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Development towards commercialization of marine fish larvae feeds – Microdiets

Final FRDC Report – Project 2004/258

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Fish for the future

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2004/258 Development of commercialization of Marine Fish Larvae Feeds – Microdiets

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Objectives

1. To optimize formulated marine fish larvae diets and to foster commercialization.
2. To serve as service centre for any larvae problems and product development.
3 To further develop and commercialize automatic feeding system for microdiets.

1.0 Non-Technical Summary

The current project 2004/258 was initiated based on the success of previous FRDC funded projects. Several larvae systems and a basic formulation prototype larvae diet were developed.

Outcomes achieved to date

The project specifically focused on developing formulated diets for marine fish larvae, specifically targeting more physical aspects of microdiets such as feeding methods, feed availability and particle recognition. A commercially ready microdiet formulation was developed that achieved better ingestion, digestion and survival for yellowtail kingfish than other commercially available diets. Two manufacturers of aquaculture feeds showed interested in the formulations, however neither of the companies have started producing the diet commercially.

A previously developed world first automatic microdiet dispenser (AMD) was further developed to commercial standards. Previously handmade, the feeders were redesigned as injection molded units that could be made on mass. A touch screen controller was also developed, which allowed individual control of each feeder. To date 15 feeding systems have been sold to both research and commercial hatcheries around the world, with reports of substantial cost savings in the more efficient use of microdiets and labor.

In general, the Australian and more specifically Western Australian aquaculture industry is benefitting from the flow on effects of developing these feeding techniques and microdiet formulations through ongoing consultation with the principle investigator and collaborative research efforts into both finfish and now octopus culture. The benefits of these outcomes flow directly to marine hatcheries, commercial and R&D aquaculture centers in Australia and overseas.

Microdiet development

Different microdiet preparation methods and nutritional parameters were tested and optimized during the project. It was found that the current method of micro-binding (MBD) achieved better stability and less leaching of amino acids compared to micro marumerisation (MEM). The MBD particles sunk significantly slower than the MEM particles, giving the larvae a longer time for ingestion.

Characterization of the chemical and physical properties such as leaching and sinking rates were made, to enable the determination of the ‘window of opportunity’ for larvae to successfully ingest the food particles.

Different feed attractants and inclusion methods were compared in terms of the effects on ingestion rates and larvae growth and survival. This determined the best attractants and the preferred method of inclusion. Higher observed ingestion rates and better survival of yellowtail kingfish larvae resulted from feeding this diet.

Ridley Pty Ltd, an Australian aquaculture feed producer, expressed interest in manufacturing the microdiet for the Australian market, however with no outcome to date. Skretting Pty Ltd, a global aquaculture feed manufacturer has also expressed interest in the attractants that were used in the diet, but have not taken it further. The main outcome to date is the development of

a commercially ready microdiet that can be manufactured in Australia using local and imported ingredients for the Australian aquaculture industry that would provide a competitive advantage.

Research & Development Plan 2005-10

Without a reliable supply of seed stock from marine hatcheries the aquaculture industry in Australia cannot reach its full potential. Reliable, environmentally and economically sustainable hatchery production remains a key limitation in many sectors of the industry.

Following the second National Hatchery Feeds and Technology workshop convened in September 2004 and held over two days in Sydney, the latest R&D plan has been developed. Download proceedings at: http://www.fish.wa.gov.au/docs/op/op030/index.php?0400

The purpose of the current R&D plan was to briefly update the old plan (2000-2005, (www.aims.gov.au/hatchery-feeds) and to indicate new priorities and goals for R&D. It was planned that these priorities would, hopefully, be used by the relevant funding agencies to set research priorities and to evaluate R&D proposals. The priorities and R&D needs are direct summaries from the workshop and some discussion with industry that took place after the workshop.

Feeding system

Until recently, there was no commercial feeding system available that could cope with microdiet particles in relatively small accurate doses, which is imperative for water quality and larvae health. The automatic feeding system developed by the group during the current project provides a solution for this problem. Initially, the feeding system was developed to reduce labor requirements and provide more appropriate feeding regimes for finfish larvae. This unique system has now been patented and is in operation in many commercial fish hatcheries and research facilities worldwide.

Using the feeding system enables any commercial and/or R&D hatchery to optimize their feeding regimes by providing small amounts often, rather than manually feeding large amounts several times a day. Small doses of microdiet delivered more frequently throughout the day and outside the normal working hours, provide a constant presence of microdiet particles in the water column. This increases the bioavailability of nutrients and gives the larvae the best opportunity to ingest the diet, resulting in better water quality and reduced incidence of disease. An economic advantage is gained as a result of better growth rates and larval health.

During the project the feeding system was registered as a patent (under the FRDC and the Department of Fisheries WA). The production of the feeders and the control panel was optimized and is currently contracted to a local molding company and control instrument manufacturing company.

The systems are currently being sold around the world to commercial hatcheries and R&D centers.

KEYWORDS: Microdiet, attractants, automatic, dispenser, ingestion, digestion, rotifers *Artemia*.

2.0 Background

During the past three decades, extensive efforts have been made to develop microdiets (MD) as a complete or partial substitution for both rotifers and *Artemia*. The majority of the focus has concentrated on the larvae's nutritional requirements. Despite substantial achievements, complete replacement of live feeds for most marine species is still not feasible.

In addition to MD quality, important physical characteristics of MD particles within the water column such as sinking rates, nutritional stability, leaching rates and leachate profiles all contribute to the MDs attractiveness. They influence ingestion and digestion rates and can greatly affect larvae growth, however these factors have received very little attention.

Larvae feeding methods and feeding systems play a crucial role towards the way MD is received by the larvae. The best MD is only as good as the method used to dispense it into the larvae tank. However, this area of research is undeveloped compared to feeding systems and methods for on-growing fish. Only a handful of automated MD feeding systems exist and very limited work has been published in this area.

This work intends to review these aspects of MD properties, current developments, MD feeding systems and feeding methods.

2.1 Food Identification and Ingestion

The first contact between food item and larvae occurs in the water column, where the particle is recognized as food and accepted, or rejected. Therefore, it is essential that this interaction be optimized to achieve a maximum likelihood that this feeding process is successful. There are many factors affecting this process including particle/organisms concentration, frequency and duration of interactions, and chemical and physical recognition.

Various substances, such as free amino acids, nucleotides, nucleosides and ammonium bases, are known to be 'feed attractants' for fish larvae. A practical way to increase the ingestion rates of MD would be to incorporate or coat the diet particles with extracts or hydrolysates of marine organisms. A comparison between incorporating and coating with feed attractants (krill hydrolysate) showed better success by coating the diet (Kolkovski et al. 2006), while both methods of inclusion preformed significantly better than no hydrolysate. Squid hydrolysates have also been found to improve ingestion and growth (Kolkovski et al., 2009). It is assumed that inclusion of hydrolysates as partial protein replacement benefits the larvae in two ways:

1. Higher ingestion rates by improving attractiveness, and
2. Higher assimilation of free amino acid and short peptides.

2.2 Microdiet characteristics

Leaching

An important issue concerning MD particles is amino acid leaching rates, which varies according to the method of MD binding and manufacture. Kvale et al. (2006) reported leaching of protein molecules (9-18 kD) after 5 minutes immersion in water (3% NaCl, 12°C) at a rate of 80-98%, 43-54% and 4-6% for agglomerated, heat coagulated and protein encapsulated microdiet. Yufera et al. (2003) determined the rate of different types of amino acids leaching from micro

bound diet (MBD) and microencapsulated diet (MED). The authors found that hydrophilic amino acids leached more from MBD and hydrophobic amino acids leached more from MED particles. The leaching rates of the two diets were also significantly different. Other authors tested the leaching rates from several different microdiets made with different techniques and found similar results (Kolkovski et all. 2009). Although some amino acid leaching is necessary for food particle recognition, a balance between rates of leaching, stability of MD particles in water and the digestibility of the diet need to be made.

Buoyancy

One of the most significant problems with microdiet particles is their negatively buoyant inert state, contrary to the movement of live zooplankton that acts as a visual stimulus for increased feeding activity (Kolkovski et al., 1997a,b). Excessive sinking rates of MD can lead to bacterial proliferation and deterioration of water quality when particles accumulate on the bottom of the tank and decompose. Jackson and Nimmo, (2005) demonstrated significant differences between sinking rates of several commercial MDs. Different attempts have been made to increase microdiet particle buoyancy by adjusting oil levels, varying manufacturing methods as well as using rearing systems with up welling currents (Kolkovski et al., 2009). Knowledge of sinking and leaching rates of microdiets can and should be used to optimize feeding procedures. Ideally very small quantities of diet should be fed as often as practicable when using sinking diets.

Feeding system

Although commercial MDs are now thought to be nutritionally adequate for marine finfish larvae, none are used solely without rotifers and/or *Artemia*. Part of the reason is the microdiet distribution method or manner of presenting the diet to the larvae.

Hand feeding is the simplest and still the most widely used method with relatively long periods between feeding events (30 to 60 minutes). However, the high metabolic rate of larvae demands continuous feeding over long photoperiods, which presents logistical difficulties when performed manually. Moreover, it results in an inefficient use of a relatively expensive product.

Continuous availability of MD is optimal for larvae and is best achieved by dispenser automation. Only a handful of automated microdiet feeding systems exist and almost no scientific papers have been published (Papandroulakis et al., 2002)..

Dosage system

The first requirement from a mechanical microdiet dispenser concerns its capacity to deliver one stable quantity per feeding event. Once a reliable dose is established, its repetition throughout the tank is necessary to provide continuous larvae/particle interaction. Several feeding systems currently exist including, belt feeders, horizontal drums with pneumatic pistons, rotating disks and more recently, dynamic solenoid operated slotted-plates. While some were specifically designed for microdiet, others were adopted from large pellet feeders.

MD delivery generally takes place directly above the water surface, however there is a risk of aggregation of particles into clumps that immediately sink. To avoid this, Raunes, a cod hatchery in Norway, has developed an intermediate vessel ("spider") where the microdiet is premixed with water before being distributed into the water column through several small rigid plastic pipes at different points of the tank. This method of dispersing micro-particles is very efficient and avoids trapping the micro-particles in surface skimmers. However, premixing

MD with water may cause very strong leaching of the nutrients, the extent of which is yet to be determined.

Another way of evenly allocating microdiet throughout the tank is by using an air-blade above the water surface and under the MD dosing point, which propels the particles within a controlled area. The air current separates clumped particles that then distribute evenly over the surface. This method is simpler than the "spider" device, but requires tanks without skimmers for particles less than 150μm that tend to float and become trapped, rather than sink.

Sparing on the quantity of microdiet

Microdiets are expensive (up to 200€/Kg) and are likely to remain so, due to expensive raw ingredients, difficult manufacture techniques, and relatively small-scale production leading to high fixed-cost contributions. Therefore, it is necessary to optimize yield from such a product. In the on-growing sector of fish farming, feed conversion ratio (FCR) is of primary concern to farmers and feed producers. FCR should also be used as an indicator to help hatchery managers improve their methods and efficiency when substituting for live feed. However, FCR figures for larvae feed – live or formulated do not exist, and estimating FCR with larvae is extremely difficult. Although 'commercial batch FCR' is calculated by dividing total food inputs (rotifers, *Artemia* and microdiets) by harvest biomass for specific cultures, ongoing FCR measurements during larvae production are always difficult to determine on a daily basis. Actual MD FCR (ingested MD / weight gain) could be as low as 0.6:1 due to the high digestibility of the MD (Kolkovski, 2009; Leclercq D., personal communication). However, this is rarely achieved because of excess feed spoilage, usually caused by inadequate feeding strategies that lead to practical FCR values above 3:1.

Using the right feeding system to reach a precise and controlled distribution of microdiet improves FCR values considerably. Some European hatcheries quoted 20-40% less feed being used when shifting from hand distribution to automation, with additional benefits of increased larvae survival and growth (Leclercq D., personal communication; Aquastream hatchery, France, personal observations).

Although larvae nutritional requirements are now recognized, MD properties and feeding technologies are neglected. Therefore, the current project took a more integrated approach to microdiet development; taking into account the effect of physical and chemical properties, manufacture techniques, feeding systems, tank hydrodynamics and water quality on ingestion, digestion, and the resultant better growth and survival of larvae.

3.0 Automatic Microdiet Dispenser

3.1 Description and Development History

3.1.1 Introduction

In 2002, the Mariculture Research & Advisory Group headed by Dr Kolkovski was working with *Seriola lalandi* (Yellowtail Kingfish) and *Pagrus aurata* (Pink Snapper) researching larvae nutrition and feed attractants. Some adjunctive work was being done on the bacterial loading of larvae culture tanks and *Artemia* enrichment tanks. Significantly high levels of pathogenic bacteria were found in both areas of the hatchery. Yellowtail kingfish are a pelagic fish and are particularly susceptible to bacteria, thus a solution was needed.

The main cause of bacterial loading in the larvae tanks was identified as a combination of inefficient cleaning and bad feeding practices. Improving tank hygiene was simple, however a solution to stop microdiet building up on the tank bottom was more difficult. The larvae were being hand fed every 30 minutes. This was very labour intensive and led to large masses of inert diet in the water column at feeding time that the larvae could not possibly eat. The wasted diet inadvertently sank to the bottom of the tank and out of reach for the larvae. Automatic feeding was obviously the solution; however there was nothing on the market that could cost effectively feed 24 experimental tanks simultaneously, in very small amounts, distributed across the whole tank surface, every few minutes of an 18-hour day.

Weaning fish larvae from live food to dry microdiet (MD) continues to be a major challenge when rearing marine finfish larvae. In most research and commercial hatcheries, the feeding of MD is usually done by a combination of belt feeders and manually hand feeding several times a day, which demands high labour requirements. Both methods have their disadvantages. MD tends to stick to the belt feeder especially in humid conditions (like most areas near culture tanks), while hand feeding is labour-intensive and can easily result in uneven distribution and over-feeding. Furthermore, fouling can easily result from few and relatively large doses, whereby a large proportion of uneaten MD particles will sink to the bottom of the tank. Overfeeding promotes bacterial growth, which reduces dissolved oxygen levels and increases larval stress and the risk of infection.

Therefore as part of a focus on the development of new and innovative culture systems for marine hatcheries the AMD – Automated Microdiet Dispenser was designed as a reliable and efficient method to dispense fine particulate feeds (100 μm – 2000 μm) to larvae culture tanks. A Programmable Logic Controller (PLC) was developed in parallel to operate any number of AMD units and complete the AMD system. To date, the system is the only one that caters for both research and commercial larvae culture. Apart from the AMD there are only very small and unreliable automatic feeders for aquariums or huge ones designed for feeding large fish during the grow-out phase. The feeders that were available were not precise enough and couldn't cope with small quantities of very fine MD. There is no equivalent system available "off-the-shelf" anywhere in the world.

The need was recognized for such a system to be available to researchers and fish culturists around the world. After manufacturing hand made units for various research fellows, in 2005 a need to develop the feeding system to commercial production was recognized, so that the benefits of using such a system would be available to anyone. Therefore an injection mould was developed that could quickly produce any number of identical feeders, along with an easy to use touch screen controller.

The AMD is designed to periodically administer a small amount of MD (≥ 100 mg) to larvae rearing tanks, creating a situation that more closely resembles live feed, and which leads to more efficient weaning. A small quantity of MD delivered more frequently increases its availability to larvae by increasing the frequency of encounters. This enables them to consume a greater proportion of the MD and therefore reduces the opportunity for bacteria to proliferate. MD can be distributed evenly across the photoperiod or alternatively can be dispersed in higher amounts during periods of high larval activity, such as the morning. The use of AMD's also leads to reduced labour requirements and facilitates feeding outside of working hours. This not only reduces production costs by more efficiently using expensive microdiets but also results in better quality larvae by promoting healthier culture conditions and freeing technicians to do other tasks.

The development of the AMD system was a component of the FRDC funded project 2001/220 and is further being refined under the current FRDC project. The Department of Fisheries Western Australia and the FRDC jointly registered the AMD system as an international patent in 2005, with Mr John Curnow, Mr Justin King and Dr Sagiv Kolkovski listed as the inventors.

3.2 AMD Innovations

There are 6 aspects of the feeder design, which are innovative and different from other feeders already on the market.

1. The use of a solenoid in order to pull the moving parts in a uni-planer direction, effectively using one articulated moving part. A stainless steel spring around the solenoid piston pushes the plate back into position. This enables relatively simple maintenance and longevity for the unit within the marine hatchery environment.
2. The piston and plate are connected loosely for a loose action of the plate, in order to stop sheer compression of the diets that are passing through. Sheer action on the diets causes them to compact at the end of the tube and between the plates, which would otherwise block the feeders.
3. The two plate design, where slots and bars overlap in both resting and fully pulled positions in order to block microdiet from falling freely through the units when they are not activated. When the plate is in motion the slots through the plates line up and allow a uniform amount of diet to pass through. This gives precise repetitive allocation of the diets being processed.
4. The angled feed delivery tube set at between 30 and 60 degrees from vertical, (depending on the feed type) stops the feed being compressed at the bottom of the container. This allows the feed to sit on the inside of a cylinder shaped container along the inside bottom side of the tube, and prevents compression onto the plates by minimizing the bulk of feed directly above the moving parts. Angling the feed delivery tube prevents a plug of compressed microdiet from forming, which would otherwise block the feeder.
5. The feed is evenly distributed onto the water surface and clumps are broken up by using air jets to pass a stream of air across and below the base plate. Aeration is passed through an internal manifold in the bottom part of the feeder housing which passes air parallel to the feeder plates.
6. The flexible hanging bracket allows the feeder to vibrate freely when the mechanism is activated, in order to aid the movement of microdiet down the feed tube.

7. Computerized PLC operation for flexible and precise feed allocation is used. The PLC can be used to energize the solenoids for fractions of a second to accurately control the amount of microdiet passing through the feeder. The PLC has facility to adjunct automatic lighting and system maintenance equipment such as pumps, which allows the complete synchronization of the aquaculture system as a whole.

8. 12 VDC operations at delivery are used for safety reasons.

3.3 Development Steps

3.3.1 Conceptual development.

The need to automate the process of feeding 24 experimental tanks was evident after running the first experiment in the larvae rearing system. Feeding was being done every 30 minutes to 1 hour and usually leading from one feeding event lead into another. This required that at least 2 technicians were present for a given experiment, in order to cope with all tasks that are required of larvae rearing. Therefore a number of concept feeders were designed and tested.

Concept descriptions:

a. The first prototype involved a geared electric motor rotating a vertical shaft with a sweeping arm on the end of it. The arm would push microdiet under a cowl, from where the diet was to drop through an opening. This was modelled on a working feeder designed for large pellets. However, the microdiet failed to pass through the opening.

b. The second design consisted of a rotating slotted barrel, situated directly underneath a hopper containing microdiet. The barrel was rotated using a small electric motor and the diet was meant to fall from the slots in the barrel and into the tank. However, it was found that the slots quickly packed with sticky particles and then stopped dispensing food.

c. A rotating motorized auger situated below the hopper was designed to extrude the diet into the tank. The blades of the auger were too small and tended to block, so the microdiet stopped flowing. A larger auger was not practical because of the precise handling of the diet that was needed.

d. The present design had a number of manifestations, the first of which had a single hole drilled into a moving plate situated directly under the hopper. The moving plate had a solid plate beneath it to stop diet from freefalling through the hole. A solenoid was used to pull the plate aside from the hopper and allow the food to fall into the tank. A spring was used to replace the plate beneath the hopper to fill the vacated hole once again. This was quite successful with larger and more free-flowing diets, however the finer (<500 mm), stickier diets tended to compact on the top of the plate, which prevented them from falling into the hole.

e. The precursor design to the commercial AMD, using two slotted plates, one above the other and situated beneath a vertical hopper worked for most diets in the laboratory, where it was not humid. However, as soon as the feeder was positioned over a tank it would stop working because of compaction of the diet on top of the plate and humidity sticking the diet together. Then the hopper was repositioned on a 45° angle, which stopped the compaction problem and allowed the diet to flow unrestricted. Furthermore, an air manifold was positioned underneath the exit point, which was dryer than the hatchery air and kept the diet from becoming wet. This air manifold also broke up any clumps of diet before they fell so

that an even distribution of diet was blown across the water surface. Initially a 20mm clear tube was used for the hopper, which only held 20g of diet and needed to be filled more than once a day for most experiments. The tube diameter was then increased to 25mm and the length extended so that it held 80g of diet, and only had to be filled once a day.

Most of the conceptual designs required a small geared down electric motor in order to work. This was considered a weak point, because of the unreliable nature of electric motors in humid and salty environments. The solenoid had no mechanical parts that could fail and even worked after being dropped into seawater and flushed with ethanol and dried. The solenoid was cheaper to manufacture and easy to source, whereas electric motors were relatively more expensive and less reliable.

3.3.2 *Final concept proven. Manufacturing feeders and controllers 'in-house'.*

The first AMD microdiet feeding system that was manufactured was intended to provide the Marine Finfish Larvae Research group at DoFWA with an in-house reliable feeding system for the 24 tank larvae rearing system (described by Kolkovski et al, 2004), in order to undertake larvae research at the cutting edge of science. Associate researcher in the larvae research field from Tasmania, Dr Stephen Battaglene observed this system and raised funds through the cooperative Research Centre CRC to commission the manufacture of a system for the Tasmanian Aquaculture and Fisheries Institute (TAFI), Hobart. Funds from this project paid for two technicians to construct a 24 tank feeding system and install it in situ at TAFI, for the Striped Trumpeter research program (Fig. 1-4).

Figures 1-2. DoFWA staff installing the system.

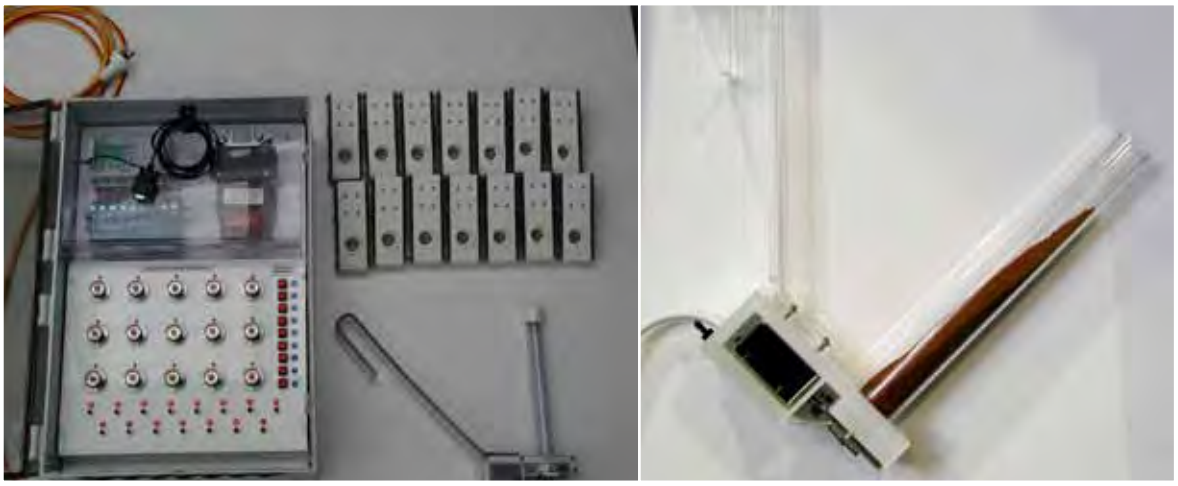
Figure 3. TAFI and DoFWA staff.

Figure 4. Feeding system control panel.

Thereafter, Sagiv Kolkovski saw the potential for such a system in other facilities that he was associated with, and offered to manufacture the feeding system on a cost recovery basis. However, at this stage the demand from Grand Canaria, Spain, Singapore, Norway, the USA and New Caledonia, proved to be too time consuming for the DoFWA technicians to undertake. Therefore the manufacture of the feeder body was outsourced, in order to allocate resources to core research activities.

3.3.3 Out sourcing feeder manufacture.

Two local companies, a local plastics fabrication company and an engineering company, were contracted to manufacture feeder bodies and the mechanism plates, while the feeder assembly and controller manufacture was still conducted 'in-house' (Fig 5-6).



Figures 5-6. Feeding system control panel and feeders.

These contracts were taken in order to allow the Fisheries WA technicians more time to conduct core research, rather than manufacture feeders. The feeders manufactured by BCJ Plastics were of a reasonable standard; having been cut on a CNC cutting machine, however the staff didn't appreciate the need to make each feeder identical, and therefore the workmanship was not of a high enough standard. Quality control processes exposed problems associated with cutting precision, assembly accuracy and consistency of materials. The materials such as 12mm acrylic sheet was also not uniform across a whole sheet, having variances of up to 1mm in the thickness and therefore didn't allow small enough tolerances for the feeders to operate within precision constraints of 0.1 mm. In order to address this problem, sheets of acrylic were laminated together and then machined down to within the allowable tolerances. This increased the price of construction and ultimately made this method of construction invalid.

The manufacture of the two plates was outsourced, to Access Engineering in order to achieve a standard design, which was replicated accurately. The plates were trialled in 3 different materials, PVC, Polyethylene and 316 Stainless Steel. The Stainless Steel plates proved to be most accurately manufactured and more reliable than the other materials. The heat generated by the cutting machine caused slight malformation in both of the plastics, while the Stainless Steel plates were unaffected (Fig 7.).

Figure 7. Side view, solenoid and plates.

Further experiments were done using different width slots in order to vary the shot size. The plates were made with 3mm, 4mm and 5mm wide slots, so that an operator could select the amount that was needed for each feeding event. In the final design, the number of slots was varied to give different output amounts for each feeding event, because the smaller slots proved to be unreliable.

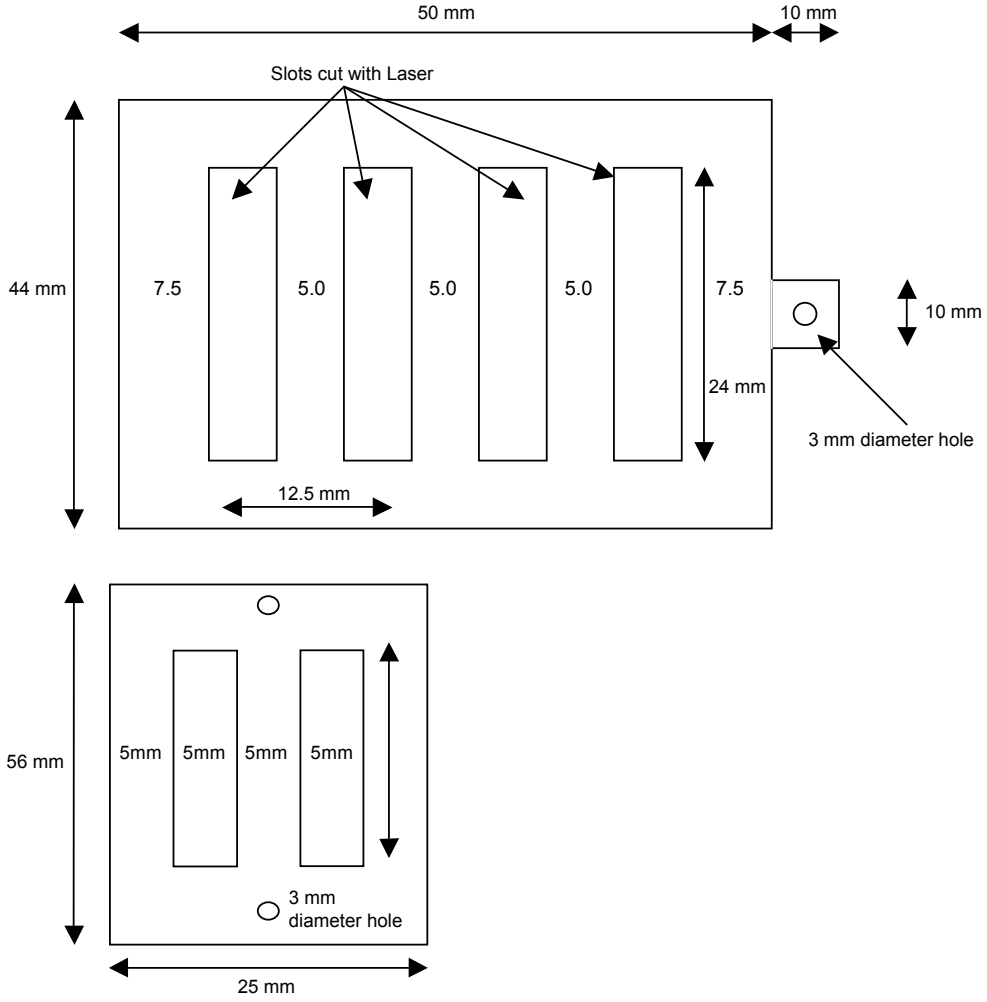


Figure 8. Plate Design diagram for the original AMD.

3.4 Original System Description

3.4.1 Automated Microdiet Dispenser operated by Programmable Logic Controller

24 Automated Microdiet Dispensers (AMD) were supplied to feed up to 8 different experimental or commercial micro diets (MDs) for larval weaning and/or nutrition experiments. Each treatment would have 3 or more replicate tanks, which are fed simultaneously via a single output from the Programmable Logic Controller (PLC). Specific PLC output programs are directed to operate particular AMD's within each feeding regime, via a network of 8 input – single output rotary switches. Each rotary switch is specific to an AMD that enables the program output from the PLC to be selected. This then connects a 12 VDC power supply via a solenoid switch that is operated by the PLC. The PLC, power supplies and switches are all situated within a single control station, which is hardwired using 2-core insulated flexible cable to the AMD's (Fig. 5).

3.4.2 Commercial Production.

Following extensive testing and the sale of 6 systems the decision to go the next step towards commercialization was taken. A number of manufacture options were investigated for instance machining individual feeders: rota moulding and injection moulding. Injection moulding the feeder bodies was considered the only alternative that allowed exact replication and dimensional tolerances to within 0.1 mm, which was an acceptable level of precision for replicate feeders and at a reasonable price.

After receiving feedback from the agencies that had already bought feeding systems we decided to modify the feeder design before committing to an expensive mould. The clients were pleased with the way the feeders worked and their reliability, however most people needed a larger hopper. Therefore a new feeder design was conceptualized with a larger hopper and tested. The concept was quite different from the first feeder design as the plates were reorientated from being on a 45° angle to level, and the hopper was aligned with the top of the feeder sloping at 45° with the opening facing back towards the operator, instead of protruding out from the front of the hopper. With these changes, a prototype feeder was made "in-house" and tested extensively over tanks, and with all types of microdiet. At the time, Gemma Micro 150 by Skretting was considered the hardest diet to automatically feed because it was fine and sticky. Therefore, most of the testing was done with this diet being used as a benchmark. The feeder actually proved to work better than the original design, more reliably and with smaller dose sizes.

A local injection moulding company, "Mouldtek" was contracted to produce a prototype feeder within the guidelines outlined by DoFWA staff. The prototype was machined from a block of acetyl plastic with precision. Further testing was conducted using the new prototype, with mixed success. Some modifications needed to be done such as increasing the hopper height, increasing the gap between the hopper and the plate and shielding the solenoid to stop microdiet being drawn into the piston chamber. After DoFWA technicians were satisfied that the prototype was acceptable an injection mould for the plastic components of the feeder was engineered. These components included the feeder body, the hanging bracket and hopper attachment bracket, an over centre clip to hold the hopper in place, 4 different moving plates and one base plate, an air manifold built into the base of the feeder body and a shield for the front of the solenoid. Brass threaded inserts were included in the moulding process to allow the

body to be screwed together using 4 X 50mm M3 304 SS screws, the base plate to be screwed in place using 2 X 8mm M3 304 SS screws and a 40mm M5 304 SS screw with a moulded plastic top to be screwed through the hanging bracket in order to attach the feeder to a range of materials while positioned over aquaculture tanks.

The moulds took 3 months to complete and then the first batch of 6 feeders were tested.

The following is a letter to the moulder outlining the issues relating to quality control of the initial moulded product.

27/07/07

Mouldtech Plastics

Re: Feeder Test Results

Dear Phil,

Generally the feeder performs to the required standard, similar to the prototype, which is to be expected. However, there were a couple of problems encountered during assemblage and they are as follows:

1. The fixed plate position is too far to the front of the feeder, around 0.5mm. This is not the position of the threaded inserts; it is caused by the body of the feeder. The two angled side covers that the fixed plate buts up against are slightly too long. I was able to shave about 0.5mm off the ends of these sections and then the fixed plate located perfectly.

2. When screwing the body of the feeder together the piston cover tends to be pushed too far up along the slides on tightening, which results in the piston being jammed. I scraped off the daggy bits on the bottom end of the cover and this seemed to fix the problem on 3 of the 4 feeders. I had to leave two of the long screws slightly loose on the 4th feeder to prevent the piston from jamming.

3. The air manifold leaks quite a bit around the cover inside the feeder. I'm not sure if this happens when the feeder is assembled because I have only tested the manifold under water when it is separate.

4. The plates that have 3 slots are a little out of shape; do you think that a more rigid plastic would prevent this from happening? Again I'm not sure how important this is to the performance of the feeder.

5. The plates don't seem to sit on the fixed plate in parallel, because the front end of the moving plate is lifted up. I suspect that this is caused by the part of the plate that stops it in position, as it would naturally push the plate that way by pushing back at the top of the plate cover above the piston. But it looks like the plate travels in parallel with the fixed plate and only kicks up at the end of the movement, which may also be of some benefit to the way the food is agitated and falls.

6. There are a few points on the moulded product that have been gouged or malformed in the process:
   - On the hanger bracket
   - On the single slotted thick plate stopper
   - The bolt holes are not lined up square

- The leading edge of the triangular section in front of the piston

7. The sparkarated finish isn't uniform all over the body of the feeder.
8. Is it possible to join the 3 main body parts with flush joints?

Apart from these minor things the feeder looks great, and seems to work well so far on the bench. We will test it further as we go and look forward to testing the feeder with the moulded hopper.

Regards

John Curnow

Senior Technical Officer

Department of Fisheries WA

The mould was modified to correct the faults that had been identified and the next batch of feeders worked very well, within 2% error per shot for individual feeders and to within 5% error between feeders, for any given commercial diet and also experimental diets that were manufactured by DoFWA staff.

3.4.3 The Automatic Microdiet Dispenser (AMD)

The AMD body is moulded from ABS and it is extremely strong and splash proof. The AMD mechanism operates by using a low voltage piston solenoid to pull a slotted plate across the bottom opening of a hopper containing the diet. The moving plate rests on another smaller, stationary slotted plate. When not in operation, the slots on each plate overlap with the bars on the other plate, thus preventing the microdiet from falling through (Fig. 9, 10)

Figures 9-10. Feeder side and bottom views.

When the AMD operates, the moving plate is pulled horizontally between the hopper and stationary plate, followed by a return movement facilitated by the stainless steel spring. The feeder releases a small quantity of microdiet through the openings at the moment the slots line up, when passing in both directions. The plate can be pulled up to 19 times in any one feeding event, as often as every minute all day.

The AMD can cope with MD particle sizes from 100 μm to 2mm. As little 100 mg ± 2 %, depending on the microdiet, is released without the need to weigh the diet each time. An air manifold constantly jets beneath the plates in order to scatter the MD across the water surface within a target area. The AMD's are controlled by a programmable logic controller (PLC) and can be programmed to any desired feeding schedule.

3.4.4 Solenoid Supply

Following the finalization of the mould to specific requirements, the wholesale supplier for the original solenoid stopped importing that specific solenoid. As part of the manufacturing process DoFWA staff ordered 400 solenoids for future orders, however when the solenoids were delivered they didn't fit the recently finalized moulded feeder. Inquiries were made into the change and after much negotiation DoFWA was given the name of the company that manufactured the solenoids, UFO Top Tech Co., Taiwan. They explained that they had deleted that particular solenoid and that they could no longer supply it.

The main issue for the feeders was that the replacement solenoid was only half as strong, so that it didn't operate the feeders adequately and it had slightly different external dimensions. Therefore, DoFWA staff made experimental modifications to the new solenoid in order to increase its pulling force. After 3 prototype modifications, we managed to increase the pulling force higher than the original solenoid. UFO Top Tech Co. was approached in order to get the modification made in their factory before being exported to Australia. The company produce and supplied 500 solenoids using DoFWA new specifications, as detailed below.

Furthermore, the mould for the feeder body was modified slightly to accommodate the new dimensions of the solenoid. The final drawings of the modified solenoid design are shown in Fig. 13.

3.4.5 Solenoid Modification

The Department of Fisheries Western Australia has compared and tested two solenoids acquired from Electus Distribution, Australia.

The first of these two solenoids is a superseded model of solenoid that we were using as part of an automatic feeding devise. The design was completed and DoFWA started to market the feeder worldwide. However, the last batch of solenoids received from Electus was a different model from the original solenoid that were used, which made the feeder inoperable.

This new solenoid is different in design, such that the outside dimensions are larger and the piston protrudes 3.5 mm further out of the body.

Also, the pulling power of the new solenoid was tested and found to be half that of the old model. However, modifications were made to the new model that have improved its' pulling power to better the old model.

3.4.6 Test Description

The solenoid test comprised of a horizontal surface to which the solenoid body was attached and a tray resting on the same surface in front of the solenoid (Fig. 11).

The tray was attached to the solenoid piston using a solid draw bar.

The surface was made from Hard PVC plate and the tray was made from polypropylene.

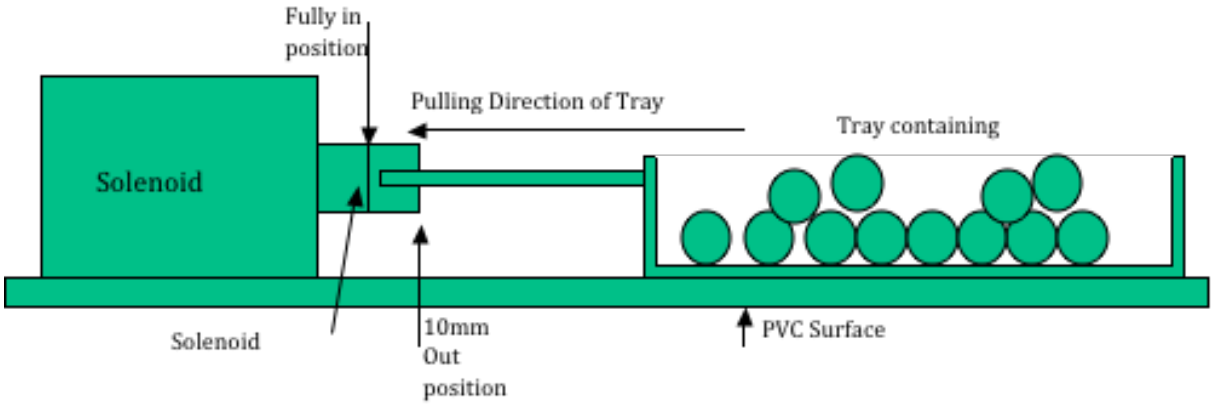


Figure 11. Solenoid testing.

In order to test the pulling strength of the solenoid we loaded the tray with weights and then energized the solenoid in order to pull the tray towards the solenoid body using the in-pulling action of the solenoid piston.

The same tray and PVC surface was used for each test to exactly replicate the friction co-efficient between the tray and the surface. A 10 Amp 12 VDC regulated power supply was used for all tests.

Table 1. The results of the solenoid modification tests.

Model	Pulling distance	Maximum weight pulled	Modification
Previous model	10mm	806g	None
New Model	10mm	380g	None
New model 1	10mm	350g	Piston shortened 3.5 mm
New Model 2	10mm	500g	Stopper insert shortened 3.5 mm
New Model 3	10mm	850g	Modified taper on piston and stopper to allow the piston to rest inside the body with only 8.5mm of the piston protruding

The new model was able to pull a little less than half of what the old model could pull. Modification 3 resulted in the new model being able to pull slightly more than the old model a distance of 10 mm.

The modification that resulted in this huge increase in pulling power is detailed below.

Modification 3.

The taper on the leading end of the solenoid piston was lengthened. The stopper insert internal taper was also lengthened to match. This allowed the piston to rest 3.5 mm further inserted into the solenoid body, when pulled fully in, and decreased the distance between the piston and the stopper when 10 mm extended from being fully in. This simple modification doubles the effective pulling power from 10mm.

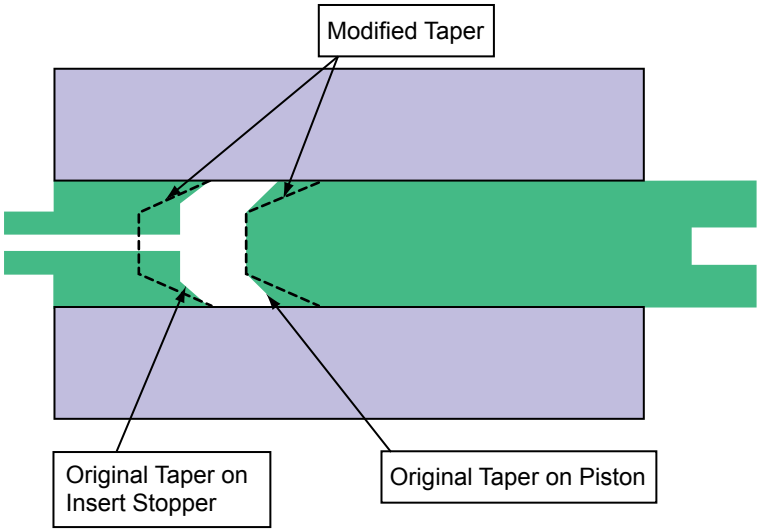


Figure 12. Solenoid modifications.

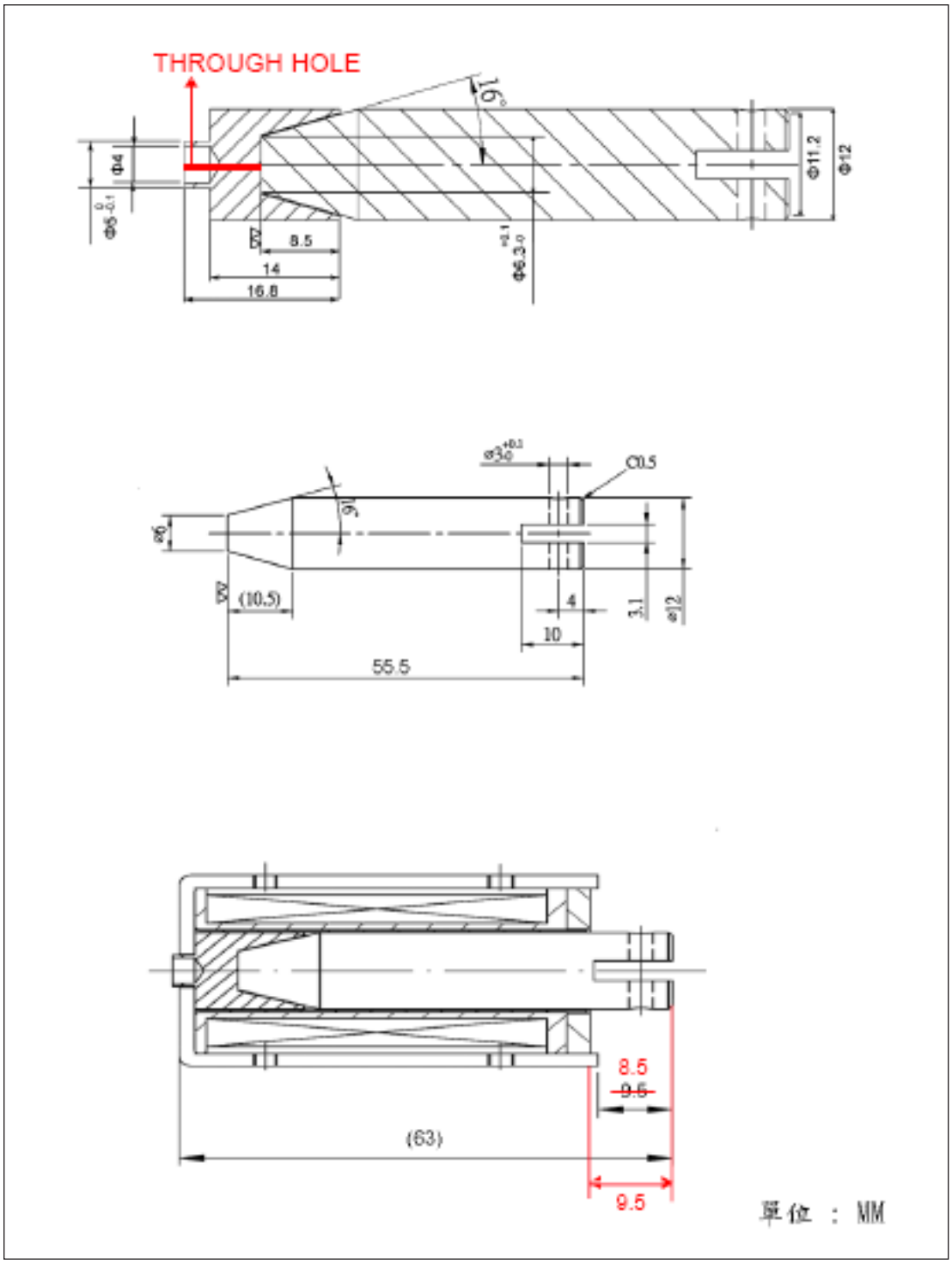


Figure 13. Final solenoid design

3.4.7 Controller Manufacture

A local manufacturer, Hinco Instruments, Perth WA, using touch pad interface and PLC control has refined the controller design for themselves to produce (Fig. 14).

The design brief was outlined as below.

Figure 14. Touch pad control panel.

3.4.8 Automatic Microdiet Dispenser (AMD) – Controller Requirements

The AMD is a devise that dispenses small amounts of fish microdiet, (particle size range 100 μm to 2000 μm) periodically throughout the day.

The requirements of the controller would be to operate the feeders individually, or as groups of not more than 4 at a time, by using a 12 VDC pulse to energize the feeder solenoid (12 VDC, 4 A, 50 Watts each).

When the solenoid is energized the piston draws a moving plate across a stationary plate, which momentarily aligns two slots and allows MD to drop through from a hopper situated above the moving plate. The moving plate is returned to the resting position by a stainless steel spring, ready for the next event. When not in operation, the slots on each plate overlap with the bars on the other plate, thus preventing the MD from falling through.

Each time the feeder performs this action it is called a feeding event.

3.4.9 Software Requirements

Feeding events:

- Single operation 0.3 seconds on and then off until the next event
- Double operation i.e. 0.3 seconds on, 0.2 seconds off, 0.3 sec on, and then off until the next event

These feeding events are usually timed to occur during the day, either evenly spread across the day or in periods of varying rates of feeding.

Possible feeding event patterns:

- Feeding events evenly spread across the feeding period i.e. from 9:10 to 22:30 at intervals of 650 sec., or
- Split into multiple periods, i.e.
  - 9:10 to 10:30 at intervals of 300 sec,
  - 10:30 to 16:30 at intervals of 1200 sec,
  - 16:30 to 18:00 at intervals of 600 sec,
  - 18:00 to 22:30 at intervals of 200 sec.

These patterns need to be easily programmable by the user, with the use of a touch pad and with the option of PC download.

***NB**. The software should be written so that no more than 1 feeder is operated at one time. This is to allow the use of a small power supply and to prevent it being overloaded at inrush current, when the solenoids are being energised. Progressively longer on-delay functions for each feeder program (1 sec, 2 sec, 3 sec etc.) can be used to stop all the feeders operating at one time (especially at the beginning of the feeding period), and still allow the correct number of feeding events for the day.

3.4.10 Hardware Requirements

- Attractive wall mounted polycarbonate enclosure with appropriate properties rating for a moist environment, with a hinged clear front panel (small as possible).
- Multi voltage operation (100-250 VAC) for controller.
- Mains voltage operation PLC (100-250 VAC).
- 24 output (expandable) PLC to allow individual operation of 24 feeders (up to 48 if required).
- At least 6-inch touch screen with clear and simple operation for the end user.
- Greater or equal to 8 Amp 12 VDC Switchable Power Supply (100-250VAC).
- Circuit Breaker and RCD protected electrical circuits.
- Twenty-four slave relay operated 12VDC outputs.
- Quality (gold plated) RCA connectors located on the underside of the enclosure.
- RCA outputs numbered for feeder program identification.

3.4.11 Requirements for controller manufacture

The whole unit should be neatly assembled in an aesthetically pleasing manner. The controllers will be sold onto third parties who will be purchasing a whole package of feeders with a controller. It is intended to become a commercial product and the controller design should reflect an international marketplace. It includes a 6” touch screen; minimize box size with a clear front panel and RCA connectors underneath the box, so that the cables can hang.

Software Options for Touch Screen

- Feeders need to be easily fully programmable through the touch screen.
- The format should be self explanatory, with a cover screen having basic operations and a menu to lead to other programming screens.
- The cover screen should have a large Logo, or perhaps all the screens could have a logo as a background

Cover Screen

- The cover screen appears when the power is turned on.
- This screen should be simple, with logo, and a Menu button (could have a start or stop button depending on the status of the feeders).
- If the touch screen is left for 5 minutes the cover screen auto appears.

Menu

1. Start Feeding
2. Stop Feeding
3. Feeder Test
4. Set current time and date
5. Feeder operation
6. Feeder calibration

Feeder test screen

The user can manually test each feeder from this screen by keying the appropriate number and then a test button. This could set the feeder off for what ever is programmed on the relative Feeder Program Screen. e.g. a single or multiple shot for Feeder 14.

Set current time and date

Set time and date, like the MS Windows format.

Feeder Operation

This screen needs to be able to turn ON or OFF any of the feeders individually and select to program the feeder.

Eg. This screen could be a table of numbers with all the feeders listed as numbers (or a 0 to 9 keypad), to select the feeder "enter". Once the feeder is selected it leads to another screen where the options are: ON, OFF and PROGRAM with the feeder number at the top.

Feeder "XX" Program

User inputs for each feeder program are:

1. Calibration result
2. Daily feed ration

3. Starting time
    a. First period duration
    b. First period % of ration
    c. Second period duration
    d. Second period % of ration
    e. Third period duration
    f. Third period % of ration
    g. Forth period duration
    h. Forth period % of ration
4. Gain % per day (increased ration)
5. Expected mortality rate per day (% reduction of ration)

Feeder Calibration Screen

A touch screen window should be set up so that the user can push "go" and initiates a set calibration operation sequence for a chosen feeder. This would be over a period of eg. 5 minutes, activating the feeder every 10 seconds, so that the feeder activates 30 times. The touch screen will indicate when the calibration sequence has finished. The user will collect the feed dispensed and input the weight in grams into the feeding program screen that is controlling that particular feeder. The PLC would then calculate how often to operate the feeder in order to feed the required daily amount from this standard calibration.

Example operation

1. Calibration amount is 5.79 g, when the calibration period is 40 shots over 10 minutes, average shot is 0.14475g,
2. Daily amount is 50 g,
3. Number of shots per 50g is 346 shots,
4. Day length is 14 hrs,
5. Frequency of feed is then every 145 seconds for the 14 h period,
6. For known number of fish 250,000,
7. Gain is 5% per day, so the following day is automatically increased to 52.5g, then 55.125g and so on. The frequency will automatically adjust to feed the increased amount,
8. Expected mortality rate 1%, so the feeding rate will decrease by 1 %, after the feeding gain the feed will actually increase by 4% each day,
9. Start feeding time 08:00.

These figures are entered into boxes on the screen and the PLC feeds the required amount over the day.

The day could be split into 4 periods whereby the feeding can be weighted. These periods are assigned a length of time in hours.

Period 1 is 2 hrs long and feed allocation is 30%.

- Day 1: 15g over 7200sec = Feed every 69 sec for first 2 hrs.
- Day 2: 15.75g over 7200sec = Feed every 66 sec for first 2 hrs.
- Etc.

Period 2 is 6 hrs long and feed allocation is 10%.

Period 3 is 4 hrs long and feed allocation is 20%.

Period 4 is 2 hrs long and feed allocation is 40%.

Touch screen recipes need to prevent the user from selecting more than one recipe for a given channel.

Remote operation

There are a couple of options for remote operation.

Option 1.

The control box is set up as a remote operational transmitter, and we supply receiver modules with 2 to 4 outputs. These receiver modules would have their own power supply and can be connected to mains power and have slave relays to operate the feeders for the required duration.

Option 2.

The control box is manufactured to the same specifications however, the RCA outlets are removed and a data cable is used to talk to modules that operate 4 to 6 individual feeders. This could be sold as an alternative to purchasing the main controller equipped with RCA outputs.

3.5 Conclusion of PLC software design

The result was a simplified version of this description that covered the basic needs for controlling 24 feeders, as individuals or in groups up to 24 (as outlined in Appendix 1). The entire ensemble of requirements were possible, however too costly for commercial application.

3.6 Commercialized controller description

The AMD system presents a wide range of versatile feeding regimes for larvae and juvenile fish. The operator can easily customise for specific feeding requirements on a daily basis through a user-friendly touchpad interface. Alternatively, a series of feeding regimes can be pre-programmed to cater for changing larvae requirements, which can be used repeatedly for successive larval production runs.

The controller operates 24 AMD units, either individually or as a group of identical feeders. Each AMD unit periodically administers an operator-determined number of MD doses during one to four feeding periods. This allows the culturist to provide MD evenly across the whole day, or provide periods of relatively high and low intensity feeding that cater for diurnal larvae requirements. A more constant availability of MD is provided when the larvae need it.

A full description of the commercialized controller and how to operate it is in Appendix 1, AMD Instruction Manual.

3.7 Patent application

As an initial step to register the feeding system as a patent, a provisional patent application was submitted on 31 May 2005 by WRAY & Associates (patent and trade marks attorneys) on behalf of the Department of Fisheries, WA and FRDC as the applicants. The inventors on the patent application were registered as: Dr Sagiv Kolkovski, Mr John Curnow and Mr Justin L King.

Following the successful international patent search (Appendix 2) and approval of the provisional patent application (after amendment of the claims), an international patent application was submitted on 31 May 2006 under the Patent Cooperation Treaty (International application No. PCT/AU2006/000735). The patent was published on 7 December 2006 (Publication No. WO/2006/128234, World Intellectual Property Organisation, Appendix 3).

The patent was registered in most European countries, Asia, South and Central Americas and USA.

The patent is currently maintained by the DoFWA.

While written this final report, the new FRDC project application aimed at further development of the feeding system as well as, establishing framework for the patent registration and sales of the system, was not approved. Therefore, due to lack of funding from FRDC or any other source, the patent registration was stopped.

3.8 Marketing developing and assessments.

Two marketing assessments were carried out. Internal market developing and assessment plan by Dr Kolkovski and his team and external market opportunity testing by Synovate, a business consulting company (Appendix 4).

Both assessments achieved the same conclusions that the feeding system is likely to be viable commercially.

The marketing and following systems sales followed the developing plan by Dr Kolkovski and his team. Links with an agent that distribute the system in Europe was established (Mr Didier Leclercq, ACUI-T, France). These links enable direct sales to hatcheries in Europe including technical support.

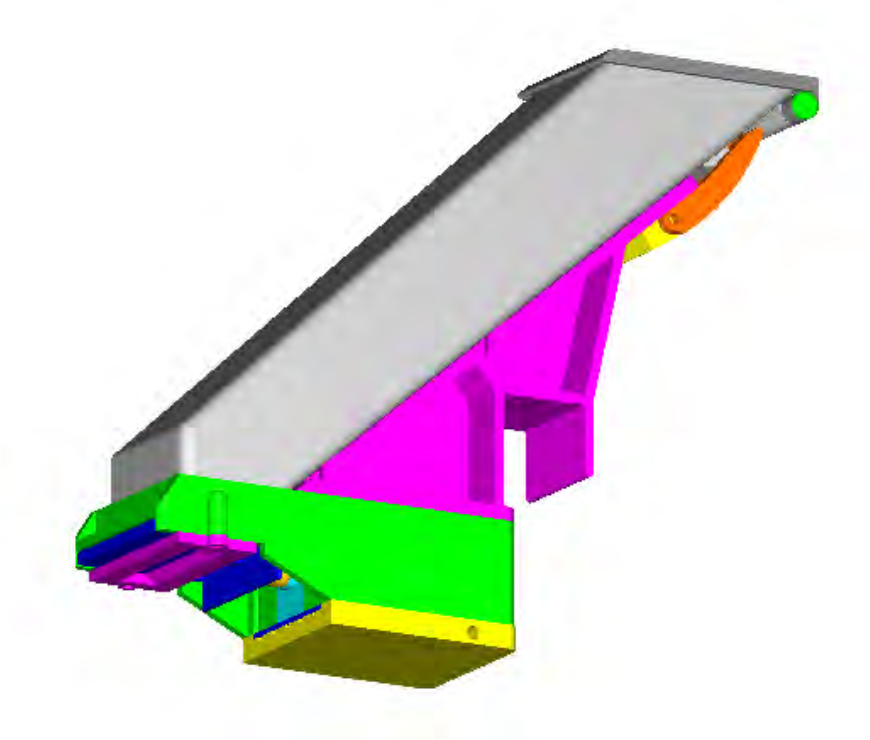
Advertising and articles in international professional journals (Hatchery International and Fish Farming International, Appendix 5) were carried out, resulting in direct sales.

During 2006-08, the system was also presented in several symposiums including World Aquaculture 2006 (poster), 2007 (display booth, Fig. 15), 2008 (poster), Australasian Aquaculture (2008, display system).

Figure 14. Display booth at World Aquaculture Symposium, Istanbul, Turkey 2007.

Automated Microdiet Dispenser System

Development and Marketing Plan



Prepared By

Dr Sagiv Kolkovski, Mr John Curnow and Mr Justin L. King

September 2007

Department of Fisheries, Western Australia

Marine Finfish Aquaculture and Aquaculture Systems

1. Background

The Mariculture Research & Advisory Group is focused on the development of new and innovative culture systems for marine hatcheries. As part of this focus we designed the AMD – Automated Microdiet Dispenser as a reliable and efficient method to dispense fine particulate feeds (150 µm – 1500 µm) to larvae culture tanks. A computerized controller was developed in parallel to operate any number of AMD units, and complete the AMD system.

Currently, there is no equivalent system available "off-the-shelf" anywhere in the world. The development of the AMD system was a component of the FRDC funded project 2001/220 and is further being refined under this FRDC project 2004/258.

The Department of Fisheries Western Australia and the FRDC jointly registered the AMD system for patent (patent pending) in 2005, with Mr John Curnow, Mr Justin King and Dr Sagiv Kolkovski listed as the inventors.

2. Development steps

1. Conceptual development. A number of feeder designs were conceptualized and tested.
2. Final concept proven. Manufacturing feeders and controllers 'in-house'.
3. Out sourcing feeder manufacturing. A local plastics fabrication company was contracted to manufacture feeder bodies. Assembly was still 'in-house'. Quality control exposed problems associated with precision cutting, accurate assembly and consistency of materials.
4. Commercial production. A local mould manufacturer was contracted to produce an injection mould for the plastic components of the feeder based on a tested and finalized engineered prototype.
5. Controller manufacturing. A local manufacturer using touch pad interface and PLC (Programmable Logic Controller) control has refined the controller design and are now producing the controllers.

3. Industry profile

Scope

Aquaculture and especially marine aquaculture is sustaining considerable growth around the world. The European community produced more then 1.4 million tonnes of fish in 2006. Global marine aquaculture is growing at 10%-15% annually. New species are continually being investigated and business is constantly expanding to new locations. For example, Turkish aquaculture has grown by 300% in the past 5 years.

The target market for the AMD system is biased towards relatively more sophisticated and advanced aquaculture facilities. During the past decade, modern hatcheries in developed countries have become bigger and more industrial. The cost of production of juveniles has gone down substantially, with improved technology and increased production rates per unit effort. However in all cases, labour represents a large part (40% to 50%) of the total costs. Therefore, any reductions in this area can have a significant impact on hatchery profitability, and the AMD system is aimed at providing hatcheries huge savings in this sector.

It is envisaged that marine aquaculture will continue to grow strongly in order to supply the increasing demand for marine fish and to compensate for the decreasing yield from commercial fisheries. Automation and up scaling is currently the main developmental focus in European and other developed hatcheries around the world.

Market share

In general, feeding systems for larvae and juveniles in intensive hatcheries are considered as being within a niche market.

Potential Byers:

- Finfish hatcheries
- Prawn hatcheries
- Universities and R&D centres
- Ornamental fish distributors and public aquariums

Location

Potential markets are in developed countries including Europe, USA, Scandinavia, Japan and Taiwan. A combination of the migration towards high tech hatchery systems, intensive and super-intensive methods and high labour costs, makes the system very attractive and commercially viable in these countries.

Developing countries (South East Asia, China and India) are not considered as a significant potential market. Although, the aquaculture in many of these countries is booming, a combination of the relatively high system cost, low labour costs and the lack of technological development makes the system prohibitively expensive.

Based on estimates of industry contacts and scientists around the world, Table 1 shows estimates of sales in different regions.

These estimates are only for marine hatcheries. They do not include fresh water hatcheries (for example, there are 200 yellow perch (red fin) hatcheries in the northern US and many other species around the world). The numbers are based on personal estimates from reputable industry contacts and consultants. There is no official information on the number of hatcheries in the world.

Table 1. Global sales estimates

Regions	Europe (Existing hatcheries and potential buyers)	Middle East including Turkey	USA, Canada	Japan, Taiwan	Chile, S. C. America	Other	TOTAL
Marine finfish hatcheries (not including Salmonids)	120	20	15	100		10	275
Other hatcheries (ells, crustaceans)	15	2	5	50	100	10	182
R&D Centres and universities	65	5	20	15	10	5	120
Others (public aquariums etc.)	10		5	5		2	22
Total	210	37	45	170	110	27	599
Estimated sales (based on 20% of the market)	**44**	**7**	**9**	**34**	**11***	**5**	**110**

* Chile and South, Central American market penetration was calculated as 10%.

Competition

There are several automatic feeders being offered in the market place. The operation of these feeders is based on mechanical belt, pneumatic action, disk rotation and other mechanisms. All of these feeders are aimed at dispensing larger feed particles (>1mm). Currently, there is no feeder designed specifically for microdiets (< 1mm).

Therefore hatcheries are using a combination of hand feeding and other feeders. None of the current feeding techniques are accurate, they result in overfeeding and lead to large amounts of waste diet in the tank, which causes water fouling and diminished profit (microdiets cost up to AU$350 kg^{-1}).

The AMD system represents a unique advantage in microdiet feed control in terms of aliquot size, feeding frequencies, and dispersion of the feed. The only commercial feeder that is potentially a competitor is the Arvo Tec feeder. This feeder is aimed at the nursery stage of fish development, dispensing diets of particle size range from 0.3 mm to 6.0 mm, but is not accurate dispensing particle sizes <1 mm. When compared to the AMD system:

- Advantages- copes with larger diets.
- Disadvantages
    - Not accurate within target size range 100 µm to 1000 µm.
    - Significantly bulkier.
    - 3 times the cost compared to the AMD feeder (Table 2). Arvo Tec controller costs AU$5 700, and each feeder costs AU$715, total for 24 feeders AU$22 860 compared to $10 000 for AMD system.

Cost of production

The initial developmental stage of the system was funded by the FRDC. The commercialization of the system (i.e. development of a plastic injection mould, sub-contracting the manufacture of the prototype feeder and the controller and the initial patent registration) was funded by FRDC, DoFWA and revenue raised by system sales.

The system is comprised of a controller and a number of individual AMD units (up to 24 on the base model with optional expansion to 64 feeders in 8 units incriminates).

Table 2. Production costs, sale price and profit of the system.

Item	Manufacturing Costs, $	Sale price, AU$	Profit margins, %	Profit AU$
Control Box	2 900	4 000	37.9	1 100
Feeder	50	250	400	200
Total per full system (24 feeders)	4 100	10 000		5 900

* Manufacturing costs does not include DoFWA staff time. This includes, testing the systems before shipping, liaising with sub-contractors, packaging, marketing etc.

Service and warranties

The system will be supplied with a 12-month warranty on parts. It might be possible to have service through a sales representative.

Future investment

Regional and country registration of the patent - AU$30-40 000. One-off payment.

On-going patent registration (annually or every few years) - AU$3-5 000.

Marketing (on-going) – depending on the scale of marketing but is estimated around $10-15 000 / year.

Table 3 shows estimated costs associated with the registration and future sales of the system.

Table 3. Future costs.

Costs	Patent Registration	Marketing	Total	Systems sales to cover costs
First year (2007-8)	40 000	10 000	50 000	10
2008-9	5 000	20 000	25 000	5
Every year after	5 000	10 000	15 000	3

Marketing Strategies

The marketing strategies includes the following:

Personal contacts. To date, 6 systems have been sold (Tasmania, Spain, US, New Caledonia, Singapore and Norway) without any advertising. Another 5 orders have been confirmed and will be manufactured as soon as the new mould is finalized.

Advantages – free of costs, disadvantages – *limited circulation.*

Internet and Email. Creating a dedicated website to promote the system under the Department of Fisheries Website. The website will be linked to other aquaculture portals.

Creating email list of potential buyers collected through personal contacts, symposiums and trade shows.

Advantages – low costs (creating the website) or free (email). Large exposure to specific potential clients.

Professional symposiums and trade-shows. Probably the best 'value-for-money'. Presenting the system in a trade-show will expose it to hundreds or even thousands of people involved in the aquaculture industry. Can also create immediate sales since the potential buyers can see the full-scale system and get a 'first hand' impression.

Advantages – large exposure, Disadvantages – expensive

Advertising in professional journals. There are several professional aquaculture journals. The two that are likely to have the most impact are; Fish Farming International and Hatchery International. The first one is looking at general aquaculture and has, by far, the largest distribution in the world. Hatchery International is more specific to hatchery production of any marine organism and is aimed at the targeted potential buyers for the system.

Advantages – *large exposure to targeted markets*, Disadvantages – *expensive*.

Agent or representative. A representative in specific regions will be needed to help with market penetration. This is specifically important in Japan where most of the hatchery managers solely speak Japanese. It is proposed that a sales representative will work with

commission (around 10-15%) that will be added to the cost of the system.

Advantages – assist in penetrating difficult and closed markets, local support, Disadvantages – increased system costs.

Costs

Professional symposiums and trade-shows. It is difficult to assess cost of attending and displaying at trade-shows due to each trade-show charging different rates. Different locations mean different costs i.e. air fair, accommodations etc.

Estimated costs may include:

- Booth design (one off) - $1 000 (design will be carried out 'in-house' by the DoFWA)
- Booth materials, publications, posters etc. - $3 000
- Shipment - $1500
- Airfare (@ $2 200 x 3 persons) - $6 600
- Accommodations, meals, etc. (@ $200/day x 7 days x 3 persons) - $4 200
- Booth hire - $4 300

Total - $20 600

This amount can be reduced significantly after the first trade-show, as all the advertising materials will be re-used.

Future Symposiums

- Aquaculture Europe 2007 symposium, Turkey (October 2007)
- World Aquaculture Symposium, Busan Korea (May 2008)
- Australasian Aquaculture (July 2008)

Advertising in professional journals.

Initially, a relatively large advertisement will be purchased prior to the trade-show / symposium in Turkey (October 2007). The two magazines will be distributed free-of-charge at the trade-show; this will create a good opportunity to effectively advertise the system.

Cost: Fish Farming, Quarter page B&W - $1 400

Hatchery International, Full page, colour - $1 864

On-going advertising

Cost: Hatchery International, ½ page, colour, 6 issues per year - $1 180 each issue.

Fish Farming International, ¼ page colour, $2 400, or mono, $1 300, 12 issues.

4.0 Microdiets and Larvae Rearing

4.1 The effect of different micro algae as 'green water' on barramundi larvae growth and survival.

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Aquaculture and Fish Health, Department of Fisheries, WA.

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4.1.1 Introduction

The addition of micro algae is reported to have beneficial effects on cultured marine finfish larvae, with improved growth and survival of fish supplied with unicellular algae (Cahu *et al.*, 1998). This practice is known as the 'green water method' and is used for most species from tropical to cold-water (Moreti *et al.*, 2005). Different algae strains and species are added at different concentrations to create various levels of transparency through the water column.

There are various theories as to why and by what mechanism algae are beneficial to marine finfish larvae. Cahu *et al*., 1998 found that the presence of algae promotes earlier development of brush border membranes by triggering digestive enzyme production, at both the pancreatic and intestinal levels. Larval behaviour can also be influenced by algae. Naas et al, 1992 found that clear water caused halibut larvae to concentrate at the surface and near the tank walls, whereas in green water their time in the water column searching for prey increased. Furthermore, live micro-algae have been found to stabilize water quality, contribute to nutrition and reduce the bacterial levels in larval culture tanks (Naas *et al.*, 1992; Salvesen *et al.*, 1999; Reinertsenet al, 1993).

Algal production for larval rearing adds high costs in the requirement for space and time for culture. Moreover, there is always a risk for contamination and the algal culture crashing. Algal pastes overcome or reduce the problems and limitations associated with live algal cultures. In recent years several algae pastes have become commercially available for larvae culture with varying degrees of success. The current experiment compares a local red algae paste *Dununiella salina* with two commercially available pastes and one live alga used in the 'green water method'. This red alga paste is cultured at high salinity, which is relatively bacteria free and promotes naturally high lipid and ß-carotene levels in the algae, characteristics that are potentially beneficial for marine larvae.

In the current experiment growth and survival of barramundi larvae were assessed using four types of algae as the "green water".

4.1.2 Materials and Methods

Experimental design

The effect of four alga's used for 'green water' on growth and survival of barramundi larvae to 26 days post hatch (dph) was assessed. Larvae were reared according to a standard rearing protocol (Curnow *et al.* 2006, Table 1). Four algae treatments, each with 4 replicates, were compared for barramundi growth and survival, at 28°C and a salinity of 32 ppt.

Diluted microalgae pastes were periodically dosed from three 270 l holding tanks into 3 replicate experimental larvae tanks each. Algae were evenly distributed by pumping through a

manifolds of equal length using a small centrifugal pump, at a flow rate of 750 mls min^{-1}. Live algae, *Nannochloropsis oculata* (NL) was pumped at a rate of 0.75 l min^{-1} from a 1000 l tank, from 08:00 to 09:00 and then 15 minutes off and 12 minutes on, until 19:00. The algae pastes were pumped from 08:20 until 09:00 and then 12 minutes off and 1 minute on, until 19:00. Algae paste dilution rates for *Chlorella* paste, *Chlorella* company, Japan (CP), *Nannochlorpsis* paste (NP) from Reed Aquaculture, USA, and *Dununiella salina* paste (DP) from Australia are shown in table 2. The dosing delivery was designed to achieve secchi disc depth in all tanks shown in table 1 and 2.

Larval rearing

Barramundi larvae at 1 dph were sourced from Darwin Aquaculture Centre and evenly distributed into 16 x 270 l conical tanks (Kolkovski *et al.* 2004), at a density of 7810 larvae / tank (34 larvae / l). The tanks had an up-welling flow through water delivery system (Kolkovski *et al.* 2004). The tanks were supplied with fresh temperature controlled marine bore water at the rates of 1.5 l min^{-1} per tank. The water temperature averaged 28°C ± 0.3°C. The physio-chemical parameters were maintained at pH 7.85 ± 0.02, DO 6.5 ± 0.5ppm (mean ± SD), salinity 32.0 ppt and a photoperiod of 10 h light / 14 h dark at light intensity of 500 Lux at the tank surface.

Growth and survival were monitored during the experiment and at its conclusion. Initially at 2 dph, 25 larvae in total were sampled and thereafter periodically 10 larvae every 3 days from each tank were randomly removed and sacrificed using iced water. These larvae were measured for standard length (SL) and wet weight (WW). At 23 and 26 dph, 25 larvae were sampled and measured from each tank. The developmental stage of fish was defined following Wellford and Lam (1993).

Rotifers were enriched using Roti Selco (Inve) as per manufacturers instructions and fed to the larvae from 1 to 16 dph, *Artemia* were enriched using DHA Selco (Inve) as per manufacturers instructions for 24 h and fed from 13 to 20 dph at a low rate, and Microdiet (MD) (Gemma Micro, Skretting) was fed from 9 to 26 dph (Table 1).

Microdiet was fed to the larvae using automatic feeders and the feeding intervals were adjusted to the feeding behaviour of the larvae during the day (10 min of intense feeding in the morning, followed by feeding at 20 minute intervals). The tanks were monitored throughout the day using a secchi disk to ensure consistent algae concentrations in all tanks (Table 1 and 2).

Daily growth coefficient

Daily Growth Coefficient (DGC) (Kaushik, 1998) was calculated using the following equation:

$$DGC = \frac{[(Final\ weight)^{0.3} - (Initial\ weight)^{0.3})]}{Number\ of\ Days}$$

Survival

Survival percentage was calculated as the fraction of the initial number of that contributed to the final biomass [total average ww 90g of larvae per tank], corrected for larvae that were sampled during the trial.

Bacterial assay

Duplicate culture media samples from each tank were collected before and after algal addition in the morning and used to inoculate agar plates for further total bacteria counting, and identification. Sampling equipment was pre-sterilized using ethanol spray. Culture media samples were serial diluted (Quinn *et al.*, 1994) and a 100µl sample was spread onto MSA-B and TCBS plates (Marine Salt Agar-Blood, composed of Trypticase Soya agar, 3% horse blood and 2% NaCl). Dilutions for *Artemia* were 10^{-5} dilution, and culture media samples were diluted to 10^{-4}, in order to get a suitable number of CFU (Colony Forming Units) plate^{-1} for counting.

All agar plating was carried out in a laminar flow cabinet in sterile conditions. All equipment was sterilized using flame, ethanol spray or autoclave. Dilution media was tested for microbial activity to guarantee sterility of media and methods. The plates were left at room temperature (≈25°C) with a temperature data logger for 24 h before counting colonies formed per plate (Quinn *et al.*, 1994).

Stress test

The final day of the trial 26 dph, stress tests were conducted by pouring 200 ml of water containing 10-15 larvae from each tank using a plastic beaker through a mesh (400 µm) screen in order to capture the larvae on the screen. The screen was then blotted dry from below with paper towel. The larvae were left on the dried screen for 9 min and then returned to 8 l of aerated water in a plastic bucket. The proportion of survivors after 30 min was recorded. The value for each protocol was calculated by averaging the outcomes from 4 replicates.

Table 1. Rearing protocol, temporal input table

Date	Day	DPH	Algae density Secchi depth (cm)	Rotifers per ml	Artemia per ml	MD (g/1000larvae)	MD (g/1000larvae)	MD/tank g/tank	GM150 MD g/tank	GM300 MD g/tank	G0.3 MD g/tank	GM150 total	GM300 total	G0.3 total	total rotifers million	total Artemia million
29-Apr-05	Fri	Day 1	~50	15											259.2	
30-Apr-05	Sat	Day 2	~50	15											259.2	
1-May-05	Sun	Day 3	~50	20											345.6	
2-May-05	Mon	Day 4	~50	20											345.6	
3-May-05	Tue	Day 5	~50	20											345.6	
4-May-05	Wed	Day 6	~50	20											345.6	
5-May-05	Thu	Day 7	~50	20											345.6	
6-May-05	Fri	Day 8	~50	25											432	
7-May-05	Sat	Day 9	~50	25		0.70	4.4	4.4				70.16			432	
8-May-05	Sun	Day 10	~50	25		0.77	4.7	4.7				75.24			432	
9-May-05	Mon	Day 11	~50	25		0.85	5.0	5.0				80.70			432	
10-May-05	Tue	Day 12	~50	15		0.93	5.4	5.4				86.55			259.2	
11-May-05	Wed	Day 13	~50	15	1	1.02	5.8	4.4	1.5			92.82	92.82		259.2	4.32
12-May-05	Thu	Day 14	~50	15	2	1.13	6.2	3.1	3.1			99.55	99.55		259.2	8.64
13-May-05	Fri	Day 15	~50	10	3	1.24	6.7	1.7	5.0			106.77	106.77		172.8	12.96
14-May-05	Sat	Day 16	~50	10	4	1.36	7.2		7.2				114.51		172.8	17.28
15-May-05	Sun	Day 17	~85		4	1.50	7.7		7.7				122.81			17.28
16-May-05	Mon	Day 18	~85		3	1.65	8.2		8.2				131.72			12.96
17-May-05	Tue	Day 19	~85		2	1.82	8.8		8.8				141.27			8.64
18-May-05	Wed	Day 20	~85		1	2.00	9.5		9.5				151.51			4.32
19-May-05	Thu	Day 21				2.20	10.2		10.2				162.49			
20-May-05	Fri	Day 22				2.42	10.9		10.9				174.27			
21-May-05	Sat	Day 23				2.66	11.7		8.8		2.9		186.91	186.91		
22-May-05	Sun	Day 24				2.92	12.5		6.3		6.3		200.46	200.46		
23-May-05	Mon	Day 25				3.22	13.4		3.4		10.1		214.99	214.99		
24-May-05	Tue	Day 26				3.54	14.4				14.4			230.58		
25-May-05	Wed	Day 27				3.89	15.5				15.5			247.30		
26-May-05	Thu	Day 28				4.28	16.6				16.6			265.23		
27-May-05	Fri	Day 29				4.71	17.8				17.8			284.46		
28-May-05	Sat	Day 30				5.18	19.1				19.1			305.08		
29-May-05	Sun	Day 31				5.70	20.4				0.0					
30-May-05	Mon	Day 32				6.27	21.9				0.0					
31-May-05	Tue	Day 33				6.89	23.5				0.0					
1-Jun-05	Wed	Day 34				7.58	25.2				0.0					
2-Jun-05	Thu	Day 35				8.34	27.1				0.0					

Table 2. Algae paste administration rates

Larvae days post hatch	Nanno paste put directly into larval tank (mls)	D.salina paste put directly into larval tank (mls)	Chlorella paste put directly to larval tank (mls)	Nanno paste put into dosing tank (mls)	D.salina paste put into dosing tank (mls)	Clorrella paste put into dosing tank (mls)	Dosed Nano per larvae tank (mls)	Dosed D.salina per larvae tank (mls)	Dosed Chlorella per larvae tank (mls)
2	25	100	25						
3	25	100	25						
4	15	60	15						
5	25	100	30						
6	20	97.5	20						
7	10	75	12.5	50	150	50	12.5	37.5	12.5
8				60	300	80	15	75	20
9				60	450	85	15	112.5	21.25
10				60	450	85	15	112.5	21.25
11				60	450	85	15	112.5	21.25
12				60	450	85	15	112.5	21.25
13				60	450	85	15	112.5	21.25
14				60	450	85	15	112.5	21.25
15				60	450	85	15	112.5	21.25
16				60	450	85	15	112.5	21.25
17				40	300	60	10	75	15
18				40	300	60	10	75	15
19				40	300	60	10	75	15
20				40	300	60	10	75	15
21									
22									
23									
24									
25									

4.1.3 Results

Larvae reared within the DP environment demonstrated significantly better (P<0.05) growth compared to the NP. The DP produced similar results to the NL and CP reared larvae , the differences were not significant (P>0.05).

Barramundi larvae grown in tanks with NP added were significantly smaller and weighed significantly less (P<0.05) than larvae in the other treatments at 26 dph (Fig. 1 & 2). There were no significant differences in survival shown between the treatments (P>0.05). Total biomass at the end of the experiment was as follows: *Chlorella* paste 442.8 g, *D. salina* paste 403.8 g, *Nannochloropsis* live 443.7 g and *Nannochloropsis* paste 369.9 gr. There was no significant difference in terms of total biomass between the treatments.

The stress test (fig. 4) revealed no significant difference in larvae rigor between the different treatments (P>0.5).

Rearing tanks supplied with *Nannochloropsis* paste had significantly higher (P<0.05) levels of *Vibrio* sp. at 20 dph, while *Nannochloropsis* live tanks showed significantly less total bacteria (Fig. 5). The bacteria levels increased in the morning after addition of the NP, CP, and DP and decreased after the addition of the NL.

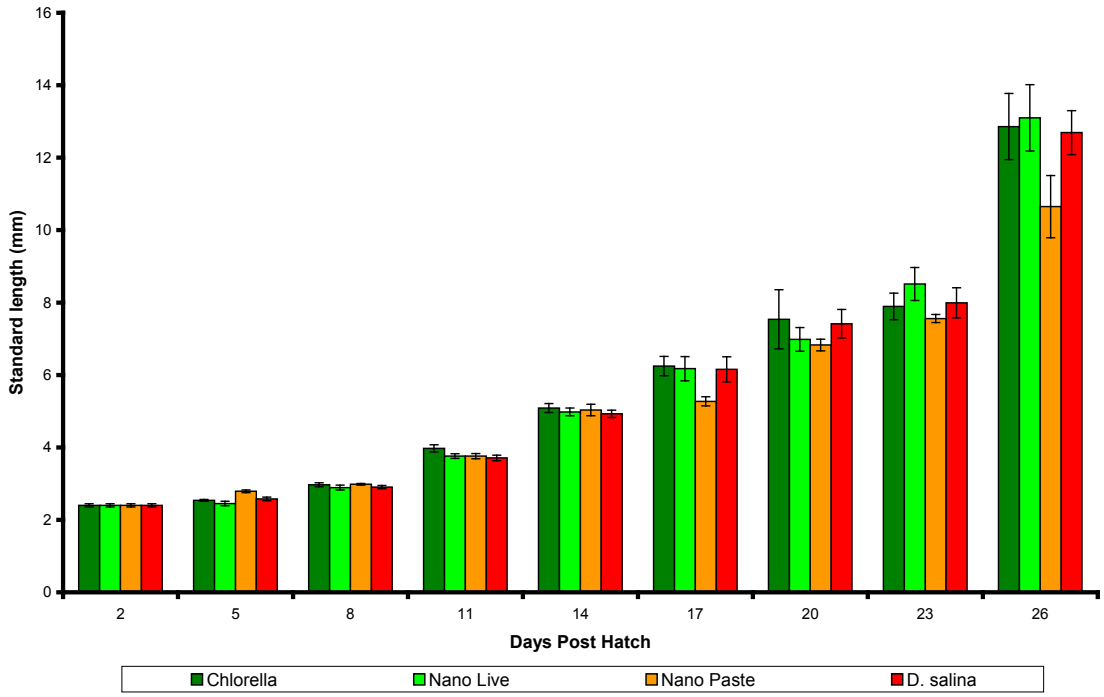

Figure 1. Averaged standard length of larvae taken from four replicate tanks in each algae treatment, across progressive larval ages.

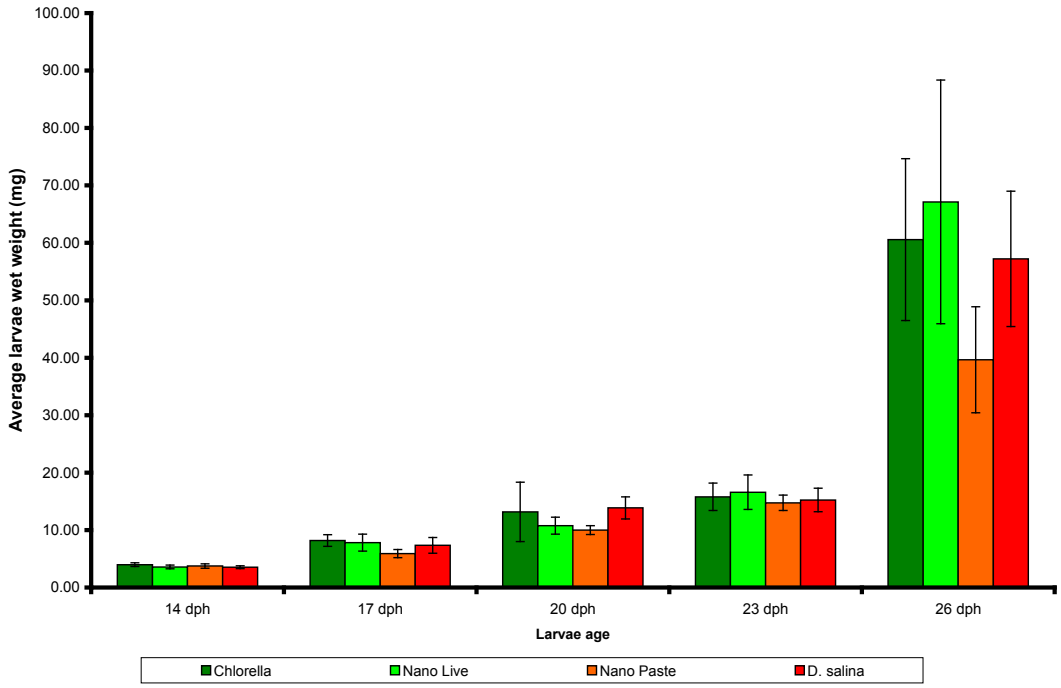

Figure 2. Averaged Wet Weight (mg) I.S.D., N=4, of larvae taken from four replicate tanks in each algae treatment, across progressive larval ages.

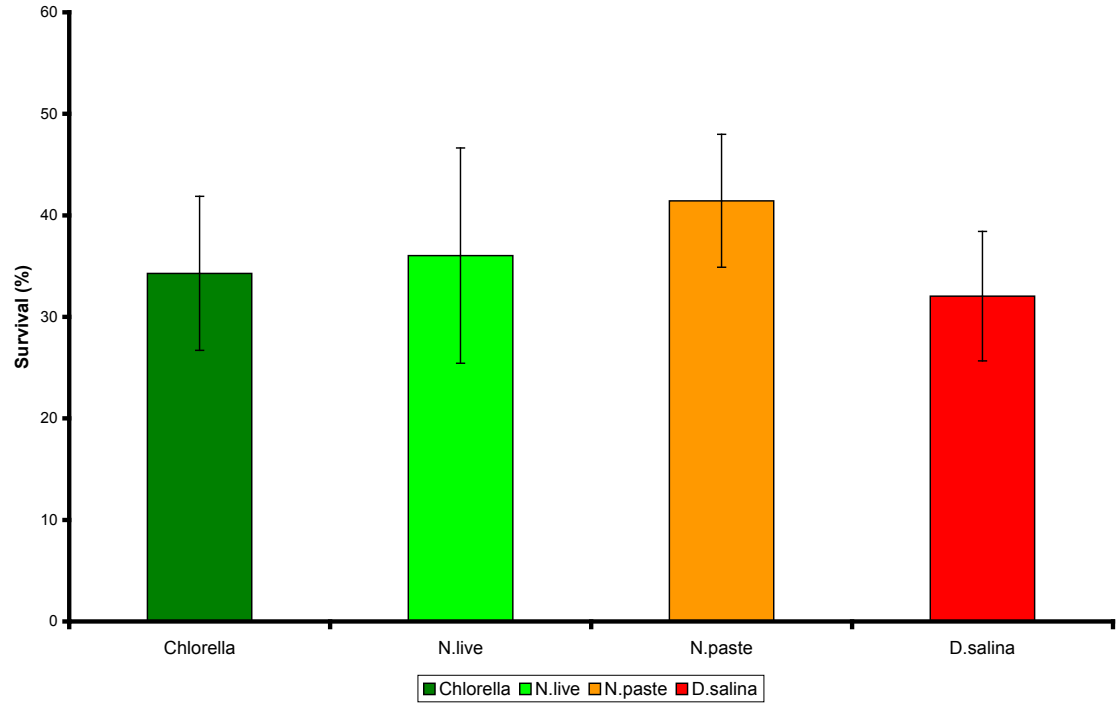

Figure 3. Survival as a percentage of initial tank stocking rates at 26 dph, not including sampled larvae.

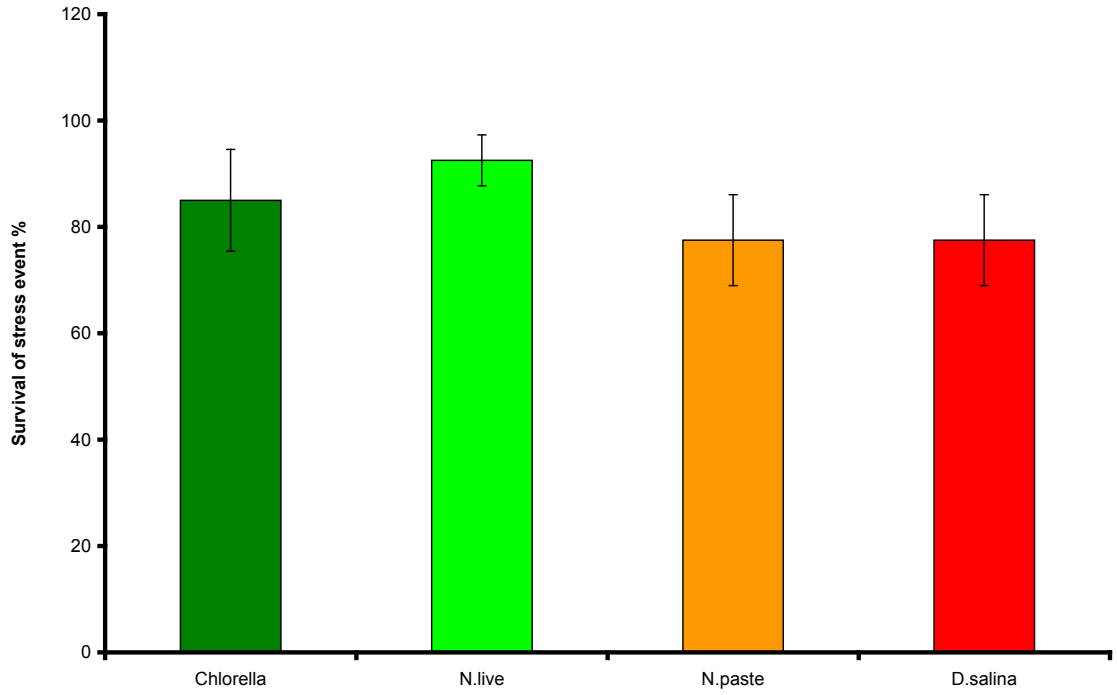

Figure 4. Percentage survival of larvae subjected to stress, as an indication of larval health.

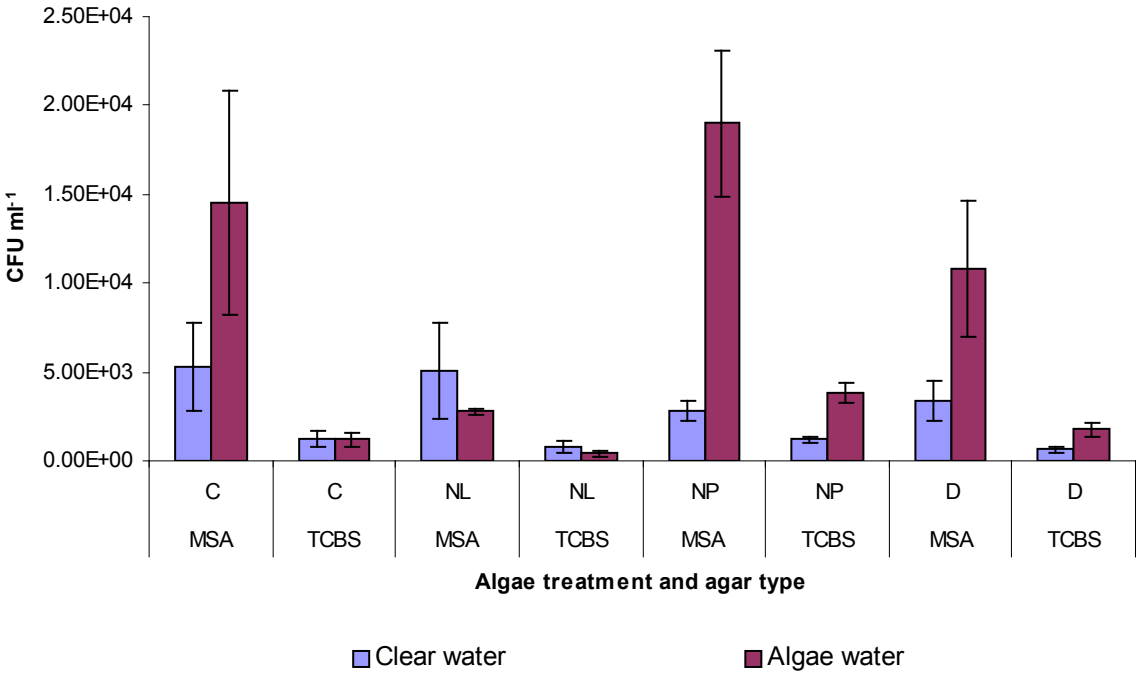

Figure 5. Bacteria count per ml of larvae culture media, before and after algae addition to the larvae culture tanks. Total bacteria counts are indicated by those cultured on MSA and the Vibrio sp. fractions are estimated by culturing on TCBS.

Table 3. Algae volume and value assessment (as purchased in Australia). Live Nannochloropsis treatment was omitted from the table since daily use was variable according to algae density and price may fluctuate between hatcheries according to rearing systems and methods.

Algae	Average daily use (mls)	Total used per tank (litres)	Cost AU$/lt	Total cost per larvae tank, AU$
Chlorella paste (CP),	19.5	0.39	70	27.30
Nannochlorpsis paste (NP)	15.375	0.3075	130	39.97
Dunaliella salina	92.25	1.845	14	25.83

4.1.4 Discussion

Different algae used as 'green water' performed very similar in terms of growth and survival and bacterial profiles. *Nannochloropsis oculata* paste was the only treatment that showed any significant differences, resulting in significantly smaller larvae. Significantly higher levels of total bacteria and *Vibrio* species were found in this treatment and may have contributed to an overall lower growth (Reinertsenet al, 1993). Both algae and bacteria in the tank is known as being important in establishing the gut flora in larvae, which could have had negative impacts in this treatment (Cahu *et al.*, 1998).

Vibrio anguillarum in larval cultures has been found to lower the mean survival rate of larvae, however the presence of *Vibrio alginolyticus* has been found to lower ammonia concentrations in cultures leading to increased survival of the larvae (Munro *et al.*, 1995). This could explain the higher survival rates in the NP treatment, however ammonia levels were not measured and the types of *Vibrio* sp were not identified. Although it is feasible to conclude that the higher levels of *Vibrio* sp. had an adverse effect on growth, (Munro *et al.*, 1995)

Higher survival in the NP treatment could also be attributed to higher levels of cannibalism in the other treatments. Barramundi larvae are known to be highly cannibalistic even in the larval stages (Curnow *et al.*, 2005). Better water quality and lower bacterial levels in the other treatments may have resulted in stronger larvae, leading to higher incidences of cannibalism. This could lead to the removal of smaller larvae and skew the population towards larger cannibalistic larvae, and would explain the higher size variation in the three treatments when compared to the NP.

The introduction of live micro algae is reported to lower bacterial levels in larval culture tanks (Reinertsenet *at al.*, 1993; Salvesen *et al.*, 1999), which is supported by the bacterial assays performed in the current experiment. The introduction of live algae in the morning lowered bacterial levels, which is in contrast to the introduction of the pastes that increased the total bacterial levels. Live algae promoted the largest larvae in terms of length and weight, although this treatment had the largest variation in size. However no significant differences were demonstrated in overall production.

The main objective when producing hatchery-raised fish is to reliably supply high quality juveniles, while minimizing the costs. It is clear that algae pastes can be a very viable alternative to live algae. Several algae pastes are currently imported to Australia. *Dunaliella salina* is the only algae paste produced in Australia and the paste is different to all the other green algae pastes since it consists of broken cells rather than whole cells. *D. salina* cell membranes are very soft and fragile and are destroyed during harvest. However, this didn't affect the performance of the larvae or increase the bacterial levels in the tanks.

Due to its long shelf life (≥24 months), *D. salina* paste presents a viable alternative for live algae or other algae pastes. Commercial comparisons still need to be performed comparing the

concentration of the algae that is needed when used in larvae tanks. A commercial comparisons between the algae sources comparing the amount and concentration of the algae that is needed when used in larvae tanks and the price per unit will vary between countries due to the cost differences, an estimated comparison was conducted for the Australian market (Table 3). It is clear that the *D. salina* paste is the cheapest option followed closely by the *Chlorella* paste. Taking into account that the growth (wet weight) was highest with larvae in the *Chlorella* paste treatment with similar survival to the *Nannochloropsis* paste, it seems that it is the most viable option. However, shelf life of the *Chlorella* paste is very short – around 25-28 days (from arrival). On comparison the *Nannochloropsis* paste shelf life is 6 months (when frozen) and the *D. salina* 24 months (at room temperature). Therefore, one should look at the time frame for using the algae. For example, in a commercial hatchery that can plan larvae rearing periods, *Chlorella* paste might be suitable. However, if shelf life as well as cost is an issue, the alternative might be the *D. salina* paste.

4.2 Feeding rates and growth of yellowtail kingfish *Seriola lalandi* juveniles fed on two commercial and experimental weaning diets

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4.2.1 Introduction

Yellowtail kingfish *Seriola lalandi* is a highly valued fish in many Asian countries such as Japan, Hong-Kong, Singapore and Korea. It is considered one of the best fish for sushi and sashimi in Japan (called 'Hiramasa', 'Kanpatchi' or 'Buri').

Several species of fish from the family Carangidae are currently being cultured around the world (Kolkovski 2007; Kolkovski and Sakakura, 2007). These species include, yellowtail *S. quiqueradiata* (Japan and Tawian), amberjack *S. dumerili* (Japan and Hawaii), yellowtail kingfish *S. lalandi* (Australia, New Zealand), horse mackerel *Tracurus japonicus* (Japan), and striped jack *Caranx delicatissimus* (Hawaii, Japan).

Yellowtail kingfish are distributed from temperate waters of the southern hemisphere across the equator to the northern Pacific. The production of *Seriola* sp. in Japan (2003) is around 150,000 mt / annum. However, until recently most of the production was based on wild caught juveniles. Only in the last few years, commercial hatcheries have started producing YTK juveniles (Nakada, 2000).

A few industry initiatives are currently being implemented in Australia and New Zealand. Two commercial hatcheries are currently operating in South Australia. The estimated production from South Australia is around 1 500 mt / annum.

Yellowtail kingfish is a fast growing fish that can wean to dry diets with very few known problems. In Australia, due to quarantine limitations, the variety of micro diets and weaning diets is limited and currently, there is no (locally-made) specific weaning diet for yellowtail kingfish larvae and juveniles.

The present study compared two commercial diets available in Australia, Proton (INVE, Belgium) and Gema Micro (Skretting), which are both designed for temperate species such as gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*), and an experimental diet (Department of Fisheries, WA).

4.2.2 Materials and Methods

Experimental design and system

Twelve 270 l conical tanks were used in this experiment. The tanks had an up-welling flow through water delivery system (Kolkovski et al., 2004). The system was designed for nutritional experiments using formulated feeds and the use of an up-welling water inlet method extends the suspension time of inert particles in the water column. The system (refer to Kolkovski *et al.*, 2004, for system details) supplied fresh temperature controlled marine bore water at the rates of 1.5 l min^{-1} per tank. The water temperature averaged 22.2°C ± 0.3°C. The physiochemical parameters were maintained at pH 7.85 ± 0.02, DO 6.5 ± 0.5ppm (mean ± SD), salinity 35.0 % and a photoperiod of 12 h light / 12 h dark at light intensity of 500 Lux at the tank surface.

Yellowtail kingfish eggs were obtained from F1 broodstock. The fertilized eggs were hatched in a 1000 l conical tank (Kolkovski et al. 2004) and then transferred to a 5000 l round tank and reared until 22 days past hatching (dph) using standard rearing protocol comprizing of 'green water' (Moretti et al. 1999), enriched rotifers followed by enriched *Artemia*. Green water (*Chlorella* sp.) method was used and a secchi depth of average 80 ± 17 cm was maintained and reduced proportionately with age. The hydrodynamics, live food and algae distribution, seawater inlet and outlet were described in Kolkovski et al. (2004) and aimed at reduced larval deformities and stress.

At 22 dph larvae were transferred from the rearing tank, counted and re-stocked into the experimental system. Each tank was stocked with 460 larvae (average wet weight 9.34 mg ± 1.5 mg) at total biomass of 4.296 g ± 0.22 g. Each diet treatment was replicated with 4 tanks.

Artemia was co-fed during the first 3 days. Each day the amount of *Artemia* fed was reduced by half. The feeding rate was allocated at 30% of body weight per day.

Microdiet was fed to the larvae using automatic feeders (Department of Fisheries, WA) and the feeding intervals were adjusted to the feeding behaviour of the larvae during the day (10 min of intense feeding in the morning, following by feeding at 20 minute intervals).

At 37 dph the larvae were harvested, graded to 2 different size groups, counted and the length and weight were determined. 25 fish from each tank were sample for length and wet and dry weight measurements. Larvae were rinsed with deionised water and individually measured for length. The larvae were then dry blotted with paper towel and weight was determined using a 4 decimal place balance (AMD). The larvae were then dried (100°C for 24 hr) and dry weight was determined.

Diets

Two commercial diets were used, Proton (INVE), a Belgium diet initially developed for gilthead seabream *Sparus aurata* and Gema Micro (Skretting) initially developed for European sea bass *Dicentrarchus labrax*. Diet particle sizes were 250 – 500 µm. The diet particle size range was adjusted by mixing smaller particles with larger ones in order to appropriate the range for larvae at different developmental stages.

An experimental diet made by the Department of Fisheries, WA was also tested. The diet was manufactured using a micro-binding method. All the ingredients were blended using a mechanical mixer (Hobart). The binder (gelatine) was dissolved in hot water (80°C) and was added to the mixed ingredients. Additional cold water was added until a uniform paste was formed. The paste was then extruded and dried in an oven at 45°C for 48 hr. The resultant dry 'spaghetti' was then ground and sieved to provide feed particle size ranges of, 300 - 500 µm and 500 - 710 µm.

Table 1. Chemical analysis of experimental diet.

Protein	65%
Fat	18%
Ash	7%
Moisture	9%

Diet Leaching

Soluble amino acid levels were determined by adding approximately 1 g (weighed to 4 decimal places) of each diet into a beaker containing 500 ml of marine bore water at 21°C. The samples were stirred using an automatic overhead stirrer (IKA Labortechnik, RW20) with a 25 mm propeller type agitator set to 60 rpm. The stirrer was set to keep the majority of microdiet particles suspended in the water column for the duration of the leaching process (Yúfera et al., 2002). The microdiets were mixed for 8 min less filtering time, so that the moisture was removed from the diets at 8 min ± 5 sec. Deionised water was used to wash the sample at the end of the filtering process. Paper filters with 11 µm pore size were used to filter the microdiets. The supernatant was then reserved (frozen) and analyzed to determine the concentration of free amino acids.

Amino acids were separated on a reverse phase amino acid HPLC column using an 1100 series HPLC system (Agilent, USA) and measured by precolumn derivatization using *o*-phthalaldehyde (OPA) and 9-fluorenylmethylcloroformate (FMOC) (Sigma-Aldrich). These results were then used to calculate the amount of free amino acids (FAA) per gram of microdiet (expressed as $nmol{\cdot}gr^{-1}$), using the following equation:

$$FAA\,(nmol.g^{-1}) = \frac{FAA\ \text{conc.}\,(umol.ml^{-1}) \times \text{supernatant volume}\,(ml)}{\text{microdiet dry weight}\,(g)}$$

Proximate Analysis

A proximate analysis of Diets was conducted by 'Chemistry Centre WA' according to the methods specified by the AOAC (1990). Protein levels were calculated from the determination of total nitrogen by Leco auto-analyzer, based on N x 6.25. Phosphorus was determined spectrophotometrically using the vanadomolybdate method. Nitrogen free extractives were calculated based on the difference between the dry matter content minus the protein, fat and ash content of the samples. Organic matter was calculated as the total dry matter minus the ash content of all samples. Gross energy was determined by calculation of the energetic value of key nutrient components (protein x 23.6 MJ/kg, fat x 39.5 MJ/kg, NFE x 17.3 MJ/kg) of the samples.

Digestibility

In vitro protein digestibility of the microdiets was determined using a modification of the pH-stat titration method described by Dimes and Haard (1994). Briefly, at the end of the feeding trial, individual juveniles were euthanized and placed on cold dissecting tray. Using a dissecting scope, the gut was quickly and carefully dissected with a scalpel and placed in a centrifuge tube on dry ice. Once all guts were extracted, the centrifuge tube was stored at -50°C for 24 hours. The frozen guts were homogenized with distilled water using a tissue grinder. Dry ice was used to prevent thawing of tissue. The homogenized guts were collected in a vial and frozen at – 50°C and immediately lyophilized pending further analysis. To reconstitute the digestive enzyme extract for the digestibility assays, lyophilized

homogenates were re-suspended in distilled water at 4°C using a ratio of 1:10 (dry weight : water), re-homogenized and centrifuged for 30 minutes at 11000 rpm and 4°C and stored at - 20°C until the digestibility assay. Alkaline protease activity of the gut-enzyme extracts was measured using the method described by Kunitz (1947) as modified by Walter (1984) using 0.5 % casein in 50 mM Tris-HCl, 10 mM CaCl2 (pH 8.0 at 25°C) as a substrate. To determine the degree of hydrolysis (DH) of proteins, the microdiets were finely ground to pass through a 120 µm mesh screen and homogenized in distilled water to give a final protein concentration of 8 mg mL^{-1}. This solution was then adjusted to pH 8.0 with 0.1 N NaOH at a temperature of 25°C. The digestion was initiated with the addition of 53.8 ±4.4 U of yellowtail digestive extract to 10 mL of protein solution. The digestion solution was maintained at a constant temperature of 25°C and under continuous agitation using a water bath and a magnetic stirrer. The extent of the protein digestion was automatically recorded using a 718 Stat Titrino (Methrom Ltd, Switzerland). The degree of protein hydrolysis (DH) was calculated from the following algorithm (Adler-Nissen, 1986);

$$DH\ (\%) = B \times N_B \times 1/\alpha \times 1/M_P \times 1/h_{tot} \times 100\%$$

where B (ml) is the volume of NaOH required to maintain the pH of the reaction at 8.0; N_B is the normality of the titrant (0.1 N NaOH); $\alpha = (10^{pH-pK}) / (1 + 10^{pH-pK})$; M_P is the mass (g) of protein in the reaction mixture; and h_{tot} is the total number of peptide bonds in the protein source. If the amino acid composition is unknown, an average value of 8.0 meq g^{-1} protein can be assumed (Alarcon et al., 2002). All assays were performed in triplicates.

4.2.3 Results

Survival

Survival to 36 dph for larvae fed on the experimental diet was significantly ($P<0.05$) better at 43%, than Proton and Gemma Micro at 16.7% and 14.5% respectively (Fig.1 & 2).

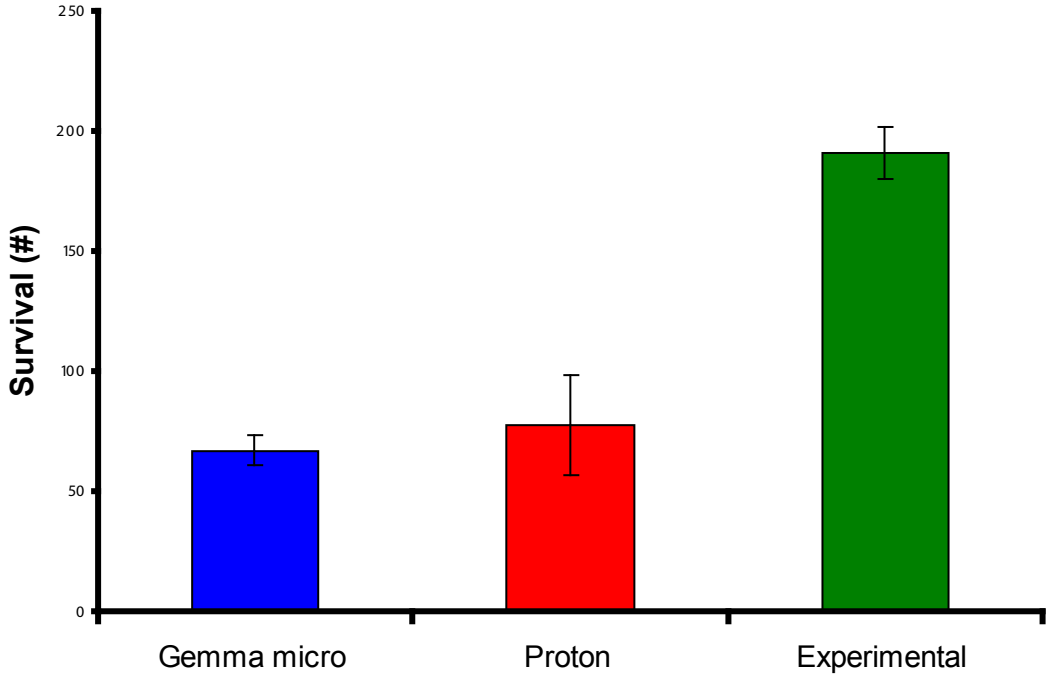

Figure 1. Survival of yellowtail kingfish larvae fed 3 different weaning diets.

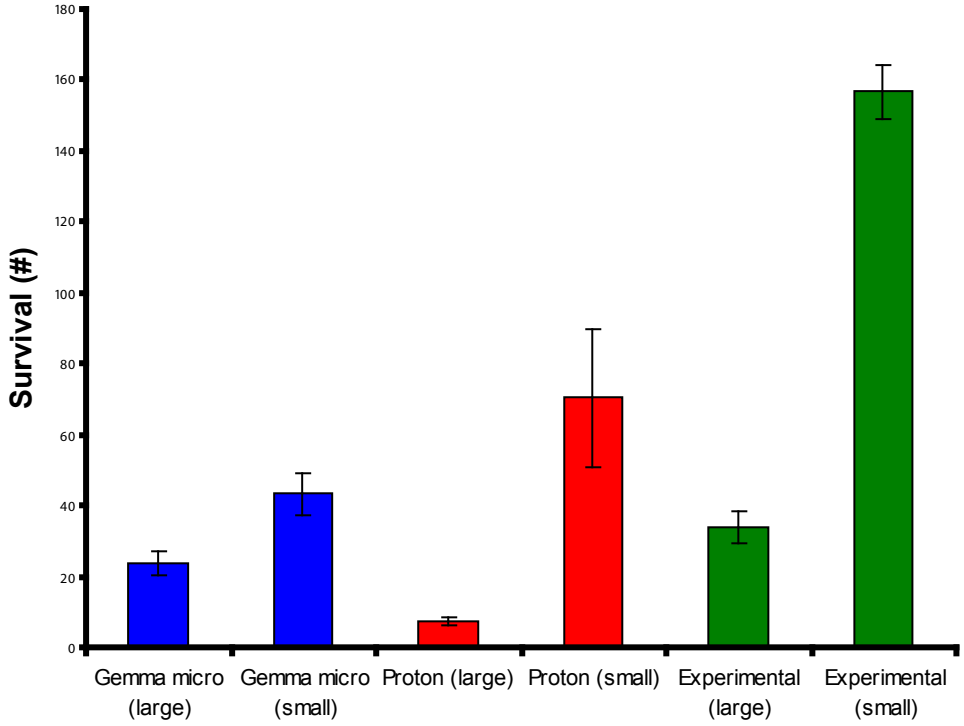

Figure 2. Survival of yellowtail king fish larvae after grading to two groups.

Growth

Length was not significantly different between Gemma Micro and the experimental diet fed larvae (24.6±0.7 mm and 22.9±0.8 mm for Gemma Micro and experimental diet, respectively) while Proton fed larvae were significantly smaller than larvae fed the other two diets (19.7±0.96 mm) (Fig. 3)

Final larvae weight had similar patterns as larval length. Both Gemma Micro and the experimental diet fed larvae were not significantly different from each other, although Gemma Micro fed larvae were slightly bigger (58.9±6.7 mg and 57.2±4 mg, for Gemma Micro and experimental diet, respectively) (Fig. 4).

Diet type had a significant and very strong effect on the total tank biomass (calculated by multiplying the survival by final average weight). The experimental diet treatment resulted in the highest biomass, which was more than double that of the Gemma Micro treatment, and three times the biomass of the Proton treatment (10.8±1.14 kg, 4.3±0.69 kg and 2.9±1.45 kg for the experimental, Gemma Micro and Proton diets, respectively) (Fig. 5).

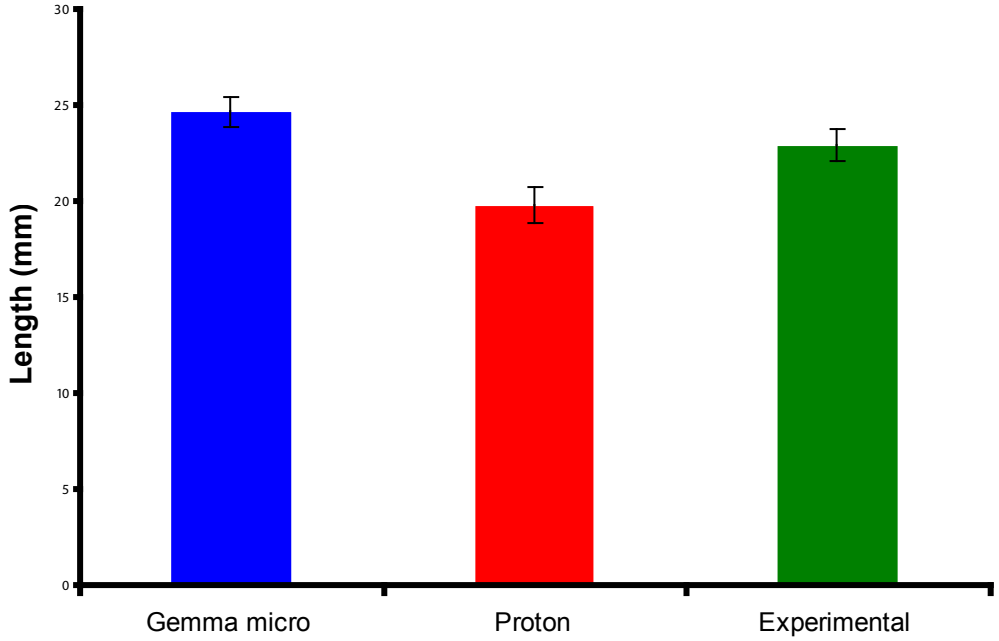

Figure 3. Final length of yellowtail king fish larvae at 36 dah.

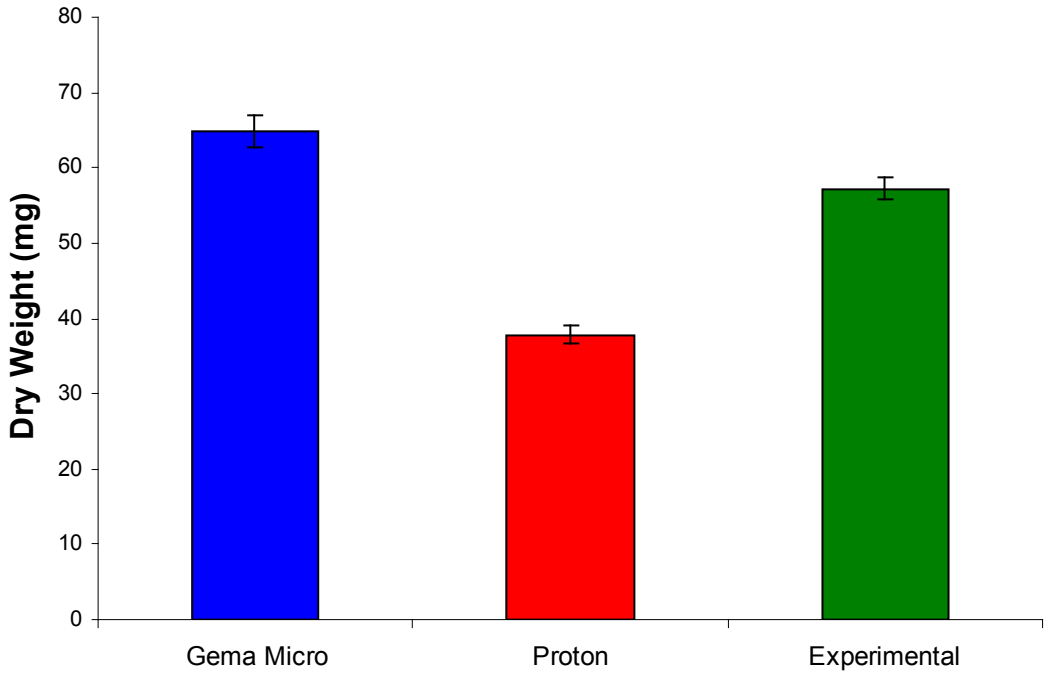

Figure 4. Final dry weight (mg) of yellowtail kingfish at 35 dah.

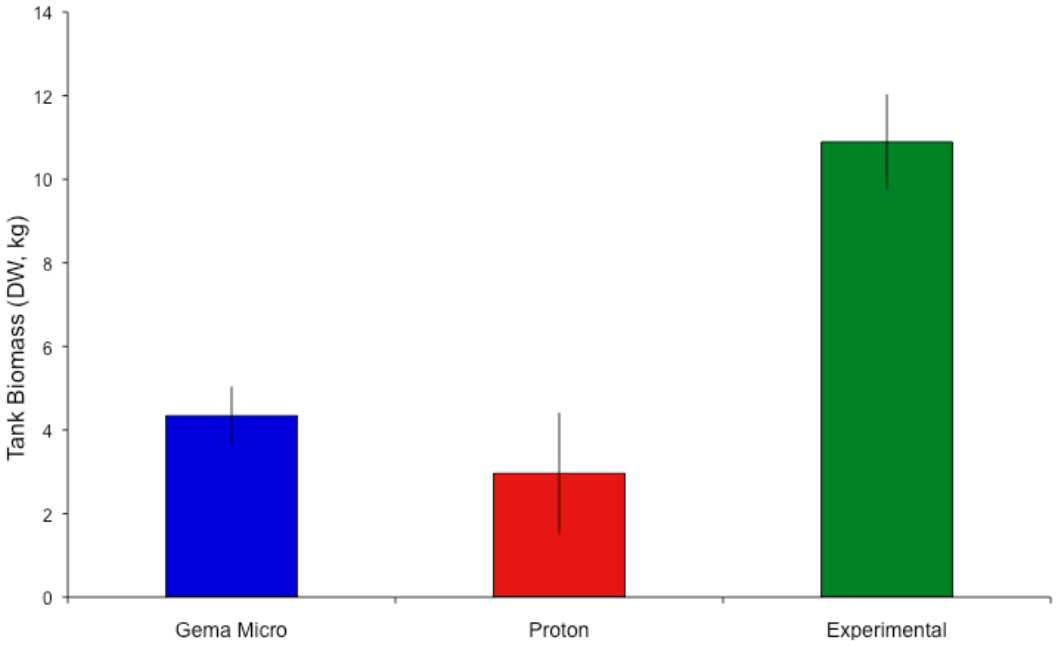

Figure 5. Final average tank biomass.

Diet leaching

Most of the diets demonstrated similar amino acid leaching patterns while the majority of the leaching occurred with hydrophobic amino acids such as leucine, isoleucine, taurine and valine. A stand out was the experimental diet that although demonstrated a similar pattern of leaching with hydrophobic amino acids leached few other amino acids that none of the other diets did. These include arginine, lysine, glycine and alanine.

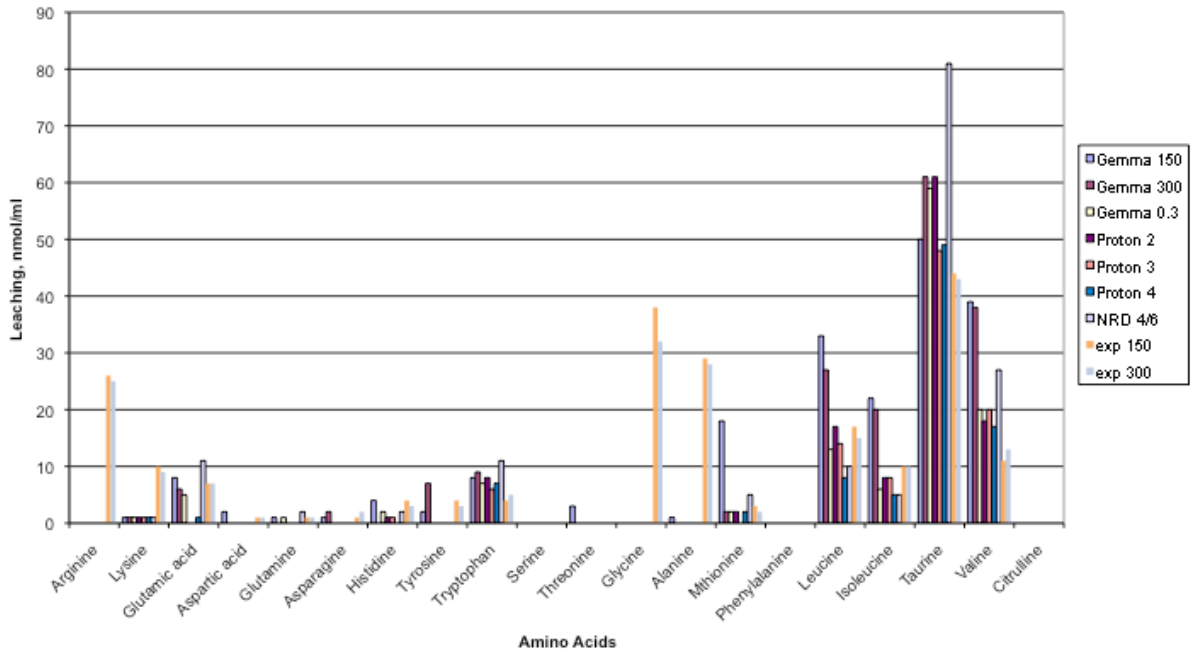

Figure 6. Amino acid leaching profile.

Proximate analysis

All three diets had a similarly high protein content with the experimental diet the highest (58.8%, 56.9% and 60.1% for Gemma Micro, Proton and experimental diet, respectively). Lipid content was similar for Gemma Micro and the experimental diet (19.9%) while Proton was lower (12.9%). However, Proton diet had higher percentage of EPA and DHA and ARA compared to Gemma Micro but lower then the experimental diet. The ratio of DHA:EPA:ARA was also different with experimental and Proton diets having, 1.7:1:0.075 while Gemma Micro having a ratio of 1.25:1:0.06.

Table 2. Fatty acid analysis

Fatty Acid	Sample Notation	Gemma	Proton	Exp
		Percent of Total Fatty Acids		
Myristic	C14.0	2.9	2.8	2.7
Pentanoic	C15.0	0.3	0.3	0.3
Palmitic	C16.0	17.3	14.7	15.4
Palmitoleic	C16.1 cis-9	3.1	4.1	3.2
unknown		0.4	0.4	0.4
Margaric	C17.0		0.4	0.1
Stearic	C18.0	3.8	3.2	3.3
unknown		0.4		
Oleic	C18.1 cis-9	9.2	14.3	9.1
Vaccenic	C18.1 cis-11	2.2	3.4	2.4
Linoleic	C18.2 cis-9,12	27.5	12.5	21.4
Linolenic	C18.3 cis-9,12,15	4.5	3.5	4.1
Arachidic	C20.0	1.8	1.7	1.9
cis-11-Eicosenoic	C20.1 cis-11	5.1	4.8	5.5
Eicosadienoic	C20.2 cis-11,14		0.3	
Arachidonic	C20.4 cis 5,8,11,14	0.4	0.6	0.8
Behenic	C22.0		0.2	
unknown We believe this to ba a C22.1 cis compound		6.4	5.5	5.9
Eicosapentaenoic	C20.5 cis-5,8,11,14,17	6.0	8.5	9.3
Docostetraenoic	C22.4 cis-7,10,13,16		0.5	0.3
Nervonic	C24.1 cis-15	0.5	1.2	0.6
Docasapentenoic	C22.5 cis-7,10,13,16,19	0.6	2.2	3.1
Docosahexenoic	C22.6 cis-4,7,10,13,16,19	7.5	14.6	16.2

Digestability

The digestibility of the experimental diet was significantly higher (2.64±0.17%) than the tested commercial diets followed by Gemma Micro 150 (2.24±0.09), Gemma Micro 300 (1.71±0.29), Proton 2/3 (1.44±0.23) and NRD 4/6 (1.42±0.16). It is interesting to point that while the two Gemma micro diets were significantly different from each other (suggested that they have different nutritional ingredients) the proton and NRD diets had similar digestibility suggesting that it is similar diet sieved at different particle sizes (as the experimental diet).

Feeding observation

Although the experiment was not designed to accurately evaluate ingestion rates, it was clear that feeding rates of both Gemma Micro and the experimental diets were higher than the Proton diet. Initial response to the diet at the beginning of the experiment (22 dph) was much stronger for the Gemma Micro and experimental diets. During the initial addition of the diet (9:30am), 2-3 larvae responded to the Gemma Micro and experimental diets. The response to the diets increased with each feeding event until 12 pm where larvae could be seen swimming actively towards and feeding upon the diets. The response to the Proton diet was milder and in the first day only a few larvae actively ingested it.

On a regular basis, more 'leftovers' were noticed on the bottom of the Proton fed tanks (all tanks were fed the same amount of diet).

4.2.4 Discussion

The experimental diet produced clearly superior results compared to the commercial diets being tested. Although Gemma Micro demonstrated slightly higher growth compared to the experimental diet, it resulted in significantly lower survival.

Total biomass data highlights the differences between the diets. The experimental diet promoted double the production of Gemma Micro and triple that of the Proton diet.

These results can be explained by several factors: diet attractability, digestibility and nutritional composition.

Clear significant differences in the larvae's feeding behaviours and attraction towards the three diets were observed. Higher ingestion rates of the experimental diet resulted from increased feeding activity. Different amino acid leaching patterns may be the cause for this outcome. Four amino acids, arginine, lysine, glycine and alanine were leached only from the experimental diet. These four amino acids are known to be strong feed attractants (Kolkovski et al. 1997, 2001). This would contribute to the overall attractiveness of the diet and better facilitate the larvae's food particle recognition and consequently lead to increased ingestion. The specific ingredients of the commercial diets are not known, however Gemma Micro also showed superior attractant leaching profiles, which would contribute to a higher relative intake compared to Proton.

Amino acid and fatty acid profiles in combination with their respective digestibility values, are some of the most important parameters to considerer when formulating diets for weaning marine fish larvae. The most common method used to asses digestibility of the diets involves in *vivo* assays based on the collection of fecal samples in order to quantify directly or indirectly some inert marker to estimate the amount of nutrient digested and absorbed by the digestive system. However, considering the small size of marine fish larvae and early juveniles, these analyses would be expensive, extremely laborious and difficult to perform. An alternative method is the use of assays to measure *in vitro* digestibility to rapidly, accurately and cost-effectively evaluate the quality of proteins in the diet.

Of the techniques commonly used to evaluate *in vitro* protein digestibility, a practical, rapid and reliable assay is the use of the kinetic pH Stat method, which typically results in a high correlation between the Degree of protein Hydrolysis (DH) and the apparent protein digestibility coefficients estimated *in vivo*, in addition to the growth rates obtained (Dimes *et al.* 1994). This correlation is particularly high when digestive enzymes extracts of the particular species been

evaluated are used. Thus, measurement of DH can be used as an estimation of biodisponibility of dietary proteins to larvae and early juveniles of yellowtail kingfish.

In the present study, the experimental diet resulted in a significantly higher degree of hydrolysis compared to the other diets. A significant correlation was found between the *in vitro* protein digestibility of the diets (in terms of degree of hydrolysis) and the performance of the fish. This results helped to explain the higher survival and final total biomass produced using this diet. Rungruangsak-Torrisen *et al*. (2002), suggest that differences in the *in vitro* digestibility values between diets or ingredients could be useful in predicting potential differences in growth in vivo. This would be very advantageous for future studies, if it is necessary to discriminate protein sources rapidly with low biodisponibility without the need to perform time-consuming experiments.

Another possible explanation of the significantly higher digestibility of the experimental diet compared to the two commercial diets is the result of different ingredients, binders or manufacture techniques, which are largely unknown, and/or the inclusion of pancreatin (porcin pancreatic extract, Sigma) in the experimental diet (Kolkovski, 2001). Pancreatin is a digestive enzyme mixture that the undeveloped larvae are yet to endogenously produce and would aid in the digestion and assimilation of the diets other ingredients.

An important factor to realize when using *in vitro* digestibility assay, is the enzyme/substrate ratio used in reaction assay. DH changes greatly if this ratio is modified, decreasing when ration is increased, which could be associated to loss of enzyme activity, substrate exhaustion and end-product inhibition (Alarcón *et al*. 2002). In the present study, a E/S ratio of 0.67 U protease activity mg^{-1} protein was used, which is similar to physiological E/S ratio of 0.8 U protease activity mg^{-1} protein employed in analysis to Seabream *Sparus aurata* larvae (Alarcón *et al.* 1999). The DH reported in the present study are very similar to degrees of hydrolysis reported by Alarcón *et al.* (1999).

Both Gemma Micro and the experimental diet had a significantly higher lipid content, approximately 35% more compared to the Proton diet. This difference in the lipid content might have led to better larval growth. Higher lipid content can better satisfy the maintenance energy requirements of the larvae, allowing more of the protein fraction to be used for growth. Several authors described the importance of lipids, lipid groups and fatty acids to the development of marine larvae (Kovem et al. 2001; Iziqeurdo et al. 2000). Moreover, yellowtail kingfish larvae have a specifically high requirement for fatty acids due to their fast developmental rates (Sakakura et al. 1999). It is interesting to note that the fatty acid profile of Proton seems to be more appropriate in terms of the essential fatty acids, EPA, DHA and ARA, which was similar to the experimental diet. However, the total fatty acid inclusion rate in Proton is significantly less than both Gemma Micro and the experimental diet, and may have led to an insufficient fatty acid intake.

It is assumed that diet attractability (leading to higher ingestion rates), digestibility (leading to better digestion and assimilation of nutrients) and nutritional profile resulted in stronger larvae and therefore, lower cannibalism (Curnow et al, 2006). These factors combined led to higher final survival in the experimental diet fed larvae compared to larvae fed Gemma Micro and Proton. The overall result was a more uniform sized and larger population that made up significantly higher biomass production.

4.3 The effect of Krill and experimental hydrolysates on growth, survival and ingestion rate in yellowtail kingfish *Seriola lalandi* larvae

Kolkovski, S., Curnow, J., and King, J.

4.3.1 Introduction

A key factor in weaning fish larvae onto dry microdiets is the attractiveness of the food particles. It was found that different substances released by prey organisms have strong effects on triggering larval feeding behavior. Larvae rely on chemicals emitted from live or dry feeds in order to initiate a search response that eventually leads to ingestion.

The chemical stimuli are not only involved in increasing food search activity, leading to increased tasting and food intake frequency, they also activate the digestive system and endogenous enzyme secretion (Kolkovski et al. 2009). Feed attractants trigger the release of digestive system neurohormones that consequently activate the release of digestive enzymes, in much the same way that smell triggers digestive processes in humans. The result is an enhanced ability to digest the food particles (Kolkovski et al. 1997a,b).

During the last decade, many substances were identified as feed attractants and attempts have been made to include them in fish larvae and juveniles diets. These substances include specific amino acids, ammonium bases and different nucleotides. It was found that some substances have a synergetic effect and the ingestion rates of feeds by larvae may be further enhanced if several of these substances are given as a mixture (Kolkovski et al. 1993, 1995, 1997a).

While the effect of a specific compound as a feed attractant can be determined, it is more difficult to standardize the effect of mixtures of extracts or hydrolysates of different organisms on ingestion rates. This is due to the fact that extracts and hydrolysates, even from the same organism, differ from each other in protein composition, free amino acid levels and the inclusion of other substances. This can be caused by factors such as differing season, food sources of the organism being hydrolyzed, and the processing methods.

Different methods of introducing feed attractants to the fish larvae have been developed. These include direct dripping into the rearing tanks, inclusion during diet production and through coating the diet particles (Kolkovski 2004).

The role of feed attractants in larvae weaning diets and a comparison of different feed attractants and their effect on the growth and survival of yellowtail kingfish larvae were tested.

4.3.2 Materials and methods

Experimental Design

Yellowtail kingfish larvae were reared from 14 days post-hatch (dph) to 29 dph using one of six experimental microdiets with 4 replicates. Protein hydrolysates were compared using a two-way matrix of treatments, which assessed both hydrolysate type and the method of its incorporation into the diet. A single protocol that progressively excluded live feeds (rotifers and *Artemia*) was used to wean the larvae onto experimental microdiets. Treatments were compared for larvae growth and survival at the end of the experiment.

Experimental diets

A single microdiet formula (prototype diet, 56% protein, 22% lipids, 11% ash) was used as the basis for all treatments and acted as the control microdiet. The microdiets were manufactured and either krill hydrolysate or experimental hydrolysate was added to the basal microdiet, which were incorporated primarily as feed attractants. A comparison of two methods of incorporation was made, whereby the basal microdiet was either coated with a solution of ethanol and hydrolysate supernatant or this solution was incorporated during the process of manufacture to make up 3% dry matter. Additionally, a second control microdiet was prepared by spraying with an ethanol/water mix in order to better assess whether the manufacturing process had an effect.

Table 1. Experiment treatments.

Treatment	Microdiet Description
Control (C)	The basal formula for all the diets.
Control Coated (CC)	The basal diet that has undergone the coating procedure (spraying with ethanol and water), without hydrolysate being added.
Krill Incorporated (KI)	The basal formula with additional 3 % Krill hydrolysate being added as an attractant during manufacture and prior to drying.
Krill Coated (KC)	The basal diet that has been ground to size (250-500 µm) and then sprayed with an attractant (Hydrated Krill Hydrolysate – ethanol mix), to make up 3 % of the diet's dry weight as dry Krill Hydrolysate.
Experimental Incorporated (EI)	The basal formula with additional 3 % experimental hydrolysate being added as an attractant during manufacture and previous to drying.
Experimental Coated (EC)	The basal diet that has been ground to size (250-500 µm) and then sprayed with an attractant (experimental hydrolysate – ethanol mix), to make up 3 % of the diet's dry weight as dry Hydrolysate.

Larvae

Yellowtail kingfish larvae were reared using a green water technique (Moretti *et al*., 1999) and fed enriched rotifers in a 5 m^3 culture tank (Kolkovski et al. 2007). At 14 dph the larvae were evenly allocated into the experimental tanks by counting individual larvae into each tank.

A total of 72 000 larvae were transferred into twenty-four 270 l up-welling conical tanks (3000 fish $tank^{-1}$) in the system described by Kolkovski et al. (2004) and were fed one of the six experimental diets according to the rearing protocol. Initially 100 larvae in total and thereafter every 3 days, 10 larvae from each tank were randomly removed and sacrificed using iced water. These larvae were measured for standard length (SL), wet weight (WW) and dry weight (DW). WW was measured using a four decimal place balance (AND, Japan) after rinsing larvae with de-ionized water on a 400 µm screen and blotting excess moisture away from behind the screen using lint free paper towel. DW was measured after drying for 24 hours at 100°C. At the final observations (29 dph) 24 fish were sampled from each tank.

Rearing Protocol

Algae at 300 ppm (concentrated *Chlorella vulgaris*, DHA-enriched SV12®, *Chlorella* Industry Co. Ltd., Japan, and *Nannochloropsis occulata*, Instant Algae, mixed 50:50, Vol:Vol) and rotifers (cultured with *Nannochloropsis occulata*, yeast and instant algae) were automatically supplied by the feeding system described by Kolkovski et al. (2004) for the period that protocols received rotifers. For the initial 6 days following their introduction at 12 dph newly

hatched *Artemia* spp. (GSL, INVE) nauplii (instar I) were manually supplied 4 times daily and then enriched (DC DHA Easy Selco®) *Artemia* spp. were fed to all protocols, and microdiet was administered automatically every 6 – 12 min (increasing frequency with increased daily ration) using automatic feeders (Department of Fisheries, A). At 9 am and 4 pm the feeders were checked and a manual feed was given to the tanks from the daily ration.

Temperature controlled marine bore water was supplied to each tank at a flow rate of 1.0 l min^{-1} and microalgae (and rotifers when required) every 15 min at 0.7 l min^{-1}. The physiochemical parameters (average ± SE) were maintained at water temperature 20.9 ± 0.8°C, pH 7.98 ± 0.02, DO 6.5 ± 0.4 ppm, salinity 35.0‰ and a photoperiod of 14 h light / 10 h dark. The green water method (Moretti et al., 1999) was used and a secchi disk depth of 70 ± 17 cm was maintained.

Proximate Analysis

Total nitrogen was determined with a Technicon segmented flow analyser using the Berthelot colorimetric determination (Searle, 1984), after a sulphuric/salicylic acid/hydrogen peroxide digestion of the organic material (Bradstreet, 1965). Crude protein was determined by multiplying the nitrogen content by a conversion factor of 6.25 (Sosulski and Imafidon, 1990).

A Soxhlet n-hexane extraction (method 963.15, AOAC, 2000) was used to determine crude fat.

Statistical analyses

Statistical analyses of protocol effects were performed using SPSS 8.0 statistical software package. One-way ANOVAs (Sokal and Rohlf, 1969) were done along with descriptive statistics, regression analysis and Tukey's post hoc test ($P<0.05$). Variances were tested for normality and homogeneity. Results are presented as mean ± standard error (SE).

4.3.3 Results

Fish in different treatments at 29 dph were not significantly different in length, wet weight or dry weight.

Table 2. Summary of results at 29 dph.

Treatment	Avg Larvae Length (mm)	± SE (mm)	Avg Larvae WW (mg)	± SE (mg)	Avg Larvae DW (mg)	± SE (mg)
C	8.68	0.28	21.35	3.04	4.51	0.64
CC	8.09	0.25	17.22	2.30	3.57	0.48
EI	8.48	0.13	21.32	1.40	4.48	0.32
KI	8.60	0.16	22.06	1.35	4.59	0.28
EC	8.45	0.09	19.75	1.02	4.12	0.25
KC	8.45	0.11	18.94	1.06	3.89	0.23

Survival was shown to be significantly ($P<0.05$, Fig 1) better in the treatment that received microdiet that had been coated with experimental hydrolysate (EC) than all other treatments. Microdiets that were coated with hydrolysate (EC, KC) performed relatively better than the microdiets containing additional hydrolysate incorporated during the manufacturing process. The inclusion of hydrolysate within either of the manufacturing processes improved survival compared to the control diet without inclusion of hydrolysates.(C). It is interesting to point out that the manufacturing process of spraying the diet with an ethanol solution improved larvae survival compared to the control diet. No differences were found between the ethanol

sprayed diet and the diet with the inclusion of hydrolysate (CC, EI and KI). Tank production (Fig 2) reflected the survival results in all treatments. There was significantly ($P<0.05$) higher production when using the microdiet coated with experimental hydrolysate. The krill hydrolysate coated microdiet was not significantly better than all other treatments except the basal control diet.

Table 3. Survival (as % of stocked numbers) and tank production.

Treatment	Average Survival	± SE	Tank Production (mg)	± SE (mg)
C	6.2%	2.4%	3387	1020
CC	10.0%	0.9%	5101	561
EI	9.6%	1.1%	5989	301
KI	9.5%	1.5%	6271	1155
EC	15.8%	1.9%	9207	715
KC	12.5%	1.6%	7049	752

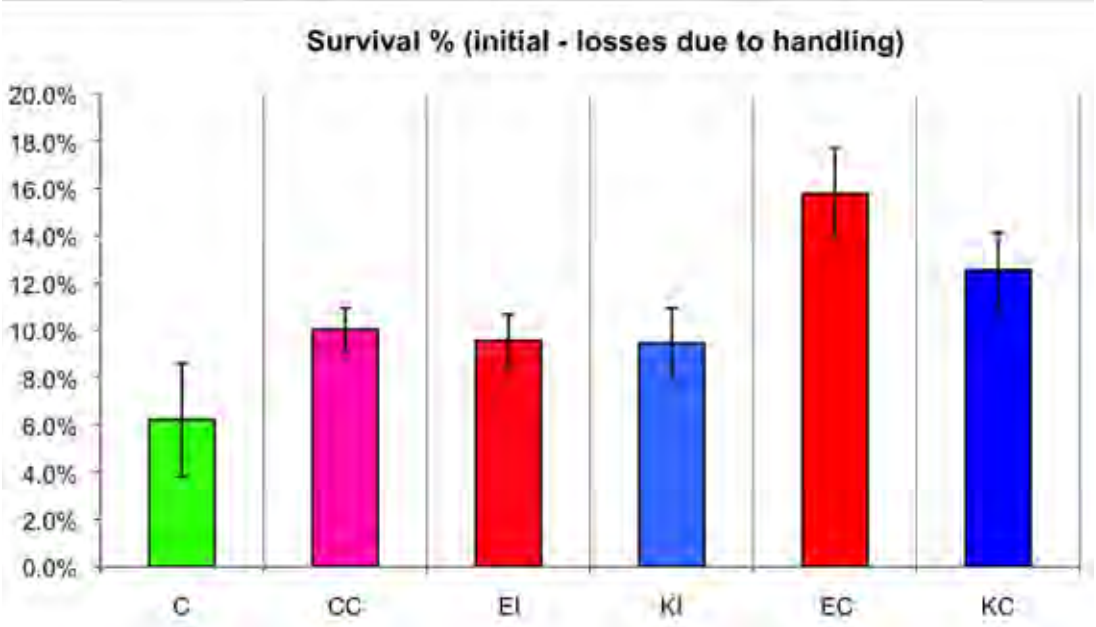

Figure 1. Larvae survival as percentage of stocking number.

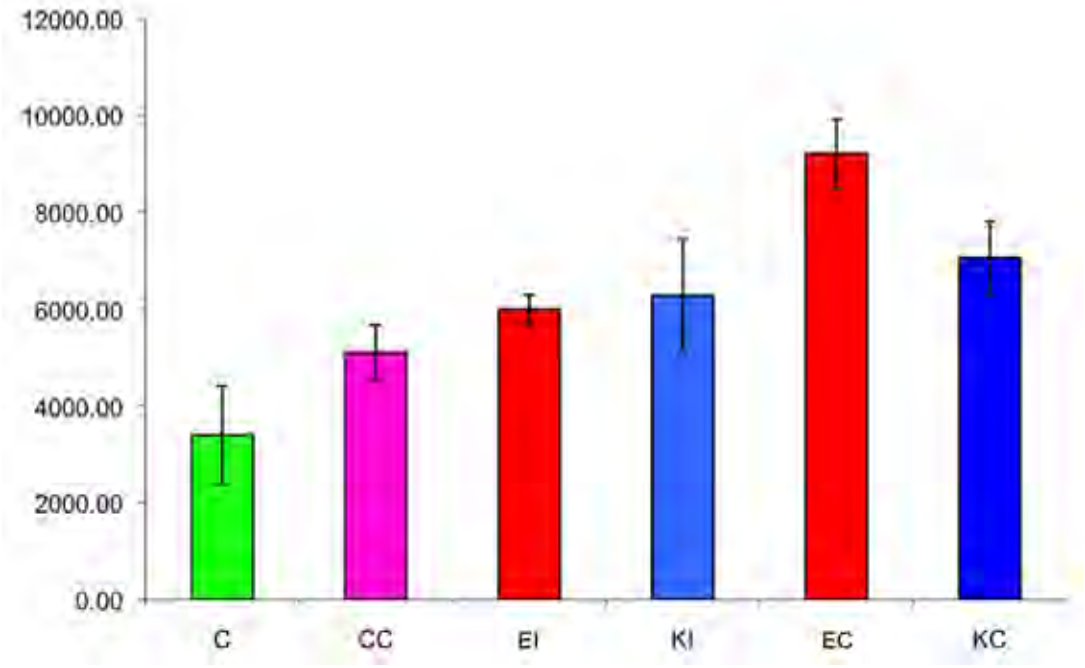

Figure 2. Tank production (final biomass, Average wet weight x survival).

4.3.4 Discussion

The first interaction between food particle (live or inert) and larvae occurs in the water column, where the particle is accepted or rejected. Therefore, it is essential that this interaction is maximised and optimised. There are many factors affecting the feeding process including food particle and larvae concentrations, frequency of encounters, food identification through chemical and physical cues and many others.

Feeding behaviour involves several steps that the larval undertake in the process of finding and ingesting food particles (Kolkovski et al. 2009):

1. General and not specific reaction, initiation of search movements involving chemical and electrical stimuli.
2. Identification of the food particle location involving chemical stimuli.
3. Close identification of the food particle, involving chemical and visual stimuli.
4. Tasting and/or actual feeding requiring chemical and physical stimuli (taste buds).

Various substances, such as free amino acids, nucleotides, nucleosides and ammonium bases are released from prey organisms and are potent inducers of feeding behavior in marine (Knutsen, 1992; Doving and Knutsen, 1993, Kolkovski 2006) and freshwater fish larvae. Generally planktonic organisms concentrate in 'patches' that attract the fish larvae (Cassie, 1959). Kolkovski *et al.* (1997a, b) identified some of the active substances in *Artemia* rearing water and added these substances to the larvae-rearing tank. The authors then analyzed the effect that individual substances had on ingestion rates by eliminating one substance at a time and observing the differences in feeding activity. When microdiet ingestion rates dropped, the missing substance was regarded as being an active feed attractant. The authors found four amino acids that induced increased feeding activity; glycine, alanine, arginine and ammonium

salt – betaine. Furthermore a synergistic relationship was reported between the amino acids and betaine, which when combined produced a stronger effect than the sum of the individuals. These and other amino acids as well as other substances were also found to stimulate other marine species.

A practical way to increase the ingestion rates of microdiets would be to incorporate these substances as extracts or hydrolysates into the diet. Kolkovski *et al.* (2000) tested the effect of krill hydrolysate as a feed attractant on yellow perch *Perca flavescens* and lake whitefish *Coregonus clupeaformis*, by coating commercial starter diet with 5% krill hydrolysate. Fish fed the coated diet experienced similar growth to fish fed live *Artemia* and significantly higher growth than fish fed the control diet. Results in the current experiment concur with this outcome, where tested hydrolysates, krill and experimental contributed to significant increases in larvae performances.

There are several ways to introduce the attractants to the larvae (Kolkovski *et al.* 2009). These include the following:

- The addition of attractants directly into the water uses large amounts of these substances, but maintains a constant concentration.
- Coating the diet particle results in unknown leaching rates, but can contribute to higher palatability and more specifically identifies particles as food.
- Incorporation into the diet, as part of the protein source also results in an unknown leaching rate (depending on the microdiet type), however only a low amount of attractants are needed, part of the protein source in the diet is replaced, and digestion and assimilation is improved.

In the current experiment, coating the diets with hydrolysate led to significantly higher growth rates compared to larvae fed diets with hydrolysate incorporated. Although coating resulted in smaller mean individual weight, it resulted in significantly higher survival, whereas excluding hydrolysate entirely from the diets reduced their performance significantly. Other hydrolysates such as squid hydrolysate have also been found to be effective in increasing both ingestion and growth (Kolkovski *et al.*, 1997a, Kolkovski and Tandler, 2000; Lian *et al.*, 2008; Lian and Lee, 2003). It is assumed that inclusion of hydrolysates as a partial protein replacement benefits the larvae in two ways:

1. Higher ingestion rates due to the hydrolysates feed-attractability properties.
2. Higher assimilation due to the availability of free amino acids and short peptides.

Currently, several commercial microdiets include different hydrolysates such as krill, fish and squid hydrolysates in their formulations.

The result from the current experiment demonstrated that coating the attractant may result in better larvae performances compared to inclusion of the attractant into the diet. However, the hydrolysates were supplied and added solely as feed attractants and not as a protein replacement. It might be that the incorporation of higher fractions of hydrolysate to the diet will result in a compounded effect – partial protein replacement that may increase the digestion and assimilation of amino acids and short peptides as well as feed attractants. The level of the inclusion should be monitored carefully, as higher inclusion rates of hydrolysate can result in a negative effect on larval performances (Kolkovski, 2001).

4.4 Comparison between two manufacturing methods for marine fish larvae microdiets

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4.4.1 Introduction

During the past decade, efforts have been made to develop formulated diets to replace or, at least, reduce the use of live foods for marine fish larvae (Kolkovski, 2001, 2004, 2006; Koven et al., 2001). Currently, none of the commercially available microdiets (MDs) match the performance of live food organisms. Although weaning the larvae from *Artemia* onto a MD can be achieved at metamorphosis in many species (Dabrowski, 1984; Foscarini, 1988; Hardy, 1989; Kolkovski et al. 2009), the early introduction of prepared diets as the sole replacement for live food has met with limited success (Adron et al., 1974; Barnabe, 1976; Kanazawa et al., 1989; Appelbaum and Van Damme, 1988; Walford et al., 1991).

The efficacy of utilisation of feed particles (either live or inert) by marine larvae is affected by many external and internal factors. Primarily the searching, identification and ingestion process is influenced by physical stimuli including colour, shape, size and movement and at molecular level olfactory stimuli.

Substances secreted by live food organisms that act to stimulate a feeding response in larvae belong to a group of chemicals known as 'feed attractants' and some have been identified (Kolkovski et al., 1997). Moreover these chemical and physical factors affect the soft palette and influence the ingestion process, which is the precursor to the digestion process. Feeding behaviour (Mackie and Mitchell, 1985) includes several steps in the process of finding and ingesting food particles:

1.) General and not specific reaction, initiation of search movements involves chemical and electrical stimuli.

2.) Identification of the food particle location involves chemical stimuli.

3.) Close identification of the food particle involves chemical and visual stimuli.

4.) Tasting and/or actual feeding require chemical stimuli (taste buds).

Based on these steps, microdiet particles need to be attractive to the larvae by leaching out feed attractant, usually free amino acids. However, the rate of leaching should be slow to prevent the particle from disintegrating in the water.

4.4.2 Diet Types

Four types of microdiet particles are currently being used:

1. Micro-bound diets (MBD),
2. Micro-coated diets (MC),
3. Micro-encapsulated diets (MED) and
4. Merumerisation (MEM) and particle assisted rotational agglomeration (PARA).

All have been used extensively in nutritional studies with finfish larvae. MBD's are manufactured using the simplest method, where all the ingredients are mixed with a binder (activated by temperature or chemically) and then dried, ground and sieved to the required size. MC particles are actually MBD particles coated with oils or other binders to reduce leaching. MED particles are taken one step further and have a membrane or capsule wall, which separates dietary materials from the surrounding medium. The capsule wall helps maintain the integrity of the food particle until it is consumed and helps maintain water quality. However, this attribute may restrict leaching of water-soluble dietary components and therefore reduce the larvae's attraction to the food particles. The capsule wall is also thought to impair digestion of the food particle (Yufera et al., 1998).

Currently, the manufacture process for MBD's is the most commonly used method of preparation. It consists of dietary components held within a gelled matrix or binder (Lopez-Alvarado et al., 1994). They do not have a capsule and it is suggested that this facilitates greater digestibility and increased attraction through greater nutrient leaching (Partridge and Southgate, 1999). Many different binders have been used in MBD's including polysaccharides from seaweed such as agar, carrageenan and alginate and proteins such as zein and gelatine (Meyers et al., 1972; Adron et al., 1974; Hashim and Mat Saat, 1992). Binders vary considerably in their nutritional value and binding characteristics and the choice of binder can significantly influence the MBD's stability in water, rate of ingestion and nutrient assimilation (Partridge and Southgate, 1999). Heinen (1981) assessed water stability of formulated diets made from 11 different binders; MBD made from agar and alginate were amongst the most stable in terms of integrity, while carrageenan was amongst the poorest.

Both MED and MBD are generally dried prior to use and this may hinder their digestion.

Relatively new methods of preparing microdiets, initially used in the pharmaceutical industry, are cold extrusion) or marumerization and particle assisted rotational agglomeration (PARA (Barrows and Lellis, 2006). These methods are based on mixing the ingredients with the binder (as with MBD), extruding the paste through cold extrusion (marumerizer). The resultant 'spaghetti' like material is then put in the PARA device (spinning disk) which then fractures, shapes and increases density. The final particles can vary between short rods to complete spheres. This method can usually produce particles >300µm.

4.4.3 Microdiet characteristics

Leaching

As mention above, one of the problems of MBD particles is the high leaching rate of amino acids. Yufera et al. (2003) determined the rate of different amino acids leaching from both MBD and MED. The authors found contrary patterns between the two diet types. While

hydrophilic amino acids leached the most from MBD, hydrophobic amino acids were found to leach from MED particles at a higher rate. The leaching rates of the two diets were also significantly different. For instance, 70% of free lysine leached from MBD particles after less than 5 minutes, while less than 7% leached from MED particles after 60 minutes.

Buoyancy

One of the most significant problems concerned with microdiet particles is their negatively buoyant inert state. MBD particles don't move like living zooplankton, which is a visual stimulus for increased feeding activity. Furthermore they sink to the bottom where they are no longer available to the larvae and accumulate there, leading to bacterial proliferation. This further necessitates the need to effectively wean the larvae onto the MBD, in order to both modify their digestive capacity and their feeding behaviour. A change in behaviour is illustrated by the larvae's ability to recognize the inert particles as food and to more actively hunt for them during a relatively smaller window of opportunity, as the particle passes down through the water column. Different attempts have been made to increase the time the microdiet particle spends in the water column including buoyancy (air in the particle), oil levels, manufacturing methods and different rearing systems using up welling currents.

The aim of the current experiment was to compare the physical properties of two microdiets manufactured using identical formulation with two different production methods, MBD and MEM/PARA.

4.4.4 Methods

Diet preparation

Two microdiets were manufactured using the same formulation ('prototype' diet). All ingredients were ground and sieved using 150µm mesh. The dry ingredients were mixed (Hobart mixer) together followed by the addition of the oil fractions. After homogenizing the mixture, a solution of binder dissolve in warm water was added to the mixture. The wet ingredients were mixed for 20 minutes to a completely homogenous dough.

The dough was then split into two fractions to be use for the different particle preparation methods. The dough was made in two batches labelled 1.2.1 and 2.1.

MBD

The dough was extruded using a wet extruder (Fuji Paudal, 5 mm die) and the resultant 'spaghetti' was dried in an oven (40°C, 48 hr). The dry spaghetti was then ground (little green grinder) and sieved to within several particle size ranges, 700-500µm, 500-300µm, 300-200µm, 200-100µm.

MEM/PARA

The MEM (micro extrusion marumerization) /PARA (particle-assisted rotational agglomeration) diet was prepared at AIMS, Townsville, Qld. The wet dough was cold extruded using the MEM extruder with 0.3-0.4 mm dome die (Fig. 1a). The resultant spaghettis were transfered to the PARA for spheronizing (Fuji Paudal, Fig. 1b). The PARA consists of a round container with a spinning disk located at the bottom. The disk has a variable roughness on the contacting surface, and can spin at different speeds. The combination of the spaghetti diameter, spinning speed and disk surface determines the final diameter of the spheres.

The resultant diet spheres were sieved and frozen for storage, while some were dried in an oven (40°C, 48 hr) and then sieved to the appropriate particle sizes for investigation of their physical properties.

Figure 1a, b, c. Marumerizer and the diet noodles.

Figure 2a, b. PARA (spining disk).

Proximate Analysis

Total nitrogen was determined with a Technicon segmented flow analyser using the Berthelot colorimetric determination (Searle, 1984), after a sulphuric/salicylic acid/hydrogen peroxide digestion of the organic material (Bradstreet, 1965). Crude protein was determined by multiplying the nitrogen content by a conversion factor of 6.25 (Sosulski and Imafidon, 1990).

A Soxhlet n-hexane extraction (method 963.15, AOAC, 2000) was used to determine crude fat.

4.4.5 Sinking rates

Materials

The sinking rate testing apparatus consisted of a vertical clear acrylic tube of 140 mm diameter, with a cone shaped bottom. The tube was marked to indicate the surface level of a 5 l volume of water above the outlet valve in the apex of the cone. A second line at a vertical distance of 1072 mm above the first mark was drawn to indicate sinking distance, and then the tube was filled with seawater (35ppth, at 21°C), so that the water surface was aligned with the upper mark.

Method

Triplicate samples (≈0.5 g) for each time period, from each particle size range of each diet were weighed to 4 decimal places (AND, Japan).

Each sample was placed on the surface of the seawater and quickly sprayed 3 times with water, using a trigger-pump spray bottle. Time periods of 2, 4, and 8 minutes, minus the sampling time of 6 seconds, were allowed to elapse and then a 5-litre sample was taken from the bottom of the test equipment to collect any diet that had passed the lower mark. Each time period was repeated 3 times with a discreet sample for each.

Sinking rates were calculated as a DW percentage of the calculated sample DW to reach the distance within each time period.

Diet Leaching

Soluble amino acid levels were determined by adding approximately 1 g (weighed to 4 decimal places) of each diet into a beaker containing 500 ml of seawater at 21°C. The samples were stirred using an automatic overhead stirrer (IKA Labortechnik, RW20) with a 25 mm propeller type agitator set to 80 rpm. The stirrer was set to keep the majority of microdiet particles suspended in the water column for the duration of the leaching process (Yúfera et al., 2003). The microdiets were mixed for 5, 15 and 30 min less filtering time, so that the moisture was removed from the diets at the set time ± 10 sec. Deionised water was used to wash the sample at the end of the filtering process. Paper filters with 3 µm pore size were used to filter the microdiets. The supernatant was then reserved (frozen) and analyzed to determine the concentration of free amino acids.

The filter paper with the microdiet was then dried in an oven (100°C, 24 hr). The leaching weight from the diet was calculated as:

$$\textit{Microdiet weight lost,\%} = \frac{\textit{(Initial microdiet dry weight (g) – Final microdiet dry weight (g)x100}}{\textit{Inital microdiet dry weight (g)}}$$

Amino acid analysis

Amino acids were separated on a reverse phase amino acid HPLC column using an 1100 series HPLC system (Agilent, USA) and measured by precolumn derivatization using *o*-phthalaldehyde (OPA) and 9-fluorenylmethylcloroformate (FMOC) (Sigma-Aldrich). These results were then used to calculate the amount of free amino acids (FAA) per gram of microdiet (expressed as $nmol \cdot g^{-1}$), using the following equation:

$$FAA\,(nmol.g^{-1}) = \frac{FAA \text{ conc.}\,(umol.ml^{-1}) \times \text{supernatant volume}\,(ml)}{\text{microdiet dry weight}\,(g)}$$

4.4.6 Results and Discussion

The two preparation methods resulted in different particle shapes. While the MBD particles were irregular 'miniature rocks', the PARA particles were smooth and either short rods or complete spheres (Fig. 3).

Fig. 3a. PARA particles.

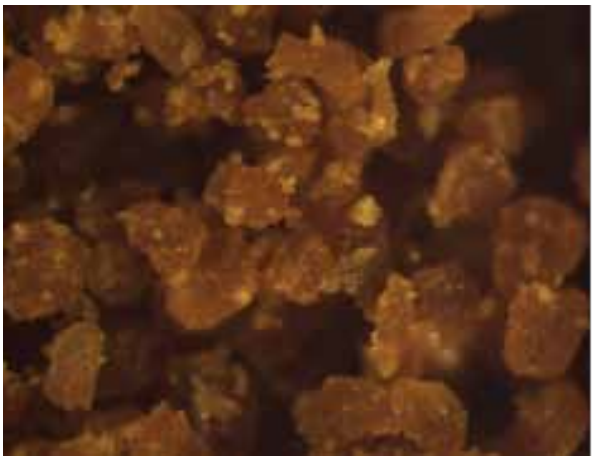

Fig. 3b. MBD particles.

Both diets have relatively narrow distributions of particle size with the narrowest distribution within PARA dry diets (Fig. 4). In general PARA diet wet and dry had narrower distribution in the range of 300-700 µm (89%) particle size with almost no particles smaller then 200 µm. The majority of the MBD diet particles were also in the range of 300-500 µm (50%) with around 20% of the particles in the upper (500-700 µm) or lower (200-300 µm) groups and 4-5% in the smallest (200 µm) or largest (>700 µm). The MBD particle range distribution largely depends on the sieving process. Due to the manufacturing methods, small particles or 'dust' are created during the crushing and milling of the dry diet. Effective sieving reduces the 'dust' around the particles and narrowes the particle size range. The PARA/MEM particles have very little 'dust' or small particles and the ones that happen tend to stick to the wet particles and therefore won't sieve properly.

It is interesting to point out that after drying, the PARA diets particle distribution changed. While in the wet form around 40% of the particles were in the range of 300-500 µm, after drying this portion increased to 80% with a narrower distribution pattern (Fig. 4).

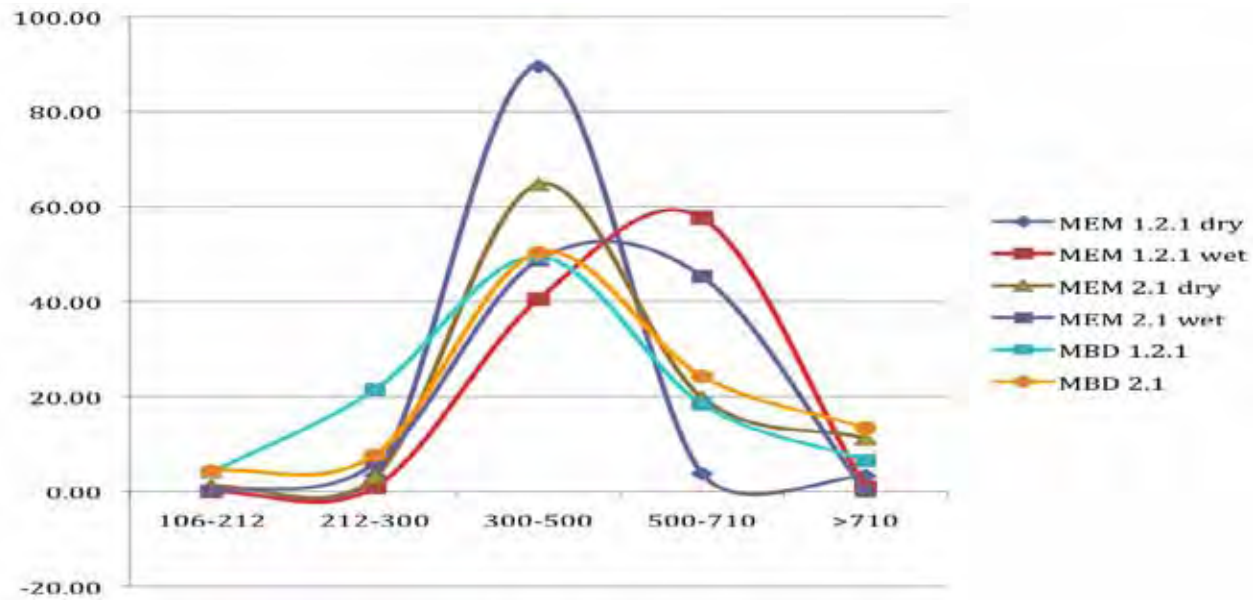

Figure. 4. Distribution of microdiet particles (%).

Proximate analysis

Since the raw mixture was common for both diets and no other process was carried out that may change the proximate analysis, it was assume that there were no differences between the two diets.

The proximate analysis was as follow: Protein 59%, lipid 18%, and ash 9%. Moisture: MBD 2%, PARA 24±3% (varies with size).

Leaching rates

Although the two diets were identical in their composition and formulation, the two methods of manufacture led to different leaching rates. All diets lost between 25% and 40% dry weight in the initial 5 minutes of being submerged in seawater, with only 5% additional leachate being released after 30 minutes. Micro-bound diet leached less across all the diet particle size groups compared to MEM diets (both wet and dry). The wet MEM diet resulted in the highest loss of weight, with more than 40% lost weight in the initial 5 minutes.

There was an inverse correlation between leaching rate and particle size, whereby smaller particles (200-300 µm) were found to leach at the fastest rates, followed by the medium size group (300-500 µm), and the larger particles (500-700 µm) leached the slowest (Fig. 5a,b).

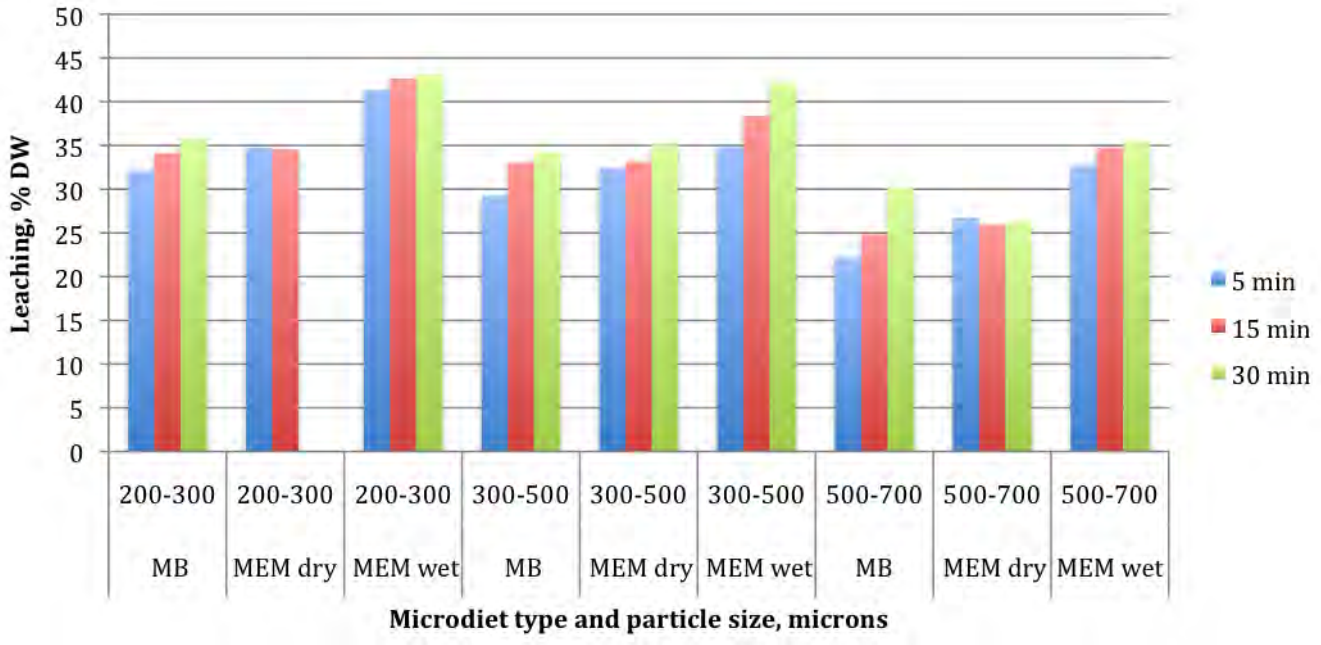

Figure 5a. Microdiet leaching rates as % of DW, sorted by particle size.

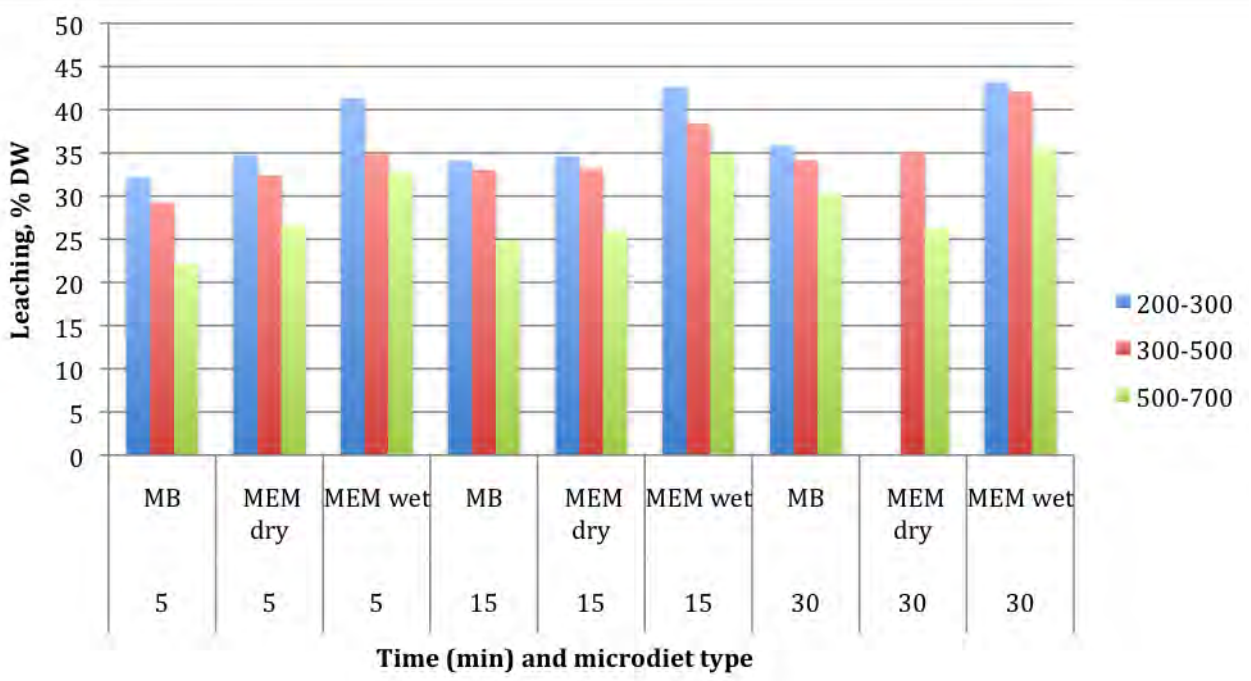

Figure 5b. Microdiet leaching rates as % of DW, sorted by time.

As mentioned above, one of the problems of dry microdiets particles is the high leaching rate of amino acids. Kvale et al. (2006) reported higher leaching rates for PARA (agglomerated) diets compared to the results obtained in the current experiment. Leaching of protein molecules (9-18 kD) after 5 minutes immersion in water (3% NaCl, 12°C) at a rate of 80-98%, 43-54% and 4-6% for agglomerated, heat coagulated and protein encapsulated microdiet. López-Alvarado *et al*., (1994) tested the leaching rates from several different microdiets made with different techniques and found losses of 85-91% with MBD using different binders, significantly higher losses compared to the current experiment of 30-35% leaching after 30 min.

Similar results were obtained by Hamre (2006). Measurements of leakage rates from two commercial and two experimental larval feeds showed that 18–42% of the protein leaked from the feed within 2 min of hydration. This probably corresponds to the fraction of water-soluble protein in the feeds. Free amino acids leak from formulated diets at a higher rate than protein, due to their lower molecular weight. The author reported that more than 50% of radio-labelled amino acids leaked from a MBD after 1 min of hydration and, after 5 min, less than 10% was left in the feed. Similar leakage rates have been found for water-soluble vitamins (K. Hamre, unpublished) and will probably apply to minerals as well.

A diet particle needs to achieve a fine balance between leaching amino acids and other nutrients to act as feed attractant and digestibility of the particle to suit the undeveloped larvae digestive system. A particle that will be hard and leach resistant will also present a challenge to the larvae digestive system, whilst, a particle that will digest easily in the gut will also disintegrate relatively quickly in the water (Kolkovski, 2006a; Yufera et al., 2000)

Sinking rates

Different patterns were found between the sinking rates of the three different diets: MBD, MEM wet and MEM dry with the same particle range of 300-500µm (Fig. 6). The dry MEM particles sunk almost immediately and after 3 min 80% were collected from the bottom of the cylinder. The wet MEM and the MBD both had slower sinking pattern with the MBD sinking significantly slower with 47.4%±3.8% and 61.55%±5.4% after 3 min for the MBD and wet MEM diets respectively. Following by 62.8%±0.6% and 73.54%±1.5% after 6 min for MBD and wet MEM diets, respectively.

Interestingly, there was a significant difference between sinking rates of the wet and dry MEM diet with dry MEM particles sinking at a faster rate than the wet particles. This phenomenon might be explained through the distribution of the diet particles before and after drying (fig 4). The dry particles are more uniform and are narrower in their distribution compared to the wet particles. This was probably caused by shrinking of the particles during the drying process, making them denser.

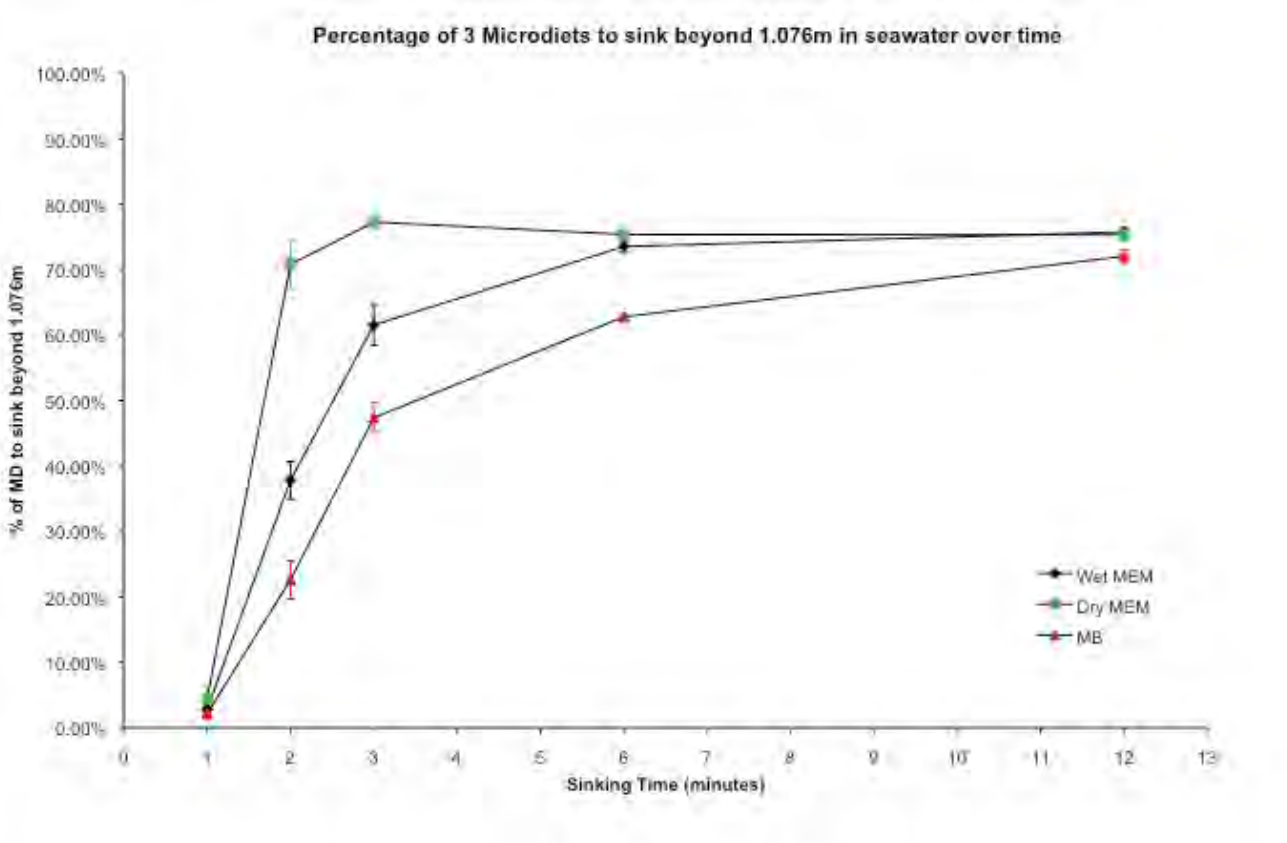

Figure 6. Microdiets sinking rates.

One of the most significant problems with microdiet particles is their negatively buoyant inert state. However, very few scientific studies have investigated this issue. In addition, formulated microdiets particles don't move like live zooplankton. This specific movement act as a visual stimulus for increased feeding activity (Kolkovski et al., 1997). Furthermore the particles sink to the bottom of the tank where they are no longer available to the larvae and accumulate there, leading to bacterial proliferation and deterioration of water quality. This further necessitates the need to effectively wean the larvae onto the MBD, in order to both modify their digestive capacity and their feeding behaviour. A change in behaviour is illustrated by the larvae's ability to recognize the inert particles as food and to more actively hunt for them during a relatively smaller window of opportunity, as the particles pass down through the water column. Different attempts have been made to increase the time the microdiet particle spends in the water column including increasing buoyancy by adjusting and modifying oil levels, manufacturing methods and also using rearing systems with up welling currents (Kolkovski et al., 2004; Teshima et al., 2004). Knowledge of sinking and leaching rates of microdiets can and should be used to optimise feeding time in the larvae tank. The faster the diet particle sinks the shorter the feeding intervals should be coupled with smaller quantities of diet, in short, feeding less more often.

Conclusions

From the current experiment results it seems that although PARA/MEM manufacturing method can offer more homogenous diet particles in terms of shape and size distribution and probably with less raw material lost (as 'dust'), the chemical and physical properties of it are less desirable in larvae diets. Faster sinking and higher leaching rates make these diets less available to the larvae.

5.0 Development of marine fish, crustacean and mollusc hatcheries Strategic Research and Development Plan 2005 – 2010

(as submitted to FRDC in 2006)

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5.1 Background

Without a reliable supply of seed stock from marine hatcheries the aquaculture industry in Australia cannot reach its full potential. Reliable, environmentally and economically sustainable hatchery production remains a key limitation in many sectors of the industry. The FRDC and other funding agencies have long supported hatchery research on a sector by sector basis. However, in 1999 it became apparent that more coordination was required across sectors. A National Hatchery Feeds Workshop was held in Cairns, Qld in 2000. From this workshop a strategic Research and Development Plan was prepared. This plan was made publicly available through the AIMS website (www.aims.gov.au/hatchery-feeds). The plan provided researchers with guidance and direction when seeking funds from FRDC and a number of projects were initiated to address national research priorities. However, it is fair to say that many researchers were disappointed that there was not more discussion with FRDC and other agencies regarding the R&D priorities.

It is against this background that the latest R&D plan has been developed. It follows a similar process with the second National Hatchery Feeds and Technology convened in September 2004, and held over two days in Sydney (see FRDC project no. 2001/220 final report). Over fifty participants from state and national research centres, universities, and key industries attended. The key output from the workshop was a comprehensive book and CD that provides an up to date snapshot of the current R&D activities, several reviews given by international keynote speakers and industry profiles.

5.2 Objectives of the workshop

To determine the current status of hatchery feeds research and development and to provide the vision for R&D for the next five years, by:

1. Reviewing the priority areas for research and development in the key sectors:
    - Microalgae
    - Rotifers
    - Artemia
    - Copepods
    - Formulated feeds.
2. Identifying any new constraints to the continued development of Australian aquaculture

in the area of marine hatcheries. Including any expansion / increased efficiency required in any of the existing sectors and to identify potential new sectors / commodities.

3. Identifying opportunities to enhance collaboration and information exchange amongst researchers and industry.
4. Developing a new R&D plan including technology transfer and communication strategies.

The purpose of the current document was to briefly update the old plan (Appendix 1) and to indicate new priorities and goals for R&D. These priorities would, hopefully, be used by the relevant funding agencies to set research priorities and to evaluate R&D proposals. The new plan outline is simpler and broader than the old plan and reflects the workshop participants desire to not be bogged down in detail. The priorities and R&D needs are direct summaries from the workshop and some discussion with industry that took place after the workshop. The plan is broken down into three key research areas of nutrition, systems and health and hygiene. Then follows a Communication and Technology Transfer Plan outline and a summary sheet comparing the status of important issues in 2000 with 2004 and the new vision for 2010. The current version is a draft to be endorsed following circulation to all workshop participants and industry representatives who have expressed an interest.

Note. The R&D plan was distributed to all stakeholders and final draft was submitted to FRDC. However, the plan was not integrated into FRDC strategic plan. Some priorities were addressed and incorporated into the CRC Seafood program (mainly addressing yellowtail kingfish Seriola lalandi larvae problems) mainly as request from industry.

5.3 Research and Development Plan 2005 - 2010

Goals:

1. To remove nutritional limitations to juvenile production.
2. To meet the changing needs of end-users.
3. To ensure good health and high performance in juvenile production using sustainable methods.

1) Nutrition

a) Live Foods

i) Reduce reliance on live feeds by:

(1) Optimizing weaning diets and protocols

(2) Identifying alternatives to live feeds

(3) Developing novel methods of delivery

ii) Optimize production of live feeds by

(1) Increasing access to algal pastes

(2) Gaining access to a wider range of rotifers

(3) Gaining access to high quality *Artemia*

(4) Developing cheap non-pond methods of producing copepods

b) General

(1) Optimizing nutrient specifications

(2) Determining nutrient requirements of emerging species

(3) Promoting better feeding and nutrition of broodstock

2) *Systems*

c) Improve hatchery systems through:

(1) Optimizing energy consumption, reducing labour inputs and capital costs

(2) Developing automatic process control and monitoring

(3) Optimising stocking density

(4) Developing new systems to reduce variability, deformities, and cannibalism.

(5) Use and adopt overseas technology (when relevant)

d) Define, document and refine management protocols

3) *Health and hygiene*

e) Develop and promote methods for the control of microbial populations through:

i) Understanding and delivery of water treatments (eg ozone)

ii) Probiotics

iii) Immune stimulants

f) Understand and manage factors contributing to the onset and proliferation of disease and malformations

g) Encourage disease and malformation identification and epidemiology studies

h) Development of vaccines

i) Formulation and acceptance of protocols for translocation and risk minimisation

4) *Communication and Technology Transfer Plan*

a) To promote a more highly skilled workforce through:

i) Personnel exchanges, both national and international

ii) Refresher and up-skilling training

iii) Technical workshops (eg. *Artemia* processing)

iv) Development and distribution of Technical manuals

v) Improved industry feedback to ensure relevant raining

b) To improve communication between industry sectors via:

i) A secure website and e newsletter

ii) Biennial workshops

iii) Working groups under Asia-Pacific Chapter of WAS

iv) Developing group demonstration projects

v) Maintaining a contact database

c) To improve communication between Research Institutes sectors

d) To improve technology transfer from Research Institutes to the Industry

i) Developing an extension service network (intra and inter state), which can mean having scientists or research technicians spending time working within a commercial hatchery to assist them in the transfer of technology.

Communication and technology transfer was highlighted as the most important issue by the industry. It was felt that outcomes of R&D projects are not always delivered to industry. The R&D community also felt that results developed in research hatcheries are in many cases not adopted by industry because they prefer to stick to 'safe' and known methods. Therein lies the challenge to researcher and industry.

Status in 2000	Status in 2004	Vision for 2005	Vision for 2010
High production costs of traditional hatchery feeds	In most hatcheries, same production systems and costs as in 2000. Some hatcheries implemented more intensive systems.	Improved efficiency, reliability and quality	Dramatically reduced costs and improved production (output, hygiene)
Dependence on Artemia	Significantly reduced usage of Artemia. Still essential in most hatcheries.	Short term: alternative diets Long term: formulated diets	Short tem: early weaning to micro diet without jeopardising performances. Long term: species specific formulated diets
Global Artemia shortage	No shortage of low quality Artemia. However, high quality is hard to find.	Brine shrimp in abundance	Local production of high quality Artemia for supply to Australia aquaculture.
Inadequate feeds for emerging sectors	Still a major problem that impacts not only on growth and survival but also deformities.	New and alternative feeds developed to suit each sector	Tailor-made specific feeds and additives to suit Australian species requirements.
Inefficient technology transfer	No change, still a major bottleneck in many hatcheries around Australia.	Improved communication between research agencies and industry	Frequent meetings to exchange information. Regular exchanges of technical and research staff.
Hatcheries seeking individual solutions	No change, the industry is still an emerging industry were each player is looking for his own solution rather sharing problems and solutions.	Benchmarked code of best practice	Information network between hatcheries, to help solve specific problems.

Appendix 1.

Extract from the HATCHERY FEEDS

Research and Development Plan 2000 - 2005

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Executive Summary

With the expansion of aquaculture in Australia there is a need to improve coordination between and within both the R&D and industry sectors with regard to the study of hatchery feeds, and to identify opportunities and priorities for future research. The recent world shortage of the brine shrimp *Artemia* has precipitated a crisis situation in aquaculture hatcheries. Accordingly, in late 1999 FRDC commissioned a Hatchery Feeds R&D Plan, which was developed at a workshop held in Cairns, Queensland, on 9–10 March 2000. The objectives of the workshop were:

- To assess the status of hatchery feeds, including live and compounded feeds, and to identify research in progress.
- To assess priorities for research and development needs in the area of hatchery feeds.
- To identify constraints to the continued development of Australian aquaculture in the area of hatchery feeds.
- To identify opportunities to enhance collaboration and information exchange amongst researchers and industry.

To develop a national R&D plan for hatchery feeds.

The aquaculture community was widely polled to establish industry priorities for future research. A questionnaire was sent to all stakeholders, together with an invitation to attend the workshop, which was held in Cairns on 9-10 March 2000. Researchers were invited to present the results of work in progress, and industry needs were canvassed in open forums.

For convenience, the subject was divided into 5 main areas of research: microalgae, rotifers, brine shrimp, copepods and formulated diets. Status reviews were commissioned in each of these areas, and priorities in each defined in the workshop. In all areas, the need to benchmark best practice and to more efficiently transfer research results to industry were highlighted. In addition to these common priority areas, the following specific areas were identified as worthy of further research:

- Microalgal production systems
- The role of microalgae in green-water systems
- Assessment and production of Australian rotifer strains and alternative feeds
- Production of brine shrimp in Australia rather than depending on imported product
- Early weaning of larvae on to formulated feeds
- Scaling up existing systems for copepod production
- Development of a knowledge-base for copepod production
- Improvement of diets for copepod production
- Identification of appropriate copepods as food for individual species
- Development of local microdiets

In recognition of the need to improve communication between researchers and industry in the field of hatchery feeds development, we have implemented an e-mail discussion group (hatchery-feeds@aims.gov.au) and have developed a web site detailing the outcomes of the Hatchery Feeds Workshop (http://www/aims.gov.au/hatchery-feeds). Aquaculture conferences and workshops will be utilised for future meetings of researchers and industry involved in the development of hatchery feeds.

Hatchery Feeds R&D Plan 2000-2005

This R&D plan was developed at the request of FRDC, in response to a need to better coordinate hatchery feeds research in Australia. A questionnaire was circulated to all identified aquaculture stakeholders in January 2000, together with an invitation to attend a workshop held in Cairns on 9-10 March 2000. Forty eight people attended the workshop, including 17 from industry. The current status of R&D was then used as a baseline for development of a 5-year plan for hatchery feeds research. For convenience, this research was divided into four key areas: microalgae, rotifers & brine shrimp, copepods, and formulated diets. During the workshop, breakout groups were formed to discuss issues and research needs in each topic area. Chairs and reporters for each group were:

Topic	Chair	Reporter
Microalgae	Malcolm Brown	Frances D'Souza
Rotifers and Brine shrimp	Stewart Fielder	Stephen Battaglene
Copepods	David McKinnon	Rob Rippingale
Formulated diets	Sagiv Kolkovski	Peter Appleford

The synthesised discussion of the breakout groups forms the basis for the R&D plan.

Mission Statement

To provide strategic research to improve the nutrition of early life history stages of aquaculture species, and to facilitate efficient technology transfer between research agencies and industry.

Status in 2000

High production costs of traditional hatchery feeds

- *Dependance on Artemia*
- *Global Artemia shortage*
- *Inadequate feeds for emerging sectors*
- *Inefficient technology transfer*
- *Hatcheries seeking individual solutions*

Vision for 2005

- *Improved efficiency, reliability and quality*
- *Short term: alternative diets*

Long term: formulated diets

Brine shrimp (Artemia or Parartemia) in abundance

New and alternative feeds developed to suit each sector

- *Improved communication between research agencies and industry*
- *Benchmarked code of best practice*

PRIORITY AREAS FOR RESEARCH AND DEVELOPMENT IN HATCHERY FEEDS IN AUSTRALIA

2000-2005

Key to Symbols

👍	High priority	➤	Links to
💰	High return	⏳	Longer term

Group A: Microalgae

👍 A1. PRODUCTION

💰

Transfer of existing technology: Cost-efficiency of algal production can be greatly improved with better transfer of existing technologies. Training-workshops such as the 'Microalgae for Mariculture' workshops operated by CSIRO but with more emphasis on current mass-culture production, specialised advice, or installation of commercial 'packages' for algal production are possible mechanisms.

On-site production versus remote production, including microalgal concentrates: There are economies of scale with algal production. Algal production constitutes the major hatchery cost: up to 30–50%. for small- to medium-sized hatcheries. It could be more cost-efficient if these hatcheries purchased their microalgae from a larger, centralised algal production facility (or larger hatcheries) able to produce cheaper biomass and to transport their product as concentrates or pastes. The potential for Australian industry to mass produce microalgae *per se*, not just as an adjunct to hatchery production, still needs R&D to identify the best mass cultivation technologies; for example, use of photo-bioreactors for mass production of the range of aquaculture strains.

Heterotrophic production: A limited number of microalgae (e.g. *Tetraselmis* strains) are capable of heterotrophic growth which offers the potential for much cheaper biomass than conventional phototrophic production. The cost and specialised equipment required probably prevent this technology being used routinely by hatcheries, though perhaps this also might be a system to be adopted by a large, centralised producer of algal biomass for on-selling of concentrates to hatcheries. Thraustochytrids — algal-like micro-organisms — are also capable of heterotrophic growth and these have valuable nutritional profiles as they are rich in the essential fatty acid DHA. There are already commercial products derived from heteretrophically-grown thraustochytrids; that is, AlgaMac 2000 and Docosa Gold (dried preparations of *Schizochytrium* sp.) and these are finding popularity in hatcheries for *Artemia* enrichment.

A2. ECOLOGICAL SYSTEMS

Microalgae for ecological systems, e.g. green-water: There is a need to identify microalgae that are 'good food' species, and that form sustained, stable blooms. Australian isolates are preferred for this application due to endemic species and ecosystem issues, and species selection would need to be targeted to the animal being cultured.

Managing pond and tank systems: A better understanding of pond dynamics will assist in the manipulation (i.e. management) of systems to promote the natural blooming of favourable microalgal species. Parameters that will influence the phytoplankton ecology will include fertilisation (i.e. nutrients), temperature, light, salinity and turbulence. This understanding may need to be developed for each specific site.

Substrate and structure (e.g. AquaMats): We need a better understanding of the influence of pond substrate and structure on the phytoplankton ecology.

A3. REFERENCE COLLECTION AND SUPPLY SERVICE

Supply of larger volumes: Some hatcheries have requested larger volumes of microalgae (e.g. 1–5 L), or even concentrates, to use as starter cultures.

Broader choice of species, including local strains and temperature-tolerant strains. Specific industry sectors or hatcheries desire more species than are currently available. However there is an issue regarding the use of new imported strains. Local strains, more suited for specific locations or application are needed — especially strains that are tolerant to the high temperatures encountered in tropical hatcheries and tolerant to wide temperature fluctuations.

Mixtures of algae: There may be some benefit in supplying mixtures of specific microalgae. Some research has been done in this area, but as yet the application of mixed cultures has not been successful. The best approach has been to mass culture individual species separately, and then apply them as mixtures at the stage of feeding to animals as a 'multi-species' diet. The composition of multi-species diets for specific target animals needs to be established.

Non-toxic strains: Use of non-toxic strains from algal groups often associated with toxic species needs more evaluation. In particular, more research is needed on strains for new live feeds; for example dinoflagellates for copepods (see Section 4)

Taxonomic guides: A taxonomic guide for good and problem algal species, perhaps including nutritional properties, temperature tolerances and mass culture suitability, would aid hatcheries undertaking mixed culture or green-water applications outside (e.g. prawn and oyster ponds).

Cost: Starter cultures are currently supplied to the industry by CSIRO on a cost-recovery basis. This can constitute a significant cost to hatcheries utilising many starter cultures; but the cost is generally accepted as reasonable by the industry. Costs for starter cultures are equivalent to cultures supplied by other collections overseas.

Non-axenic isolates: The industry does not necessarily want axenic strains as is the case for many of the imported 'traditional' strains. This is reflected in both the desire by some hatcheries to use new imported strains and, conversely, the need to find equivalent endemic strains.

➢C3

A4. ASSESSMENT/APPLICATION

Growth trials — ongoing assessment: Generally speaking, we have a good understanding of nutritional profiles of microalgae, from both Australian and overseas research. What is lacking is information on the nutritional needs of larvae, so we can match the microalgal diet to the target species. Growth trials, where microalgae of a defined composition are fed to larval food organisms, would assist in a better understanding of larval needs.

Transfer of microalgal nutrients through the food chain: Most research in this area has focussed on the transfer of polyunsaturated fatty acids from microalgae or other enrichment products to rotifers and *Artemia*. Microalgae are an important source of other key nutrients, for example vitamins, sterols and free amino acids. A better understanding of the transfer of these nutrients through different zooplankton to larvae is needed to provide more information on the requirements of larvae.

Assessment of off-the-shelf products: New commercial microalgal-like products such as dried thraustochytrids, AlgaMac 2000 and Docosa Gold are becoming more widely used by industry. Compositional analysis has shown that zooplankton enriched with these products have high concentrations of the essential fatty acid DHA. More commercial testing of these products as enrichments for zooplankton fed to target larvae is warranted.

A5. PRODUCTION OF ZOOPLANKTON

Intensive production of rotifers, brine shrimp: Only several species of microalgae are used in the routine production and/or enrichment of zooplankton, including green-water applications. An examination of a broader range of species might reveal alternative microalgae that improve zooplankton production and/or nutritional characteristics.

Microalgal diet selection for copepods: Copepods are recognised as having superior nutritional qualities, yet difficulties in their intensive culture limits their utilisation in hatcheries. Alternative microalgae, especially dinoflagellates, might assist in improving their production.

Group B: Rotifers and brine shimp

B1. BENCHMARKS FOR ROTIFER/BRINE SHRIMP PRODUCTION

Many different techniques are used to feed rotifers and brine shrimp in Australian hatcheries. We need to identify the best practices being used overseas for live food production and to translocate this technology to Australian hatcheries. In order to provide a benchmark for Australian production, we need information from individual hatcheries, such as the quantity of *Artemia* used and the level of fish production. Methods to improve feeding efficiency and reduce the use of *Artemia*, such as on-growing of nauplii, need to be introduced to hatcheries.

B2. TECHNOLOGY TRANSFER

➢B8

There is a great deal of information on the production of live feeds throughout the world. The degree of technology used in Australian hatcheries is extremely variable. In order to raise the standard of live feed production across-the-board in Australian hatcheries, we must increase the level of technology transfer. This may be achieved by introducing regular workshops, producing manuals (printed and electronic) and reports, and initiating a website or mailing list through the internet.

B3. ASSESSMENT AND PRODUCTION OF AUSTRALIAN STRAINS AND ALTERNATIVE SPECIES

There is a need to establish a research program to identify new strains of endemic rotifer species that may be suitable for culture in Australian hatcheries. The program will require that we (a) initially isolate rotifers and (b) then develop techniques to mass culture them. If this is possible, the suitability of the rotifer as a live feed for marine fish will need to be evaluated in research and commercial hatcheries. New rotifers could be selected for size (very small for first feeding fish larvae; very large as a potential *Artemia* replacement) or productivity. Improvements in rotifer size and productivity may also be sought by initiating a selective breeding program for rotifer strains already cultured in Australian hatcheries.

B4. AUSTRALIAN PRODUCTION OF BRINE SHRIMP IN PONDS

Australia has a large resource of saline ponds situated on the coast (usually for salt manufacture) and in inland Australia (natural ephemeral saline lakes; man-made saline evaporation basins as part of rising saline groundwater interception schemes). These lakes may be suitable for culture of either endemic brine shrimp such as *Parartemia* spp. or previously introduced *Artemia* spp. There is a need to evaluate the potential for commercial production of brine shrimp in Australian salt lakes.

B5. WEANING AND CO-FEEDS

There is a need to reduce the use of *Artemia* as a live food for marine fish. This may be achieved by developing new feeding strategies for fish larvae, such as an extension of the rotifer feeding phase and early weaning of larvae with formulated diets.

B6. ENRICHMENT

Rotifers need to be enriched, particularly with (n-3)HUFA's prior to feeding to marine fish larvae. Several commercial enrichment diets are readily available to Australian hatcheries; however, the efficacy of the enrichment protocols used in Australian hatcheries is in doubt. Procedures need to be developed to regularly analyse HUFA content of enriched rotifers to ensure that target concentrations are being reached. Best practice methods currently in use in overseas hatcheries need to be determined and translocated to Australia. Flow-on effects of the enrichment of rotifers with vitamins etc. on the production of Australian marine fish larvae need to be quantified.

B7. EVALUATION OF ALTERNATIVE SPECIES FROM OVERSEAS

Possible alternative species to rotifers as live feeds may be cultured overseas. A program to identify new genera (e.g. the cladoceran *Moina*) that may be suitable for marine hatcheries would be useful. Once overseas genera are identified, similar species endemic to Australia may be isolated and evaluated.

➢B2

B8. EVALUATION OF NEW ROTIFER SYSTEMS

Large-scale batch culture of microalgae is generally expensive and can account for 30–40% of total hatchery costs. Continuous microalgal production systems may reduce the cost of producing algae to feed rotifers. This technique should be investigated. Development and commercialisation of microalgae concentration in Australia may also provide an off-the-shelf feed for rotifer production. This may reduce the cost and increase the reliability of rotifer production. Significant mass-culture technology of rotifers has been developed overseas. For example, ultra-high-density production systems have been developed in Japan, which are based on feeding concentrated freshwater *Chlorella*. Final harvest densities in these systems can be 100 times greater, and production costs can be 65% less, than those of traditional culture methods. This technology needs to be transferred or adapted to Australian hatcheries.

B9. EVALUATING ONGOING TECHNOLOGY

Large-scale production of juvenile rock lobsters requires large quantities of *Artemia*. To reduce costs and increase production we need to develop techniques for reliable production of advanced (on-grown) brine shrimp.

B10. CULTURE SYSTEMS

The development of extensive, fertilised-pond larval rearing techniques may overcome the need to conduct large-scale live-feed production in Australian hatcheries. Extensive larval rearing has been used successfully for a number of marine fish species. The suitability of this technique for larval rearing may be highly species specific, and this needs to be evaluated. There may be advantages in having an initial 10–14 day rearing phase in an intensive hatchery, followed by on-growing in extensive ponds. This would reduce the dependence on *Artemia*, and could significantly reduce the cost of fish production.

Group C: Copepods

C1. SCALE-UP OF EXISTING SYSTEMS

Considerable effort has been made in the development of copepod production systems around the country (e.g. *Tisbe* in Tasmania, *Gladioferens* in Perth, *Acartia* in Darwin and Cairns). These systems need to be scaled up and tested for effectiveness with other copepod species where necessary, and made available to industry. The effectiveness of these systems in suppling copepod food in commercial hatcheries needs to be assessed.

➢C6

C2. DEVELOPMENT OF A KNOWLEDGE BASE

There is a need to assemble available information on copepod culture into a central knowledge base to facilitate attempts by individual hatcheries to grow copepods. Though there is information available on the culture of specific copepods, at present this is difficult to identify and locate.

C3. FOOD TYPE AND FEEDING REGIMES

Further research is required to identify better diets for copepods (e.g. dinoflagellates) as opposed to more conventional microalgal species. Improved feeding regimes need to be established, by determining the appropriate amounts and mixtures of various food items to maximise copepod production. Alternative diets, such as formulated diets, also need to be investigated.

➢A4

C4. MATCHING SPECIES

Fish species differ in their requirements for live feeds. Copepod species which are appropriate for each fish target and climatic zone (tropical, temperate, etc) need to be identified. For example, groupers and tropical snappers require sub-100µm food at first feeding, but this size requirement is soon passed by the nauplii of larger copepod species.

C5. ESTABLISHMENT OF BENCHMARKS FOR ECONOMICAL PRODUCTION

With the development of different production techniques for different copepod species, benchmark performance indices should be developed to identify the most cost-effective and efficient systems. Benchmarks should be established for production of nauplii for larviculture, for adults as *Artemia* replacements or supplements, and for matching the suitability of specific copepods to specific fish.

➢C1

C6. THE ROLE OF COPEPODS IN POLYCULTURE VERSUS MONOCULTURES

Green-water systems provide dietary diversity for larval fish. The effectiveness of presenting copepods as monocultures, as opposed to presenting them as part of a suite of potential food items, should be compared for each copepod species in cultivation. In addition, the ability of copepod species to persist in green-water situations needs to be assessed.

C7. STORAGE

There is benefit in being able to store live copepod material until needed. Spawning of broodstock can be fickle, and there would be considerable advantages in stockpiling copepod eggs and nauplii until the time which they are required. Usually, these copepod life stages are only required for a period of days, yet the production of sufficient numbers can take weeks to months. Promising short-term results have been obtained by refrigerating nauplii and adults but there is continued interest in being able to control the development of resting eggs. However, research to date has been opportunistic.

C8. CENTRAL ZOOPLANKTON REFERENCE CENTRE

A central facility should be established from which seed copepod cultures could be obtained by hatcheries. Without such a facility it is unlikely that individual hatcheries would develop the infrastructure to maintain copepods for their own intermittent use. Questions relating to the legality and appropriateness of relocating animals must be considered.

Group D: Formulated diets

D1. TEST CURRENTLY AVAILABLE DIETS

➢B5

A survey of the larvae and weaning diets commercially available in Australia is needed. The survey should focus on the main commercial species currently reared (i.e. barramundi and snapper).

The survey should use standard protocols and include the following topics:

- cost versus profit
- growth versus cost
- labour efficiency

D2. DEVELOP STANDARD TESTING SYSTEMS

A standard system for testing microdiets needs to be developed. The performances of a given microdiet are greatly affected by the shape, size and volume of the larval tanks. The inert movements of the diet particles depend on hydrodynamics in the rearing tank. A standard testing system for both tropical and temperate areas will have the advantage of testing different diets with different fish species in the same conditions.

The testing system should be on a commercial scale to allow immediate transfer of results to industry, without the need for up-scaling. A standardised system would also improve hatchery skills in general.

D3. DEVELOP LOCAL DIETS

In the short term the development of local diets should focus on co-feeding, using both dry and live feeds. Research in this area should aim to shorten the weaning period and decrease the amount of *Artemia* being used. In the longer term research should aim at the complete replacement of *Artemia* with microdiets. Local microdiets will need to compete with overseas diets in terms of cost and performance.

The R&D of local microdiets will need to focus on improving:

- ingestion by using feed attractants
- digestion by using easy-to-digest proteins, binders and dietary enzymes etc.

Communication between hatcheries, research institutions and feed providers needs to be improved to aid development of local diets and to get feedback from the hatcheries that are using a particular diet.

6.0 Benefits and outcomes

The project benefits, outputs and related outcomes can be divided into several sections:

I) System development

II) Microdiet development

III) Feeding protocols

In general, outcomes and benefits will (and in fact already) flow to marine hatcheries (finfish and other marine organisms), industry and R&D centres.

Feeding system development

The development of the Microdiet Feeding System involved innovated solutions in terms of automated systems, delivery, controllers as can be seen in the registered patent. This system allowed better control over time and money. In fact, currently, it is the only feeding system in the world designed specifically to solve the problems of continuous feeding microdiets to larvae. The system is commercially available and it is being manufacture in WA by local companies. During the project life, the systems were sold to Australian and overseas R&D centres as well as commercial hatcheries around the world.

The outcome of this activity is a better-controlled environment for marine larvae, reduced feeding time and manpower resulting in better larvae survival and growth and more efficient hatcheries.

Microdiet development

Several different microdiet preparation methods were tested during the project. Chemical and physical parameters were identified for different diet particles both commercial and experimental. The experimental diet matched and even outperformed commercially available diets and therefore, presents a commercial opportunity for microdiets that can be tailor-made to specific species and/or specific conditions. Moreover, characterising the chemical and physical parameters as leaching and sinking rates enable hatcheries to decide on specific feeding methods and rates i.e. small amounts in short intervals for fast sinking diets etc.

Comparing manufacturing processes resulted in evaluation of the methods that can be useful for future microdiet manufacturing.

Comparing the commercially available and the experimental diet may assist the yellowtail kingfish hatcheries to decide on the best product for weaning the larvae.

The outcome of this activity is optimising food and feeding practises in commercial hatcheries, resulting in better larvae growth and reduced tank fouling by the inert diets.

Feeding protocols

Developing weaning protocols and rearing methods (i.e. green water) resulted in recommendation on different algae products that can be use in commercial hatcheries during the larvae rearing period.

The outcome of this activity is optimising the larvae rearing and reducing the reliance on live algae and substitute it with algae paste with no effect on larvae performances.

7.0 Further development

Project 2004/258 initiated and continues research & development activities in two major areas: microdiet feeding system and microdiets. The outcomes from the project lead the way to the commercialization in these two areas. A follow up FRDC project is currently being negotiated aiming at capturing the commercial benefits of system sales to continue the patent registration (under FRDC and the Department of Fisheries, WA).

As part of a new project, a negotiation with commercial feed manufacturer will be carried out to commercialize the production of the microdiet.

Several new items related to the feeding system (i.e. larger hopper, extension modules etc) will be developed in the new project utilizing the revenue from the system sales.

8.0 Intellectual properties

During the current project, the feeding system was patented worldwide See Appendix 3. The patent is currently maintained by the Department of Fisheries, WA funded by sales revenue.

An IP agreement will be signed between FRDC, the Department of Fisheries, WA and feed manufacturer prior to commercialization of the prototype microdiet.

9.0 Conclusion

The current project focused on the development of marine fish larvae feeds and feeding system. The project achieved all the objectives set in the project proposal. An innovative microdiet feeding system available commercially worldwide. This development also resulted in worldwide patent (first for both FRDC and DoFWA).

Microdiet prototype that outperformed commercially available diets is ready for up-scale and commercialization by feed manufacturer in Australia or overseas.

Weaning and rearing protocols for yellowtail kingfish and barramundi were developed using commercially available and experimental diets as well as commercially available algae pastes. These protocols can be used in commercial hatcheries.

Chemical and physical aspects of microdiets were looked at, including those related to different preparation methods. Different feed attractants were tested to determine the optimal ingredients in terms of ingestion and the experimental diet was compared to commercially available diets and was proven to have the same and, in some cases, better performances. Methods of attractant incorperation were tested, showing significant diferences.

R&D plan was developed based on input from stakeholders during the Second Hatchery Workshop that was held in Sydney (2004).

During the project life span, strong links to R&D centres and commercial hatcheries overseas were developed. Collaborations with several R&D institutes in Australia (Tasmanian Aquaculture and Fisheries Institute) and overseas (HUBBS – Sea World, San Diego, CA, USA, University of Nagasaki, Japan, CIESE and UABC, Mexico) were initiated.

10.0 Publication

Peer review

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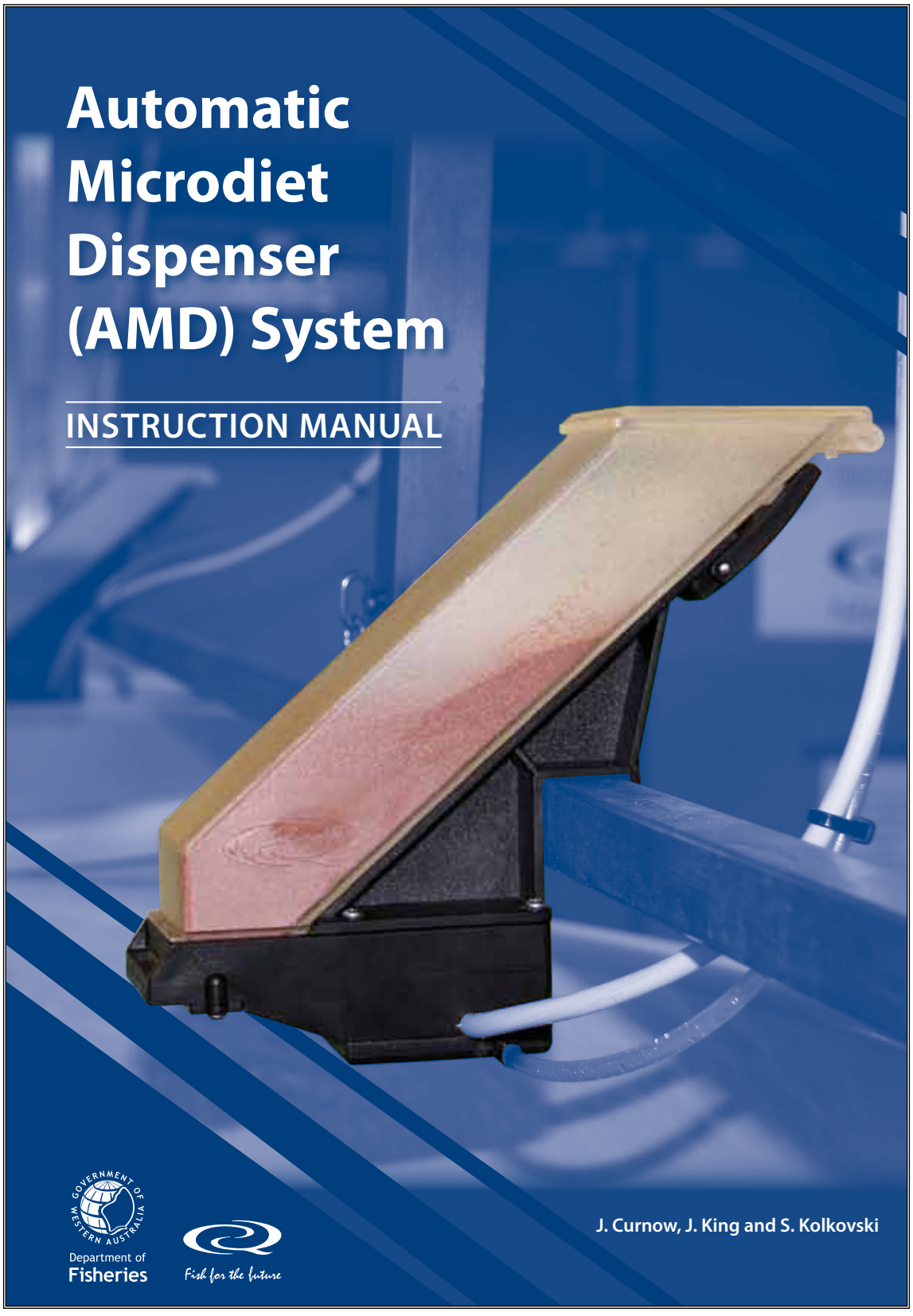
12.0 Appendices

Appendix 1 AMD Instruction Manuals



Automatic Microdiet Dispenser (AMD) System®

This book covers the use and care of the Department of Fisheries Western Australia Automatic Microdiet Feeding System

Please read these instructions carefully and retain for future reference.

J. Curnow, J. King and S. Kolkovski

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Contents

System Description

The AMD system is designed to give the aquaculturist more versatility when dealing with the task of feeding larvae and juvenile fish. The programmable logic controller (PLC) is easily operated through a user-friendly touchpad and controls up to 24 AMD units (base unit), either individually or as a group of identical feeders.

The software allows the user to pre-program a series of feeding regimes that cater for the changing larvae requirements and can be used repeatedly for successive larvae production runs. Alternatively, programs can be easily customized for specific feeding requirements on the day.

The AMD periodically administers a small amount of microdiet to larvae culture tanks, which evenly spreads the daily feed allocation across the whole feeding period, and outside of working hours. This prevents the need to manually feed the larvae, and provides a more constant availability of microdiet when the larvae need it.

The AMD feeding regime reduces opportunities for bacterial proliferation on unconsumed feed particles, by considerably increasing the probability of fish larvae ingestion. Furthermore, it minimizes diet wastage and reduces costs.

The AMD mechanism operates by using a low voltage piston solenoid to pull a slotted plate across the bottom opening of a hopper containing the diet. The moving plate rests on another smaller, stationary slotted plate. When not in operation, the slots on each plate overlap with the bars on the other plate, thus preventing the microdiet from falling through.

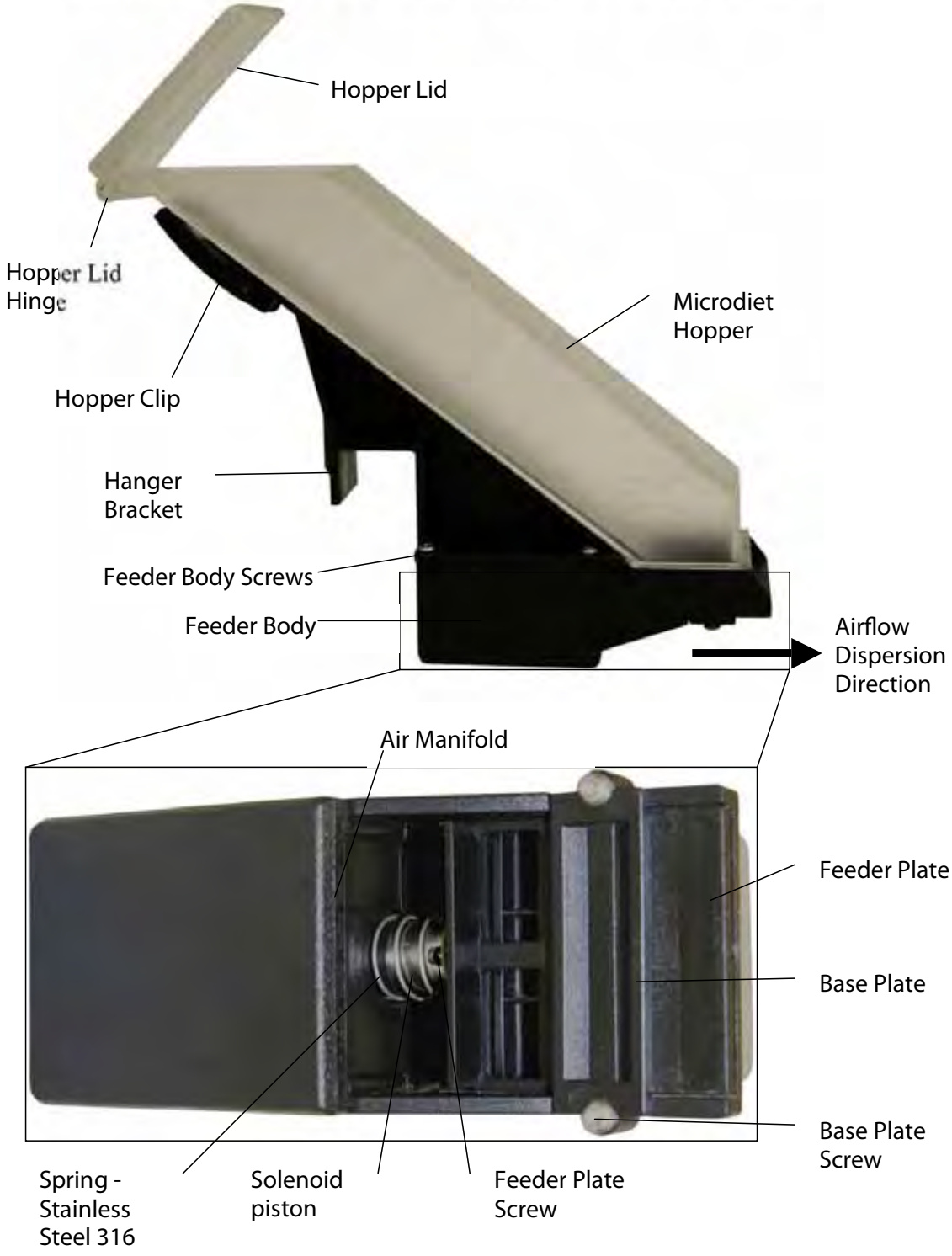
When the AMD operates, the moving plate is pulled horizontally between the hopper and stationary plate, followed by a return movement facilitated by the stainless steel spring. The feeder releases a small quantity of microdiet through the openings at the moment the slots line up, when passing in both directions. The plate can be pulled once or twice in any one feeding event, as often as every minute all day. The requirement for two consecutive operations depends on the microdiet flow characteristics and the amount of microdiet required.

The AMD is supplied with 4 feeder plates with different characteristics.

1. Plate 0, delivers the least amount per shot (≥0.05 g shot^{-1}) and with the lowest variability.
2. Plate 1, delivers slightly more than the plate 0, is less precise, but is better for small amounts of difficult diets.
3. Plate 2 delivers twice the amount of diet than plate 1.
4. Plate 3 delivers the most diet in one shot (≤ 2.5 g double-shot^{-1}).

When the most appropriate feeder plate is used a diet particle size range of 100 µm up to 2.0 mm can be dispensed through the AMD.

Automatic Microdiet Dispenser Features



Feeder Plate Description

Plate 0 The first feeder plate has a 0.5 mm gap below the hopper, a single slot and is blank on the end. This plate delivers the least amount per shot (≥0.05 g shot^{-1}) and with the lowest variability.

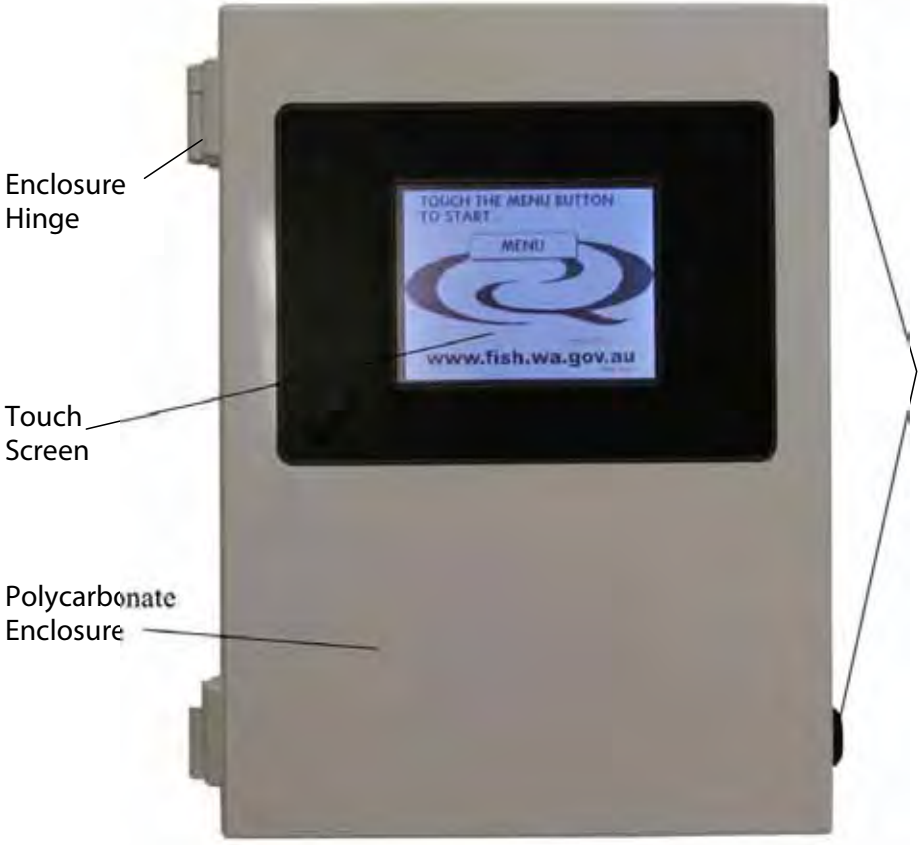
Plate 1 The second feeder plate has a 1 mm gap, a single slot and has a single line on the end. This plate delivers slightly more than the first plate. This plate is less precise, but is better with difficult diets.

Plate 2 The third plate has a 1mm gap, 2 slots and has two lines on the end. This plate delivers roughly twice the amount of diet than plate 1.

Plate 3 The forth plate has a 1mm gap, 3 slots and three lines on the end, which delivers the most diet in one shot (≤2.5 g double-shot^{-1}).

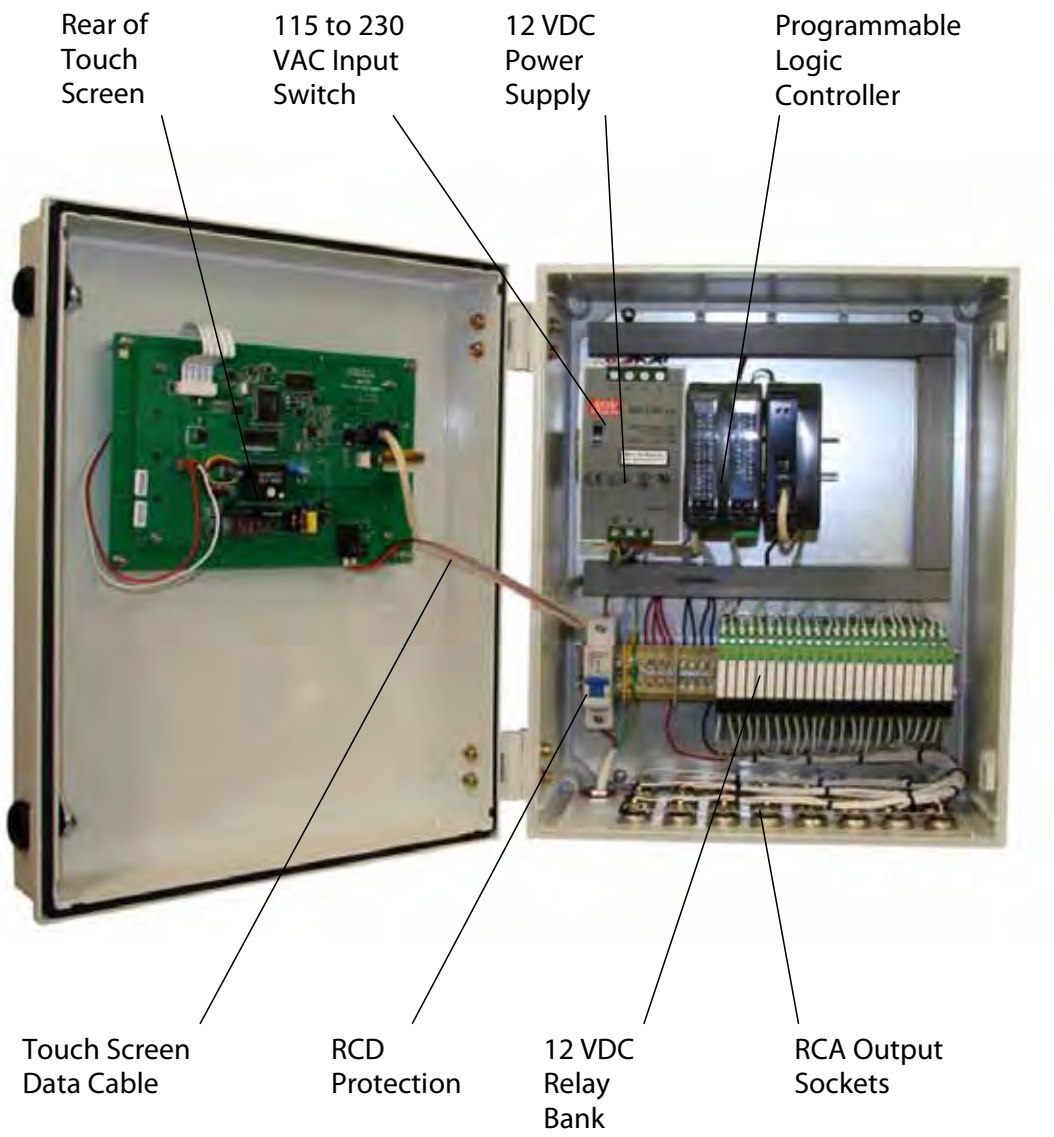
Controller Features

Controller Face



Controller Underside

Internal Controller Components



Section 1 **SAFETY**

- 115 - 230 Volt AC power is used and is a shock hazard.
- Avoid opening the front panel unless the power is turned off at the general power outlet and the plug is disconnected.
- Power leads must be kept clear of any water, and connections kept dry to avoid any short circuits.
- A circuit breaker is installed inside the controller enclosure in the event of overload or short circuit, if the controller has no power, check for the cause and fix, then reinstate circuit breaker to power on.
- Do not install controller in areas with:
  - Excessive or conductive dust,
  - Corrosive or flammable gas,
  - Moisture or rain,
  - Excessive heat,
  - Regular impact shocks,
  - Excessive vibration.
- Ensure the connectors are covered to prevent contact with "live" wires.
- The 12 Volt DC power supply has a fuse installed, ensure power is off when replacing a blown fuse.
- It is recommended that all AMD cords are located above head height to prevent trip hazards and being dropped into water.

Section 2 **BEFORE YOU START**

i) Power

- The power source is **manually** switchable 115 -230 VAC, 10 A, ensure the correct voltage is selected.
- The power source must be earthed.
- The relays and feeders are operated by a 10A - 12 Volt DC power supply.
- AMD units are rated at 24 – 50 Watts each.

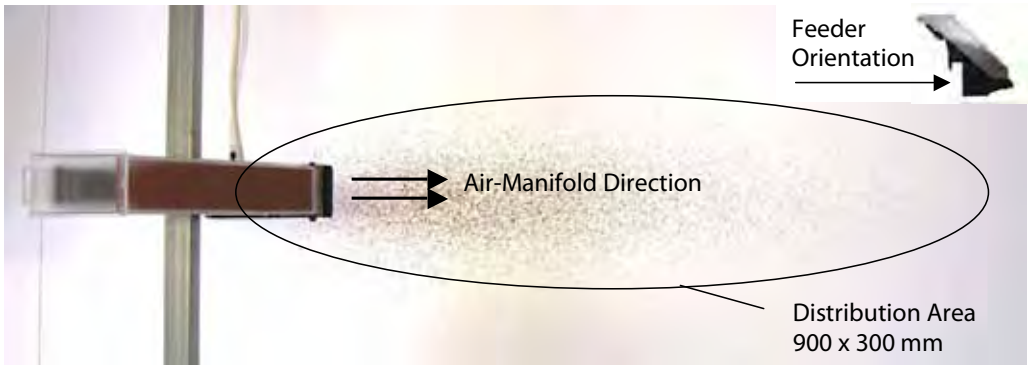
ii) Installation

- The control panel enclosure should be located off floor and bench surfaces by mounting on a wall or bracket to allow free access to the bottom of the enclosure.
- The control panel should be placed in a clean dry environment away from potential water hazards.
- AMD units should be installed a minimum of 200 mm above the water surface and should not be placed directly above aeration. AMD units should be hung from a bracket made from 25 mm square tubing that is fixed securely and level. Aluminium or stainless steel is recommended for the construction of the bracket, which can be hung from the ceiling or suspended on stands secured to the floor.
- The AMD units are supplied with a 1.5 m cord, which can be connected to an extension cord of maximum length 20 m to the control panel. Extension cords will need to have RCA plugs soldered to the ends, one female and the other male, preferably gold plated for high corrosion resistance. It is suggested that extension cables be kept dry and secure. 1mm double insulated cable is recommended for use when manufacturing these cables, in order to prevent an unnecessary voltage drop along the length. However, if shorter lengths are required a lesser-sized cable may be suitable, as long as it can carry 4 amps of 12 VDC current with negligible resistance.
- When disconnecting RCA plugs, do not pull them out using the lead, ensure that the plug is held.

Section 3 **SYSTEM OPERATION**

Setup

The AMD units should be placed ≥ 200 mm above the water surface, away from splashing and not directly over aeration or air diffusers. Each AMD unit should be installed over the tank directly above and to one side of the microdiet distribution area, as shown in the diagram below. The distribution area should be forward of the AMD unit.



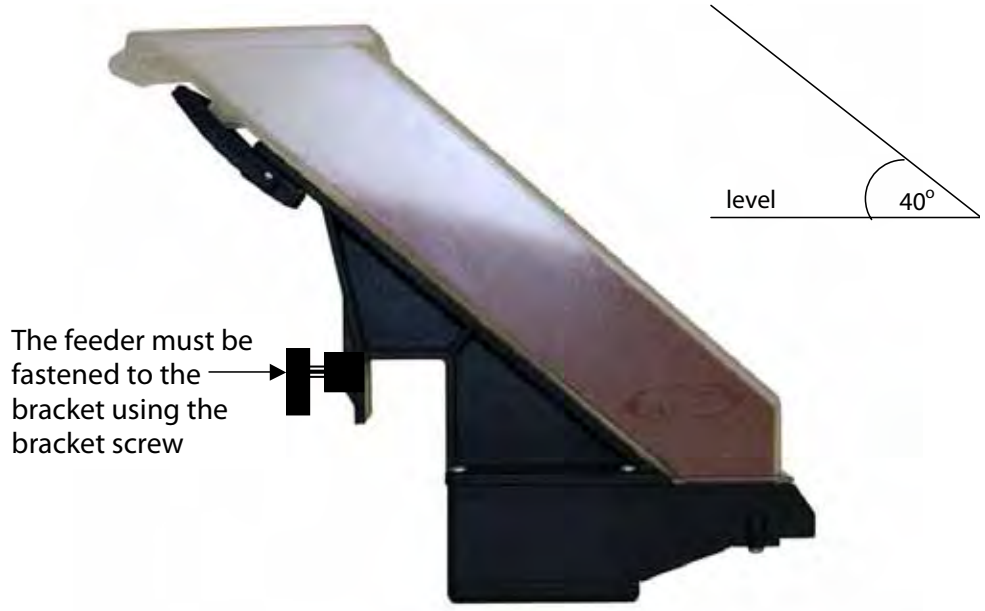
The feed distribution area for micro diet (300 µm to 500µm particle size), for an AMD set at 250 mm above surface and with 10 l min^{1} airflow to manifold.

The AMD should be directed towards the distribution area with sufficient airflow supplied to spread the microdiet evenly over the target area. If the airflow is insufficient the microdiet may clump and sink through the water column too rapidly.

The air supply should be dry in order to prevent the microdiet from clumping and sticking to the plates. If a larger microdiet distribution area is needed the AMD unit can be raised, or the airflow increased. The area covered may also depend on the microdiets physical characteristics.

When the AMD bracket is hooked over a beam ≥ 10 mm wide, it is designed to hold the feed hopper at a 40° angle from horizontal. This prevents the microdiet from being compacted onto the feeder plate and allows a more uniform allocation of microdiet for the duration of the feeding period. The beam needs to be fixed securely and level to ensure that the AMD units perform as replicate dispensers.

The microdiet should rest loosely in the feed hopper as shown in the diagram below. The microdiet should be prevented from compacting onto the feeder plate or reduced performance may occur.



Feed Types

All commercial feed types listed below have been tested and can be administered by the AMD:

- Micro particulate diets within the size range 100 µm to 2.0 mm.
- Extruded pelletized diets within the size range 100 µm to 2.0 mm.

Touch Pad Control

The controller has been factory set with the feeding program that can be manipulated through a user-friendly touch pad interface.

Display

The cover sheet will appear when power is turned on.

Press menu and select from the options available.

1. *Home* - returns to the cover page.
2. *Clock* – opens the date and clock edit page.
3. *Test Feeders* – allows you to select a feeder and manually operate a single or double action.
4. *Program* – enters the program select page that selects a program to edit.
5. *Feeders* – allows you to select a feeder and allocate a program to operate it.
6. *Start* - runs all feeding programs.
7. *Stop* - discontinues all feeding programs.

Aliquot Delivery

The aliquot size inherently varies between microdiets and is dependant on each of the individual microdiets flow characteristics. This can change with moisture content, freshness, environmental conditions, the microdiet particle type and size, the ingredients in the diet (i.e. % of lipid inclusion), and the method of manufacture. Despite these differences the aliquot size of most diets is able to be determined and therefore the amount fed over an entire feeding period can be controlled.

Generally, the diet will flow through the AMD at the slowest possible rate when a single-action feeding program is used, in conjunction with a single slot (plates 1 and 2). This program is appropriate for diets that have relatively fast flow characteristics. A double-action feeding program may be necessary when dispensing <150 µm particle sized microdiets. These diets can be sticky and have proven to be the most difficult to uniformly dispense. Maintenance on the feeders may be needed daily when using sticky diets. This involves simply shaking the diet

loose back up the hopper and allowing the diet to naturally fall back into place. A fine brush can be used to clean the plates and allow the diet to fall more freely through the openings.

Calibration

If accurate microdiet feeding is required it is suggested that the average aliquot weight of each microdiet and size class be determined. This will allow the user to calculate the required number of feeding events to dispense the daily ration within the desired time. The sum total number of events in 4 feeding periods should be equal to one day's allocation.

Note - *In order to determine an accurate average aliquot weight the first 10 to 20 aliquots should be disregarded, as they will generally be unreliable as an indication of average aliquot weight.*

The feeder should be operated 20 or more times over a container that is positioned to catch the dispensed diet. The average aliquot is then calculated.

Average aliquot weight calculation:

Average Aliquot Weight (g) = Dispensed weight (g) ÷ Number of Shots

Number of shots required per day:

Daily Shot No. = Daily Food Ration (g) ÷ Average Aliquot Weight (g)

Loading Microdiet

When loading the microdiet into the feeders there are a few simple rules that need to be followed in order for accurate and reliable allocation of the microdiet to occur.

1. The microdiet must be gently allowed to fall down the hopper and onto the feeder plate. This prevents the microdiet from being compacted onto the plate, which can cause a plug of diet to form and stop the feeder from operating. This is most important when very fine (<300 μm) diets are being used.

2. There is a maximum amount of each diet that can be reliably fed in one session. No commercial diets tested have stopped flowing through the feeder, however if a diet does stop flowing it is usually because there is too much in the hopper, which causes compaction. Therefore, if the feeding period requires more microdiet than is able to reliably flow through the AMD, then two feeding sessions in that period must be used (i.e. the feeder can be loaded in the morning and then in the afternoon to prevent overloading). The afternoon allocation is generally given more than is needed to finish off the days feed, which leaves some microdiet in the AMD units for first feeding the next day.

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Section 4 **TOUCH SCREEN CONTROLS**

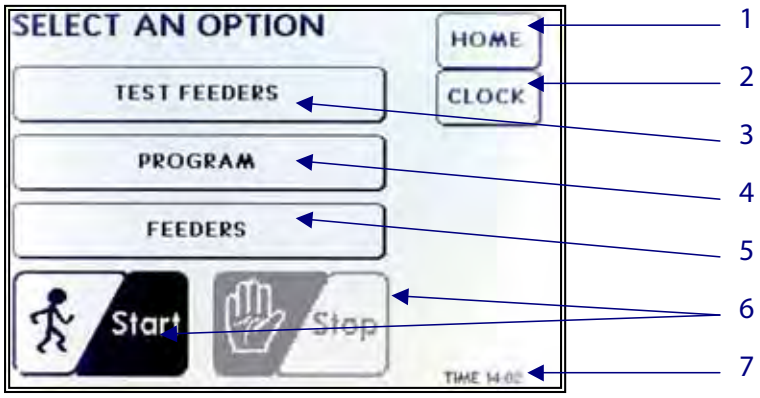
The PLC has been factory set with the feeding program that is manipulated through a touch pad interface.

Home Page Display

The home page will appear when power is turned on.

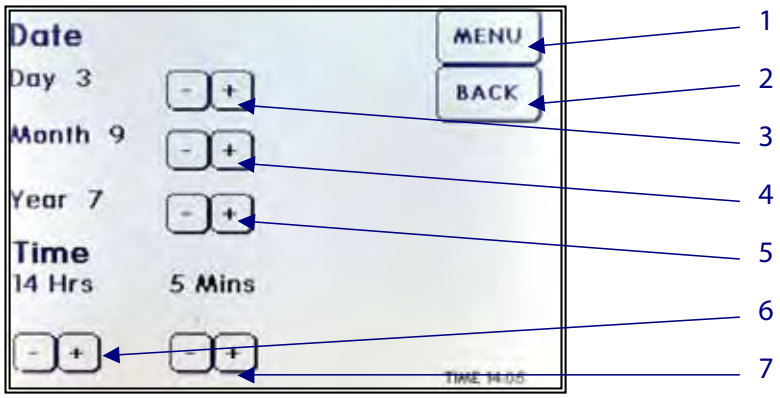
Press menu and select from the options available in the main menu.

Main Menu



1. Links to the Home Page
2. Links to the clock edit page
3. Links to the test feeders page
4. Links to the program select page
5. Links to the feeder allocation page
6. Start/Stop button.
7. Current system time is displayed on all pages.

Clock Edit



1. The menu button returns to the main menu page and has the same function on all pages where it appears.

2. The back button returns to the previous page. It has the same function on all pages that it appears.

Set date, using the "–" and "+" buttons corresponding to each value

3. Edit Day of month

4. Edit Month of year

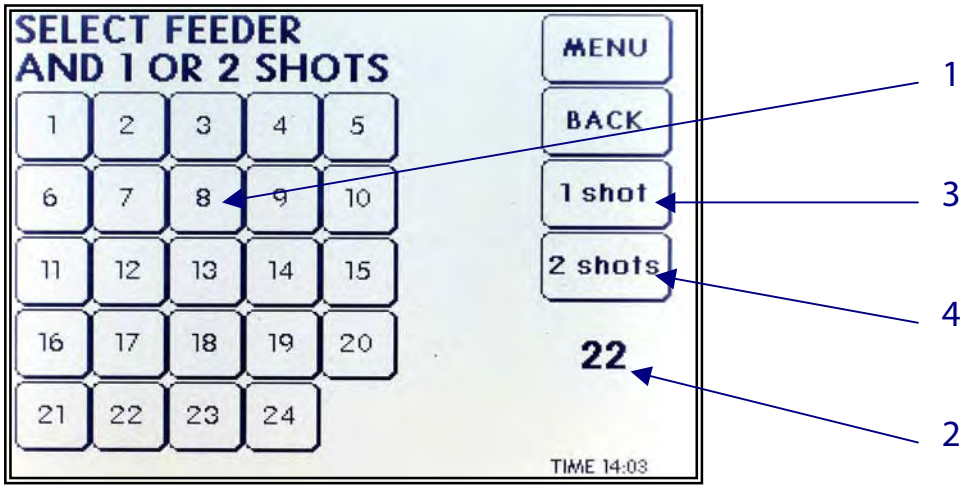
5. Edit Year

Set Time, using the "–" and "+" buttons corresponding to each value

6. Edit Hours

7. Edit Minutes

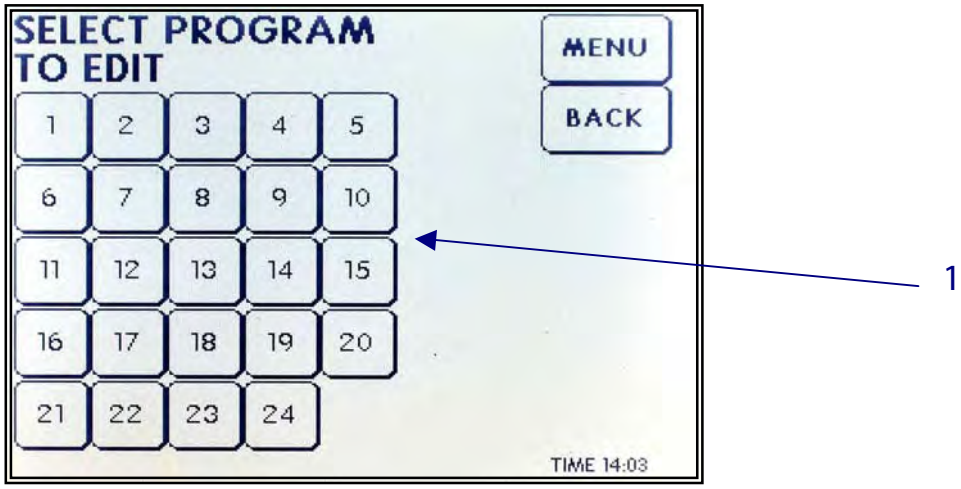
Test Feeders



1. Select AMD 1 to 24 to test.
2. The field on the bottom right will change to the currently selected feeder.
3. Press “1 Shot”, for single AMD operation
4. Press “2 Shots”, for double operation

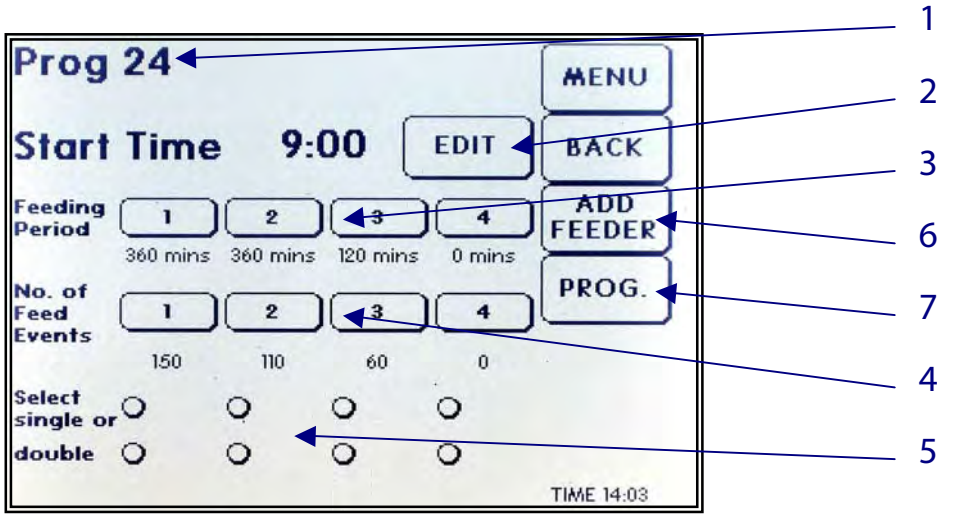
Program Edit

Program Select



1. Select program 1 to 24 to enter the corresponding program edit page

Program Page



1. Program display
2. Links to the start time edit page.

6. Links to the feeder program select page
7. Links to the select program page. It has the same function on pages where it appears.

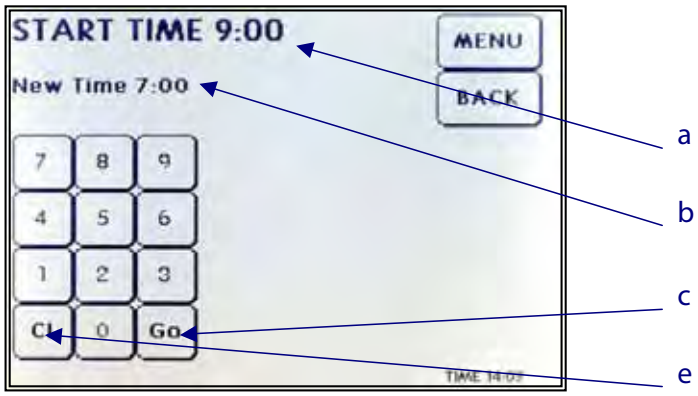
Program Display

The program number being edited (currently 24) is displayed in this field.

Start Time Edit Page

A numeric keypad allows you to enter real time 0:00 to 23:59 hrs.

Note– *If 24 hr feeding is required the start feeding time should be 0:00*



a. Current start time

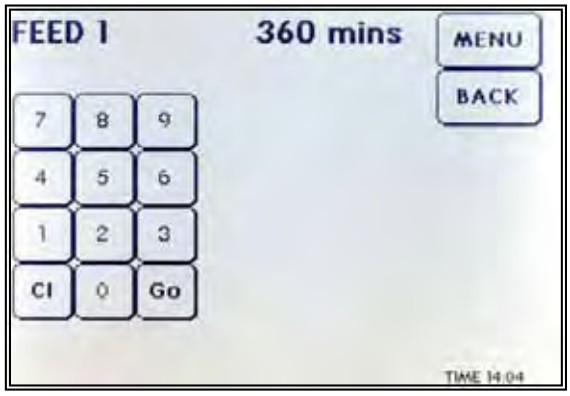
b. New start time being entered

c. "Go", confirms the new value displayed and returns to the previous page and has the same function on all pages that it appears.

d. "Cl", clears the entered value and has the same function on all pages that it appears.

Feeding period edit page

A numeric keypad allows you to enter the duration of a feeding period between 0 and 1440 minutes, for each of the feeding periods 1 to 4.



Note-

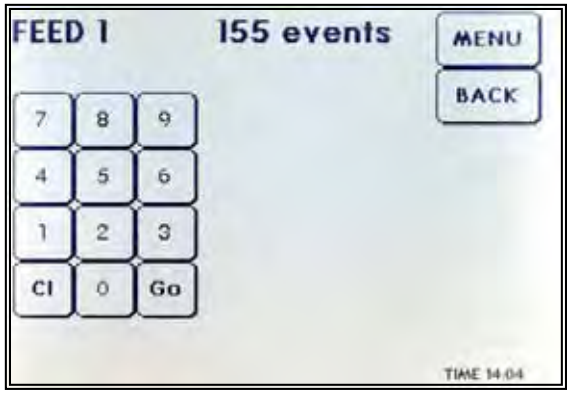
a. *The sum total of all 4 feeding periods should not be more than 1440 minutes (24 hrs).*

b. *If a single feeding period with evenly spaced feed events is required for the whole day, set periods 2, 3 and 4 to 0 min.*

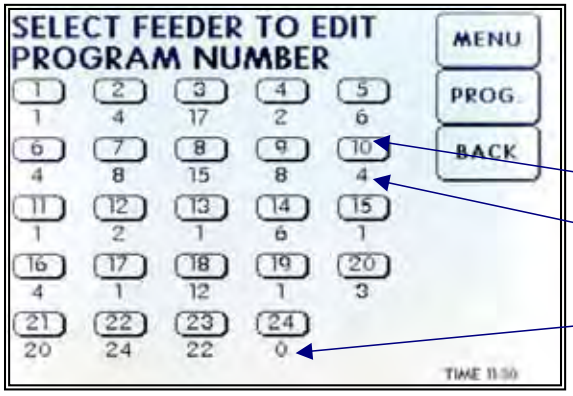
Number Of Feed Events Edit Page

A numeric keypad allows you to enter the number of feeds required in the period displayed.

Note– *The number of events in a period should not exceed the number of minutes.*



Feeder Program Allocation

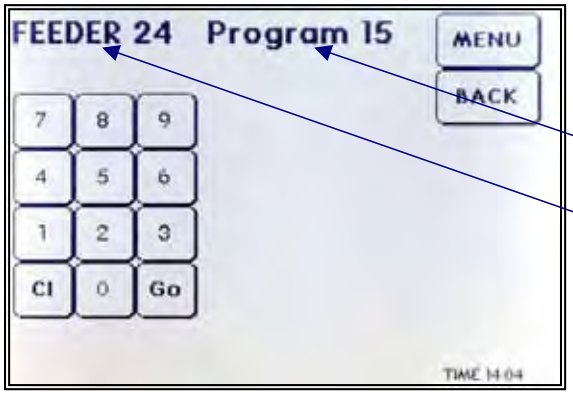


1. Links to the feeder program edit page for the AMD corresponding to the numbered button.
2. Field that displays the current feeder program allocation.
3. An individual feeder that is displaying zero is off.

Feeder program edit page

A numeric keypad allows you to allocate the selected AMD to a program.

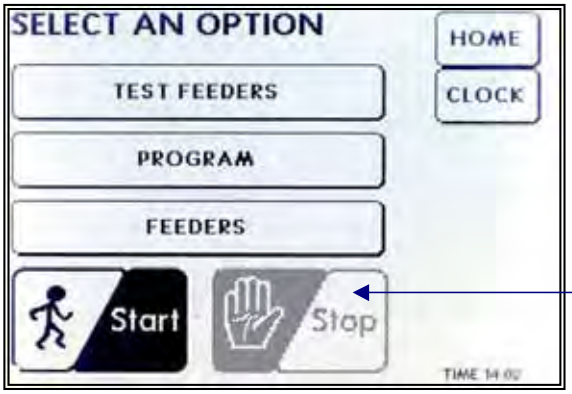
Note- *0 to 24 AMD units can be allocated to each program, however having more than 3 grouped feeders a llocated to one program may cause weak feeder operation (refer to Troubleshooting Section)* .



1. The program to which the current AMD is being allocated
2. The number of the AMD being allocated

If a value is entered outside of the allowable range a warning message will appear. To return to the edit page press back.

Start and Stop



1. When a button is "greyed out" this is the active state of the controller.

Select "Start" to begin all feeding programs.

Select "Stop" to discontinue all feeding programs.

Section 5 **MAINTENANCE**

Cleaning

Cleaning should be done every day in order to ensure reliable operation of the AMD units and prevent the build up of old diets on the working parts.

Best results are achieved with a high-pressure air nozzle. Safety glasses should be worn during this procedure. The hopper should be removed and blasted through with air, and the feeder plate and base plate should also be cleaned using this method. Any loose microdiet in the AMD can also be removed using this method.

Alternatively a soft brush can be used to clean the plates and a bottle brush for the hopper. **Do not** use a wire brush.

- If an AMD is accidentally submersed in water, turn power off, unplug AMD from control panel and flush the solenoid with deionised water to remove any salts and then use alcohol to disperse water. Dry as quickly as possible and spray with non-toxic water dispersant and anti corrosion spray.

Storage

The AMD units should be disconnected and stored in a clean dry environment when not in use. Thorough cleaning of the units should be done before storing them.

The AMD solenoids are expected to last at least 1 million repetitions, therefore correct use and maintenance should ensure long lasting service.

Section 6 **TROUBLESHOOTING**

Symptom	Fault	Solution	Notes
No Power to Controller	*Not Plugged in properly*	Check the power cord is correctly plugged into the general power outlet.	
No Power to Controller	*Circuit Breaker*	Check the circuit breaker switch is turned on. If the circuit breaker switch has been tripped check for electrical faults or short circuits (live/neutral or live/earth) before turning back on.	There is no Residual Current Device installed in this controller, therefore power should be turned off at mains before opening the enclosure.
Controller Not Operating	*Switchable power supply*	The switchable power supply needs to be manually set to the local mains voltage output 115 VAC or 230 VAC **before** operating.	The power supply should be set to the correct voltage **before** operating. Power should be turned off before opening the enclosure.
Feeder Not Dispensing	*Plate jammed*	The plate mechanism may need cleaning. The solenoid piston may need cleaning.	
Weak Feeder Operation or Not Operating	*Excessive resistance in circuit*	Ensure the connectors are clean. Ensure the connectors are completely pushed in. Check solder joins in connectors. (All feeders factory checked). Cable should be heavy enough to carry 4 Amps of current, 12VDC.	**Note**. *Cable lengths up to 35 m of 1.0 mm^2 copper core should not present any significant voltage drop (<5%), providing the solder joints are correctly soldered.* Check the circuits for resistance it should be <1.5 Ohms. Ensure that connections are made away from wet or humid environments and are kept dry and clean.
Weak Feeder Operation or Not Operating	*Overloaded Program Output*	Reduce the number of feeders operating on the individual program. Avoid operating more than 3 feeders that are grouped together on one program (refer to notes). A capacitor is in series with each feeder that supplies the initial 'inrush' current needed to energize the feeder solenoid. This prevents the power supply being overloaded. Once the solenoids are energized the power supply can cope with all feeders at once.	The programs do not operate at the same time, a 0.1 second delay mechanism is written into the software to stop this from happening. But if all 24 feeders are operating on one program the following groups of eight will operate simultaneously 1, 4, 7, 10, 13, 16, 19, & 22 together, 2, 5, 8, 11, 14, 17, 20, & 23 together and, 3, 6, 9, 12, 15, 18, 21, & 24, together.

Symptom	Fault	Solution	Notes
Weak Feeder Operation or Not Operating	*Relay switch*	Check that the relay for the output is properly inserted into the base. Check that the relay is operating and not faulty. Ensure the capacitor is functioning.	
Diet Not Being Dispensed Properly	*Diet is too large*	Ensure diets particle size is between 100 μm and 2.0 mm.	A 2.0 mm pellet diet is the largest food particle that will pass through the feeder, larger diets may result in jamming the plate dispensing mechanism.
Diet Not Being Dispensed Properly	*Diet is wet*	Ensure the feeder is not splashed by any water. Ensure the plate mechanism is kept dry. Ensure the feeder is mounted >300mm above the water surface. Ensure the feeder is not mounted above tank aeration. Only clean feeder plates with a dry soft brush or with a blast of dry compressed air. Only pass dry air through the microdiet-dispersing manifold.	
Diet Not Being Dispensed Properly	*Overloading hopper*	Ensure that the diet is not compressed into the hopper. Ensure that the hopper is filled (especially very fine diets) only to the natural fill level when the hopper is at 40 degrees from horizontal.	The food may be overly compacted onto the plates from within the hopper caused by compressing the diet into the top of the hopper or shaking the feeder excessively once loaded. The diet should be allowed to fall naturally onto the feeder plate as the hopper is filled.
Varying dispense amounts	*Low power to the feeders*	Ensure the feeder circuit is less than 1.5 Ohms. Ensure the connectors are clean. Ensure the connectors are completely pushed in. Check solder joins in connectors. (All feeders are checked before sending). Check for loose wires or dry solder joins causing intermittent faults.	

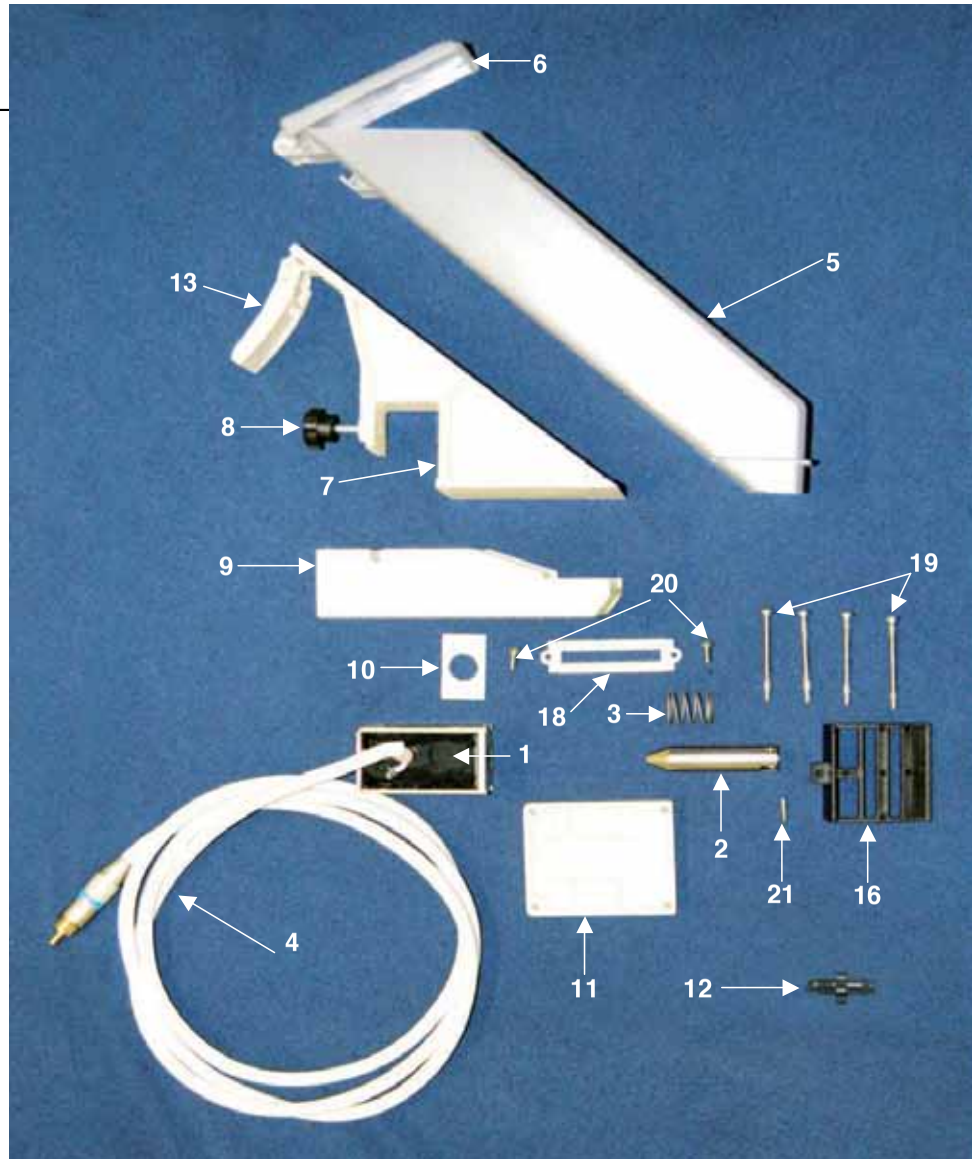
Symptom	Fault	Solution	Notes
Varying dispense amounts	*Gap variation*	Ensure the base plate screws are not loose. Ensure the feeder plate is sitting parallel with the base plate. Ensure the feeder plate screw can freely move inside the solenoid piston attachment point.	The gap is set for the plates: Thick (4,0 mm) single slot plate (blank end), 0.5 mm gap Thin (3,5 mm) single slot plate (marked I), 1.0 mm gap Double slot plate (marked II), 1.0 mm gap Triple slot plate (marked III), 1.0 mm gap A smaller gap dispenses less diet, with less variation. A larger gap dispenses more, and is less likely to fail.
Varying dispense amounts	*Feeder bracket angle*	Ensure the feeder bracket is installed so that it holds the feeder level.	40 degrees up from horizontal is the optimal angle for the hopper. If this angle varies, by as little as 5 degrees, it will make a difference to the way the feed is resting on the plate and could result in a different delivery amount. Ideally feeders should be mounted on a bracket manufactured using 25mm square steel tube, so that they are securely resting over the tank.
Varying dispense amounts	*Condensation on feeder plates*	Ensure that the feeder is mounted > 300 mm above the water surface and away from aeration bubbles. Ensure the air manifold supply is dry air.	The feeders are generally located over water, which can cause condensation on the mechanism and therefore feed can build up on the plate. This may change the amount of diet that is dispensed. It is recommended that the feeder plates be cleaned daily with a soft brush, or compressed air. This will stop build up of diet, particularly with very sticky and fine particulate diets.

Section 7 **SPECIFICATIONS**

Specifications	Controller	Feeders	Hopper
Voltage, volts	100-240 AC	12 DC	
Power, Watts		50 (max)	
Cable	1mm, double insulated, 3-core flex	1mm, double insulated, 2-core flex	
Connections	3-pin plug, 10 Amp, 240 VAC, flat earth	Male, Gold Plated RCA Connector	
Dimensions, mm	320 x 400 x 188	180 x 160 x 65	225 x 50 x 65
No of feeders	24 (base)		
Expansion modules	8		
Weight, grams	7884	450	100
Capacity, grams			250
Diet particle size, μm		100 – 2000	100 – 2000
Colours	Grey	Variety available	Opaque
Materials	Polycarbonate with touch pad screen	Body- ABS Plates- Acetal	Acrylic

Section 8 **PARTS LIST**

Part No. 1	Solenoid
Part No. 2	Solenoid piston
Part No. 3	Solenoid Spring
Part No. 4	Cable with RCA Connector
Part No. 5	Hopper
Part No. 6	Hopper lid
Part No. 7	Feeder Bracket
Part No. 8	Bracket Screw
Part No. 9	Feeder Body
Part No. 10	Piston Cover
Part No. 11	Air Manifold Feeder Base
Part No. 12	Air Manifold Nipple
Part No. 13	Hopper Clip
Part No. 14	Feeder Plate 0 (Page 5)
Part No. 15	Feeder Plate 1 (Page 5)
Part No. 16	Feeder Plate 2 (Page 5)
Part No. 17	Feeder Plate 3 (Page 5)
Part No. 18	Base Plate
Part No. 19	Body Screw (x4) (SS 304, M3 X 40 mm)
Part No. 20	Base Plate Screw (x2) (SS 304, M3 X 8 mm)
Part No. 21	Feeder Plate Grub Screw (x1) (SS 304, M3 X 10 mm)



Appendix 2 Patent international review results

PATENT COOPERATION TREATY

From the:
INTERNATIONAL SEARCHING AUTHORITY

To:

WRAY & ASSOCIATES
Level 4 The Quadrant
1 William Street
PERTH WA 6000

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

	Date of mailing *(day/month/year)* 11 JUL 2006
Applicant's or agent's file reference 118158:JHK	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/AU2006/000735	31 May 2006	31 May 2005

International Patent Classification (IPC) or both national classification and IPC
Int. Cl.

A01K 61/02 (2006.01) ***A01K 5/02*** (2006.01)

Applicant

DEPARTMENT OF FISHERIES A BODY CORPORATE EXISTING UNDER THEFISH RESOURCES MANAGEMENT ACT 1994 et al

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [X] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the IPEA/AU	Date of completion of this opinion	Authorized Officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	23 June 2006	**ALLAN SMAILES** Telephone No. (02) 6283 2154

Form PCT/ISA/237 (Cover sheet) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/AU2006/000735

Box No. I Basis of this opinion

1. With regard to the **language,** this opinion has been established on the basis of:

   [X] The international application in the language in which it was filed

   [ ] A translation of the international application into, , which is the language of a translation furnished for the purposes of international search (under Rules 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

      [ ] a sequence listing

      [ ] table(s) related to the sequence listing

   b. format of material

      [ ] on paper

      [ ] in electronic form

   c. time of filing/furnishing

      [ ] contained in the international application as filed.

      [ ] filed together with the international application in electronic form.

      [ ] furnished subsequently to this Authority for the purposes of search.

3. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Form PCT/ISA/237 (Box No. I) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/AU2006/000735

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	**13**	**YES**
	Claims	**1-12, 14**	**NO**
Inventive step (IS)	Claims	**13**	**YES**
	Claims	**1-12, 14**	**NO**
Industrial applicability (IA)	Claims	**1-14**	**YES**
	Claims		**NO**

2. Citations and explanations:

D1: US 6715442 B1 (BELLOMA) 6 April 2004
D2: US 4437595 A (Stevens et al.) 20 March 1984
D3: JP 10-098975 A (DAINICHI KOGYO KK) 21 April 1998
D4: US 5199381 A (MASOPUST) 6 April 1993
D5: US 2800256 A (DI NUZZO) 23 July 1957

A. NOVELTY – Claims 1-12, 14

A.1. Claims 1, 9, 14 lacks novelty in the light of each one of D1-D4, for example:

A.1.1. D1 discloses a food dispenser (10) for delivering food material in particulate form (120), comprising a receptacle for holding the food material (90), a shutter means (plates 110, 114 and holes 152, 154) at the lower end of the receptacle, moveable between an open and a closed position (by aligning or mis-aligning the holes) to provide a passageway for delivery of the food from the receptacle past the shutter when in the open position (see figures & abstract).

A.2. Claim 2, 12 lacks novelty in the light of each one of D1-D3, for example:

A.2.1. D1 discloses the shutter having two plates for relative slidable movement as outlined at A.1.1.

A.2.2. Note that for D2 the sloped sides (16) of the hoper comprise one of the plates having two apertures (18) as seen in figure 4, that align with two holes (28) in sliding plate (26).

A.2.3. Note that for D3 the two shutter plates are the flat base of the hopper (2) and the sliding plate (5). The two plates have "a plurality of apertures" one hole (3) in the base of the hopper, and two in the plate (5) that can be aligned with hole (3).

A.3. Claim 3 lacks novelty in the light of each one of D1-D3, for example D1 discloses the features of claim 3 as already outlined at A.1 and A.2 above.

A.4. Claim 4 lacks novelty in the light of each one of D2-D3. Each disclose wall of the container extending upwardly at an angle from the vertical.

A.5. Claims 5, 11 lacks novelty in the light of each of D1 and D3:

A.5.1. D1 discloses a spreader (16) for dispersing the food material once it has passed through the passageway.

A.5.2. D3 discloses an air blower (11) to disperse the particulate material.

A.6. Claims 6, 10 lacks novelty in the light of D1: The receptacle of D1 is mounted so as to shake from side to side as the shutter holes are aligned and mis-aligned.

Continued in supplemental Box

Form PCT/ISA/237 (Box No. V) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/AU2006/000735

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. Claim 4 lacks clarity – there is no antecedent to "the tube".
2. Claims 5 and 11 lack clarity as they are each drafted as two sentences. In each case the second sentence is taken to add nothing to the scope of the claim and is considered merely as indicating an optional feature.

Form PCT/ISA/237 (Box No. VIII) (April 2005)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International Application No.
PCT/AU2006/000735

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of: **BOX V**

A.7. Claim 7 lacks novelty in the light of each of D1-D4, for example, D1 discloses pneumatic ram (26) for repetitively moving the shutter means between open and closed positions.

A.8. Claim 8 lacks novelty in the light of each of D1-D4, for example, D1 discloses a controller coupled to the actuator (see column 6, line 28-33).

B. INVENTIVE STEP – Claims 1-12, 14

B.1. Claims 1-12, 14 as above.

B.2. Furthermore, claim 4 lacks an inventive step in the light of D1. It is considered that having the food receptacle at an angle to improve the flow of the food material would be an obvious improvement to make to the teaching of D1.

B.3. Claims 5 and 11 lack an inventive step in the light of either one of D2 or D4 in combination with D3. D3 teaches the use of a fan to disperse the food particles, and this would be an obvious feature to add to the food dispenser of either D2 or D4 to better disperse the food.

B.4. Claims 6 and 10 lack an inventive step in the light of D2 when further combined with the teaching of D5. D5 column 2, line 47-54 and column 3 line 20-25 discloses a food dispenser which is mounted such that the operation of the sliding dispenser plate induces vibrations in the food receptacle. It would be obvious to add this feature to the teaching of D2 to improve the flow of material in the food receptacle.

Form PCT/ISA/237 (Supplemental Box)(April 2005)

Appendix 3 Feeding system patent as registered

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

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PCT

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A01K 61/02 (2006.01) *A01K 5/02* (2006.01)

(21) International Application Number:
PCT/AU2006/000735

(22) International Filing Date: 31 May 2006 (31.05.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2005902784 31 May 2005 (31.05.2005) AU

(71) Applicants *(for all designated States except US)*: **DEPARTMENT OF FISHERIES A BODY CORPORATE EXISTING UNDER THE FISH RESOURCES MANAGEMENT ACT 1994** [AU/AU]; 168 St Georges Terrace, Perth, Western Australia 6000 (AU). **FISHERIES RESEARCH AND DEVELOPMENT CORPORATION A BODY CORPORATE EXISTING UNDER PRIMARY INDUSTRY AND ENERGY R & D ACT 1987** [AU/AU]; Po Box 222, Darkin West, Australian Capital Territory 2600 (AU).

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(74) Agent: **WRAY & ASSOCIATES**; Level 4, The Quadrant, 1 William Street, Perth, Western Australia 6000 (AU).

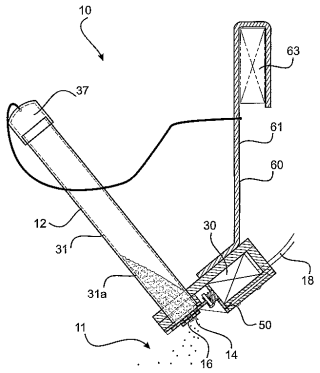
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FOOD DISPENSERS AND AQUACULTURE SYSTEMS



(57) Abstract: A food dispenser (10) with particular application to delivering a controlled amount of food material (11) in particulate or powdered form to fish and other aquatic animals, and also a method of delivery of such material. The food dispenser comprises a receptacle (12) for holding the food material and shutter means (14) located at a lower end of the receptacle. In its open position, the shutter provides a passageway (16) from the receptacle past the shutter for delivery of the food material. A dispersing unit (50) supplies an air flow for dispersing the food material. A flexible mounting bracket (60) enables vibration of the dispenser during operation to assist the flow of the food material.

WO 2006/128234 A1

"Food Dispensers and Aquaculture Systems"

Field of the Invention

The present invention relates to food dispensers.

The invention has been devised particularly, although not solely, as a food dispenser for delivering a controlled amount of micro-particulate material to aquatic animals in an aquaculture system.

Background Art

The expansion of commercial farming of aquatic species of fish and crustaceans has seen continued research into methods and apparatus for dispensing food. In recent years there has been a general replacement of live foods with formulated feeds. This has occurred primarily because the nutritional requirements of aquatic animals have been experimentally determined and it has been possible to formulate feeds as required.

Formulate feeds for the early stages of fish are typically provided in the form of micro-particulate food material that remains soluble in the aquaculture system for a sufficient period of time, and is of a size which allows for the food material to be readily consumed.

The manner in which the micro-particulate food material is dispensed provides several problems each of which relate to how, when and in what form the material is presented. Generally, the success of a particular feeding regime is measured in terms of the growth and survival rate of the aquatic animals. The manual effort required in maintaining and monitoring the aquaculture system is also a relevant factor.

Quantitative requirements including the water temperature, water depth, food type, food particle size, feeding frequency and so on, each have an effect on the growth and survival rate of the animals being reared.

Advantageous conditions for the growth, especially for fish and crustacean larvae, remains an area of continued research in which the feeding regime is of particular importance.

It would be of benefit if it were possible to experiment with several micro-particulate feeding regimes and to determine several advantageous methods of presenting the food material for subsequent consumption. It would also be advantageous if feeding regimes could be performed with control and reliability.

It is against this background and the problems and deficiencies associated therewith that the present invention has been developed.

Disclosure of the Invention

According to a first aspect of the invention there is provided a food dispenser for delivering an amount food material in particulate or powdered form comprising: a receptacle for holding the food material; and shutter means located at a lower end of the receptacle, the shutter means being moveable between an open position and a closed position; wherein upon movement from the closed position to the open position there is provided a passageway from the receptacle past the shutter means for delivery of the food material therethrough.

Preferably, the shutter means comprises at least two plates arranged for relative slidable movement between the closed and open positions, the two plates having a plurality of apertures which when in the closed position are not aligned (comment: they are aligned in a closed position) and which when in the open position are aligned so as to provide the passageway.

Preferably, the receptacle comprises a tube extending upwardly from the shutter means such that food material container in the tube can gravity feed past the shutter means.

Preferably, the food dispenser includes mounting means configured for mounting the food dispenser to a structure with the tube extending upwardly from the shutter means at angle inclined away from vertical. The food material may rest loosely in the tube along a lower side thereof. The angle from vertical may between 30 and 60 degrees from vertical. By reducing the amount of food material directly above the shutter compaction is reduced preventing the formation of a plug of compressed feed.

Preferably, the food dispenser includes a dispersion means for dispersing the food material over an area, once the food has passed through the passageway. The dispersion means may comprise an air flow delivery means arranged beneath the shutter means for blowing the food material over the area.

The mounting means may be configured for allowing the receptacle to shake upon movement of the shutter means. This may loosen the food material to avoid compaction of the food material above the shutter means.

The food dispenser may include an actuator for repetitively moving the shutter means between the open and closed positions. The food dispenser may include a controller coupled to the actuator for moving the shutter means according to predetermined criteria so as to controllably deliver the food material. Preferably, repetitively moving the shutter means causes vibrations which are transmitted to and shake the receptacle.

According to a second aspect of the invention there is provided a method of dispensing food material in particulate or powdered form including: holding the food material in a receptacle; moving a shutter means from a closed position to an open position to provide a passageway through which the food material can pass from the receptacle; allowing the food material to pass through the passageway; and moving the shutter means from the open position to the closed position to stop the passage of the food material.

Preferably, the method includes shaking the receptacle by having the receptacle mounted to a structure such that vibrations associated with moving the shutter means travel to the receptacle and loosen the food material.

Preferably, the method includes dispersing the food material over an area once the food material has passed through the passageway. Preferably, dispersing the food material includes subjecting the food material to an airflow.

Preferably, the method includes aligning a plurality of holes in the shutter means to provide the passageway and allow for the food material to pass therethrough.

Insight into the advantages and characteristics of the present invention can be gained from the following description of preferred embodiments and the accompanying drawings. Further aspects and preferred features may be apparent.

Brief Description of the Drawings

The invention will be better understood by reference to the following description of one specific embodiments thereof, as shown in the accompanying drawings in which:

Figure 1 is a cross-sectional view in elevation of a food dispenser according to the embodiment of the invention;

Figure 2 is an enlarged cross-sectional view in elevation of the food disperser in use;

Figure 3 is a fragmentary elevational view of the food dispenser in an open condition for dispensing food;

Figure 4 is a fragmentary elevational view of the food dispenser in a first (normal) closed condition;

Figure 5 is a fragmentary elevational view of the food dispenser in a second closed condition, momentarily interrupting dispensing of food;

Figure 6 is plan view of an upper plate constituting a first component of shutter means used in the food dispenser;

Figure 7 is a plan view of a lower plate constituting a second component of the shutter means used in the food dispenser;

Figure 8 is a side view in elevation of a body constituting part of the food dispenser;

Figure 9 is a plan view of the body;

Figure10 is rear view of the body; and

Figure 11 is a front cross sectional view of the body taken along the plane designated 11-11 in Figure 8;

Best Mode(s) for Carrying Out the Invention

Referring to the drawings there is shown a food dispenser 10 according to a preferred embodiment of the invention. The food dispenser 10 is configured for periodically administering a controlled micro-diet to a larvae culture tank. The micro-diet comprises formulated feed.

The food dispenser 10 includes a receptacle 12 for holding the formulated feed. A number of commercial feed types may be held in the receptacle 12 including Gemma Micro 150 and 300; Gemma 0.3, Skretting crumble 600 and 1 mm [Skretting]; Proton 1, 2/4 and 3/5; NRD 3/5, 4/6 and 5/8 [Inve].

A shutter means 14 is located at a lower end of the receptacle 12 and is moveable between an open position and a closed position. Upon movement from the closed position to the open position, the shutter means 14 provides a passageway 16 from the receptacle 12 past the shutter means 14 for the passage and delivery of

the feed.

A control interface means 18 is provided such that the food dispenser 10 can be coupled to a programmable logic controller which is able to spread the allocation of micro-particulate feed across the whole day. This prevents the need to manually feed the larvae, and provides a more constant availability of the feed across the whole photoperiod. This reduces the opportunity for bacterial proliferation because of unconsumed feed which can spoil the aquaculture system.

The shutter means 14 comprises an upper plate 20 and a lower plate 22 which are arranged for relative slidable movement between the closed and open positions. More particularly, the upper plate 20 is movable (and hence is the active plate) and the lower plate 22 is fixed (and hence is the inactive plate). Each the plates 20 and 22 include a plurality of apertures 24 which when in the closed position are not aligned and which when in the open position are aligned so as to provide the passageway 16. In this embodiment the passageway 16 comprises two channels 28.

The upper and lower plates 20 and 22 interact loosely for a loose action therebetween in order to stop shear compression of the feed as it passes therethrough. Shear action on the feed causes the feed to compact at the end of the receptacle12 between the plates 20, 22which would otherwise block the feeder 10.

The shutter means 14 further includes a piston solenoid 30 having actuation arm 40which is connected to the upper plate 20 through a hole using pin 21. By moving the solenoid 30, the upper plate 20 can be pulled across the bottom of the receptacle 12. Typically, the solenoid 30 is a low voltage solenoid, such as 12 or 24 volts DC.

The receptacle 12 comprises a conduit defining a tube 31 which can contain the feed and which is made from transparent material allowing for the quantity of remaining feed to be readily viewed. In this embodiment, the tube 31 comprises a

PVC tube of 25mm internal diameter. Other tubes with different dimensions can be fitted. The tube is closed at its upper end by a removable closure configured as a cap 37.

Figure 6 shows the upper plate 20 in plan. The upper plate 20 provides the plurality of apertures 24 therein as four rectangular slots 32 each of 5mm in width in this embodiment. Figure 7 shows the lower plate 22 in plan. The lower plate 22 provides the plurality of apertures 24 therein as two rectangular slots 34 although in other embodiments it may include four to match the upper plate 20. The lower plate 22 also provides bars 36 adjacent the slots 34. Having four slots instead of two would increase the amount of micro-particulate material delivered in one oscillation from the closed position to the open position and back to the closed position. No it wouldn't. You could add one extra slot to the existing base plate, increase the tube aperture and get more food, without modifying the top plate. To have anymore than three slots in the base plate would require additional slots in the top plate.

The lower plate 22 includes two mounting holes 35 which allow for the lower plate 22 to be readily changed as required.

When the shutter means 14 is in a closed position, the slots 32 of the upper plate 20 overlap with bars 36 on the lower plate 22, thus preventing the feed from falling therethrough. In operation, the upper plate 20 is moved by the solenoid 30 between the bottom of the feed tube 31 and the lower plate 22. As a whole the shutter means moves between open and closed positions.

A spring 38 (not very visible in drawings)disposed around the actuation arm 40 of the solenoid 30 biases the actuation arm towards the outermost condition. In this way, the upper plate 20 is so biased that the shutter 12 is normally in the closed position, as shown in Figures 1 and 4. The extent of outward movement of the actuation arm 40 is limited by abutment with the body 41, as best seen in Figure 4. When the solenoid 30 moves the actuation arm 40 to pull the upper plate 20, the slots 32 therein align with the slots 34 of the lower plate providing the two channels 28. In this embodiment is spring 38 is made of stainless steel.

As noted the receptacle 12 comprises the tube 31 or conduit 20 that extends upwardly from the shutter means 14. In this manner, when the receptacle 12 is filled with the feed, the portion of the feed at the lower end of the receptacle 12 has a further portion of feed thereabove. Thus when the two channels 28 are provided by moving the upper plate 20 the feed material moves past the shutter means 14 with the assistance of its own weight; that is, the feed is gravity fed to the shutter means 14.

The shutter means 14 can be moved multiple times in any one feeding event. The number of times is predetermined according to the particular flow characteristics of the particular feed and the total amount required of feed required during a feeding event. The plurality of apertures and the control of the piston allows precise repetitive allocation of the feed amount per feed event.

The dispenser 10 includes a body 41 which provides a frame to support the plates 20 and 22, the tube 31 and the solenoid 30. The body 41 includes a portion 42 incorporating a socket 45 into which the tube 31can be received at the lower end thereof. The socket 45 has an annular lip 46 at its inner end against which the lower end of the tube 31 locates The annular lip 46 defines an opening 47 which corresponds to the interior of the tube 31. The portion 42 has a lower face 48 below which the upper plate 20 slides. The lower plate 22 is fixed with respect to the body 41 and is releasably attached thereto by fasteners such as screws 23 inserted in the mounting holes 35. The spacing between the mounting holes 35 is such that the upper plate 20 can be accommodated between the screws 23 without being impeded thereby.

The body 41 also includes a portion 43 defining a cavity 49 which houses the solenoid 30..

A dispersing unit 50 for dispersing the feed over an area, once the feed has passed through the shutter means 14, is provided at a lower end of the frame 42. The dispersing unit 50 includes an air flow delivery means 51 arranged to delivery an air stream beneath the shutter means 14 for blowing the food material. The air flow delivery means 51 comprises a pluratity of outlets 53 formed in a bottom wall

55 of the portion 43 of the body 41. The outlets 53 provide a relatively consistent air flow for dispersing and breaking up the feed. The outlets 53 communicate with a manifold 57 incorpoarated within the bottom wall 55. The manifold 57 receives air for supply to the outlets 53 from a flow path 58 which is incorporated in bottom wall 55 and which is coupled to a source of pressurised air (not shown) by an air supply line 59.

A programmable logic controller (PLC) is connected to the food dispenser 10 via the control interface means 18. In this embodiment, the logic controller includes a power source operating at 100 to 240 Volts AC with 10 Amps (not that important but the same Mitsubishi Alpha is also available in 24 V DC) . The PLC includes a plurality of relays for a corresponding number of food dispensers each equivalent to food dispenser 10. The relays and provide a 12 Volt DC power supply and are rated at 24 to 48 Watts in this embodiment.

The food dispenser 10 is installed above the water surface of an aquaculture tank and is spaced part from any aeration or air diffusers. A distance of about 200 mm above the water surface has been found to be appropriate. With this arrangement, the dispersing unit 50 provides a dispersion area on the water surface. The food dispenser 10 is directed towards the dispersion area with sufficient air pressure supplied to spread the feed fairly evenly over the dispersion area If the air pressure is insufficient the feed may clump and sink through the water column below the dispersion area too rapidly. The air pressure will vary with embodiments and can be generally optimized by routine trial. Micro-particulate feed particles 11 are shown being dispersed in Figures 2 and 3. The air outlets 50 serve to break up the feed.

It is also to be appreciated that the air supply should be dry so as to prevent the feed from clumping and sticking to the upper and lower plates 20, 22. If a wider dispersion area is required then the dispenser can be installed at a greater distance above the water surface of the aquaculture system. Alternatively the speed or volume of the airflow can increased or modified. The physical characteristics of the feed will determine the required amount.

The food dispenser 10 includes mounting means 60 provided in the form of a flexible bracket 61. The flexible bracket 61 is configured for mounting the food dispenser 10 to a beam 63 such that the elongate tube 31 extends upwardly from the shutter means 14 at angle inclined away from vertical. In this arrangement the bracket 61 is designed to hold the tube 31 at angle included at about 45° away from vertical. The configuration of the flexible bracket 61 ensures that the receptacle 12 is allowed to shake upon movement of the shutter means 14 deliver the feed. This has been seen to advantageously prevent the accumulation of feed in the workings of the shutter means 14. Thus while the upper plate 20 moves back and forth a number of times to deliver a quantity of feed in a feed event, vibrations associated therewith, from the solenoid 30 or plates 20 and 22, or otherwise, are transmitted to the tube 31.This is prevents the feed from being compacted on the upper plate 20 and allows a more uniform distribution and allocation of feed during of the feeding period.

Advantageously, the feed rests loosely in the tube 31 along an inside lower side 31a, as shown in the drawings. By its angular disposition the feed is prevented from compacting on the upper plate 20.

In addition to the commercial feed types mentioned above a number of experimental feed types have been passed successfully through the feeder 10. These were made by oven drying extruded pellets. The pellets were then ground and sieved to suitable particle size ranges. Particle size ranges included, 100 - 300 µm, 300 - 500 µm, 500 - 780 um, 780 µm - 1.0 mm and 1.0 - 1.5 mm. Other sizes may of course be suitable. To assist with maximizing performance compacting on the upper plate 20 should be avoided.

The programmable logic controller is factory set with a periodic feeding program for a particular fish larvae species, the feeding program providing a predetermined feeding regime. The time between feeding events can be readily changed using a personal computer software interface.

It may be desirable to stop all the feeders from operating at one time at the start of each feeding period. For this purpose an onblock delay routine is provided.

The number of upper plate 20 oscillations per feeding event may be adjusted to vary and control the amount of feed delivered to an aquaculture tank over a feed event.

The amount of feed delivered per feed event, (i.e. the aliquot size), will inherently depend on the feed flow characteristics, which are known to vary with the particle size, the constituents of the feed and so on. Despite this the controller is able to control the feeder 10 via the control interface 18 such that the aliquot size can be substantially predetermined.

Generally, when the upper plate moves over a single oscillation during a feeding event, that is when the shutter 14 moves from the closed position to the open position and back to the open position, the feed will flow through from the receptacle 12 at a relatively slow rate. A single oscillation is generally appropriate for diets that have relatively fast flow characteristics, such as Proton 2/4 or Gemma 0.3. If a double or multiple-action feeding program is used with these feeds then although they will flow quite freely through the shutter means 14 over feeding may result if the solenoid is not operated quickly.

A double oscillation per feeding event is generally necessary when delivering Gemma Micro 150 to the larvae culture tanks. In one arrangement Gemma Micro 150 is delivered at a rate of 0.025 g per feeding event using a double plate action.

With Gemma Micro 150 and some smaller experimental feeds the shaking action on the conduit has been seen to be most advantageous. The shaking has been seen to shake the feed loose back up the tube 31and then allow for the feed to naturally fall back into place. It has been seen that vibration on the first action to shake the feed down can result in more than twice the aliquot being delivered. If larger amounts are needed per feeding event, more plate actions can be used. Also the number of slots can varied coupled with the conduit 12 aperture being increased. The feeder program will need to be calibrated to individual feeds, with their flow characteristics in mind.

Some smaller particulate feeds have been seen to pass through the dispenser 10

in smaller aliquots than larger feeds. This is thought to arise because the higher surface area of the smaller particulate feeds creates a higher coefficient of friction that results in slower flow characteristics. By measuring the amount of feed in the tube 31 the amount of feed delivered per feeding cycle can be accurately predetermined. This enables the PLC to be programmed to deliver the right amount of feed within the correct time frame.

The method of operation is relatively simple. The tube 31 is opened at its upper end by removal of cap 37 and the feed gently allowed to fall down the feed tube and onto the upper feeder plate 20 such that the feed is not compacted. The use of a funnel is recommended to avoid spillage. After this the PLC is used to set the feed events by specifying a specific date and time. It is possible to set the duration or number of cycles per feeding event.

The PLC can be programmed to deliver a few larger doses at prescribed times, for example 3 morning feeding events at 15 min intervals and 3 afternoon feeding events at 20 min intervals. Typically the solenoid 30 is operated for 0.3 seconds "on" and 0.1 seconds "off" for multiple action, during any one feeding oscillation. This moves the shutter means 14 to the open position and allows the spring 38 to move the shutter means 14 back to the closed position. Multiple oscillations are performed when larger amounts of feed are required at any one time. In one arrangement feed events may occur every 10 minutes and deliver 0.2 milligrams of feed per event.

In addition to energising the solenoid 30 for fractions of a second to accurately control the amount of feed per feeding event the PLC has the facility to supply adjunct lighting and system maintenance equipment which allows for the complete synchronisation of the aquaculture system as a whole.

Thus the dispenser 10 dispenses the feed by first holding the feed in the receptacle 12. Then the passageway 26 is provided through which the food material can pass from the receptacle 12. This is performed by moving the shutter means 14 from the closed position to the open position. The feed then passes through the passageway 16 and is dispersed over an area. After the

cycle the shutter means is moved from the open position to the closed position to stop the passage of the food material. After many cycles the dispenser 10 can be readily cleaned ensure reliable operation. This can be done with a high-pressure air nozzle.

It will be understood that various changes may be made to the form, details, arrangement and proportion of the various parts and steps without departing from the spirit and scope of the invention. For example the feed may be powdered. The mounting means may be hung from a cord connected to a beam. The lower plate be connected to the solenoid and the upper plate held stationary. The feeder may be used in a marine hatchery environment. Modifications and variations such as would be apparent to the skilled addressee are, at the very least, considered to fall within the scope of the present invention, of which the preferred embodiment described herein is one specific example.

Throughout the specification, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

The Claims Defining the Invention are as Follows:

1. A food dispenser for delivering an amount of food material in particulate or powdered form comprising: a receptacle for holding the food material; and a shutter means located at a lower end of the receptacle, the shutter means being movable between an open position and a closed position; wherein upon movement from the closed position to the open position there is provided a passageway from the receptacle past the shutter means for delivery of the food material therethrough.

2. A food dispenser according to claim 1 wherein the shutter means comprises at least two plates arranged for relative slidable movement between the closed and open positions, the two plates having a plurality of apertures which when in the closed position are not aligned and which when in the open position are aligned so as to provide the passageway.

3. A food dispenser according to claim 1 or 2 wherein the shutter means comprises at least two plates arranged for relative slidable movement between the closed and open positions, the two plates having a plurality of apertures which when in the closed position are not aligned and which when in the open position are aligned so as to provide the passageway.

4. A food dispenser according to claim 3 further comprising the food dispenser includes mounting means configured for mounting the food dispenser to a structure with the tube extending upwardly from the shutter means at angle inclined away from vertical.

5. A food dispenser according to any one of the preceding claims further comprising the food dispenser includes a dispersion means for dispersing the food material over an area, once the food has passed through the passageway. The dispersion means may comprise an air flow delivery means arranged beneath the shutter means for blowing the food material over the area.

6. A food dispenser according to claim 5 wherein mounting means is configured for allowing the receptacle to shake upon movement of the shutter means.

7. A food dispenser according to any one of the preceding claims further comprising an actuator for repetitively moving the shutter means between the open and closed positions.

8. A food dispenser according to claim 7 further comprising a controller coupled to the actuator for moving the shutter means according to predetermined criteria so as to controllably deliver the food material.

9. A method of dispensing food material in particulate or powdered form including: holding the food material in a receptacle; moving a shutter means from a closed position to an open position to provide a passageway through which the food material can pass from the receptacle; allowing the food material to pass through the passageway; and moving the shutter means from the open position to the closed position to stop the passage of the food material.

10. A method according to claim 9 further including shaking the receptacle by having the receptacle mounted to a structure such that vibrations associated with moving the shutter means travel to the receptacle and loosen the food material.

11. A method according to claim 9 or 10 further including dispersing the food material over an area once the food material has passed through the passageway. Preferably, dispersing the food material includes subjecting the food material to an airflow.

12. A method according to claim 9, 10 or 11 further including aligning a plurality of holes in the shutter means to provide the passageway and allow for the food material to pass therethrough.

13. A food dispenser substantially as herein described with reference to the accompanying drawings.

14. A method of dispersing food material in particulate or powered form substantially as herein described.

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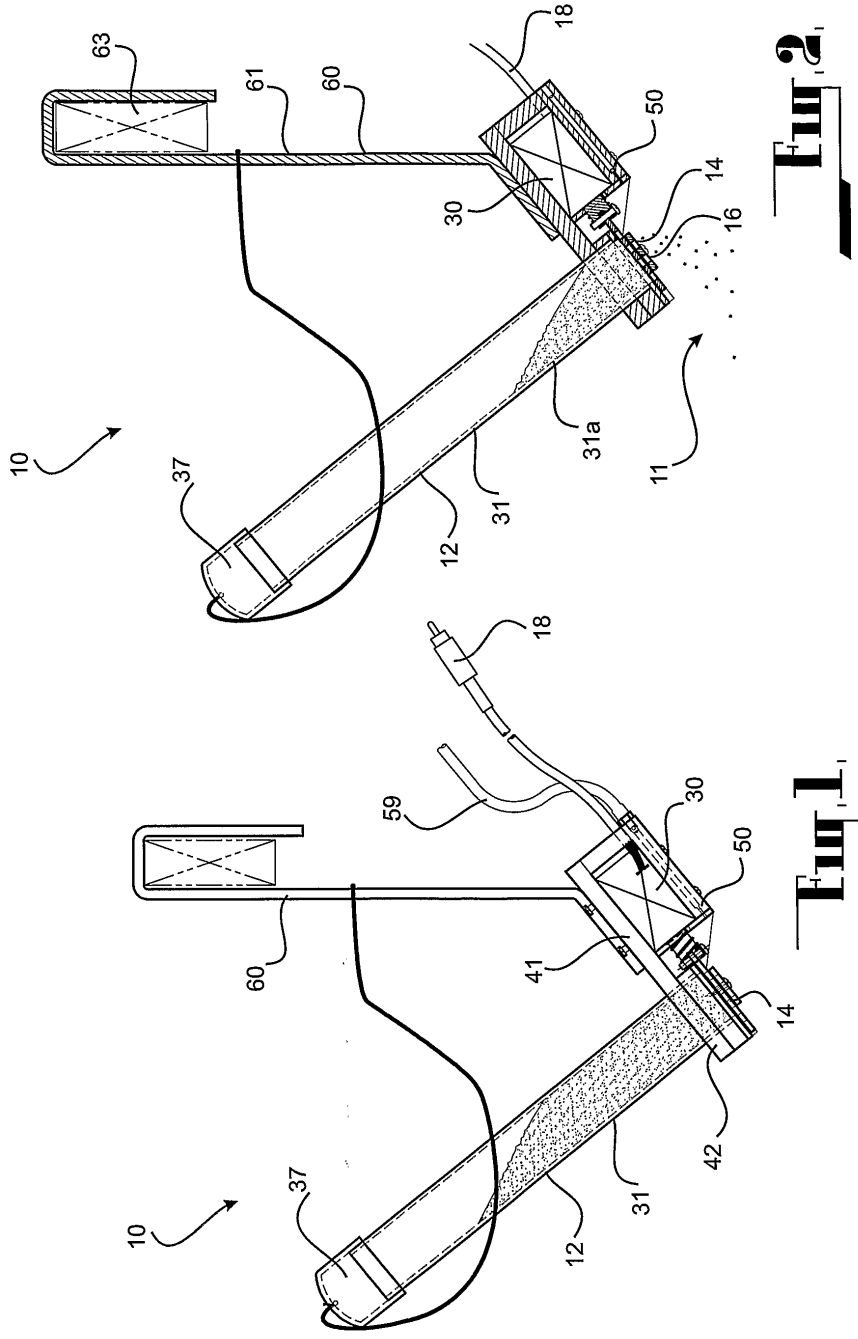
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Fig. 2

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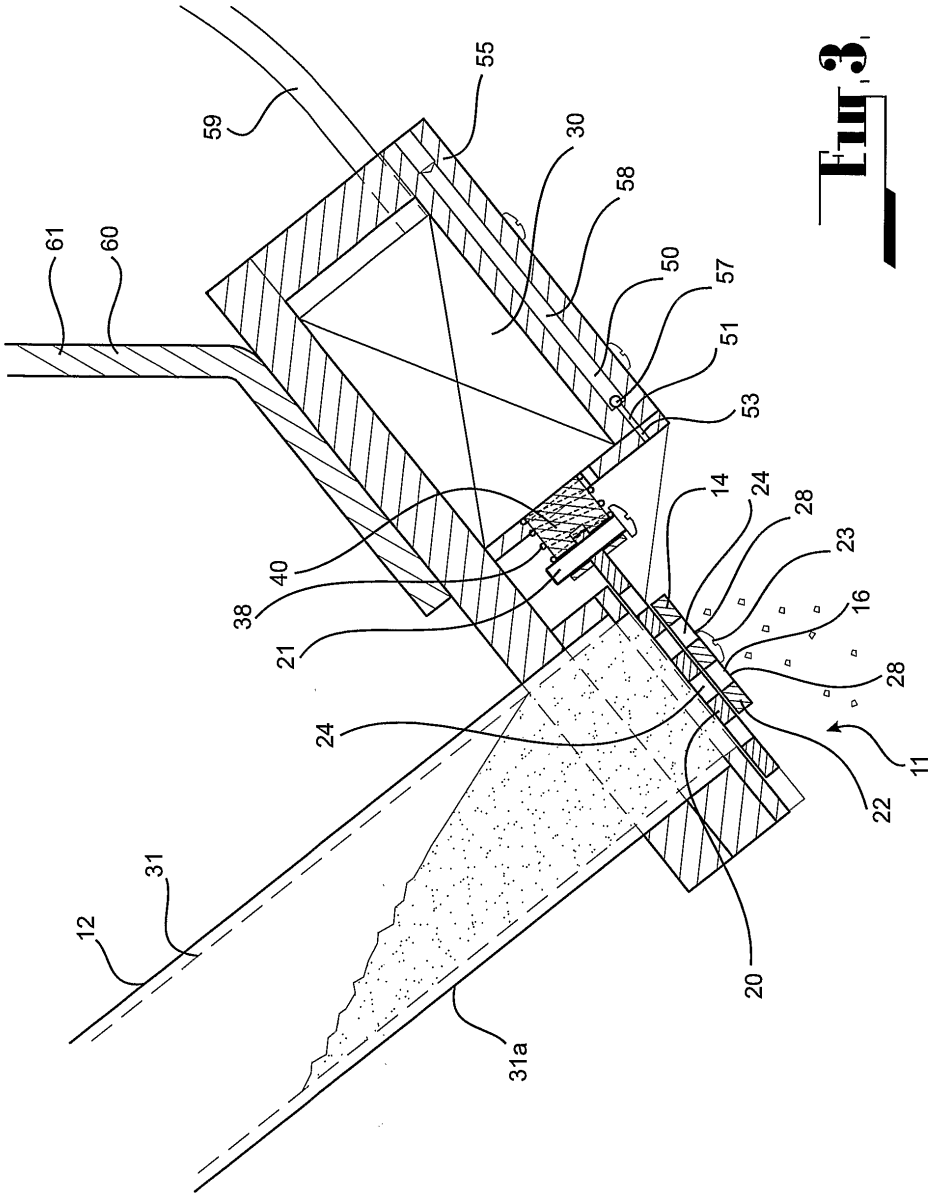
Fig. 1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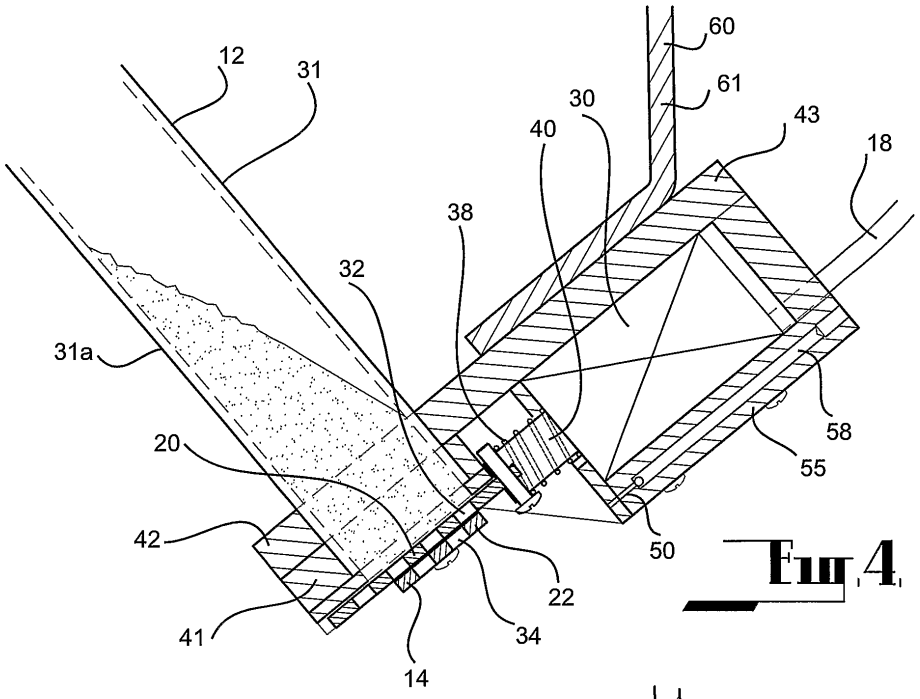
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Fig 4

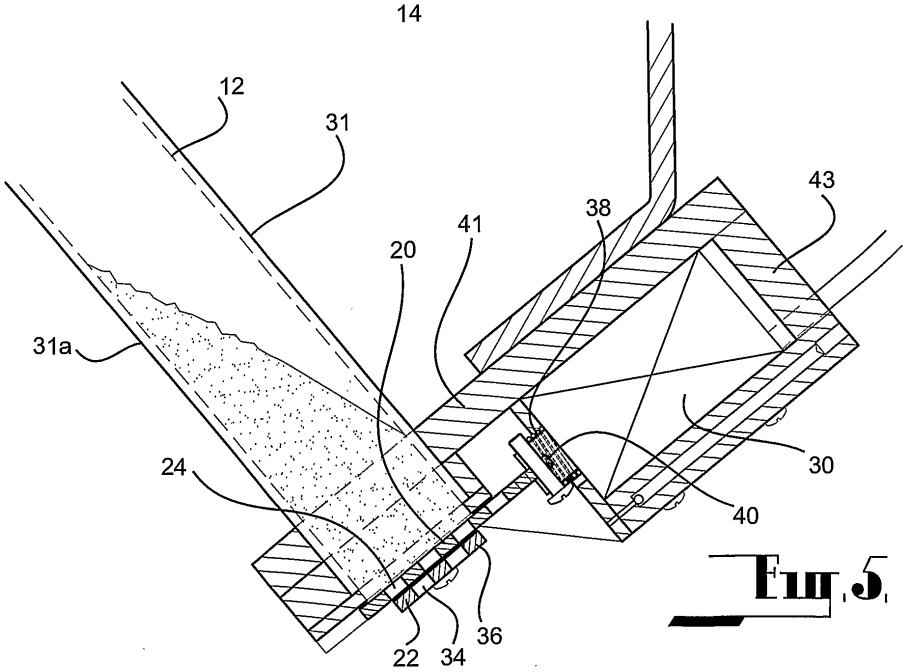
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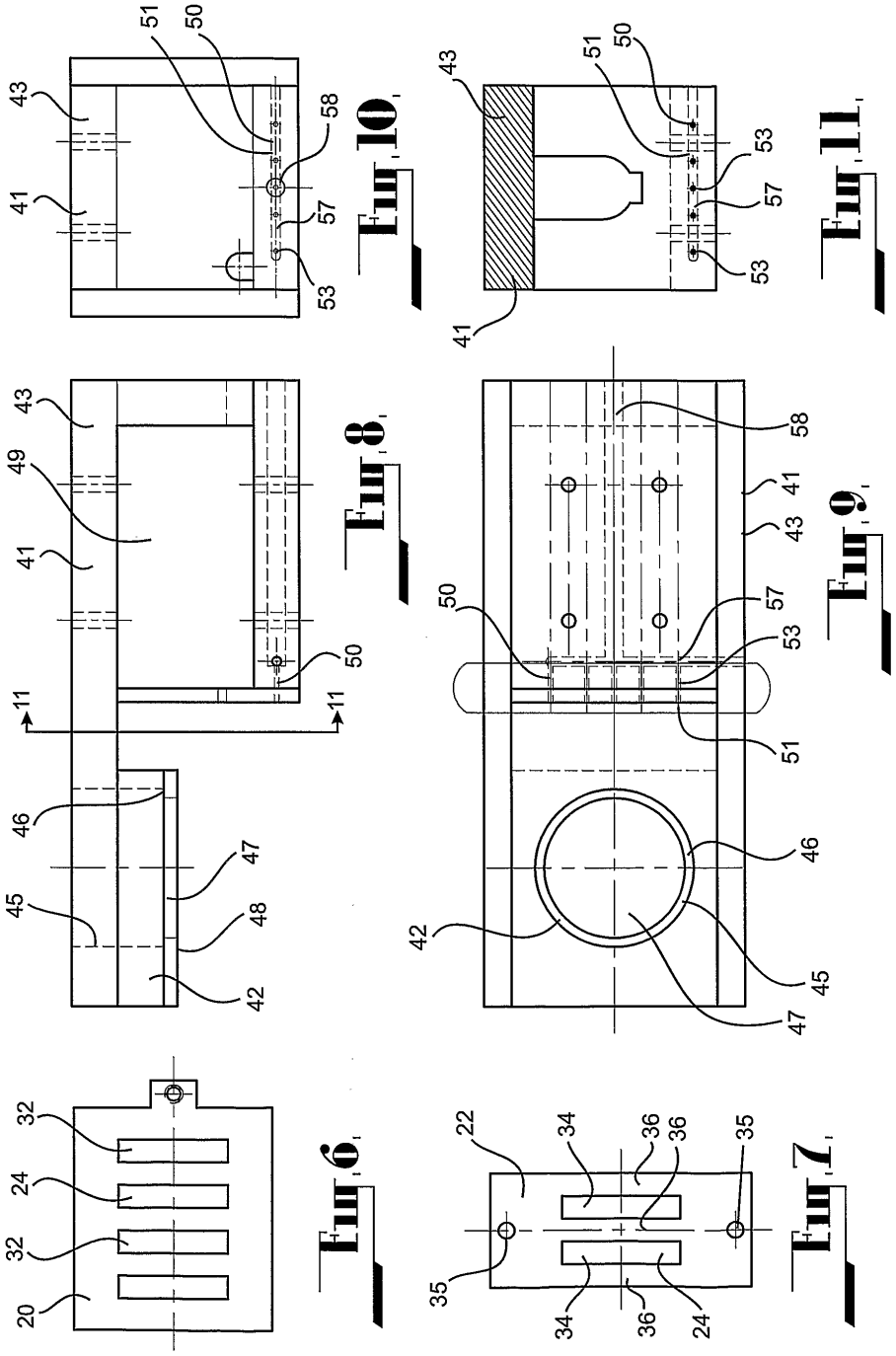
Fig 5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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2006/000735

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A01K 61/02 (2006.01) ***A01K 5/02*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DWPI IPC A01K 5/00, 5/02, 61/02, A23P 1/08, A47G 19/24, 19/32, 19/34, A47F 10/06, B65D 47/04, 47/06, 47/20, 47/26, 47/28, F16K 3/- & Keywords as below
Keywords only (Particulate, granular, powder, salt, pepper, spice, fish food, dispense, shake, pour, dose, shake, vibrate, shutter, slide, plate, perforate, hole, slot, aperture, blow, air, fan, automatic, solenoid, control, actuate, program, align, reciprocate) & like terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6715442 B1 (BELLOMA) 6 April 2004 Whole document	1-12, 14
X Y	US 4437595 A (Stevens et al.) 20 March 1984 Whole document	1-4, 7-9, 12, 14 5-6, 10-11
X X,Y	JP 10-098975 A (DAINICHI KOGYO KK) 21 April 1998 Figures 1-3 & paragraphs 5-12	1-4, 7-9, 12, 14 5, 11

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 June 2006	11 JUL 2006
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **ALLAN SMAILES** Telephone No : (02) 6283 2154

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2006/000735

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	US 5199381 A (MASOPUST) 6 April 1993	
X	Whole document	1, 7-9, 14
Y		5, 11
A		4
	US 2800256 A (DI NUZZO) 23 July 1957	
Y	Figure 1, Column 2 line 47-54, column 3 line 20-25	6, 10

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2006/000735

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member
US	6715442	NONE
US	4437595	NONE
JP	10098975	NONE
US	5199381	NONE
US	2800256	NONE

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX

Form PCT/ISA/210 (patent family annex) (April 2005)

Appendix 4 Market assessment (Synovate)

Department of Fisheries

Executive Summary

- The automatic hatchery feeder is likely to compete against the Storvik Robot, the Arvo-tec T Drum feeder and the Stemer 905 and Raunes micro feeding system. The interviewed hatchery managers were generally satisfied with the products, although the high price to purchase and install the Arvo-tech and Storvik Robot was highlighted as an issue.
- The research confirmed that the pricing assumption of A$12,000 per 24-feeder unit is realistic. Hatcheries in Denmark and the UK confirmed that they would be willing to spend from A$15,300 for an automated hatchery feeder unit.
- Although Norway produces the largest volume of farmed fish in Europe (around 650 tonnes in 2005), the market is highly sophisticated in its use of computerised feeder systems and presents less of an opportunity to the Department of Fisheries.
- The UK, Greece and Denmark offer larger opportunities.
  - The small to medium-sized, commercial, salmon hatcheries in the UK are still using a combination of hand feeding and clockwork-belt systems to feed the salmon larvae directly onto micro-diet (skipping the live feed stage). This market presents a greater opportunity than the larger, more sophisticated salmon farms that already have computerised feeder systems in place.
  - The larger, commercial sea bass and gilthead sea bream hatcheries in Greece have less sophisticated feeding methods than the UK and those interviewed were highly interested in the automated hatchery feeder.
  - Although Denmark produces a large volume of freshwater Rainbow Trout, there was interest from the commercial hatchery feeders for an automated hatchery feeder.

synovate Business Consulting

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synovate

Executive Summary

- A number of future trends in the market could also present opportunities for the automatic hatchery feeder.
  - With strong relationships already in place between aquaculture producers and dry feed producers, a large pool of potential buyers of the automated hatchery feeder could be presented, if the Department of Fisheries can establish a partnership with one of the dry feed producers.
  - Interviewees stated that machine-based feeding, particularly automated feeding is likely to increase in the future. With competition intensifying among aquaculture producers, the need to be more cost effective is becoming more imperative.
  - As the demand for sustainable farming (rather than wild catch) increases in the future, it is reported that hatcheries will look to expand operations and upgrade existing technology, particularly feeding systems.
- An opportunity also exists in the European market for a complete, lifecycle feeding system. If the Department of Fisheries can adapt its product to not only feed micro-diet but also up to 10g fish, before being taken to pen, a larger market could be presented.

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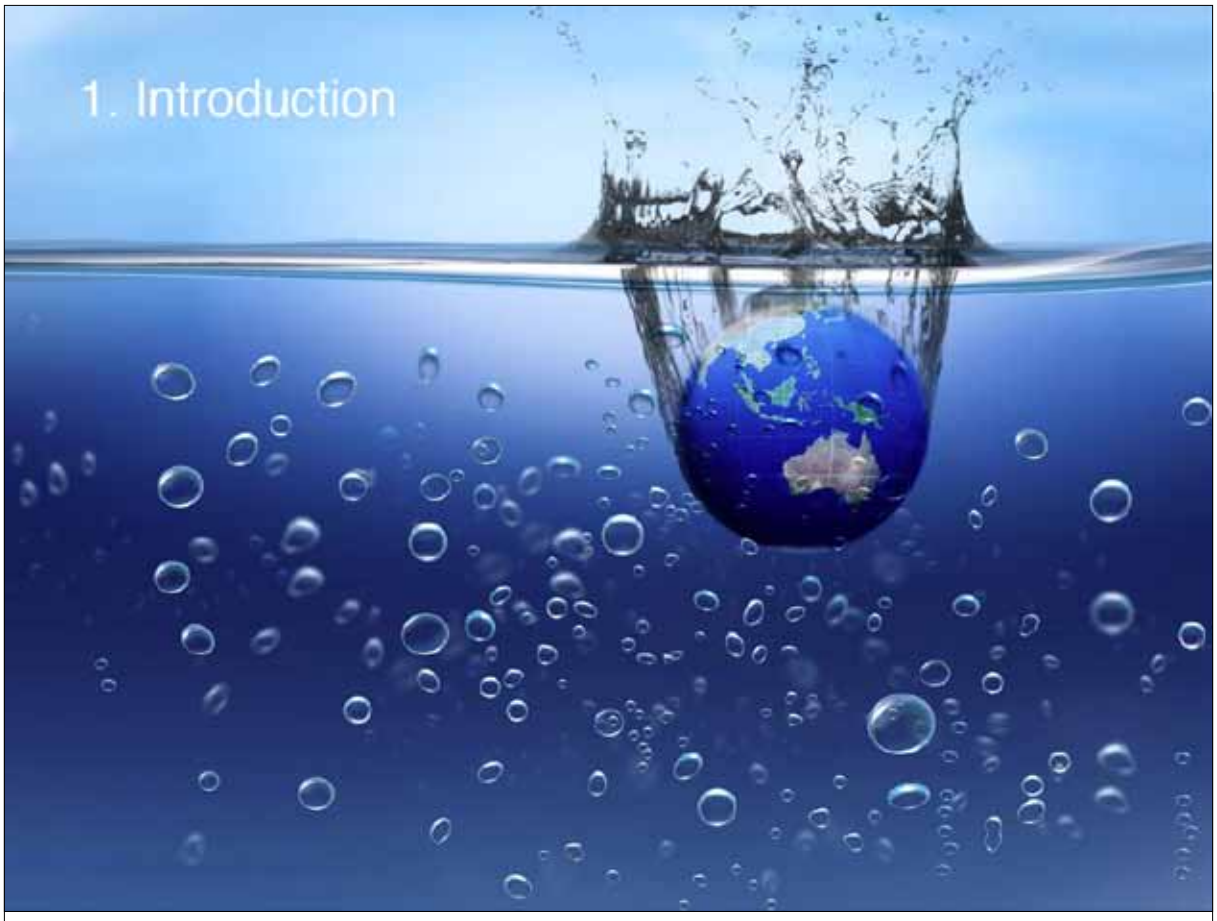

Department of Fisheries

Introduction

Background to the project

Background

- Dr Kolkovski, a scientist at the Department of Fisheries, has developed a soon-to-be patented system for feeding micro-diet to marine species larvae. The system has the potential to replace existing feeding mechanisms, which are based on either hand feeding or automatic feeders better suited to juveniles.
- Before undertaking the expensive and time-consuming process of a worldwide patent, the Department of Fisheries wishes to test the current market for hatchery feeders to gain a sense of what level of investment is appropriate to launch this technology.
- Synovate has been approached to undertake an exploratory market assessment to determine if there is a sufficiently sized market to justify further investment.

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Department of Fisheries

Introduction

Objectives and Approach

Objectives

- The primary objective of this assignment is to test the scope of opportunity for the automatic hatchery feeder. Key questions to answer are:
  - What products and methods will the feeder likely to compete against?
  - How satisfactory is the performance and price of this competition?
  - How realistic is the pricing assumption of up to AUD$12,000 per 24-feeder unit?
  - What is the likely market potential, in terms of market size and likely penetration?
- The scope of this project is limited to the European market, as it is considered to have the largest initial opportunity for the automatic hatchery feeder.

Approach and limitations

- The study was primarily conducted through a series of telephone-based in-depth interviews with industry experts.
  - Experts included industry associations and major hatchery growers in Greece, Denmark, Norway and the UK.
  - In the short timeframe that the project was conducted, it was not possible to interview industry experts in Spain, France or Turkey. Language barriers also posed some issues.
- A secondary desk research process was also undertaken to find out additional market information.

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Products and methods used in Europe

A combination of hand feeding and mechanical systems are popular feeding methods in Europe

Type of Feeding Method

Hand feeding

- Primarily used in small hatcheries that have medium to low levels of technology sophistication

Mechanical system (belt/clockwork systems)

- Used across all sized hatcheries.
- The quality of mechanical systems has reportedly improved over time and as such these systems are well established in Europe.

Fully automated systems

- Primarily used in medium to large scale hatcheries.
- Examples – Arvo-tec, Storvik System.
- Some hatcheries will also use hand feeding to monitor appetite levels.

Combination of hand feeding & mechanical systems

- With the inaccuracy of mechanical systems and the need to observe larvae, some medium to large hatcheries will also use hand feeding

synovate Business Consulting | Source: In-depth interviews conducted by Synovate, September 2007

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Products and methods used in Europe

Observation is the main identified advantage of hand feeding

Identified advantages and disadvantages of hand feeding in Europe

Advantages	Disadvantages
Observation during larvae's critical growth stages	**Expensive and ineffective**
"Actually observing how the larvae are performing is a key element of any feeding strategy."	*"The feeding staff cost money and it is expensive employing them to distribute feed 24/7."*
"As an experienced operator, it is quite useful to stand and hand feed so you can see how well the larvae is going."	*"It can be ineffective as people have different hand sizes."*
Top up inaccuracies of mechanical systems	**Causes pollution with over feeding**
"At least with hand feeding, I know if they are getting fed. We do have a mechanical system, but it just doesn't work."	*"Hand feeding does create pollution. The particles you are throwing into the tanks do blow around a bit, so having something to distribute directly into the water would help to stop this."*
"I am able to distribute the feed where the clock feeder can't reach."	

synovate Business Consulting | Source: In-depth interviews conducted by Synovate, September 2007

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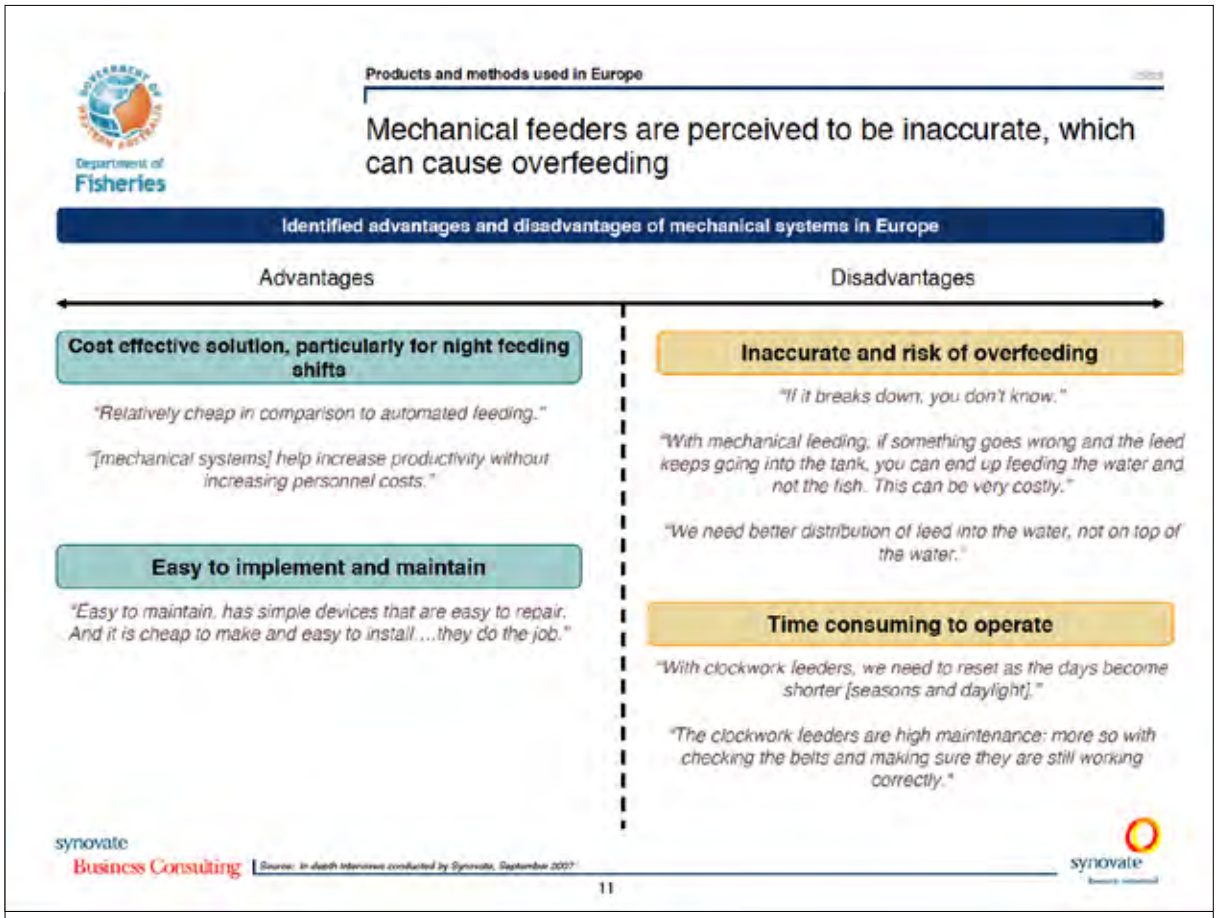
Department of Fisheries
Products and methods used in Europe
Mechanical feeders are perceived to be inaccurate, which can cause overfeeding
Identified advantages and disadvantages of mechanical systems in Europe
Advantages
Disadvantages
Cost effective solution, particularly for night feeding shifts
"Relatively cheap in comparison to automated feeding."
"[mechanical systems] help increase productivity without increasing personnel costs."
Easy to implement and maintain
"Easy to maintain, has simple devices that are easy to repair. And it is cheap to make and easy to install ...they do the job."
Inaccurate and risk of overfeeding
"If it breaks down, you don't know."
"With mechanical feeding, if something goes wrong and the feed keeps going into the tank, you can end up feeding the water and not the fish. This can be very costly."
"We need better distribution of feed into the water, not on top of the water."
Time consuming to operate
"With clockwork feeders, we need to reset as the days become shorter [seasons and daylight]."
"The clockwork feeders are high maintenance: more so with checking the belts and making sure they are still working correctly."
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Business Consulting
Source: In-depth interviews conducted by Synovate, September 2007
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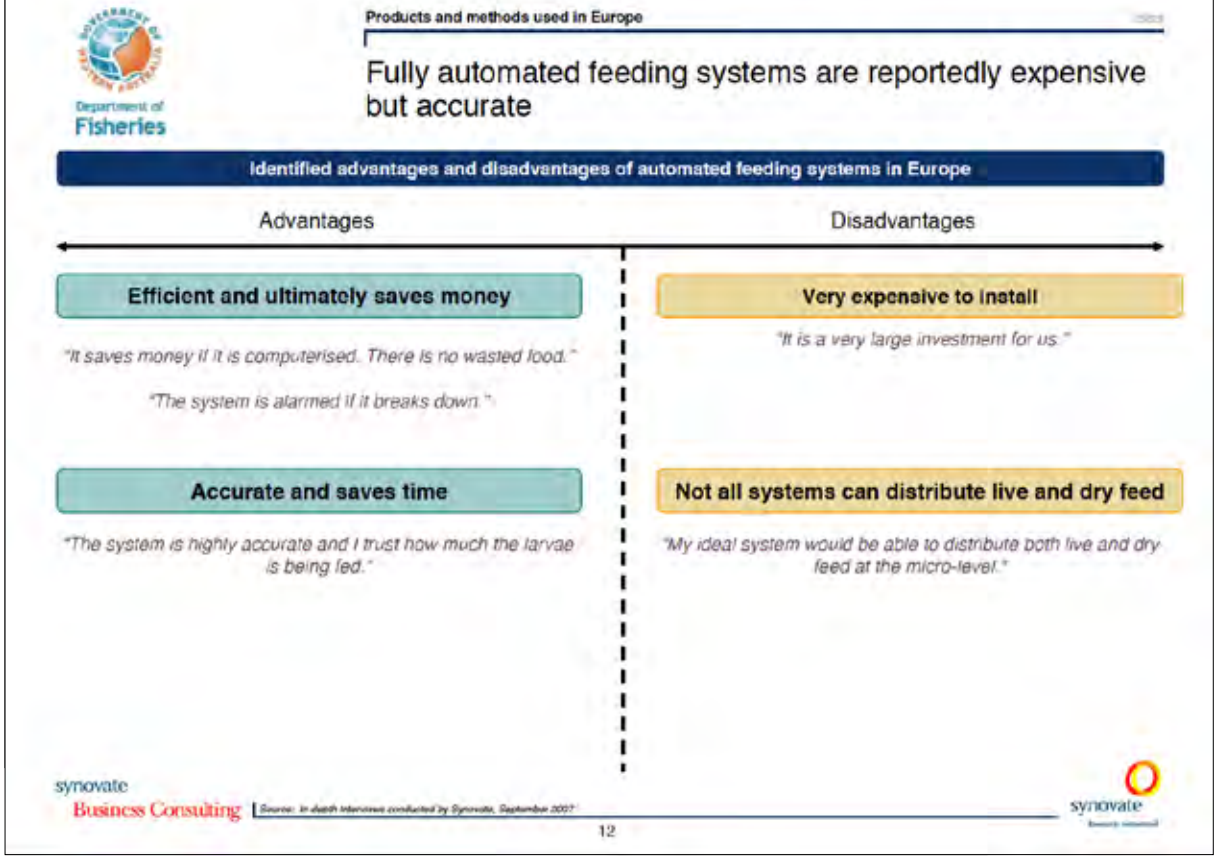
Department of Fisheries
Products and methods used in Europe
Fully automated feeding systems are reportedly expensive but accurate
Identified advantages and disadvantages of automated feeding systems in Europe
Advantages
Disadvantages
Efficient and ultimately saves money
"It saves money if it is computerised. There is no wasted food."
"The system is alarmed if it breaks down."
Accurate and saves time
"The system is highly accurate and I trust how much the larvae is being fed."
Very expensive to install
"It is a very large investment for us."
Not all systems can distribute live and dry feed
"My ideal system would be able to distribute both live and dry feed at the micro-level."
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Business Consulting
Source: In-depth interviews conducted by Synovate, September 2007
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Key competing products

Three products are likely to compete against the automatic hatchery feeder

Example images of competing feeding systems

Storvik Robot

Arvo-tec

Raunes Micro Feeder

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Department of Fisheries

Key competing products

The Storvik Robot is heavily used in Norway

Function and Features[1]

Function of Operation

- The fully automated system consists of 1 to 4 feed silos. Each type of feeding mechanism handles different types of feed, using the same railing.
- The railing is installed in the ceiling above the hatchery tanks.

Features

- The robot can accurately dose from 0.2g up to 7kg/minute.
- It can handle up to four types of feed simultaneously, including live feed.
- It offers individual feeding for each unit/tank.
- The system provides an optional fully automated refill.
- Microchips on each tank moderate temperature, with results feeding into the PC. Feed is adjusted accordingly.

Satisfaction and key issues faced by hatcheries[2]

- Overall, hatchery managers were satisfied with the Storvik Robot, with the major issue identified being the cost
  - The robot is reportedly very expensive to implement, although the price was not disclosed in interviews.

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Department of Fisheries

Key competing products

Arvo-tec feeder systems are used in several countries, including the UK, Norway, Finland, Germany and Chile

Function and Features[1]

Function of Operation

- The Arvo-tec T Drum feeder is multifunctional and suitable for micro-diet feeding in hatcheries to on-growing tanks, ponds and cages.
- Each individual feeder has a control unit that is linked to a PC.

Features

- The granule/pellet feed size ranges between 300 microns and 6mm.
- The dosage options are 0.3, 1, 5, 20, 45 or 100g.
- Arvo–tec report the drum feeders accuracy to be greater than 98% with standard trout and salmon feed.
- There is the option of a pneumatic spreader.

Satisfaction and key issues faced by hatcheries[2]

- Similarly to the Storvik Robot, hatchery managers were satisfied with the Arvo-tec feeder system. The main issues identified were:
  - The system does not perform well in humid conditions and food with high moisture content (eg EWOS food).
  - The system only handles dry food.
  - It is expensive to install, like the Storvik Robot.
- No comment was made that the Arvo-tec feeder systems struggles under 1mm.

arvotec

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[1] Arvotech website; September 2007; [2] In-depth interviews conducted by Synovate, September 2007

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Key competing products

The Sterner System is mainly used in Norway and the UK

Function and Features[1]

Function of Operation

- The Sterner 905 feeder is custom-built for the Raunes Micro Feeding system. It has internal rotating springs that stir the feed and prevent lumps/accumulation of feed.
- The Raunes Micro Feeding system mixes the dry feed in the water and distributes this feed in the tank below the surface through 12 adjustable pipes.

Features

- The system can feed 70-150 micron (10-12 g/min or 0.17-0.2 g/s) and 250-400 micron (20-24 g/min or 0.33-0.4 g/s).
- The Raunes Micro Feeding System patent application was applied for on 1 October 2002.

Satisfaction and key issues faced by hatcheries[2]

- Synovate was only able to interview one user of the Sterner/Raunes system. The hatchery was generally satisfied with the feeder system although it was stated that there was a lack of support and service with technical queries.
- Not being fully automated, it was also reported that the system requires constant adjusting.

Sterner 905 and Raunes Micro Feeder

sterner

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[1] Sterner Fish Tech website; September 2007; [2] In-depth interviews conducted by Synovate, September 2007

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Pricing

The prices that hatchery managers are willing to pay for a computerised hatchery feeder is greater than A$12,000 per 24-feeder unit

Pricing potential in comparison to other feeding methods being used in Europe

	Method of feeding	Cost	Assumptions
Current price	Clockwork system 	Between **A$5,760** and **A$15,600** * 24 feeder unit	Based on 1 feeder unit costing between €50 to €200.
Current price	Hand feeding	**A$32,000** per year	Based on estimate of €20,000 per year in Denmark for a feeder
Ideal price	Fully automated/computerised hatchery feeder	**A$15,300** to **A$23,120** * 24 feeder unit	Based on the ideal price for an automated hatchery feeder, mentioned by a hatchery manager in the UK. Control system: £2,000 to £3,000 1 feeder unit: £200 to £300 Note: these prices exclude installation costs.
	Fully automated/computerised hatchery feeder	**A$32,000** to **A$47,400**	Based on hatchery manager in Denmark willing to spend €20,000 – €30,000 on a new computerised feeder system (based on current market prices).

Although potential prices are higher than A$12,000 per 24-feeder unit, some lesser developed European countries, such as Greece, would be less likely to pay the premium prices of A$32,000 – A$47,400

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Currency conversion - €1.00: A$1.58; £1.00:A$2.27 (Westpac Currency conversion)

Source: In-depth interviews conducted by Synovate, September 2007.

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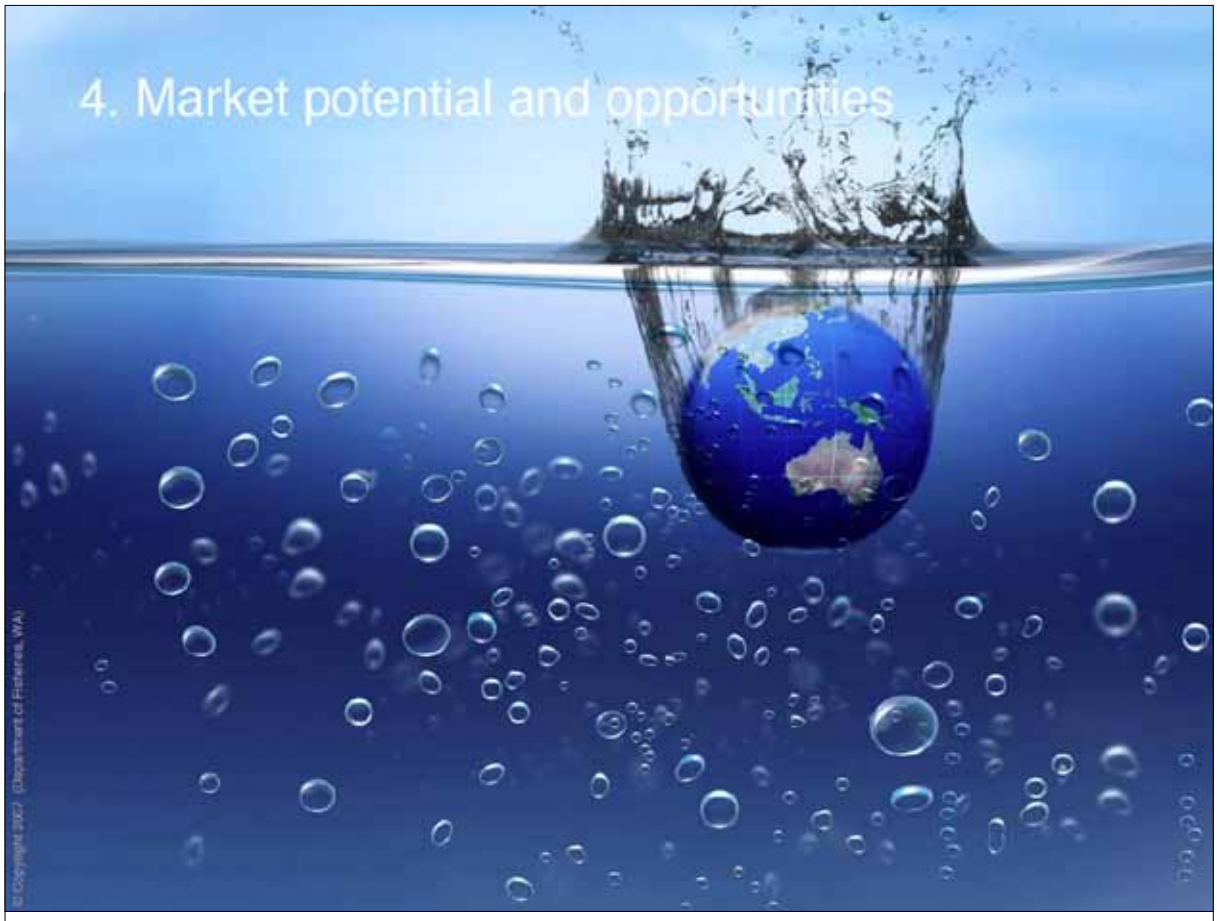
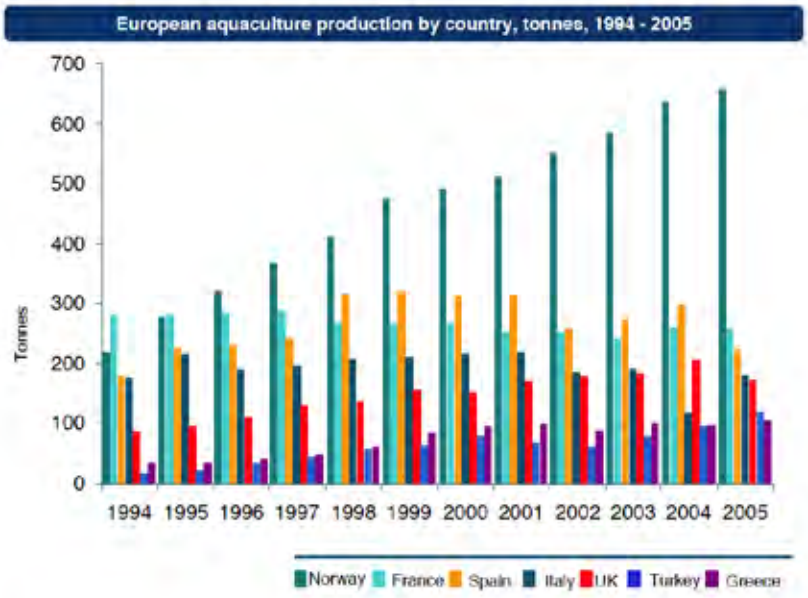


Department of Fisheries

Market potential and opportunities

Norway has led aquaculture production in Europe since 1996

In 2005, aquaculture production in Norway more than doubled France, its nearest rival



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Department of Fisheries

Market potential and opportunities

Europe has varying levels of sophistication in its feeding methods

Map showing levels of sophistication in hatchery feeding methods

While Norway is the largest aquaculture producer in Europe, it also uses the most sophisticated feeding methods

- High – PC-based systems, high level of complexity
- Medium – Some PC-based systems, some mechanical systems, and some hand feeding
- Low – Combination of non-complex processes (hand feeding and/or mechanical systems)

Although interviews could not be conducted with industry experts in Spain, France and Turkey, based on the size of aquaculture production in each of the countries, Synovate believes that Spain and France would have medium levels of sophistication in their feeding methods, and Turkey would have lower levels of sophistication.

synovate Business Consulting | Source: In-depth interviews conducted by Synovate, September 2007

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Market potential and opportunities

The UK offers potential for the automatic hatchery feeder, particularly the small to medium-sized hatcheries

UK

Commercial or research

- Interviews indicated that the **commercial** market would be more suitable for the automatic hatchery feeder.
- It was stated there wouldn't be enough funding in the research field to afford such a system.

Smaller or larger hatcheries

- The research revealed that the larger hatcheries already have computerised feeding systems in place, including Stemer, Arvo-tec and Arena*, and would not be in a position to replace their existing systems.
- The **small to medium-sized hatcheries** are primarily using hand feeding and clockwork belt systems and would therefore have more of a requirement.

Species

- **Salmon** farmers were reported to be the most likely interested market for the automatic hatchery feeder.
- Salmon larvae reportedly have highly developed digestive systems and skip the live feed stage, feeding directly on micro-diet (300 microns).

*Arena was acquired by Ocea Aquaculture solutions. No detailed information could be obtained on the Arena micro diet feeder.

synovate Business Consulting | Source: In-depth interviews conducted by Synovate, September 2007

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Market potential and opportunities

The larger, commercial hatcheries offer an opportunity for the Department of Fisheries in Greece

	Greece
Commercial or research	• There was a high level of interest from the **commercial** market for the automatic hatchery feeder. • Within the timeframe of the project, it was not possible to identify the level of potential for the automatic hatchery feeder within the research domain.
Smaller or larger hatcheries	• **Larger hatcheries** are still using unsophisticated levels of technology (primarily hand feeding and belt-driven systems) for feeding larvae. • These sized hatcheries, with larger revenues than the small to medium-sized hatcheries, will have more potential for the automatic hatchery feeder.
Species	• Sea bass and gilthead sea bream are the main species farmed in Greece and would therefore offer the largest market opportunity for the automatic hatchery feeder.

synovate Business Consulting | Source: In-depth interviews conducted by Synovate, September 2007

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Department of Fisheries

Market potential and opportunities

Denmark represents a larger opportunity to the automatic hatchery feeder in comparison to Norway

	Denmark	Norway
Commercial or research	• The **commercial** sector represents an opportunity for the automatic feeder system. • Within the timeframe of the project, it was not possible to confirm if the feeder system would be of interest to the research sector.	• The commercial and research sectors are already highly sophisticated in its use of computerised feeding systems, particularly Storvik. • Being so sophisticated and saturated with computerised feeding systems, Norway **represents less of an opportunity** for the Department of Fisheries.
Smaller or larger hatcheries	• The automatic hatchery feeder would be of interest to **medium to large-sized hatcheries**, which are still using a combination of hand feeding and clockwork belt systems.	
Species	• **Rainbow trout** is one of the main species farmed in Denmark. Although it is a freshwater species (and outside of the scope of this project), there was interest in the automatic hatchery feeder.	

synovate Business Consulting | Source: In-depth interviews conducted by Synovate, September 2007

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Department of Fisheries

Market potential and opportunities

A number of trends were highlighted during the interviews that support the introduction of the new micro diet feeder system in Europe

Future trends and opportunities

Competition will drive the demand for efficient feeding systems	With competition increasing among aquaculture producers in Europe, the need to be cost effective is becoming more important. Automatic feeders will be one way to achieve this. *"Our aquaculture production must become more cost effective, so that our cost per kilo price of production becomes cheaper."*
Growing use of technology in response to increased demand	Interviewees stated that existing players in the aquaculture industry will look to expand and update technology in the future in response to the growing demand of sustainable farming versus wild catch. It is reported that feeding systems will be one of main areas that aquaculture producers will consider.
Hatcheries were interested in a complete, lifecycle system	An opportunity exists for the Department of Fisheries to develop the feeding system to not offer not only micro diet feeding but on the whole feeding cycle up to the fish leaving the hatchery. Some interviewed hatcheries would prefer a complete system that can feed up to 10g fish, before they are taken to pen.

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Department of Fisheries

Market potential and opportunities

A potential opportunity exists for the Department of Fisheries to link with a producer of micro diet feed

Future trends and opportunities

Dry feed producers could open up a large pool of buyers for the automatic hatchery feeder	• It is reported by hatcheries that the major brands of dry feed – Skretting, EWOS and Biomar have strong ties and relationships with aquaculture producers. However, the dry feed producers, excluding Skretting*, do not have the same linkages with machine-based hatchery feeders. • If the Department of Fisheries could establish a relationship with one of the major dry feed producers, a large market of potential buyers for the automatic hatchery feeder could be presented because of the existing relationships with aquaculture producers.
A move away from live food, and the development of smaller, encapsulated pellets	• Live feed is reportedly being phased out in Europe because of the risk of disease, the high cost (in comparison to dry feed) and the difficulty in sourcing the feed (artemia, rotifers). At the same time, a major research centre in Norway stated that there is research being undertaken to develop encapsulated pellets with a higher nutritional content that are smaller than 300 microns. • These are important trends if the automatic micro diet feeder system can support smaller encapsulated pellets.

* Appears to have linkages with Raunes.

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Identified future leads

A few hatchery managers approached during the research are interested in learning more about Automatic Microdiet Feeding System

Main contacts

The following contacts were interested in receiving more information from the Department of Fisheries.

Greece

- Spiliopoulos George, Andromeda Aquaculture: +30 261 099 5613
- Banus Filippoy, Nireus: +30 210 669 8270
- Yannis - Selonda Aquaculture +30 210 372 4900

Denmark

- Tom Nilsen – Danish Trout Hatchery Manager: +45 20 30 50 32
- Oluf Myer – Danish Trout Farmer: +45 74 64 84 54

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Department of Fisheries

Appendix

Overview of the main species farmed in Europe

Main aquaculture species (fish only) by country

UK
Salmon
Turbot
Halibut
Bass
Rainbow Trout (freshwater)

France
Turbot
Sea bass
Gilthead sea bream
Rainbow Trout (freshwater)

Spain
Gilthead bream
Sea bass
Turbot
Trout (freshwater)

Norway
Salmon
Cod

Denmark & Finland
Rainbow Trout (freshwater)

Greece
Gilthead sea bream
Sea bass
Rainbow Trout

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Source: In-depth interviews conducted by Synovate, September 2007 ; FAO database, September 2007

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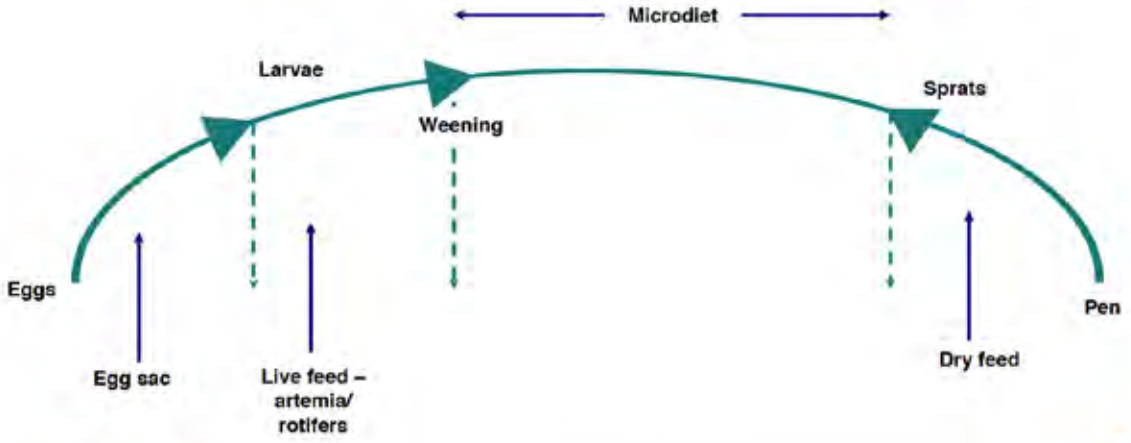
Appendix

Overview of the live feed and micro-diet development stage of fish larvae



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Source: In-depth interviews conducted by Synovate, December 2007

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Appendix 5 Advertising and articles

EWAYS

cking back

around the state, of various species and subspecies, for people to stock in local waters, says Metcalfe in a university press release.

Metcalfe said that although greenback cutthroats were declared extinct in 1937 – the victims of mining pollution, fishing pressure and competition from other trout species brought into the area – several small populations were found in tributaries in the Arkansas and South Platte River watersheds in the 1950s. Greenbacks were added to the federal endangered list in 1978, and state and federal

its goal of 20 self-sustaining populations just last year, putting the fish in a position for possible removal from the federal protected-species list.

"The new study means that we have not reached the targeted management goals, and the species is no closer to being removed from the endangered-species list than when it originally was listed," said Prof. Andrew Martin of CU-Boulder, a co-author and principal investigator on the research effort.

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AUTOMATED MICRODIET FEEDING SYSTEM For all aquatic larvae

Introducing the

AUTOMATED MICRODIET DISPENSER (AMD)

The first purpose designed microdiet feeding system for any aquatic larvae
Fitted with a computerized controller, the AMD is a simple, labour-saving system
for spreading the required daily dosage of feed in tiny, equal amounts - upwards of 100 mgs at a time.
- No more hand feeding
- No more clumps that sink and pollute the water
- No more waste of expensive microdiet

Features include:
- Large hopper containing up to 180 gr with splash-proof lid.
- Delivery of small, accurate quantities of microdiet (0.1 ± 0.01 g per shot).
- Fully programmable touch screen.
- Capable of feeding 100 µm to 2.0 mm food particle size.
- Controller for 10, 24 or more feeders.
- Individual control for each feeder.

Department of Fisheries

For more information, please contact:
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Department of Fisheries Western Australia
P.O. Box 20 North Beach WA 6920 Australia
Tel: +61-8-9203-0220
Fax. +61-8-9203-0199
Email: skolkovski@fish.wa.gov.au

Fresh Water Chlorella
(Concentrated Liquid type)

ROTICHLO

1. **Overview**
- ROTICHLO is a concentrated liquid live green freshwater Chlorella used in the culture of rotifers in hatcheries.
- Grow more than 7 billion rotifers per day by feeding 300ml of ROTICHLO per 10 million rotifers
- Using state-of-the-art technolgy ROTICHLO is produced free from viruses and pathgenic bacteria

2. **Dry weight**: 12.0 -12.5 %
3. **Crude Protein**: more than 50%
4. **Number of Cells**: 20 – 30 billion cells per ml.
5. **Shelf Life**: In a refrigerator at 4℃ will be around one month (minimum 15 to 30 days).
6. **Packing**: 17 Liter per case in a vinyl pack and a Styrofoam outer case

International Distributors Wanted

AHYUN **Ahyun AquaDream Co., Ltd**
http//www.dreamfeed.net
Tel. +82-31-551-6194/5 Fax : +81-31-551-6196 in South Korea email : hyuntae@chollian.net

Turbot hatchery buys AMD system

Above: A team at an Australian university designed the micro-diet feeder because they could not find a satisfactory one already on the market

Left: Pilar Moreno, larval production manager at Insulfa

Below left: The AMD mechanism is based on two ABS slotted plates located below a food hopper

now patented and distributed around the world directly through the Department of Fisheries, WA, or through its agent Acui-T in Europe.

Automatic Micro-diet Dispenser

The AMD body is moulded and it is extremely strong and splash proof. It can cope with MD particle sizes from 100µm to 2mm. As little 100mg ± 2 %, depending on the micro-diet, is released without the need to weigh the feed each time.

Its mechanism is based on two ABS slotted plates located below a food hopper. An air manifold constantly jets beneath the plates in order to scatter the MD across the water surface within a target area.

The AMD system presents a wide range of versatile feeding regimes for larvae and juvenile fish. The operator can easily customise it for specific feeding requirements on a daily basis through a user-friendly touchpad interface.

Alternatively, a series of feeding regimes can be pre-programmed to cater for changing larvae requirements, which can be used repeatedly for successive larval production runs.

A base controller operates 24 AMD units or more, either individually or as a group of identical feeders. Each AMD unit periodically administers an operator-determined number of MD doses during one to four feeding periods.

This allows the culturist to provide MD evenly across the whole day, or provide periods of relatively high and low intensity feeding that cater for diurnal larvae requirements. A more constant availability of MD is provided when the larvae need it.

Other applications such as dissolved oxygen monitoring, lighting and pumping can be integrated into the same control mechanism in order to ensure concurrent operation of system components.

In 2007, the system was introduced at the European Aquaculture Symposium, Istanbul, Turkey, with very strong interest from commercial hatcheries as well as R&D institutes.

"Commercial hatcheries as well as R&D centres in Europe and the United States are discovering this innovative micro-diet feeding systems and the rate of orders is increasing, despite the world economical crisis" says Kolkovski in Perth, Western Australia.

The project to develop the system, which started about five years ago, was jointly funded by the Department of Fisheries Western Australia and by Australia's Fisheries Research and Development Corporation.

■ www.fish.gov.au/amf/

Above and top: Traditional enrichments. Note dirty water and expelled enrichment micelles, which will be re-ingested

Clean water and faecal pellet, shown in the above two, with Ori-Go artemia enrichment

content, while ensuring the naupliі are active and healthy.

Skretting says it developed Ori-Go in close partnership with hatcheries by listening and trying to understand the needs of modern fry production. Indeed, this approach allowed Skretting to further enhance its co-feeding diet Gemma Wean, a cold extruded, micro-diet designed for optimising artemia usage.

"In these times of poor cyst quality, it is essential to reduce artemia reliance," says Eamonn O'Brien, who heads the Skretting marine hatchery feeds team.

"By applying our technology on cold micro-extrusion and developing micro-cutting technology, Skretting can offer a highly competitive co-feeding diet with unique properties.

"Formulated with a high protein content [60%] and a low fat content [15%], the cold extrusion allows for the production of a 'fresh' diet with superior attractability and digestibility. Heat sensitive ingredients are not harmed and the feed particles retain a soft texture."

Introduced in 2006, Gemma Wean has gradually be gaining exposure with commercial hatcheries. Skretting believes an important step has been to receive feedback from the market. As a result, in recent months it has further refined Gemma Wean to improve its physical properties.

"Clients were very impressed with Gemma Wean," says O'Brien.

"However, some asked for certain improvements in physical aspects such as sinking speed and size distribution..

"As a result, we have narrowed the size distribution curves of the Gemma Wean 0.1, 0.2 and 0.3. We have also refined the sinking speed of the diet and improved floatability."

R&D team develops microdiet autofeeder

RESEARCHERS with the Department of Fisheries in Western Australia, faced with being unable to find a reliable commercial machine, have come up with an innovative automatic microdiet feeding system for marine larvae.

The automatic microdiet dispenser (AMD) was created by the Mariculture and Aquaculture Engineering Group at the department, Dr Sagiv Kolkovski, John Curnow and Justin King, and to date their system is said to be the only one that caters for both research and commercial larvae culture.

Usually, marine larvae are fed by a combination of belt feeders and hand feeding, but both methods have their disadvantages. Microdiets tend to stick to belt feeders, while hand feeding is labour-intensive and can easily result in uneven distribution and over-feeding, resulting in uneaten feed sinking to the bottom of tanks.

Worst of all, the accumulation of the feed particles can foul a tank and promote bacterial growth, thus reducing dissolved oxygen levels and increasing the risk of larval infection.

Fitted with a computerised controller, the AMD is a simple labour-saving system designed to overcome these problems.

"The feeders that were available were not precise enough and couldn't cope with small quantities of very fine microdiets so we had to build one of our own," says Dr Kolkovski.

In 2002, the group was working with *Seriola lalandi* (yellowtail kingfish) and *Pagrus aurata* (pink snapper) on larvae nutrition and feed attractants. Some related work was carried out on bacterial loading of larvae culture tanks and artemia enrichment tanks. Significantly high levels of pathogenic bacteria were found in both areas.

As yellowtail kingfish, as a pelagic fish, is particularly susceptible to bacteria, a solution was needed.

The main cause of bacterial loading in the larvae tanks was identified as a combination of inefficient cleaning and bad feeding practices. Improving tank hygiene was simple, but stopping microdiet building up on the tank bottom proved more difficult.

The larvae were being hand-fed every 30 minutes, and this led to large masses of inert diet in the water column at feeding time that the larvae could not possibly eat. The wasted diet inadvertently sank to the bottom.

Automatic feeding was obviously the solution, but the team could find nothing on the market that could cost-effectively feed 24 experimental tanks simultaneously, in very small amounts, distributed across the whole tank surface, every few minutes over an 18-hour 'day'. So the researchers started to develop their own system – and in doing recognised that others around the world would have need of such a system.

The AMD is a flexible modular system that can be adapted to suit various aquaculture setups, including any number of feeders and an electronic programmable controller.

After manufacturing hand-made units for various research fellows, in 2005 an injection mould was developed, along with a touch-screen controller.

With a strong and splashproof moulded body, the AMD can cope with microdiet particle sizes from 100µm to 2mm. As little 100mg ±2%, depending on the microdiet, is released without the need to weigh the diet each time.

Its mechanism is based on two ABS slotted plates located below a food hopper. An air manifold constantly jets beneath the plates in order to scatter the microdiet across the water surface within a target area.

The AMD presents a wide range of versatile feeding regimes. The operator can easily customise for specific feeding requirements on a daily basis through a user-friendly touchpad interface.

Alternatively, a series of feeding regimes can be pre-programmed to cater for changing larvae requirements, which can be used repeatedly for successive production runs.

The controller operates 24 AMD units or more (via an optional expandable unit), either individually or as a group of identical feeders. Each AMD unit periodically administers an operator-determined number of microdiet doses during one to four feeding periods. This allows the culturist to provide microdiet evenly across the whole day, or provide periods of relatively high and low intensity feeding that cater for diurnal larvae requirements.

A more constant availability of microdiet is provided when the larvae need it.

Other applications such as dissolved oxygen monitoring, lighting and pumping can be integrated into the same control mechanism.

Recently, the AMD was introduced at the Aquaculture Europe in Istanbul, Turkey, and its makers report "very strong interest from commercial hatcheries as well as R&D institutes". Several systems have already been sold and the AMD is now available through the Department of Fisheries, Western Australia.

The AMD development project was jointly funded by the department and by Australia's Fisheries Research and Development Corporation. A patent registration around the world is now pending

■ **www.fish.gov.au/amf**

Automatic Microdiet Feeding System for Marine Larvae

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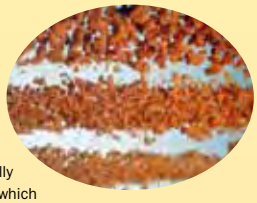
Feeding Larvae



Weaning fish larvae from live food to dry microdiet (MD) continues to be a major challenge when rearing marine finfish larvae. In most research and commercial hatcheries, the feeding of MD is usually done manually, several times a day, which demands high labour requirements. Furthermore, fouling can easily result from few and relatively large doses, whereby a large proportion of uneaten MD particles will sink to the bottom of the tank. Overfeeding promotes bacterial growth, which reduces dissolved oxygen levels and increases larval stress and the risk of infection. An automatic feeding system developed by the Department of Fisheries, Western Australia aims to resolve these problems.

The Feeding System

The automatic MD dispenser (AMD™) is designed to periodically administer a small amount of MD (from 150 mg) to larvae rearing tanks. A small quantity of MD delivered more frequently increases its availability to larvae by increasing the frequency of encounters, creating a situation that more closely resembles live feed, and leads to more efficient weaning. This also enables them to consume a greater proportion of the MD and therefore reduces the opportunity for bacteria to proliferate. MD can be distributed evenly across the photoperiod or alternatively can be dispersed in higher amounts during periods of high larval activity, such as the morning. The use of AMD's also leads to reduced labour requirements and facilitates feeding outside of working hours.

The Controller

The programmable logic controller (PLC) includes easy-to-use pro-logic programmable software that enables the operator to specify different feeding regimes for different feeders. Any combination of feeders can be assigned to as many as 10 feeding programs (depending on the PLC outputs). Other applications such as lighting and pumping integrated into the same control mechanism in order to ensure concurrent operation of system components. Specific feeding programs can be assigned to control one or several AMD's. The AMD's are operated using a safe 12VDC current.

Currently there are very few, if any, commercially available feeders that are able to intermittently or continuously dispense small amounts of MD. The ones that are available are designed mainly for the ornamental market rather than research and/or commercial fish hatcheries, and lack sufficient precision and flexibility. The AMD system is suitable for research and/or a commercial hatchery, giving flexibility for different feeds and feeding protocols.

The Automatic Microdiet dispenser

The AMD can cope with diet particle sizes ranging from 70µm to 1.5mm. At each feeding event, the same amount of MD (as little as 150 mg ± 5%, depending on MD flow characteristics) is released without the need to weigh the diet each time. The AMD's are controlled by a PLC and can be programmed to any desired feeding schedule.

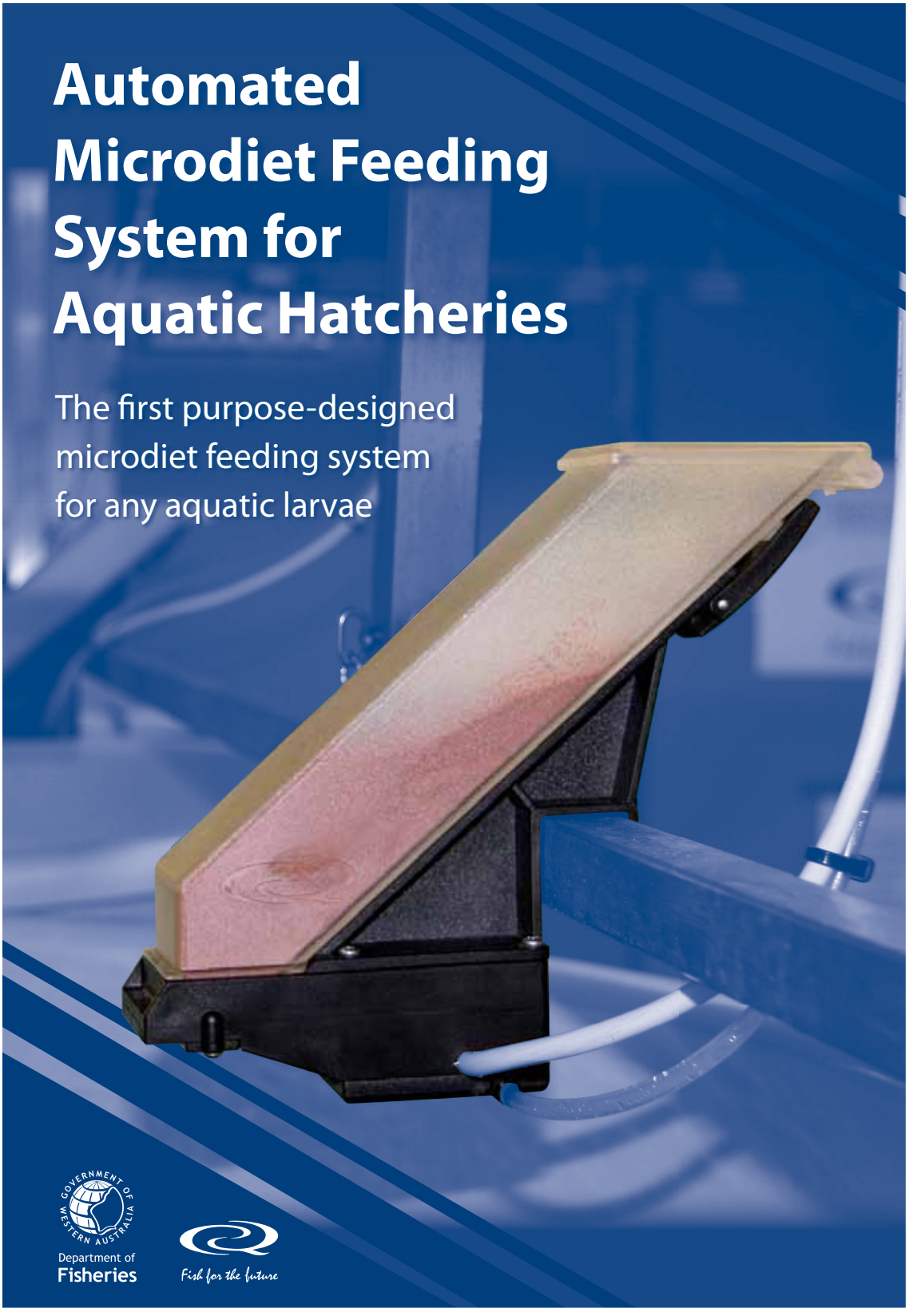
The AMD mechanism is based on two stainless steel slotted plates. The base plate is static and the feeder plate oscillates above it via a solenoid control. In the resting position the bars of the feeder plate cover the slots of the base plate preventing the MD from spilling into the tank. When the solenoid is activated, the top plate slides and aligns the slots as it passes. At that time the MD particles drop into the tank. A manifold jets constant airflow beneath the plates in order to scatter the MD particles across the water surface.

The system is now available through the Department of Fisheries, Western Australia. The AMD system was jointly funded by the Department of Fisheries and the Fisheries Research And Development Corporation (FRDC).

Department of Fisheries

Australian Government
Fisheries Research and Development Corporation

Fish for the future


Automated
Microdiet Feeding
System for
Aquatic Hatcheries
The first purpose-designed
microdiet feeding system
for any aquatic larvae
GOVERNMENT OF WESTERN AUSTRALIA
Department of
Fisheries
Fish for the future

Automated Microdiet Feeding System for Aquatic Hatcheries

The system includes an automated controller and up to 24 feeders (base system).

Controller
User-friendly touch screen interface
Fully programmable
Control 24 feeders with option of expansion
Individual control for each feeder
Feeding intervals down to 1 minute
Changeable feeding frequencies during the day

Feeder
Large clear hopper that allows easy feed level monitoring
Splash-proof lid
Delivery of small, accurate quantities of microdiet
Capable of feeding 100 µm to 2.0 mm food particle size
Air manifold to disperse microdiet evenly across tank surface
Prevents microdiet particles from clumping
Safe, 12V DC

Optional oxygen monitoring linked to feeding
Optional complete system integration including control of light, pumps, oxygen etc.

Specifications

	Controller	Feeders	Hopper
Voltage, volts	100-240 AC	12 DC	
Power, Watts		50 (max)	
Dimensions, mm	320 x 400 x 188	180 x 160 x 65	225 x 50 x 65
No of feeders	24 (base)		
Expansion modules	8		
Weight, grams	7884	450	100
Capacity, grams			250
Diet particle size, µm		100 – 2000	
Colours	grey	Variety available	Opaque
Materials	Polycarbonate with touch pad screen	ABS	Polycarbonate

Department of Fisheries

Fish for the future

The system is now available through the Department of Fisheries, Western Australia. **www.fish.wa.gov.au/amf/**
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email: skolkovski@fish.wa.gov.au
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