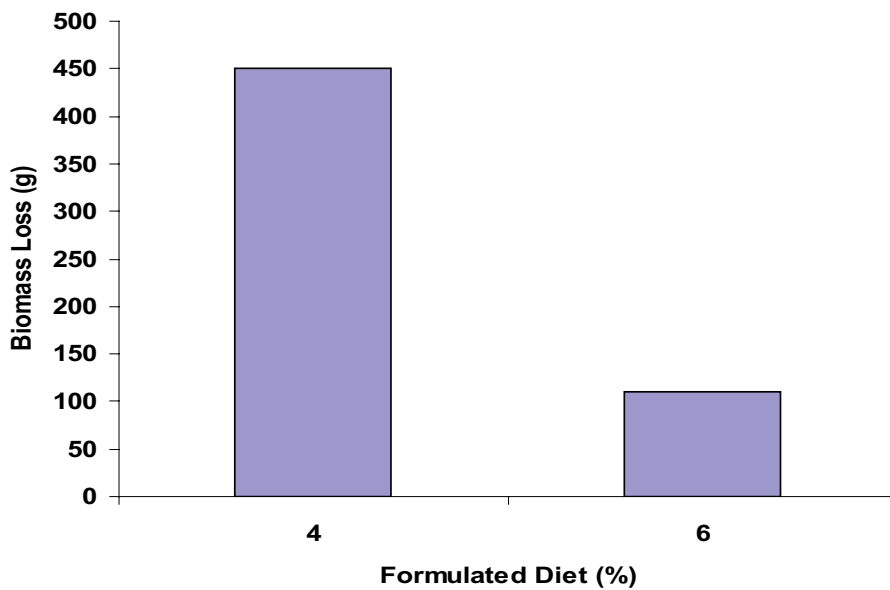

Figure 77.   Biomass decrease of two tanks fed 4% and 6% tank biomass twice daily.

Tanks fed formulated diet at both 4 and 6% of the tank biomass, recorded negative growth. Octopus fed 4% twice daily reached a final biomass of 5002.01 ± 24 g (Fig. 76) which was a reduction of 450 gr or 8.2% from the initial biomass (Fig. 77). Octopus fed 6% twice daily reached a final biomass of 3509 ± 36.54 gr (Fig.76) which was a reduction of 101 gr or 2.79% from the initial biomass (Fig. 77).

One octopus died in the 4% treatment (3.7%) while no mortality was recorded in the 6% treatment.

5.7.4   Discussion

The results were worse than the previous experiment (Section 5.6) although the same formulation was used. It was thought that the percentage of water was too high in this diet. The diet was

observed to disintegrate too fast which could have affected the octopus ability to uptake and digest it. Water quality was adversely affected by the quick diet disintegration also. It was therefore concluded that due to the low stability of the diet, the octopus were underfed, resulting in low performances.

Trials with low densities of octopus are on-going to see if positive growth can be achieved with formulated diets. Size as well as moisture and fresh feed content are all being explored to see which combination is appealing to juvenile *O. tetricus*.

5.8 Formulated diet III

5.8.1 Introduction

Due to the inability of the previous formulated diet to stay together in the tanks, this experiment was aimed at increasing the amount of binder in both of these diets so they would stay together for longer. The second diet would be varied slightly by adding krill meal while scaling back on the fish meal and mulies to see if the octopus had a preference for that.

5.8.2 Methods

The system used is described in Section 5.1.2. Flow rates were the same as described in Section 5.3.2. Water temperatures were the same as described as Section 5.5.2. Feed rates were set as 6% (Diet 1) and 4% (Diet 2) tank biomass, morning and afternoon. Biomass projected to increase 1.0% day for the purpose of projecting daily feed amounts. Uneaten feed was removed before fresh feed was given. Octopus were weighed after 7 days. The initial weight range stocked was 200 gr ± 50 gr.

Table 27.   Feed type and ingredients

Ingredient	Diet 1 (%)	Diet 2 (%)
Prawn heads (frozen)	21	21
Mulies (frozen)	30	20
Fishmeal	30	20
Krill meal		22.3
Crab extract	0.5	0.5
Vitamin mix	1	1
Binders	4.2	4.2
Fish oil	1	1
Water	10	10

5.8.3   Results
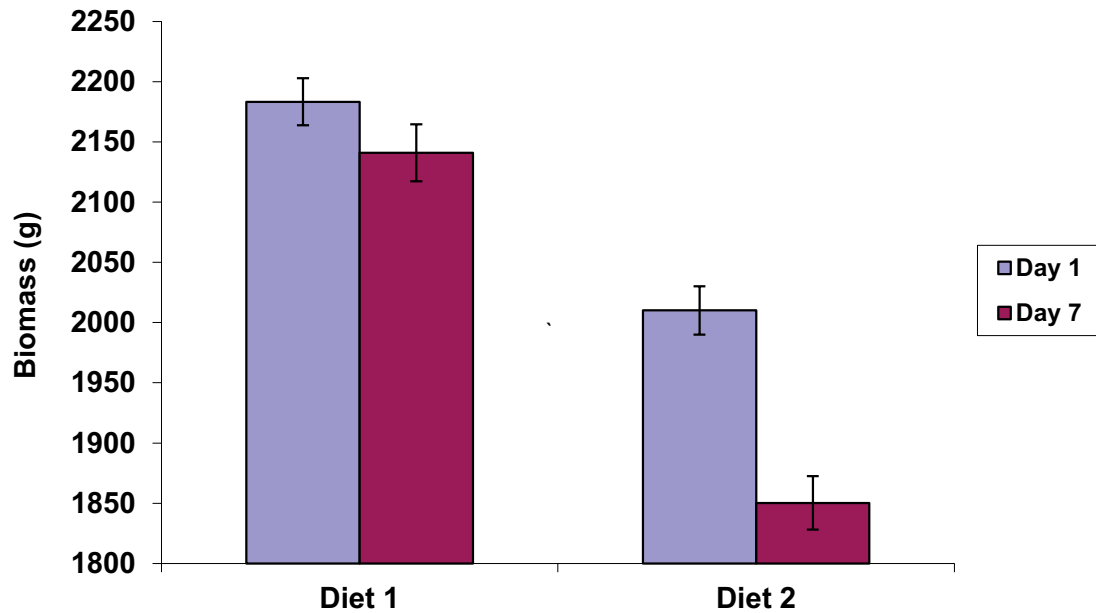
Figure 78.   Initial and final biomass after 7 days from two different formulated diets fed to juvenile *O.tetricus*.
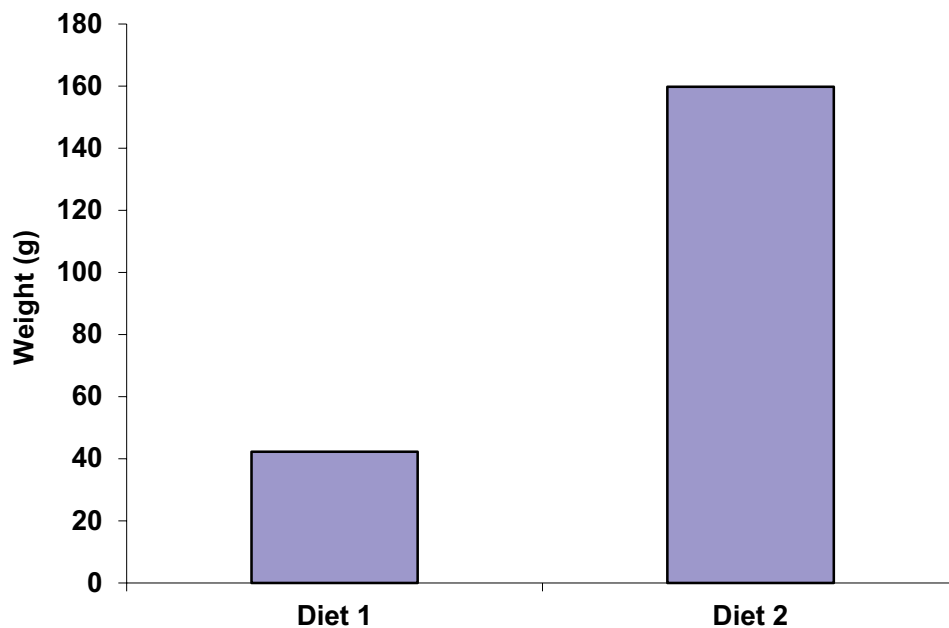
Figure 79.   Biomass decrease after 7 days from two different formulated diets fed to juvenile *O.tetricus*.

Diet 1 outperformed diet 2 in terms of weight gain (lost). However, both treatments resulted in negative growth (Fig. 78). Over a period of 7 days, the biomass of a tank fed diet 1 dropped 42.3 gr, while a tank fed diet 2 dropped 159.8 gr (Fig. 79). No mortalities via cannibalism or sickness were observed.

5.8.4. Discussion

Juveniles fed both diets were regularly observed during the trial and were not seen feeding on either diet at any stage. On some occasions, animals grabbed a piece of diet and huddled over it, but discarded it after a few seconds. Tanks became very dirty as a result. It is clear that the main problem is diet attractability and ingestion.

While krill meal considered to be an attractant in crustacean diets, surprisingly, it didn't perform the same with the octopus diets. In fact, adding krill meal and reducing the frozen mulies by 10 % resulted in a 73% drop in performance.

Work will continue to develop an edible and digestible formulated diet for juvenile octopus.

5.9 Grow-out feasibility

An initial return on investment model (ROI) was developed after the first grow-out trial were completed (Section 5.3). The model is based on data and results collected over that period. Several growth runs were conducted using a variety of tanks ranging from 1000 lt to 3000 lt. The conditions were not identical to commercial reality (pumping costs, tank design, number of tanks etc.), however this model gives some estimates on growth rates, feed amounts (natural, frozen), mortalities and costs.

Model Assumptions	
Number of animals per tank	44
Starting average weight (g)	90
Number of tanks	40
Mortality rate (10 day period)	0.725
Biomass increase/day	2.79%
Daily Feed Rate (% Biomass/Day)	6.00%
Labour Cost/Hour ($)	22
Water Cost/m³($)	0.08
Feed Cost/kg ($)	1.00
Feed Transport Cost/Week ($)	11.00
Cost of Individual Juvenile Octopus	0.63
Hourly Water Inflow Rate per Tank (lt/hr)	300.0

Mortalities	-203
Biomass Gain (kg)	727.33
Daily Water Usage (lt)	7200.00
Total Water Usage (lt)	504000
Cost Of Water Usage ($/m³ of water)	40.32
Labour Costs ($)	11550
Feed Intake (Kg)	209.69
Feed Cost net ($)	209.7
Total Feed Cost ($)	319.7
Cost Of Juvenile Octopus	1108.8
Total Costs ($)	**$13,228**
Total biomass (kg, 70 days)	885.7
Market price $/ kg	15
Total revenue	**$13,286**

Figure 80.   Initial cost assumptions and subsequent estimated revenue of octopus grow-out.

Based on the model estimation (Fig. 80), break-even figures are achieved at a stocking density of 44 octopus per tank (based on 1000 lt tanks). The commercial prototype design (Section 5.1.2) will double the capacity of the 1000 lt tanks and therefore it is predicted that for the same number of tanks, higher biomass (not necessary double) will be achievable. During the first grow-out trials, densities of up to 60 octopus were achieved. At this density, the net profit will be 26% from total revenue (assuming the same parameters as above).

The first grow-out trial was conducted during winter with ambient water temperatures ranging from 16.5 °C – 19.1 °C. It is predicted that during summer, higher water temperatures (22 ºC – 24 °C) will be conducive to higher growth rates.

The model is not taking into account capital investment and/or depreciation of equipment. The model will be updated according to these figures, as well as new growth data that will be collected during the project.

5.9.1   Growth model (updated June 2012)

Based on growth data from the high biomass experiments (Section 5.3.12), the commercial return on investment (ROI) model was updated (Fig. 81). The model is a simple presentation of a commercial farm comprising of 40 tanks. It predicts growth and biomass to market weight based on biomass achieved in the growth experiments.

For simplicity, it assumes that all octopus juveniles are stocked at the same time and the same average weight of 150 gr. However, in reality, it is more complicated. Rarely (if ever) will all tanks be stocked at the same time with the same weight of juveniles. Normally, fisherman will bring juveniles several times a week and therefore, the system will be continuously stocked with newly caught juveniles. At the same time, due to differences in initial weight and stocking times, market size octopus will be harvested at different times. This type of activity is very hard to model and/or predict since, catch can very significantly as well as, weight of juveniles.

Model assumptions	
Biomass Increase/week%	7.99%
Feed Cost/kg ($)	1.90
Number of tanks	40
Cost of Individual Juvenile Octopus	0.63
Feed Transport Cost/Week ($)	11.00
Labour Cost/Hour ($)	22.00
Daily Feed Input (Prawns)	3.00%
Daily Feed Input (Mulies)	3.00%
Initial Average Wt	150
No. Individuals/Tank	240
No. Individual/m^3	100
Mortality rate (7 day period)	2.54%
Water Cost/m^3($)	0.08
Water use (m^3/hr/10kg biomass)	1
Farm gate price 1 kg	10.00

COST - RANCHING TO 600 g	
Mortalities (individuals)	72.59
Biomass Gain (kg)	2784.08
Final Biomass (Kg)	4224.08
Total Water Usage (m3)	93764.70
Cost Of Water Usage ($)	7501.18
Labour Costs ($)	17248.00
Feed Intake (Kg)	2617.81
Feed Cost net ($)	4973.84
Total Feed Cost ($)	5127.84
Cost Of Juvenile Octopus	6048.00
Total Costs ($)	**$35,925**
Gross Income	**$42,241**
Profit	**$6,316**

COST - RANCHING TO 800.0g	
Mortalities	85.03
Biomass Gain (kg)	3879.65
Final Biomass (kg)	5319.65
Total Water Usage (lt)	129302.18
Cost Of Water Usage ($/m³ of water)	10344.17
Labour Costs ($)	20944.0
Feed Intake (Kg)	3.23
Feed Cost net ($)	6.1
Total Feed Cost ($)	193.1
Cost Of Juvenile Octopus	6048
Total Costs ($)	**$37,342**
Gross Income ($)	**$53,196**
Profit ($)	**$15,854**

Figure 81.   Updated ROI model based on the latest high biomass data.

5.10   Grow-out trials, Broodstock >1.0kg

5.10.1   Introduction

A grow out trial with larger adult octopus was conducted to determine the growth rates of octopus over 1 kg compared to juveniles octopus. While the majority of the Australian market has preference for 500-1000 gr octopus, large octopus >1 kg are also fished. This trial looked at the possibility of ranching octopus of this size. Although octopus of this size are generally wild caught, culled and value added instantly, growth rates of octopus this big in captivity may be quicker than that of wild animals of the same size.

5.10.2 Methods

The system used is the same as described as Section 5.1.2. Flow rates were set at ~ 500 lt hr^{-1} kg^{-1} octopus. Water temperature was 18°C - 20°C and an external aeration was via an air-stone. Octopus were fed frozen prawn heads and mulies. 3% tank biomass, morning and afternoon. Biomass projected to increase 2.5% per day^{-1} for the purpose of projecting daily feed amounts. Uneaten feed removed before fresh feed was given. Seventy two octopus ranging between 1 – 3 kg were obtained from commercial fisherman operating off Fremantle. Animals were weighed and stocke into 3 weight groups: 1.0 -1.5 kg, 1.5 - 2.0 kg and 2.0 - 3.0 kg. Octopus were weighed after 7 days
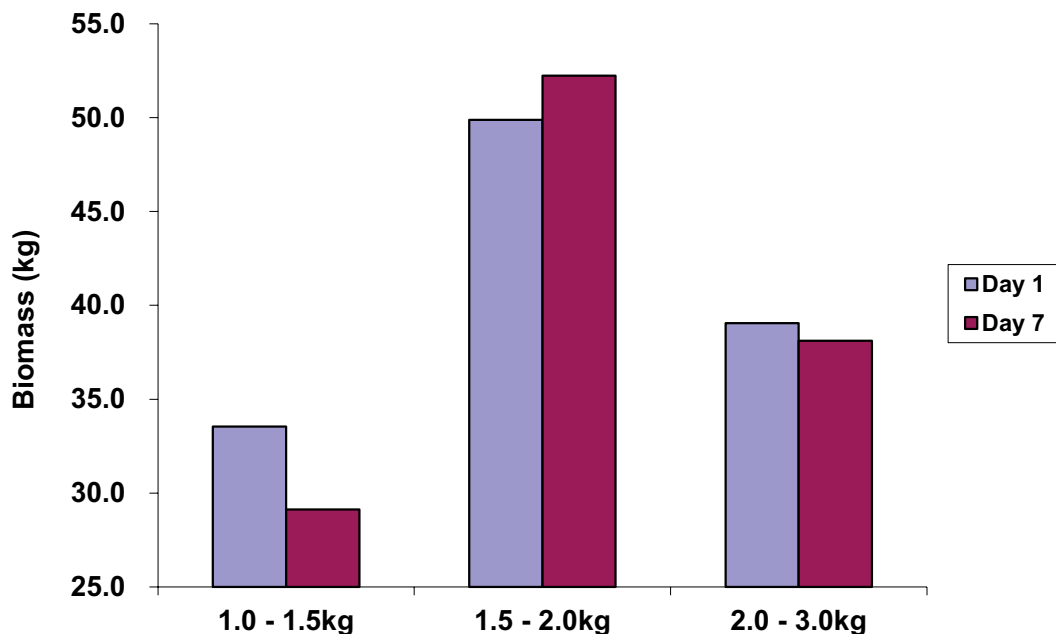
5.10.3 Results

Figure 82. Initial and final biomass after 7 days growth across 3 weight ranges of adult *O.tetricus*

Both positive and negative growth were recorded over 7 days across three weight ranges (Fig. 82) Octopus between 1.5 – 2.0 kg were the only weight group to achieve positive growth, increasing 2.36 kg (~ 5%) over 7 days. Negative growth was recorded in octopus between 1.0 – 1.5kg and 2.0 – 3.0 kg with losses of 4.42 kg (~13.4%) and 0.94 kg (~2.4%) respectively.

5.10.4 Discussion

Although positive growth was recorded in animals between 1.5 – 2.0 kg, overall results and observations indicated that grow out of large octopus normally used for broodstock was not viable. Most large octopus present in the tanks did not eat well, with a large number of mortalities experienced. When trials were concluded half way into week 2 due to high mortalities, some dead octopus were examined and their sex determined. The results indicated that we had a high number of mature females ready to lay eggs, in some cases the impending eggs being visible in the top half of their mantle after they were culled. This would account for lack of feeding in the tanks. Mating was also observed in the grow-out tanks with 2 females laying eggs in the tanks.

6.0   Ranching (*Octopus berrima*)

6.1   Introduction

Due to a shortfall in commercial catch and the subsequent market growth and increased demand for Western Australia's sub-tropical species of octopus (*O. tetricus*) since 2010, anagreement was reached between the marine aquaculture group at the WAFMRL, Occoculture Pty Ltd and a commercial fisherman operating in Coffin Bay, South Australia (Fig. 83) to transport 600 live *O. berrima* via road transport from Coffin Bay to Perth.

Figure 83.   Locality of Coffin Bay, South Australia.

O. berrima or 'Southern Keeled Octopus' is a temperate species of octopus found off the coast of south-eastern Australia including Tasmania (Norman 2000, pp-288). A major factor in the decision of the marine aquaculture group and Occoculture Pty Ltd to pursue research of this species was the fact that newly hatched larvae are immediately benthic. This would hopefully eliminate nutritional problems associated with the planktonic phase of octopus larvae culture currently being experienced with *O.tetricus* at WAFMRL.

Research was required to see if *O. berrima* juveniles could be grown-out in tanks at high densities, the same way that has seen the grow-out of *O.tetricus* juveniles become very successful during the Fisheries Research Development Corporation (FRDC) project currently in progress at the WAFMRL. At the conclusion of the grow-out trials on juvenile *O.tetricus* during the FRDC project, 50 – 600 gr octopus could be stocked at 54kg m^3 with almost no mortality recorded. It was hoped that if successful, reared *O. berrima* juveniles could be used the fill the shortfall of local product, therefore easing the pressure on the *O. tetricus* stock off the west and south coasts of Western Australia.

6.2   Transport

6.2.1   Transport system and equipment

Figure 84.   The system that was used to transport octopus from South Australia.

The system used to transport octopus from South Australia was housed in a trailer (Fig. 84). An enclosed trailer was used so as to protect the heater-chiller, pump and other electrical components while hopefully maintaining a stable air temperature, which wouldn't affect water temperature too much. The system consisted of 2 large eskies in series, which were subject to recirculated, temperature controlled sea-water via a pump and heater-chiller unit. Water leaving the eskies would pass through a filter, which would partially purify the water of organic matter before returning to the heater-chiller. Other equipment used was a dissolved oxygen meter and bottle containing pure oxygen to monitor and manually add oxygen in case levels became critically low.

Table 28.   Transport system equipment used

Link **280W self-priming pump**	Flow rate of 1200 lt hr[1] through the eskies throughout the trip.
Aqua Medic **Heater-Chiller**	Controlled temperature at 17 - 17.5 °C, brought up to 23 °C upon arrival to Perth
Filter and Filter Canister	Canister – *WaterCo* CC100 housing, Filter – Trimline C75 30 micron (0.03 mm)
Oxygen	Size 'G' industrial oxygen bottle supplied by BOC Gases.
Esky	1000 lt and 450 lt
DO Meter	Oxyguard Handy Polaris 2 Dissolved Oxygen and Temperature meter.
Travel Time	8 days to and from Coffin Bay from Perth.

6.2.2   Octopus collection and transport method.

600 live individual octopus and 8 female octopus with eggs were collected in Coffin Bay. The live individuals were tied into pre-cut onion bags (2 to the bag) and housed in oyster mesh

circular cages which separated the octopus to stop fighting and cannibalism (Fig. 85). The females carrying eggs were left in the pots they were caught in, and put into the oyster mesh circular cages individually.

Figure 85.   The oyster mesh circular cage used to separate the octopus (left) and the onion bag in which octopus were tied to the bag (right).

6.2.3   Outcome of octopus transport.

Of the 600 live individual animals transported back from Coffin Bay, only 312 animals survived. All of the 8 female octopus carrying eggs survived. The death of the octopus was due to a few factors:

- An abrupt increase in water temperature from 17.5 °C – 23 °C upon arrival at WAFMRL due to 35 °C – 40 °C air temperature in Perth on the 9th February.

- Fluctuating dissolved oxygen levels at certain times during the trip.

- A bigger than expected build-up of protein in the eskies from the octopus continually excreting faeces over the duration of the trip. This caused high ammonia levels in the water.

## 6.2.4   Initial handling of octopus at WAFMRL.

Upon arrival at WAFMRL, the female octopus were placed into 340 lt coffin tanks while the individual octopus were weighed and graded into weight classes which were (1) 30 – 50 gr (2) 50 – 80 gr and (3) greater than 80 gr into 2000 lt fibreglass tanks (Fig. 86). These tanks would be used for the initial high density grow-out trials.

Figure 86   340L coffin tank used to house the female octopus tanks with eggs (left). 2000L tanks used for the high density grow-out trials and also for separating the octopus upon arrival to WAFMRL (right).

6.3   Adult and juvenile grow-out trials (high density)

Grow-out trials on the remaining 312 octopus begun the day after arrival (10th February). A total of 3 x 2000 lt fibreglass tanks were used; one for each weight class. Each tank was fed 6% of the tank biomass morning and afternoon assuming that biomass was going to increase 1% per day^{-1}. The food given was mulie and prawn pieces rotated on a daily basis. Flow through water was set at 1000 lt hr^1, 1 exchange every 2 hours. External aeration was provided.
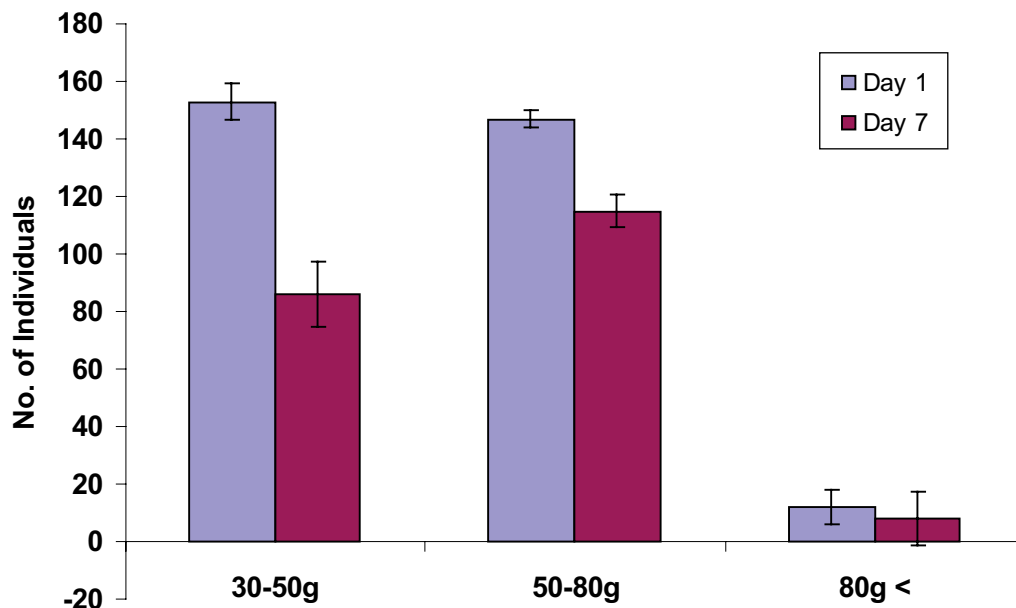
Figure 87.   Mortality represented by the number of individuals at day 1 and day 7 of different weight ranges.

High density grow-out trials of octopus in 2000 lt tanks were unsuccessful (Fig. 87). High mortality was observed across all weigh ranges tested. The largest loss of octopus was observed in the 30 – 50 gr weight range losing 67 animals after 7 days while the lowest loss of octopus was observed in the > 80 gr weight range losing 4 animals, although only 12 animals were originally stocked. Due to the resulting negative growth and mortality, the biomass of the octopus on day 7 was not obtained across all 3 weight ranges.

Some factors that may be associated with the high mortality and negative growth were;

• The already declining and ill health of the octopus from the transport from Coffin Bay.

• The lack of literature available on the culture of *O. berrima* in captivity. Having no prior information on their diet, biology or optimal temperature for culture.

• Discovering that the animals obtained were most likely mature females that were ready for spawning with a number of animals laying eggs in the culture tanks or thereafter.

• Observing no food uptake in the tanks which could have been due to (1) poor animal health (2) that fertilized females don't usually eat prior to laying eggs or (3) a distaste for the food given to them.

After the high density grow-out trials were completed, the octopus were separated into 3 x 1000 lt tanks. Here, they were fed once a day a rotating diet of mulie and prawn pieces and provided with hides which consisted of 10 x 20 cm lengths of 50 mm PVC pipe housed in an oyster mesh circular cage (Fig. 88). On one particular day, small crabs were collected from the Swan River which were accepted and eaten by the octopus. This was the only food that the juvenile octopus actively ate during this period at WAFMRL. A labour intensive process in collecting them meant it was not viable for our group to feed them to the octopus on a daily basis.

Figure 88. Hides given to the octopus in the 1000 lt tanks.

While the octopus were in these tanks, 56 individuals laid eggs in the hides. Clutches consisted of approximately 20 – 100 eggs that were laid on the roof of the hides. 48 of these animals along with the hide were separated into 340 lt coffin tanks (Fig. 30) for incubation at a temperature of 21 °C. The eggs took on average 54 days hatch at this temperature. The remaining 8 animals were incubated at 22 – 23 °C and took on average 35 days to hatch.

6.4 Larvae growth and feed suitability trials.

Hides vs. no hides

Hides which were an arrangement of 7 x 10 cm lengths of 20mm PVC pipe (Fig. 89), were trialled to see if they reduced cannibalism and territorialism.

Figure 89.   Hides which were trialled in the 50 lt larvae tanks.

6.4.1 Methods

Four 50 Litre tanks were stocked with newly hatched octopus at a stocking density of 29 animals in tank 10 and 13, 28 animals in tank 11, and 27 animals in tank 12. Tanks were operated as flow through at 100 lt hr^{-1} and temperature was maintained between 20 – 21°C. Both tanks 12 and 13 were fed a semi moist diet (Nutrakol Pty. Ltd), while tanks 10 and 11 were fed fresh feed (squid, prawn and lobster pieces) and all tanks were siphoned once daily. Hides were added to tank 11 and 12 in order to see how mortality due to cannibalism and territorial behaviour was affected.
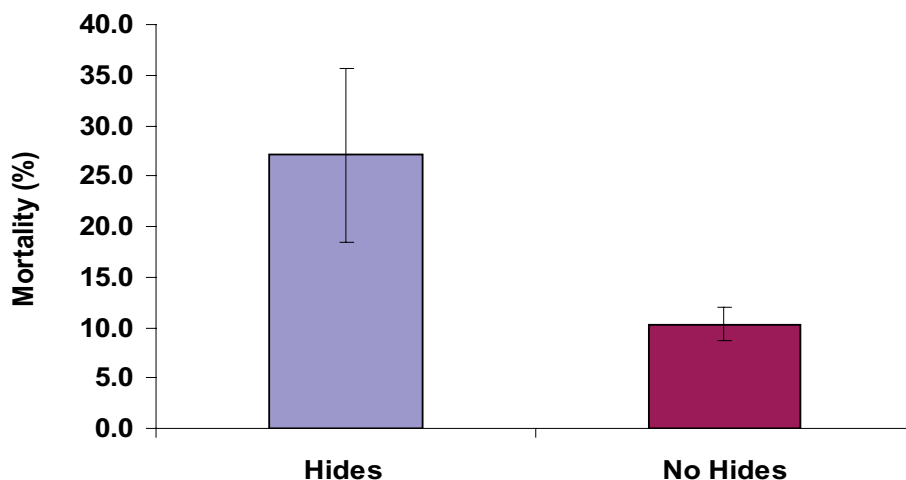
6.4.2 Results
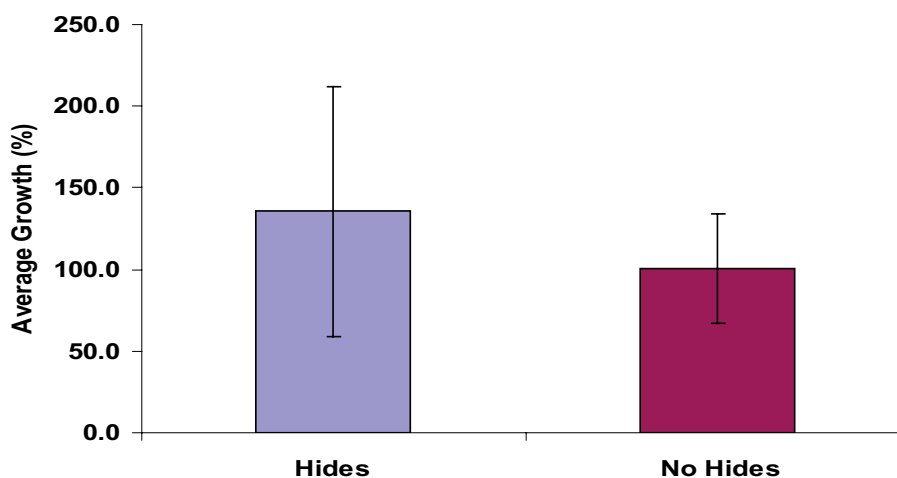
Figure 90.   Average growth (%) of octopus in tanks with and without hides over 7 days.

The average growth of octopus in tanks with hides is greater than that of tanks without hides (Fig. 90). At 7 days post hatch (dph), tanks with hides had an average growth of 135.6%, while tanks without hides had an average growth of 100.3%.

Figure 91. Mortality (%) over 7 days of octopus in tanks with and without hides.

The mortality after 7 days was greater in tanks with hides at 27.1%, while tanks without hides resulted in a mortality of 10.3% (Fig. 91).

6.4.3 Conclusion

All tanks yielded positive growth. Over 7 days, octopus in tanks with hides showed an average growth that was 35.3% greater than that of octopus in tanks without hides. However, average mortality was also 16.8% greater in tanks with hides. In terms of improving water quality, it would be preferable to remove hides from the tanks as food and faecal matter accumulates within the pipes, making them difficult to clean and facilitating bacterial growth.

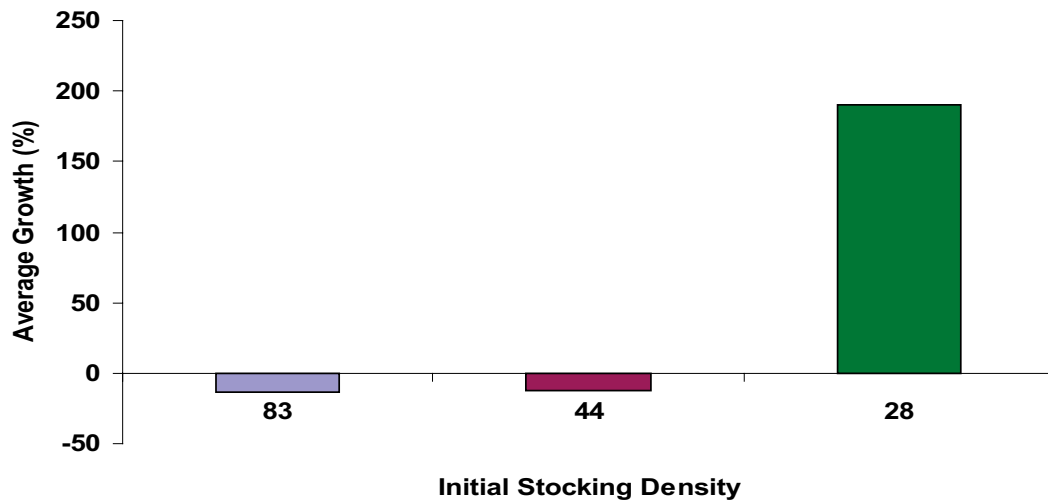## 6.5 Stocking density

6.5.1 Methods

Part A:

Three 50 lt tanks with varying stocking densities were tested to see how mortality and growth were affected. All tanks were operated as flow through at 100 lt hr^{1} and fed pieces of lobster, squid and prawn with daily siphoning of tanks. All three tanks contained hides and were maintained at $20 - 21$ °C. Tank 8 was stocked with 83 larvae, tank 9 was stocked with 44 larvae and tank 11 was stocked with 28 larvae.

Part B:

Three tanks with stocking densities greater than 65 (83, 70 and 69 larvae) were compared to three tanks with stocking densities less than 30 (29, 28 and 27 larvae) to examine effects on average growth and mortality. All tanks were 50 lt volume, fed pieces of lobster, squid and prawn with daily siphoning of tanks. Tanks were flow through at 100 lt hr^{-1} with temperatures of $20 - 21$°C.

6.5.2   Results

Part A:

Figure 92.   Average growth of *O. berrima* at different stocking densities from 1-8 days post hatch (dph)

A stocking density of 28 larvae had the highest growth of 190.45% after 8 days (Fig. 92). The tank with a stocking density of 44 larvae produced growth of -12.7% while the tank which had a stocking density of 83 larvae produced growth of -13.51%. The larvae in tanks stocked with 83 and 44 larvae lost weight.

Part B:
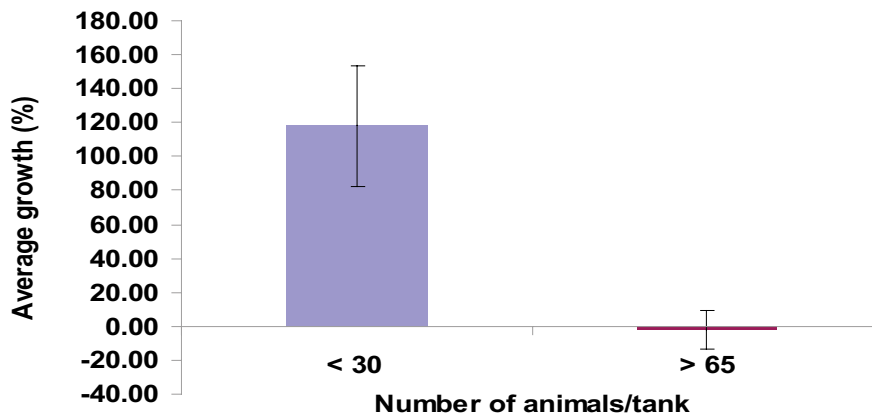
Figure 93.   Average growth (%) of octopus at a stocking density <30 compared to a stocking density of > 65.

A tank stocked with less than 30 animals produced an average growth of 117.95% (Fig. 93), while tanks stocked with greater than 65 animals produced an average growth of -1.77%.
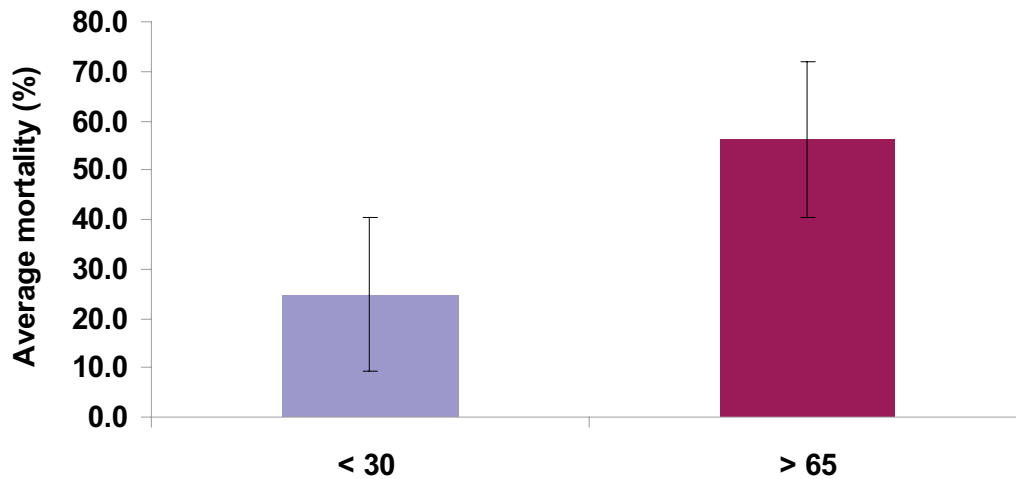
Figure 94. Average mortality (%) of octopus at a stocking density <30 compared to a stocking density of > 65.

A tank stocked with less than 30 animals resulted in an average mortality of 24.8%, while stocking tanks with greater than 65 animals resulted in an average mortality of 56.3% (Fig. 94).

6.5.3 Conclusion

The data demonstrates that stocking density is inversely proportional to growth; therefore the lower the stocking density, the higher the average growth, and the higher the stocking density, the lower the average growth. Mortality was found to increase as stocking density was increased. In order to find the optimal stocking density to maximise growth, a greater range of stocking densities need to be tested. From the data it is known that stocking densities over 65 animals is not viable with animals observed to lose weight resulting in negative growth.

6.6 Fresh feed vs. semi moist diet

6.6.1 Methods

Two 50L tanks were stocked with 29 larvae and operated as flow through at 100 lt hr^{-1}. Temperature was maintained at 20 – 21 °C. One tank was fed fresh food (squid, prawn and lobster pieces) while the other tank was fed semi-moist diet (Nutrakol Pty. Ltd) in order to see if there was any difference in growth as a result of differing food types. Both tanks were fed and siphoned daily. Neither tanks contained hides.

6.6.2   Results
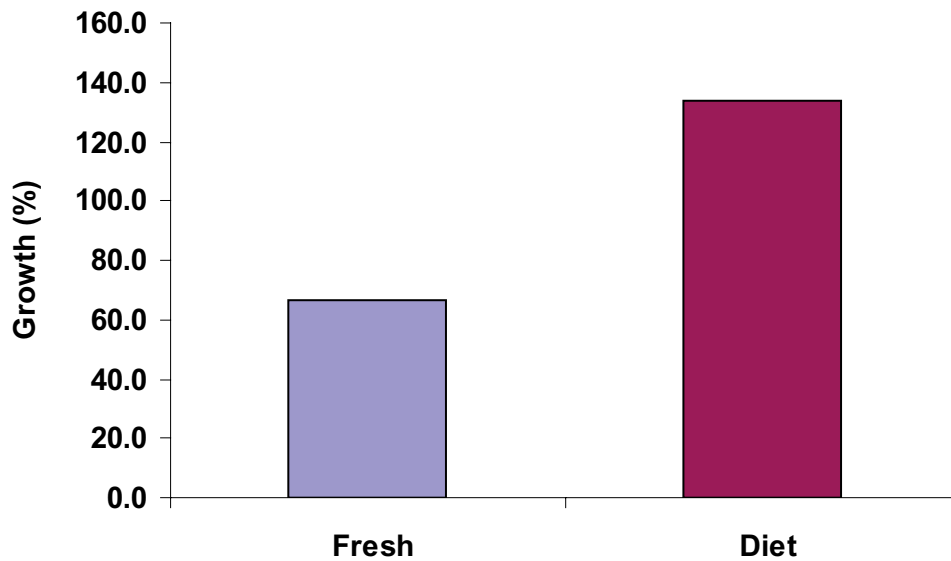
Figure 95.   Comparison of growth (%) between animals in tanks fed fresh food and semi-moist diet over 7 days.

Over 7 days a tank, which was fed semi-moist diet, produced an average growth of 133.8% while the tank which was fed fresh feed produced an average growth of 66.7% (Fig. 95).

6.6.3   Conclusion

From the data it is evident that octopus given semi-moist diet produced an average growth that was 67.1% greater than that of fresh feed. However, it should be noted that there were no replicate tanks and this experiment will be repeated in order to confirm that semi-moist diet produces better growth.

## 6.7   Tank volume and surface area

6.7.1   Methods

Two tanks of vastly different volumes and dimensions were stocked with octopus to observe any potential differences in growth and mortality. One tank was rectangular, black in colour with a volume of 340 lt, while the other tank was also rectangular, blue in colour and with a volume of 54 lt. The surface area to volume ratio of the 54 lt tank was 12.83 while the surface area to volume ratio of the 340 lt tank was 7.64. At a stocking density of 112 animals in the 340 lt tank, there were 2.31 octopus per cm^{-1} of tank surface. At a stocking density of 70 animals in the 54 lt tank, there were 0.99 animals per cm of tank surface. Both tanks contained hides and animals were given fresh feeds, which consisted of squid, pilchard, crab, lobster and prawn pieces.

6.7.2   Results
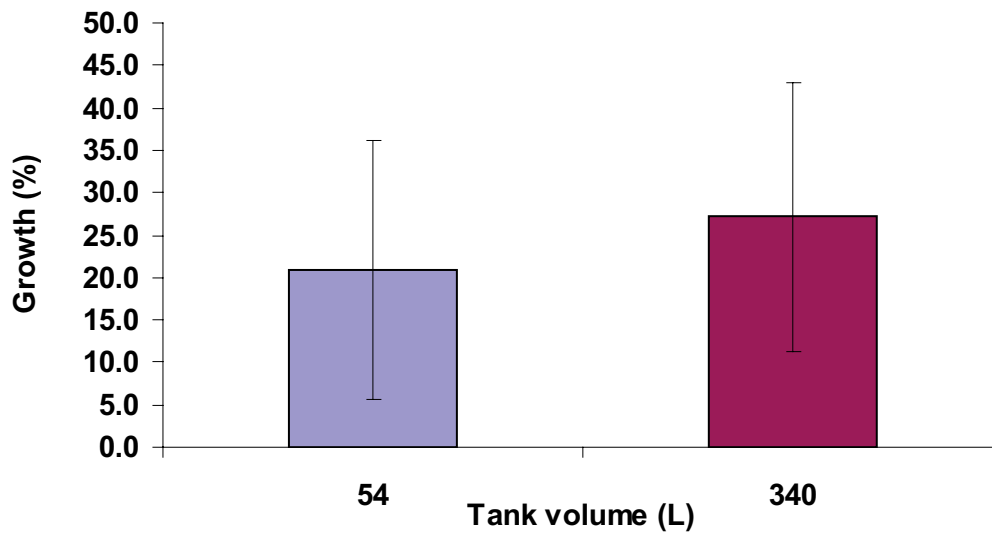
Figure 96.   Average growth (%) of a 54 lt tank compared to that of the 340 lt tank

Average growth of octopus was greater in the 340 lt tank (Fig. 96). Growth in the 340 lt tank was 27.1% while average growth in the 54 lt tank was 20.9%.
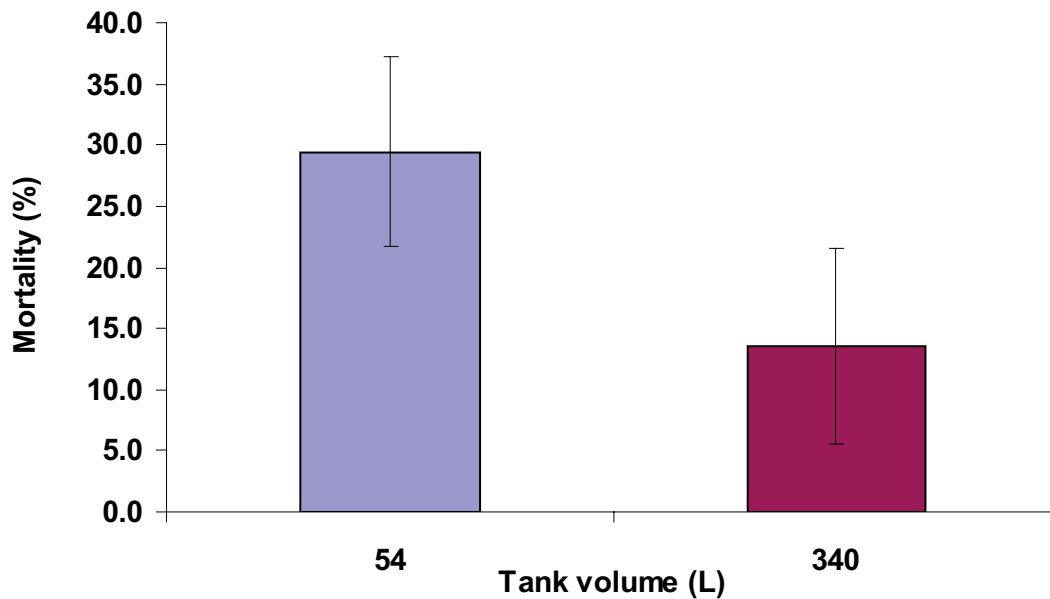
Figure 97.   Average mortality (%) over 7 days comparing a 54 lt tank to a 340 lt tank.

The mortality of octopus was higher in the 54 lt tank compared to the 340 lt tank (Fig. 97). The 54 lt tank had an average mortality of 29.4% while the 340 lt tank had an average mortality of 13.6%.

6.7.3   Discussion

The data suggests that on average, animals had a higher percentage growth in the larger 340 lt tank volume despite there being more octopus per cm in this tank. Mortality in the 340 lt

tank was 15.8% which was lower than that of the 54 lt tank. As octopus became older, more size variation among animals was observed along with increased territorial behaviour and cannibalism. The fact that animals in the 54 lt tank are now 35 dph while the 340 lt animals are 24 dph could be a factor which has contributed to this higher mortality. It would appear that a larger tank volume produced better results for growing out larvae based on these growth and mortality results. Grading of animals according to weight at weekly intervals will need to be trialled in order to hopefully reduce size variation and subsequent mortality.

## 6.8   Larvae grading trials

6.8.1   Introduction

The initial grow-out trials conducted on *O. berrima* larvae yielded some encouraging results. Although higher than expected mortality from cannibalism and natural death was observed, the octopus were observed to;

1. Eat the different feeds provided which included squid, prawn, lobster, crab and pilchard pieces, as well as formulated semi-moist diet.

2. Be able to be stocked into tanks at relatively high densities,

3. Show positive growth for the first few weeks after hatching.

Due to the high mortality resulting from these trials, a decision was made to weigh each animal individually and stock them into tanks into defined weight groups. Octopus are highly cannibalistic especially when the size difference between the largest and smallest animals in a tank is large. Defined weight groups would hopefully reduce mortality from cannibalism and increase growth.

6.8.2   Methods

All grading trials were conducted in 340 lt coffin tanks (Fig. 86). Water was flow-through at 250 lt hr[1] and temperature was set at ~ 21 °C. Octopus were fed once daily a rotating diet of prawn, mulie, squid, lobster and crab pieces in order to give them a rounded diet. Tanks were without hides and were weighed after 7 days.
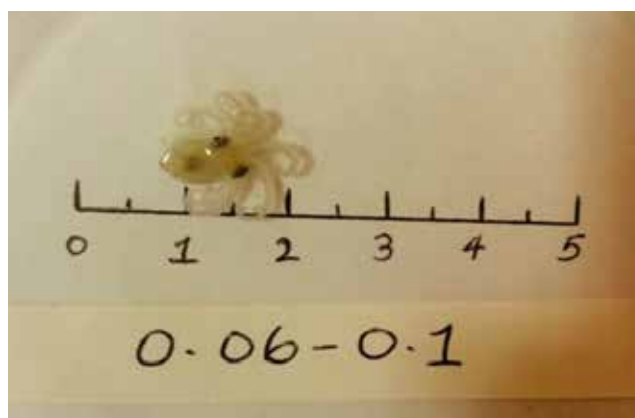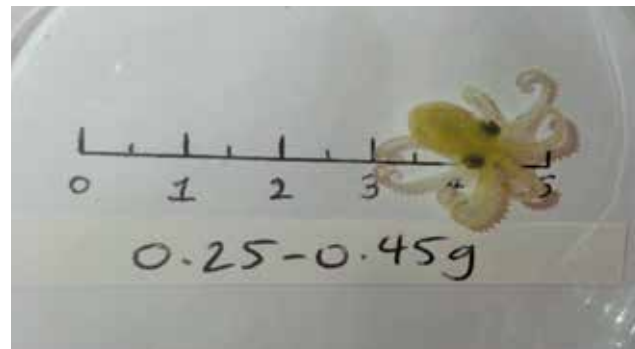

Figure 98.   Average length of octopus stocked between 0.06 - 0.1gr.
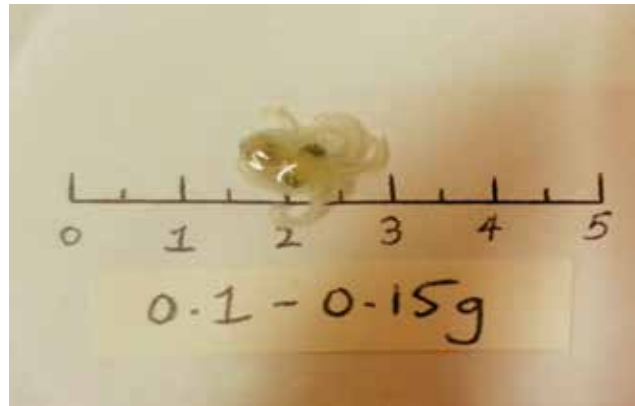
Figure 99.   Average length of octopus stocked between 0.01 - 0.15 gr.
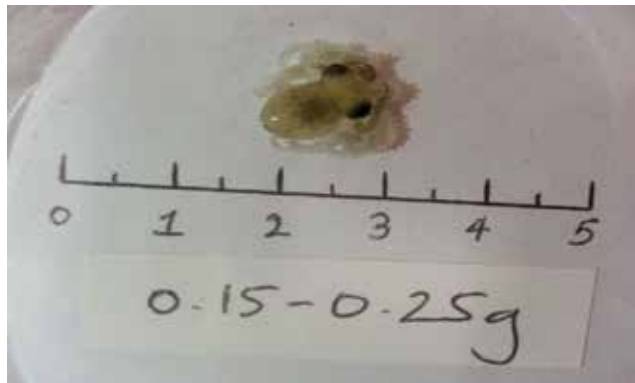
Figure 100.   Average length of octopus stocked between 0.15 - 0.25 gr.

Figure 101.   Average length of octopus stocked between 0.25 - 0.45 gr.

An indication of the size of the octopus used in these grading trials is shown above (Fig. 98 - 101.) Most of the animals were less than 0.25 gr in weight and measured ~1.0 cm. However some animals were greater than 0.25 gr and measured ~ 2.0 cm.
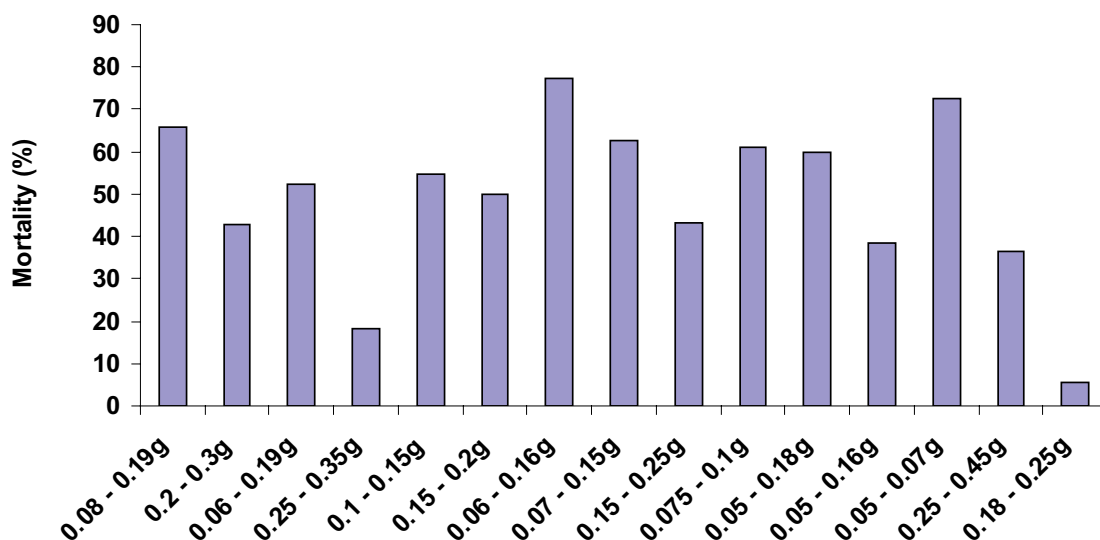
6.8.3   Results

Figure 102.   Average mortality (%) of all weight ranges that were trialled.

Like the high-density grow-out trials detailed in Section 3, the trials were also unsuccessful. The highest mortality resulted from stocking animals between 0.06 – 0.16 gr, which was 77.5 % (Fig.102). The lowest was when 0.18 – 0.25 gr animals were stocked together, which was 5.6 %. To put this into perspective, the maximum amount of animals stocked in these tanks was 40 on day 1, so losing 77.5% of the animals, meant there was 9 remaining on day 7. The average mortality across all weight ranges trialled was 49.4% over 7 days.

6.8.4   Discussion

Daily observations during these grading trials revealed that cannibalism was prolific. Octopus were observed to eat other live octopus while the animals that died naturally, were also preyed upon. Upon delivering food to the tanks, octopus were observed to huddle over the pieces giving the impression they were feeding but indeed no ingestion and growth resulted. Cannibalism indicates that the food given, although perhaps suitable for the first two to four weeks after hatching, wasn't suitable after this age. The octopus would have been constantly hungry, preying on each other to become satiated.

6.8.5   Conclusion

Observations and problems encountered.

Transport.

The fact that only 312 of the 600 (52%) animals survived the transport from Coffin Bay meant that the initial health of the surviving animals stocked at WAFMRL probably wasn't optimal. Fluctuations in usually stable water parameters such as temperature and dissolved oxygen and high ammonia levels in the eskies at various stages during transport, would have caused ill health and stress in the animals that survived. Having to acclimatise the octopus to high water temperatures upon arrival in Perth would have further deteriorated health in the surviving animals.

Feeding and growth in juvenile and adults.

Feeding and subsequent growth of the juvenile and adult octopus at WAFMRL following

transport was minimal. Multiple fresh feeds such as Prawns, Mulies, Crab, Lobster and Squid were given to the octopus with no success. Small crabs collected from the Swan River on the 15th February proved to be the only food that the octopus would actively eat, meaning perhaps a preference for live food. Factors that could have attributed to the low feed rate could have been (1) the ill health of the animals following transport and (2) that juveniles and adults of this species don't eat fresh inert foods and in fact have a preference for live prey or other prey types.

Another complicating factor in the attempted culture of this species was a large proportion of them laying eggs in the tanks. This would indicate that a high percentage of the animals caught in Coffin Bay and transported across were fertilized females ready to lay eggs. It is unsure whether this was the case or whether the females laid eggs in the tanks from stress. Female *O.tetricus* cultured as part of the FRDC project at WAFMRL did not feed when they were fertilized or had laid eggs. This would have explained the lack of feeding in *O. berrima* females during high density grow-out trials.

Feeding and growth in larvae

Despite the high mortality during the initial growth and feed suitability trials on larvae (Section 4), results for the few weeks following hatching suggested that larvae readily accepted food and grew in tanks. Positive growth resulted from;

1. Stocking tanks at high and low densities.

2. Feeding octopus either small pieces of prawn, squid, mulie, lobster, crab or semi-moist diet.

3. Supplying tanks with or without hides.

4. Culturing octopus in tanks with vastly different volumes.

However attempted culture following these initial trials proved unsuccessful with significantly increased mortality and negative growth. Like the attempted culture of juveniles and adults described in Section 6.3, live prey (Mysids) was given to the larvae on 2 separate occasions and were readily accepted. Again, a labour intensive process to collect them and the high cost to obtain them meant this wasn't pursued. Subsequently all larvae were eventually lost meaning an inability to close the life cycle of this species during this time.

6.8.6   Recommendations for future attempts at *O. berrima* culture.

A major limiting factor for the marine aquaculture group at WAFMRL during this project was the lack of available literature on the culture of *O. berrima*. Methods and protocols used to grow-out *O. tetricus* were used and subsequently were not effective for this species.

Before any more octopus are obtained from Coffin Bay:

1. Thorough research needs to be done on any available literature on *O. berrima* to obtain information on biology, ecology and food preference.

2. Consultation with industry in Coffin Bay on the preferred or available food types for *O. berrima* in Coffin Bay and surrounding areas.

3. If successful, these foods need to be located and obtained by the marine aquaculture group at WAFMRL and/or Occoculture Pty Ltd for use in any future grow-out trials.

4. More attentiveness to water quality parameters when transporting octopus from Coffin Bay. Perhaps transporting animals in the colder months when large fluctuations in water and air temperature are less likely.

7.0   References

Norman, M. 2000, *Cephalopods: a world guide.* Conchbooks, Germany.

8.0   *Further development*

Developing octopus aquaculture based on octopus ranching i.e. catching juveniles octopus and rearing them to market size, is possible and in certain conditions commercially viable. In Western Australia, ranching can only be done part of the year due to the availability of juveniles. This fact coupled with the cost of manpower and facilities might hinder the development of octopus aquaculture in Australia. However, if and when the octopus life cycle is closed and there will be post larvae and juveniles octopus available on demand, then the development of octopus aquaculture seems to be viable.

A model was designed for developing countries, which includes a combination of fishing efforts, and small-scale aquaculture, which is currently under investigation in several countries including South Africa, Sri Lanka and Mauritius. It is envisaged that octopus ranching can be developed as a combination of family unit and co-operative efforts. Local fishermen will fish for octopus and bring the juveniles back to small-scale on-shore tanks. The spouses and the rest of the family will grow the juveniles in the tanks. Harvesting and processing will take place at the octopus fishermen's co-op. This model has the potential to supply food and income to artesian fishermen and their families in rural areas without the need for large investment.

9.0 Recommendations

While the ranching of octopus was developed and the methods and systems are available for commercialization, the hatchery phase is still not complete. While significant progress was made in developing larvae systems and rearing protocols, closing the life cycle of *O. tetricus* has not yet been achieved.

The supply of octopus paralarvae is essential to be able to commercialize octopus aquaculture in Australia. It is suggested that funding will be available for R&D project aimed specifically at this objective. This project should be in collaboration with other centres in Spain and Greece and Italy allowing sharing of knowledge, skill and experience in the area of octopus paralarvae rearing. This suggested collaboration will assist with shortening the time needed to achieve this goal.

10.0 Project coverage

Two presentations were given at the World Aquaculture Symposium, Adelaide, South Australia (May, 2014).

Octopus aquaculture – advancements in protocols and systems for ranching of *Octopus tetricus*. Sagiv Kolkovski, Mark C. Natale, Justin L. King, Nicole A, Watts.

Current Status of *Octopus tetricus* paralarvae rearing. Nicole A, Watts, Mark C. Natale, Justin L. King and Sagiv Kolkovski.

An Article was published in the Fish Farming International magazine (March 2012) describing the project and its objectives.

RESEARCH

The Australian Department of Fisheries has been working closely with Occoculture, a subsidiary of Fremantle Octopus Pty Ltd, to rear octopus as a potentially lucrative limb of Western Australian aquaculture. Aided by funding from the Fisheries Research & Development Corporation (FRDC), Department of Fisheries' Principal Aquaculture Scientist Dr Sagiv Kolkovski is running a two-pronged research program into the commercial viability of exploiting *Octopus tetricus* through aquaculture.

OCTOPUS AQUACULTURE COMES TO THE FORE

Commercial farming of octopus in Western Australia (WA) is set to take a significant stride forward with the development of a model octopus farm at the Department of Fisheries' Research Division at Hillarys Boat Harbor.

O. tetricus, prized for its eating quality, displays a remarkably rapid growth rate, reaching up to three kilograms in its one-year lifecycle.

In WA, nationally and internationally, octopus is an increasingly popular seafood source, with Fremantle Octopus – Australia's biggest octopus fishing company – finding strong demand for its product.

This demand, combined with its rapid-growth rate, makes *O. tetricus* an ideal candidate for aquaculture.

The focus of the research and development project is to look at ways of ranching octopus to a suitable size for consumption in a way that returns a sufficient profit to make it a worthwhile venture.

This is working in tandem with the project's longer term and more elusive goal; to close the life cycle of *O. tetricus* and to produce octopus juveniles in sufficient numbers and to a sufficient size to make it a commercially successful aquaculture operation.

By working on the ranching side of the project, it is an effective way of fast-tracking full scale aquaculture production when hatchery-reared juveniles will be available.

Currently, similar work is being carried out in a number of countries around the world including, Spain, Mexico, Italy, Greece and Chile. However, currently, there is no commercial octopus hatchery anywhere in the world.

If and when they find the formula for rearing out the paralarvae, then the ranching side of the project will be ready for growing out the juvenile octopuses.

Currently, Kolkovski and his team are rearing juvenile octopus

at commercial densities in tanks that were designed by the group specifically for octopus ranching. Traditionally, to prevent aggressive behavior and cannibalism, octopus tanks and cages are supplied with shelters for the octopus (pvc pipes etcetera).

However, the WA octopus tanks are completely free from any shelters. "We didn't think that supplying shelters was viable in large scale operation since it required constant cleaning, limited the number of octopus

in tanks/cages and contributed to territorial behavior. Therefore, we developed ranching techniques, allowing us to stock large number of animals in tanks without any shelters, similar to the way we grow fish. We achieved almost 100 percent survival with no predation.

"The work is being carried out to imitate commercial reality as closely as possible," said Kolkovski. "We compared growth and survival of juvenile octopus at different stages using different

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Carp diet: Arafat Ahmed, John Moody, Simon Davies and Awadhesh Jha of University of Plymouth are working on the dietary chromium requirement of common carp, *Cyprinus carp*, studying the effect of trivalent chromium (as chromium chloride) on growth performance and carbohydrate utilization in common carp.

Sustainable sole: Luisa Valente, Eduarda Cabral, Sonia Batista, Margarida Bacelar, Manuela Castro-Cunha and Rodrigo Ozorio are researching on sustainable diets for Senegalese sole, focusing on the development of sustainable and practical diets for sole juveniles, minimizing the inclusion of crystalline AA.

Artemia for trout growth efficiency: Hadi Jamali, Hojatolah Jafaryan, Rahman Patimar, Mehdi Dehghan, Javad Sahandi and Hosin Adineh are researching on using artemia pathenogenetic for promoting growth efficiency in rainbow trout (*oncorhynchus mykiss*) fry by bioencapsulation with probiotic bacillus.

European eel research: A group of reasearchers are investigating the modulation of stored lipid reserves through broodstock nutrition and reproductive success in European eel. Genevieve Corraze, Josianne Stoettrup, Lars Kristian Holst, Laurence Larroquet, Sadasivam Kaushi are some of the names involved.

commercial scale anywhere in the world.

Several bottle-necks are hindering the octopus paralarvae development. Nutritional requirements are still very much an unknown with very little information available.

Susceptibility of paralarvae to bacteria in hatchery conditions is also considered a major issue.

Dracula feeding techniques

Octopus paralarvae feeding habits are quite different to fish larvae. The paralarvae sucks the internal 'juices' of its prey (usually artemia), and then releases the dead carcase, resulting in bacteria accumulation.

Therefore, both nutritional as well as engineering solutions need to be developed to match the octopus paralarvae requirements and to reduce the bacteria load in the paralarvae tanks.

If Kolkovski and his team could unlock the environmental and nutritional code required to get the octopus past its larval stage, it could open up a whole new aquaculture industry with potential global appeal.

Whatever the outcome of this next phase of investigation, one thing is for sure, aquaculture of octopus in Australia on a commercial scale is a lot closer to becoming a reality.

Octopus fisheries

In the past 11 years, octopus fisheries have boomed in Western Australia. While only several years ago, the only source of octopus was a by-catch from the lobster fisheries, today there are several fishing companies specifically targeting octopus with landed price of AU$ 10/kg (compared to around AU$ 4.50/kg only few years ago). The biggest octopus fishing company in WA is Fremantle Octopus Pty Ltd. The Fremantle-based company is controlled by the Cammilleri family which are something of seafood pioneers, having played a key part in developing the Western Australian octopus market for the last eight years. Now what they are trying to achieve, with assistance from the Department of Fisheries, could spark a revolution in octopus fishing and aquaculture industry.

In 2000, the Cammilleri's launched Fremantle Octopus, an octopus fishing company. Initially, focused on octopus fishing and developing the product for local markets, it then progressed to marketing the products in the eastern states of Australia.

Under the umbrella of Fremantle Octopus Pty Ltd, the company has evolved into a multi-limbed venture, encompassing the fishing operation, a diverse range of seafood products, octopus fishing technology and aquaculture development. "Many of

the Asian and European octopus fisheries are encountering difficulties getting enough product due to overfishing and the lack of restrictions. Add to that is the fact that, seafood demand, in general, and octopus specifically is sky rocketing. This means prices in some markets are at a premium. We're in a really strong position here, in Australia, because our octopus stocks are in excellent health due to our self-imposed and restricted management of wild octopus," said Ross Cammilleri, Fremantle Octopus founder.

Vertically integrated, the company is controlling every step of the production from fishing efforts, post-harvesting, processing and marketing of the final product.

The company is now not only vertically integrated but 'horizontally' developing the aquaculture and ranching of octopus.

With demand exceeding the sustainable catch, the company started to investigate the potential of ranching juvenile octopus to market size as well as closing the life cycle and supplying hatchery-reared octopus. Sustainable fisheries combined with the development of octopus aquaculture will result in a premium product as well as new technologies that might be applied worldwide, contributing to the sustainable of octopus around the world.

Supported by the Department of Fisheries, Western Australia and the Fisheries Research & Development Corporation (FRDC), Occoculture is currently setting up a model farm for the octopus growout and ranching. This commercial model is designed as a modular unit with the possibility of extension to a larger operation. It is anticipated that once fully operational, the model farm can be duplicated in other locations both in Australia and overseas.

To financially support the project, Occoculture is currently looking for new partners to be involved in this exciting development of the octopus aquaculture.
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feed sources, temperatures and tank conditions. We developed a commercial model taking into account all costs including food, manpower, water, electricity etc. We believe that octopus ranching, either from hatchery-reared or wild caught is very much commercially viable not only in Australia but probably anywhere around the world.

"The primary aim is to develop a business plan for similar ranching farms that can, at some point in the future, be replicated

up and down the coast – or anywhere in the world – and run by fishers who will then be in a position to supply distributors with a steady supply of fresh product."

Existing octopus ranching has attracted interest from different parts of the world, particularly in the Mediterranean, central and South America where juvenile octopus of different species are caught in large numbers. "The beauty of the growout system is that it is a modular,

easy to install system that is very simple to operate on either a flow-through or recirculating system".

Bottlenecks in closing the lifecycle

While the growout system is already in its commercial testing, closing the life cycle of O. tetricus is proving to be more challenging. Octopus paralarvae are notoriously difficult to rear in captivity, which is why it has never been achieved on a

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Appendices

Appendix 1: List of project staff

Department of Fisheries, Western Australia

Dr Sagiv Kolkovski – Principal Investigator

Justin L. King – Technician

Nicole A. Watts – Technician

Mark C. Natale – Technician

Adva Mori – Technician

Occoculture Pty Ltd

Ross Camilleri – Co-investigator

Craig Camilleri – Co-investigator