The Second Hatchery Feeds and Technology Workshop

NOVATEL CENTURY SYDNEY

September 30 – October 1

2004

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Introduction

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Aquaculture in Australia has developed rapidly over the last decade in both production and value. During recent years, research into the culture of new species has received a lot of attention and a range of species are already commercially cultured. In common with many countries around the world, production of marine fish in Australia will mainly depend on the production of seedstock from hatcheries. Because of this dependence, the continued development of Australian aquaculture will rely heavily on the development of cost-effective hatchery production technologies, with low or negligible environmental impacts. Production technologies for most cultured species are still heavily dependent on live feeds, particularly during the early life stages. A wide range of variables affect the suitability of live prey organisms for hatchery use, including ease of culture, prey size, behaviour and nutritional composition. There has been increasing interest in developing alternative feeds for hatcheries, including alternative zooplankton species and formulated diets. With the expansion of aquaculture in Australia there is a need to improve coordination among the research organisations studying feeds for larval fish, and a need to identify opportunities and priorities for future research that more closely match the needs of industry.

In 2000, the first National Hatchery Feeds Workshop was convened in Cairns. The workshop brought together a wide range of industry and research participants from around Australia together with keynote speakers from overseas. The outcomes of that workshop were Hatchery Feeds Research and Development Plan 2000-2005 and the workshop proceedings, which included reviews of major topics and state and species summaries. These outputs provided a review of the current world status of hatchery feeds and where Australian industry was placed. The R&D plan identified the gaps and research priorities for future development of hatchery feeds.

Five years later, the time has come to re-visit these priorities, review the R&D outcomes in related projects, states and species and to look at current industry status and needs for the next five years.

The Second Hatchery Feeds & Technology Workshop will be held over two days in Sydney, 30 September – 1 October 2004, immediately following the 'Australasian Aquaculture 2004' conference and trade show.

The workshop will focus on recent developments in both industry and research as related to new species, feeds and technologies. The workshop objectives are :

1. To assess the current status and advances of hatchery feeds, technologies and larvae rearing techniques in commercial hatcheries.
2. To assess the current R&D activity, advances and research capabilities made since 2000 in the major R&D centres.
3. To assess priorities for research and development needs in the area of marine hatcheries.
4. To identify constraints to the continued development of Australian aquaculture in the area of marine hatcheries.
5. To identify opportunities to enhance collaboration and information exchange amongst researchers and industry.
6. To develop a national R&D plan for marine hatcheries for 2005-2010.

The first day of the workshop will be devoted to status reviews from both industry representatives and research providers. The second day will be devoted to a round-table discussion to identify knowledge gaps and priorities for the different areas of marine hatchery production.

The workshop is organised by the Department of Fisheries, Western Australia, in collaboration with the Tasmanian Aquaculture and Fisheries Institute and the Queensland Department of Primary Industries and Fisheries and is kindly sponsored by the following organisations: Frontiers of Science & Technology Mission and Workshop component of the Innovation Access Program, part of the Australian Government's Innovation Statement, Backing Australia's Ability, Fisheries Research and Development Corporation therough its nutrition sub-program, Challenger TAFE, Western Australia, CRC – Aquafin, Cognis Australia Pty Ltd, Skretting, M.G. Kailis and Embassy of France and FEAST – France.

Organising Committee

Dr Sagiv Kolkovski

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Keynote Speakers







Nutritional requirements for finfish larvae

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Abstract

From on-start feeding, fish larvae nutritional reserves are very limited and their survival dramatically depends on exogenous feed. Hence, complete and balanced nutrition is critical of rearing success during early life stage. But most fish larvae, particularly marine ones, despite on the wild fed on a wide range of live preys, under culture conditions are forced to fed on a very limited number of preys (two or three) which frequently are not part of their natural food and hence their nutritional composition is not always the most suitable for maximum growth, development and survival of the larvae. Moreover, along larval development the fish will undertake several morphological and physiological changes which in nature are simultaneous with changes in behaviour and even habitat and type of prey fed. All these changes will affect to nutrient availability and feed utilization by the larvae in order to match their nutritional requirements. In practice, most of these problems will be simplified by the proper development of inert diets which are able to cover nutritional requirements at different moments of larval development. In order to achieve those diets we need, among many other important things, to have a complete knowledge of nutrient requirements for the different fish species.

Whereas protein composition of live preys is genetically determined, lipid qualitative and quantitative composition is greatly affected by their diet and significantly varies among batches of the same type of prey, as well as among different species. Early studies on the 80´s had determined that lipids are the most important factor affecting the nutritional quality of live preys and since then a vast amount of the research conducted on larval nutrition have focussed on these nutrients. Essential long chain polyunsaturated fatty acids, as key components of bio-membranes play many important roles in their functioning and are particularly indispensable for larval development. Their presence and quantity in the diet are determining to the efficiency of digestion, absorption, and transport of some nutrients, and to the capacity of dietary energy deposition and utilization. They markedly affect eye and brain development as well as larval behaviour. Finally, as sources of eicosanoids they regulate several physiological functions including some related with larval development, immune function and stress resistance, globally affecting larval growth and survival and rearing success. Recently, molecular studies have denote the presence and activation by the fatty acid composition of the diet of a delta-6 desaturase like gene, involved in long chain polyunsaturated fatty acids synthesis. Besides, the different essential fatty acids compete among them at many different points of fish physiology, dietary unbalances among them leading to detrimental consequences for the larvae. To complicate the picture a bit more, the molecular form in which they are administered is determinant of the utilization efficiency of the dietary essential fatty acids. Other lipids such as phospholipids are considered indispensable for fish larvae, since they distinctly promote the limited ability of larvae to absorb, to re-acylate and to transport triglycerides and provide additional sources of nutrients. Fat-soluble vitamins and pigments have also prove to play important roles along larval development and their inadequate dietary levels either by shortage or excess are negative for the larvae. In a similar manner to what is found in juveniles, dietary protein utilization has been found to be affected by the dietary source, particularly during early larval stages and playing a central role in the development and maturation of the larval gut. Besides, certain free amino acids also constitute a very important source of energy, act as attractants and play a significant role in gut function and development. Finally, despite their importance, only a very limited number of studies have focused other nutritional aspects of larval development such as water-soluble vitamin and mineral requirements and energy utilization by fish larvae.

Key words

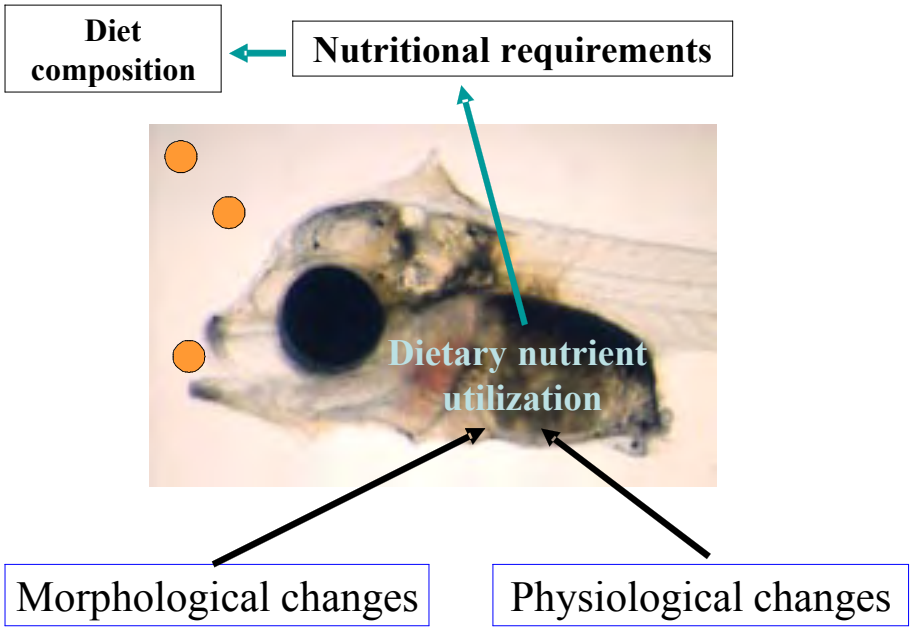
Arachidonic acid, broodstock nutrition, docosahexaenoic acid, eicosapentaenoic acid, fish nutrition, larval nutrition, essential fatty acids.

Abbreviations

EFA: essential fatty acids; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ARA: arachidonic acid; PUFA: polyunsaturated fatty acids with 18 or more carbon atoms and 2 or more double bounds; HUFA: highly unsaturated fatty acids with 20 or more carbon atoms and 2 or more double bounds; PI: phosphatidyl inositol; AA: amino acids; EAA: essential amino acids.

Introduction

From on-start feeding, fish larvae nutritional reserves are very limited and their survival dramatically depends on exogenous feed. Hence, complete and balanced nutrition is critical of rearing success during early life stage. But most fish larvae, particularly marine ones, despite on the wild fed on a wide range of live preys, under culture conditions are forced to fed on a very limited number of preys (two or three) which frequently are not part of their natural food and hence their nutritional composition is not always the most suitable for maximum growth, development and survival of the larvae. Moreover, along larval development the fish will undertake several morphological and physiological changes which in nature are simultaneous with changes in behaviour and even habitat and type of prey fed. All these changes will affect to nutrient availability and feed utilization by the larvae in order to match their nutritional requirements. In practice, most of these problems will be simplified by the proper development of inert diets which are able to cover nutritional requirements at different moments of larval development. In order to achieve those diets we need, among many other important things, to have a complete knowledge of nutrient requirements for the different fish species.



Requirements for essential fatty acids

Three very long chain polyunsaturated fatty acids, namely docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6) have a variety of very important functions in fish species, particularly in larvae. Despite freshwater fish seem to have sufficient Δ5 and Δ6 desaturases and elongases activities to produce ARA, EPA and DHA if their precursors linoleic (18:2n-6) and linolenic (18:3n-3) acids are present in the diet, such enzymatic activity is very restricted in marine fish larvae and as a consequence, DHA, EPA and ARA have to be included in the diet and are considered essential. A Δ6 desaturase-like gene has been isolated in larval gilthead sebream (Seilez et al., 2003). More recently,

experiments in our laboratory have found that its expression is affected by the diet, denoting a higher activity of this enzyme when low EFA and high 18 carbon atoms polyunsaturated fatty acids are provided in the rotifers. Inadequate contents of those EFA in the diet give rise to several behavioural and morphological alterations such as poor feeding and swimming activities, poor growth and dropping mortality, fatty livers, hydrops, deficient swim bladder inflation, abnormal pigmentation, disgregation of gill epithelia, immune-deficiency and raised cortisol levels (Izquierdo, 1996; 2004).

Since environmental factors such as temperature, salinity and light affect lipid composition of fish tissue (Izquierdo, 2004), EFA requirements could be also affected by environmental conditions. For instance, larvae of the euryhaline species *Galaxias maculatus* have been found to be higher in EPA, DHA and ARA acids when they were obtained from marine environments in comparison with those from freshwater (Dantagnan et al., submitted), denoting the important role of some of these fatty acids in osmotic regulation. Moreover, before first feeding, synthesis of those EFA was activated in larvae from freshwater environment but not in those obtained in the estuary, suggesting the influence of environment salinity on activation of elongation and desaturation enzymes.

In the wild, types and contents of EFA differ among the different steps of the trophic chain, and EFA requirements would then rely on the trophic behaviour of each fish species. Being fish larvae visual feeders, larval trophic behaviour is closely related to the development of the visual capacity. In sparids, such as gilthead seabream and red porgy (Roo et al., 1999) the most important changes in the eye structure occur along the lecitotrophic stage as a preparation for prey capture, rod photoreceptors necessary for accurate vision at low light intensity appearing in gilthead seabream about 18th day after hatch. N-3 PUFA, and particularly DHA play a critical role in neural and retinal tissue functions. Bell and Dick (1993) found that both rod and cone photoreceptors in herring eye, accumulate and selectively retain DHA, and thus, feeding herring a DHA poor Artemia during the period of rod development resulted in impaired vision at low light intensities. Moreover, elevation of dietary DHA and eicosapentaenoic acid (EPA) increase eye diameter in gilthead seabream (Izquierdo et al., 2000; Roo et al., submitted) and this fact, together with a high density of cone photoreceptors in these larvae, implied a total higher number of cones and a potentially improved visual accuracy (Roo et al., submitted). Thus, restriction in light intensity applied in some commercial hatcheries, particularly during the first two weeks of larval development when only cone type receptors of maximum light capture effectiveness at high light intensity are sufficiently developed, may impose higher DHA requirements in broodstock and larvae than in fish cultured at higher light intensities. Besides, inadequate lighting regimes may constitute an stress factor in larval culture conditions, which in turn increase the EFA demand in this fish.

Despite the retention of EFA, particularly DHA, in seabream brain, appearance of larval swimming reaction to a visual stimulus is delayed in fish fed low EFA rotifers, suggesting the delay in functional development of brain and visual system (Benítez et al., submitted). Moreover, larvae fed low EFA rotifers showed lower cruising and escaping swimming speed than those fed high EFA.

Along larval development several authors have shown a requirement of EFA for gilthead seabream very close to 1.5 % n-3 HUFA in dry matter when larvae were fed either live preys (Rodríguez et al., 1998) or microdiets (Salhi et al., 1999), regardless dietary lipid level (Salhi et al., 1994). Much higher requirements are estimated in the literature when EPA contents are 2 or 3 times higher than those of DHA (Rodríguez et al., 1994, 1997), due to the very high incorporation of EPA into the larval polar lipids and the displacement of DHA from certain polar lipids (Izquierdo et al., 2000). However, as it happens in the other life stages, provided other nutrients such as antioxidants are also balanced, elevation of dietary n-3 HUFA up to 8 % keeping a DHA/EPA ratio of 1.7 further improves larval growth and survival (Liu et al., 2002). High n-3 HUFA requirements have been also estimated for red porgy (3.39 % at 1.35 DHA/EPA, Hernández-Cruz et al., 1999) and Dentex dentex (Mourente et al., 1999) despite in the latter the high EPA content in Artemia may have caused an overestimation of the requirements as we have seen in gilthead seabream (Rodríguez et al., 1997). On the contrary, carp larvae seemed to require as low as 0.05% n-3 fatty acids from cod liver oil (Radunz Neto et al., 1993) to cover the essential fatty acid requirements along this period of life.

The particular structure of DHA provides this fatty acid with many important functions in fish metabolism. Its incorporation into cell membranes regulates membrane integrity and function, this fatty acid being an important component of phosphoglycerides, particularly phosphatidyl ethanolamine and phosphatidyl choline,

in larvae. It is specifically retained in starved or low-EFA fed fish, possibly due to the lower cell oxidation rates than other fatty acids (Madsen et al., 1999). It is necessary for growth, survival, flat fish metamorphosis and disease prevention. It may be a substrate for some lypoxigenases and several studies have shown that it has a greater potential as an essential fatty acid for marine fish larvae than EPA (Watanabe et al., 1989; Watanabe, 1993), its requirement being more limiting for growth and survival than those for n-3 HUFA (Izquierdo, 1996). Minimum dietary levels in diets for larval gilthead seabream seem to be 0.8 (Izquierdo, 2004). In larvae, high levels (5 % in dry basis) of dietary DHA in microdiets for gilthead seabream did not caused any excess problem, but further promoted growth and larval survival (Liu et al., 2002). Regarding other sparids, requirements along larval development seem to be about 1.5 % for red porgy larvae when DHA/EPA ratios are about 1.4 (Hernández-Cruz et al., 1999) and close to 2.3 % for *Dentex dentex* fed a very low DHA/EPA ratio (0.32) (Mourente et al., 1999).

Eicosapentaenoic acid is also particularly important for larval growth (Watanabe et al., 1989) playing general and particular roles in fish metabolism. Its presence in rotifers enhances non-specific lipase activity in larval seabream (Izquierdo et al., 2000), neutral lipids esterified with EPA being a preferred substrate for this enzyme. In marine fish it is a main component of polar lipids and it regulates membrane integrity and function, indeed its incorporation into phosphoacylglycerides enhances fluidity of cell membrane (Sipka et al., 1996) in a higher degree than ARA (Hagve et al., 1998) but lower than DHA (Hashimoto et al., 1999). Moderate dietary levels of this fatty acid also enhance DHA incorporation into larval PL (Izquierdo et al., 2000, 2001), causing a sparing effect on such an important fatty acid. It is a good substrate for some cycloxygenases, being precursor of some prostanoids in marine fish and also a main substrate for some lypoxigenases, being the main precursor for leukotriene synthesis in some species. Its competition with ARA for these two types of enzymes enables it to be an important regulator of eicosanoid synthesis. Best growth, survival, resistance to stress and spawning quality have been obtained in larval gilthead seabream with EPA dietary levels of 0.7-0.8 (Rodríguez et al., 1998; Salhi et al., 1999) in dry basis. In larvae, increase of EPA up to 2.9 % in dry basis when DHA/EPA levels where high (1.72) and ARA contents were only 0.05 significantly improved growth, survival and resistance to a shock temperature stress of gilthead seabream (Liu et al., 2002), denoting its high value as EFA. However, increase of dietary EPA up to 1.8 reduced growth when ARA levels are as high as 1.8 % and DHA/EPA about 1.3, denoting how the EFA value of EPA is dependant on the dietary levels of DHA and ARA.

Arachidonic acid is a main component of a minor but very important polar lipid class, phosphatidyl inositol (PI). In vitro, ARA is a preferred substrate for most cycloxigenases, being the main precursor for prostaglandin synthesis, whereas in some marine fish in vivo, EPA is the main substrate due to its high presence in the diet. ARA also constitutes a good substrate for several lypoxigenases, its derivative hydroxi-fatty acids having important physiological functions in marine fish. Its content in the PI of cell membranes possibly regulates eicosanoid synthesis. In gilthead seabream larvae, increase of ARA up to 1 % enhances survival and growth when DHA and EPA dietary contents are 1.3 and 0.7, respectively (Izquierdo, 1996; Bessonart et al., 1999). Increase in ARA contents in the rotifers also prevent post-stress mortality (Koven et al., 2001). ARA seems to play also important roles in turbot juveniles (Castell et al., 1994) and in flatfish pigmentation (Estévez et al., 1997).

Evidences of competition among two or more of these essential fatty acids have been suggested for digestive enzymes, fatty acid binding proteins, phosphoacylglycerides synthetases, lypoxigenases and cyclooxigenases, and probably in beta-oxidation as it happens in rats (Izquierdo, 2004). Not only absolute dietary values for each of these essential fatty acids but also optimum dietary ratios among them must be define since both factors will affect at least to their incorporation into the tissue lipids and hence membrane fluidity and function, the energy values obtain from their beta-oxidation and the production of metabolically active compounds. Thus, optimum DHA/EPA ratios have been defined for turbot larvae around 2 (Reitan et al., 1994) and for seabream around 1.2 at least (Rodríguez et al. 1997). Considering both the sum of the three EFAs and the ratios among them, if we plot the dietary value of the ratio (DHA+EPA+ARA)*DHA/EPA/ARA against growth in some of our recent studies (Figure 6), we found a significant correlation. If we apply the same equation to dietary fatty acids in other gilthead seabream studies (Rodríguez et al., 1994, 1995, Salhi et al., 1998, Liu et al., 2002, Koven et al., 2001, Fernández et al., 1995 and others), we found that for ARA values higher than 0.5% the closer the value of the equation (DHA+EPA+ARA)*DHA/EPA/ARA to 50 the better the growth performance.

Phospholipids

Feeding larvae low dietary contents of PL reduces growth and lipid transport from larval enterocytes to hepatocytes (Kanazawa 1993; Izquierdo et al., 2000). For instance, feeding larval gilthead seabream diets without lecithin supplementation produces accumulation of lipidic vacuoles in the basal zone of the enterocyte and esteatosis in the hepatic tissue, both of them being markedly reduced by a 2% addition of soybean lecithin, denoting an enhancement in the lipid transport activity in gut and liver (Izquierdo et al., 2000). This reduction in lipid transport could be related with a limited capacity for "de novo" synthesis of phospholipids in the larvae. Reacilation of phospholipids in the enterocyte is known to occur through the glycerol-3-phosphate pathway in both the rough and the smooth endoplasmic reticulum (Izquierdo *et al.*, 2000). But since marine fish larvae fed microdiets show enterocytes with a poor development of endoplasmic reticulum and Golgi system, reacilation capacity may be limited in these larvae. Moreover, inappropriate dietary lipids have been found to markedly affect re-esterification pathways in seabream gut (Caballero et al., submitted), modifying the type of lipoprotein formed. For instance, addition of soybean oil promotes PC synthesis by both gycerol-3-phosphate acyltransferase and monoacylglycerol pathways, thus providing material for VLDL formation, whereas addition of rapeseed oil inhibits lipid re-esterification, particularly into TG (Caballero et al., submitted).

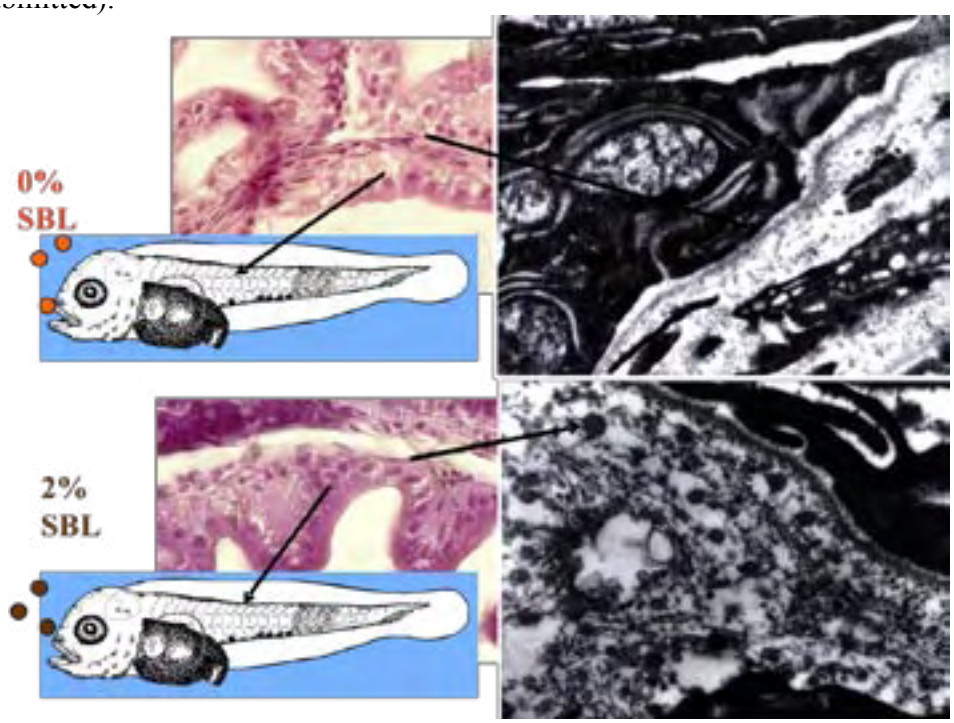


Figure 2 Inclusion of different types of phospholipids in larval microdiets markedly enhance reacilation, lipoprotein synthesis and lipid transport.

On the contrary, when gilthead seabream larvae are fed TG of marine origin, rich in n-3 HUFA it was observed an accumulation of lipid vacuoles in the basal zone of the enterocyte and hepatic steatosis, denoting the good absorption of dietary TG but also a reduced lipid transport to peripheral tissues, whereas feeding with marine PL markedly reduced lipid accumulation in both type of tissues. A higher lipid content due to accumulation of TG and cholesterol esters was found in larvae fed marine TG, whereas in larvae fed marine PL relative proportions of PC and phosphatidyl-ethanolamine (PE) were higher and richer in n-3 HUFA (Salhi et al., 1999). These results agree well with the higher incorporation into larval polar lipids of fatty acids from dietary polar lipids than from dietary triglycerides. In studies with labelled fatty acids dietary n-3 HUFA PL, significantly improved the incorporation of free eicosapentaenoic acid, but not of free oleic acid, into larval polar lipids in comparison to n-3 HUFA rich TG. This specific incorporation of eicosapentaenoic acid when dietary polar lipids are rich in n-3 HUFA could be related to the enhancement of lipid transport, mobilization and deposition in the peripheral tissues by n-3 HUFA rich dietary phospholipids. As a consequence, growth of larval gilthead seabream was improved when they were fed microdiets containing marine PL instead of marine TG despite the slightly lower dietary n-3 HUFA levels of the former (1.5% versus 1.8%, respectively) (Salhi et al., 1999).

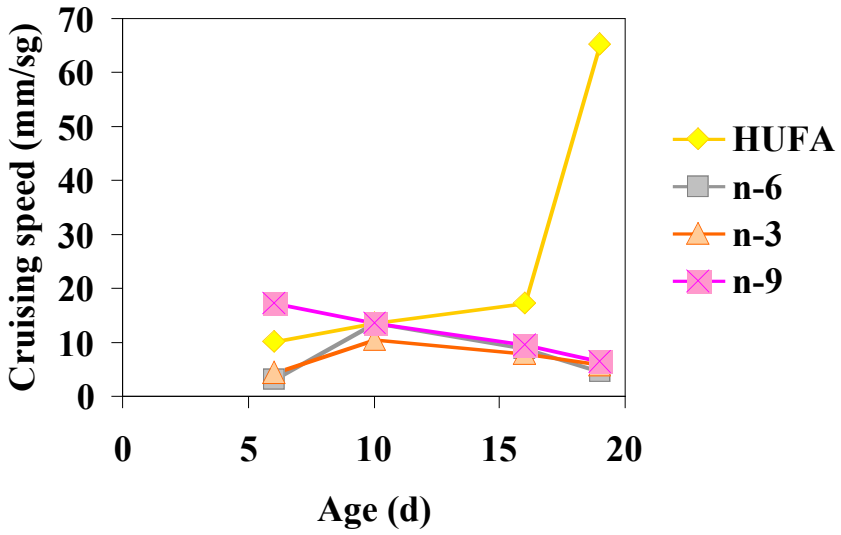


Figure 3. Effect of feeding larval seabream rotifers with different fatty acid composition.

But incorporation of dietary free fatty acids seems to be even lower than that of triglycerides. Thus, labelled oleic acid was better incorporated into both polar or neutral lipids of seabream larvae when it was provided in the diet esterified in a triglyceride than as a free fatty acid, suggesting again a limited capacity of reacilation or transport for dietary long chain free fatty acids or its preferential utilization as energy source in the enterocyte.

Enzymatic, histological and biochemical evidences suggest that marine fish larvae are able to digest and absorb n-3 HUFA rich TG more efficiently than free fatty acids, but feeding with PL, particularly if they are rich in n-3 HUFA, will enhance PL digestion and specially lipid transport allowing a better n-3 HUFA incorporation into larval membrane lipids and promoting fish growth. This confirms former studies which suggest that in addition to the dietary level of essential fatty acids, the molecular form in which they are present in the diet is also important for good growth and survival of marine fish larvae (Izquierdo, 1988; 1996; Izquierdo *et al.*, 1989).

Accumulation of lipidic vacuoles in the basal zone of the enterocyte caused by feeding diets without lecithin supplementation in gilthead seabream disappeared when 0.1% PC was added regardless of its (squid or soybean) origin (Izquierdo et al., 2000). However, squid PC was more efficient in reducing hepatic steatosis than soybean PC, suggesting a combined effect of dietary PC and n-3 HUFA to further enhance hepatic lipid utilization. Indeed both types of molecules have been found to promote lipoprotein synthesis.

Vitamins

The improvement in production of microdiets for larval feeding has greatly facilitated the determination of the vitamin requirements in fish larvae, allowing to experimentally isolate vitamin deficiencies and describing several types of abnormalities. Most described water-soluble vitamin requirements are much higher for larvae than for juveniles of the same species, not only due to the higher metabolic demand in the former, but also for the high ratio surface/volume in larval diets making the diets more prone to oxidation and leaching. Thus, whereas in juveniles vitamin premix accounts for about 2-3% of the diet, in larval microdiets they may reach up to 6-8% of the diet.

Most water soluble vitamin contents of hatchery microalgae and live prey seem to be able to match the requirements of fish larvae, except for the low levels of pyridoxine described in certain studies (González, 1997). However, fat soluble vitamin contents of microalgae and live prey greatly varied among sample batches and with culture conditions, frequently originating hypo and hypervitaminosis.

Vitamin E and vitamin A decreased in seabream from fertilization to the onset of exogenous feeding and a continuous uptake of both nutrients from live preys is observed from day 10th after hatching. However a decrease in the larvae vitamin A content is found when rotifers are substituted by *Artemia* nauplii. Enrichment

of *Artemia* nauplii with fat-soluble vitamins improves amber-jack growth (*Seriola dumerilii*) and seabream microdiet supplementation with 1756 IU of a retinol and beta-carotene mixture significantly improves larval growth. However, bioavailability of beta-carotene seems to be very poor in gilthead seabream in comparison with retinol and astaxanthin which seems to have a provitamin A function in larvae of this species. Regarding vitamin E requirements, progressive elevation of dietary alpha-tocopherol acetate levels from up to 1500 mg/kg in larval seabream diets containing free ascorbic acid significantly reduced larval survival, whereas the same increase in alpha-tocopherol when vitamin C was supplemented as ascorbic acid polyphosphate caused a significant improvement in larval growth without affecting survival, suggesting a pro-oxidative effect of alpha-tocopherol over vitamin C in the former.

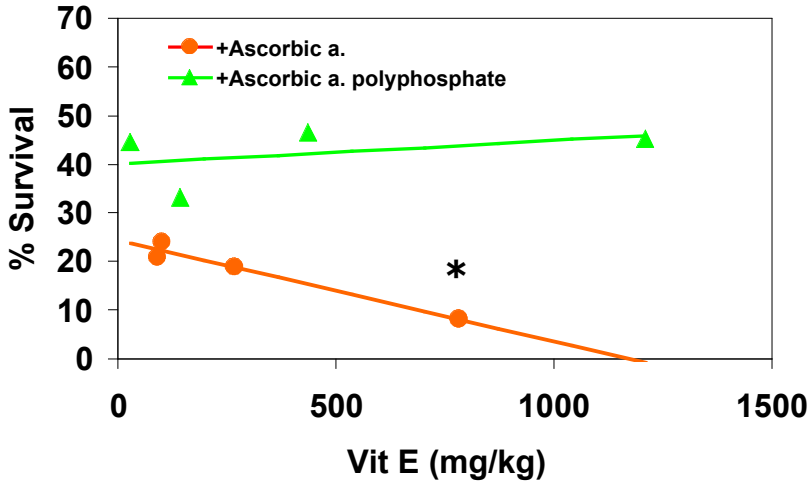


Figure 4. Effect of dietary Vit E in seabream performance in diets containing ascorbic acid in a free or polyphospate form.

Protein and amino acid requirements

Fast growing fish larvae have a high demand for protein requiring more elevated dietary contents than juveniles and adults, microdiets designed for larval rearing containing between 50 and 70 % protein. From the 20 most common amino acids 10 have been found to be essential or indispensable for all studied fish and are required for optimum growth despite fish are not able to synthesize them: Leu, Ile, Val, Thr, Phe, Met, Trp, Arg, His, and Lys. Another two amino acids, Tyr and Cys are only non essential if Phe and Met are present in the diet. At least all those amino acids should be also required by fish larvae. Moreover, the importance of other minor amino acids such as taurine, recently pointed out as essential for best growth and survival of several species of sparids should not be neglected. Methods to determine quantitative requirements of each of those aa in fish larvae include feeding microdiets with graded levels of one amino acid at a time in a test diet containing either all crystalline amino acids, a mixture of casein, gelatin and crystalline amino acids, or a semipurified diet using an imbalanced protein (zein, corn gluten) formulated so that the amino acid profile is identical to the test protein except for the amino acid being tested. As studied by Kanazawa and co-workers for fish larvae of several species, diets are designed to contain protein levels at or slightly below the optimum protein requirement for that species to assure a maximum utilization of the limiting amino acid. Hence, quantitative requirements of several aa have been determined for red sea bream and Japanese flounder larvae (López-Alvarado, 1995). Relations among aa, such as competition or common synthesis pathways, need also be considered. Moreover, aa leaching in the relatively long water staying microdiets, cause difficulties to accurately determine physiological requirements. Hence other methods previously utilized in juveniles have been applied to fish larvae. For instance, from the early 80´s it has been shown that there is not difference between the relative proportions of individual essential aa required in diet and the relative proportions of the same 10 aa present in fish carcass. Since the essential aa profile of fish muscle protein does not differ greatly between individual fish species the pattern of requirement for individual species will also be similar. Thus, analysis of the larval aa composition has been frequently used to predict its essential aa requirements (Watanabe and Kiron, 1994).

Comparison of live prey and fish larvae aa profile would also allow us to predict if such feed would cover the larval aa requirements. For instance, when turbot larvae and live food eaa profiles are compared, the profile of

the latter seems to be deficient in some eaa such as leucine, arginine, threonine or methionine (Conceiçao et al., 1997), depending on the larval age and type of prey, whereas rotifers seem to be deficient in threonine and leucine for larval seabream.

Other methods utilized in juveniles consider that when an essential amino acid is deficient in a diet the major proportion will be used for protein synthesis and only a little fraction will be oxidized to carbon dioxide to obtain energy, whereas if that amino acid is supplied in the diet in excess plasma levels will increase and it will be more available for oxidation. A force feeding method including labelled eaa has been recently developed for fish larvae (Conceiçao et al., 2003), denoting a high retention of labelled doses of eaa in the body (>60%), and low catabolism as measured by liberated 14CO2 (< 25%). In contrast, non essential aa were faster catabolized (>40%).

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Aspects of host-microflora interactions in marine aquaculture: From disease problems to microflora management?

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In most countries the efforts of the aquaculture industry to provide high quality fish and prevent diseases have been remarkable, considering the complexity of the biological systems and the relative novelty of aquatic husbandry. The dramatic decrease in use of antimicrobial agents is mainly due to health promoting measures, including vaccines. However, the production of larvae and fry is still unpredictable for some species, owing to a lack of control of the microbiota in the rearing systems. So far, conventional approaches such as the use of disinfectants and antimicrobial drugs, have had limited success in the prevention or cure of aquatic disease. It is now generally accepted that the use of antibiotics does not constitute a sustainable solution, and may result in microflora imbalance for the larvae [1], and with possible long-term effects. Better means for disease prevention or control should be pursued for intensive aquaculture production systems. Several alternative strategies to the use of antimicrobials in disease control have been proposed and have already been applied successfully in aquaculture. Further progress will depend upon an efficient microbial control, particularly in the production of larvae and during ongrowth, in order to prevent the proliferation and spread of (opportunistic) pathogens, as reviewed in [2, 3].

In marine ecosystems the intimate relationship between bacteria and their hosts, and the relatively open production systems, adds to the complexity of this problem. Intensive aquaculture provides an excellent medium for proliferation and growth of opportunistic bacteria. In the aquatic environment bacteria may travel easily between habitats and hosts, and during intensive larval production, eggs and larvae are in intimate contact with bacteria. Thus the risk of transfection and epizootics is high. Early host-microbe interactions may result in the formation of a protective microflora, or be the first step in infection. Molecular mechanisms of early host-microbe interactions have so far been poorly described. A better understanding of such factors is imperative for successful mass-production of larvae.

In intensive egg production the numbers of bacteria in incubators may be controlled by disinfection, antibiotics or other methods which may disturb the balance of microbial communities, favour proliferation of opportunistic bacteria or result in unpredictable developments. The egg chorion is a well-suited substrate for adhesion of bacteria, and fish eggs become heavily overgrown with bacteria towards hatching. In aquaculture eggs are kept at high densities in incubators with a microflora that differ in numbers and characteristics from that in the sea, and are rapidly overgrown with bacteria after fertilization [4]. The microflora that develops on the egg surface reflects the bacterial composition of the water, but species-specific adhesion to surface receptors may also affect the composition of the epiflora. Members of the epiflora may damage developing eggs, but we do not yet know whether a natural, diverse epiflora may prevent microcolony formation or domination by harmful bacteria. Factors that may protect eggs from bacterial invasion or infection are still poorly understood. Marine fish larvae ingest bacteria by drinking and are thus primed with antigens before active feeding commences [5]. This may result in the formation of an indigenous larval microflora, but at present we know few of the details of host colonisation by commensal bacteria or pathogens in the aquatic environment. Sequestering of intact bacterial antigens by newly hatched larvae may affect their development, but the mechanisms of early host-microbe interactions in aquaculture are still poorly described. The microflora of marine invertebrates resident in fish farms or used as feed may harbour bacteria that are pathogenic to the farmed fish. Thus there is also a need for better understanding of invertebrate–microflora interactions.

Successful aquaculture will rely on better insight into the complex interactions between the cultured organisms and the complex bacterial communities that develop in the rearing systems. The use of probiotics or microflora manipulation may also have a potential in aquaculture, for reviews see [3, 6].

All animals have developed the means to support complex and dynamic consortia of microorganisms during their life cycle. They maintain these societies of nonpathogenic microbes on their mucosal surfaces, and in healthy individuals microbial cells may outnumber somatic and germ cells by a ratio of 10:1. Although the stability of the microflora is important for animal health, very little is known about how its constituents communicate with us to make up stable and mutually advantageous relationships. While considerable attention has been devoted to studying the molecular mechanisms of pathogenic host–microbial relationships, relatively little is known about the molecular foundations of commensal or indigenous host–microbe relationships, and their contributions to normal animal development and physiology. The 'indigenous' microflora comprise microorganisms that inhabit body sites in which surfaces and cavities are open to the environment. However, the term 'indigenous flora' is not clearly defined, and describe the indigenous intestinal microflora composition of a given animal species as a combination of the 'autochthonous flora' present during the evolution of the animal, the 'normal flora' consisting of microorganisms that become established in practically all its members, and some 'pathogens', which are acquired accidentally and are capable of persisting in tissues**.** Today the concepts "indigenous flora" and "normal flora" are usually used interchangeably to describe the collection of microorganisms that normally inhabit the GI tract. For a discussion see [7].

The vast majority of these indigenous microbes reside in the intestine, where studies of gnotobiotic mice have disclosed that the microbiota affects a wide range of biological processes, including nutrient processing and absorption, development of the mucosal immune system, and epithelia. The intestinal tract is densely populated with microbes. The microflora of an individual varies along the gut, and as a function of development and environmental factors. The interplay between the microflora, the epithelium, and the lymphoid tissue is dynamic and reciprocal. Thus the indigenous gastrointestinal (Gl) tract microflora may exert extensive effects on the anatomical, physiological and immunological development of the host. The indigenous microflora stimulates the host immune system to respond more quickly to pathogen challenge and, through bacterial antagonism, inhibits colonization of the GI tract by overt exogenous pathogens. Indigenous GI bacteria may also include opportunistic pathogens that can translocate across the mucosal barrier to cause systemic infection in weakened hosts.

Comparisons of conventionally raised, germ-free animals, and animals that were colonized with components of the microbiota during or after completion of postnatal development (conventionalized) have revealed that a range of host functions are affected by the indigenous microbiota, such as educating the immune system and the gut-associated lymphoid tissue, affecting the integrity of the intestinal mucosal barrier, modulating proliferation and differentiation of epithelial cells and processing nutrients consumed in the diet, reviewed in [8]. Understanding how we establish and sustain mutualistic relationships with the components of our gut microflora is important in understanding the basis of health and the origins of a variety of diseases. Comparisons of germ-free and conventionally raised transgenic rats or knockout mice have established that the 'normal' microflora is an important contributor to the development of inflammatory diseases. Aggressive decontamination of the gut may help to prevent a number of reactions, such as graft-versus-host diseases or rejection. Other recent work supports the contention that structural similarities between microbial epitopes and epitopes expressed on host cells may lead to self-directed immunity, which may explain why *Helicobacter pylori* colonization of the stomach may lead to parietal cell loss, chronic gastritis, and an increased risk of gastric cancer [9].

It is generally accepted that fish contain a specific intestinal microflora consisting of aerobic, facultative anaerobic and obligate anaerobic bacteria. The composition may change with age, nutritional status, and environmental conditions, but apparently a primary transient microflora become established at the larval stage, developing into a persistent flora in juveniles or after metamorphosis. Opportunistic vibrios are common members of the indigenous microflora of healthy fish. Pathogens such as *V. salmonicida* and *V. anguillarum* have been shown *in vivo* to adhere to the intestinal epithelium of fish larvae [2]. Adhesion to mucus is usually considered a first step in the infection process. The *in situ* association of pathogenic and non-pathogenic bacteria isolated from fish to different mucosal surfaces of Atlantic salmon (*Salmo salar* L.) were tested by immunohistochemistry [10]. The majority of tested bacteria, including *V. anguillarum* serotype O1*, V. salmonicida*, *V. viscosus, Flexibacter maritimus*, and apathogenic "gut group vibrios", all adhered to mucus on salmon epithelial surfaces. In contrast, *V. anguillarum* serotype O2, did not associate with mucus, but to other tissue components. No strict surface tropism could be demonstrated as mucous-associating bacteria could be observed in all the salmon

mucosal tissues tested as well as with mucus in piglet ileum sections. Thus adhesion to mucus appeared to be a widespread trait of marine bacteria, and not restricted to pathogens or virulent strains.

It is difficult to determine whether or not a particular microorganism is truly indigenous to a particular host. Colonization begins at birth and is followed by progressive assembly of a complex and dynamic microbial society. Assembly is presumably regulated by elaborate and combinatorial microbial–microbial and host–microbial interactions relying on principles refined through animal evolution. These consortia of indigenous microorganisms may also change during the life-cycle, and in fish particularly during development from larval to adult stages. A complete view of vertebrate biology therefore requires an understanding of the contributions of these indigenous microbial communities to host development. These microorganisms also colonize an amazingly wide variety of habitats, including ecosystems where nutrients are extraordinarily scarce and where environmental stresses are extreme, and as in most natural habitats, it appears that the vast majority of our microbial partners can not be cultured with available techniques. Moreover, what we do know about the molecular foundations for commensalism comes from a very limited number of model organisms.

Fucose is well represented in mammalian cellular glycoconjugates, where it is almost always a terminal, α-linked sugar [11]. A difference between the small intestinal epithelial surface of germ-free and conventionally raised mice involves fucosylated glycans. It has been known for some time that a shift in fucosylation of epithelial cell glycoconjugates may engineer a shift in the microflora of mammals through weaning. The molecular basis for this difference revealed that a member of the normal weaning microflora uses a signaling mechanism that instructs the host to present a source of fucose that the microbe in turn can use to colonize and proliferate in this competitive ecosystem. Studies of a gut commensal of the mouse, *Bacteroides thetaiotaomicron*, has revealed a signaling pathway that allows the microbe and host to actively collaborate to produce such a foundation that can be used by the bacterium, and be mutually beneficial for the bacterium and the host. This type of dynamic molecular interaction may help to define and understand such commensal relationships [9]. Before weaning, Fuc α1,2Gal-containing glycans are not detectable in epithelial enterocytes. This was found in conventionally raised mice that have acquired a microflora or germ-free mice [11]. In conventionally raised mice, production of Fuc α1,2Gal-glycans is expressed during weaning in all enterocytes covering ileal villi. In contrast, fucosylated glycan expression is completely absent by the time germ-free animals are weaned. However, inoculation of a conventional mouse's microflora into a germ-free recipient during adulthood re-initiates Fuc α1,2Gal-glycan production, and the induction and of host α1,2-fucosyltransferase and production of Fuc α1,2Gal-glycans is sustained in ex-germ-free mice for the remainder of their lives. These results indicate that microbial signaling is required for sustaining synthesis of fucosylated glycans. Thes glycans represent a mutual benefit for both the bacterium and the host. By controlling the production of its nutrient source in the intestinal epithelium *B. thetaiotaomicron* may colonize the intestine at weaning, when this ecosystem is already densely populated with a pre-weaning microflora. Once a critical density of organisms is attained, the population can signal the host to provide a sustained supply of this carbon and energy source. It seems reasonable that this is a general strategy that may be used by other members of the commensal microflora, and may involve other glycoconjugates [9]. The host benefits by gaining some control over the composition of its microflora. The nutrient foundation may serve to help organize initial colonization by a cohort of microbes. These microbes further transform nutrient availability to allow the proliferation of other microbial partners. Consequently the resulting microbial consortium are defined by microbial–microbial and microbial–host cross-talk.

In a similar manner, the glycosylation of mucus glycoproteins changes during metamorphosis in Atlantic halibut *Hippoglossus hippoglossus* (L.). These qualitative changes included a shift in the mucus composition from predominantly neutral to a mixture of neutral and sulphated glycoproteins that occurred during the development from a pelagic larva to bottom-dwelling flatfish [12]. Numerous saccular cells were also observed in the epidermis of the yolk-sac larvae that disappeared simultaneously as the mucous cells increased in number in the epidermis of the metamorphosed halibut. These findings may help to understand the protective role of the mucus layer of Atlantic halibut during development as compared to other fish species in aquaculture, and may propose a molecular reason for a shift in the indigenous microbiota during this change in habitat.

In adult fish local mucosal and secretory immunity is important in protection against bacterial infections. Teleosts possess intraepithelial lymphoid tissue, although less organized than in mammals. Macrophages, lymphoid cells and secretory immunoglobulin-forming cells are infiltrated within the intestinal epithelium,

and intestinal epithelial cells of adult fish ingest intact antigens. However, fish apparently lack specialized intraepithelial cells for such uptake, and enterocytes may thus serve in antigen sampling [5]. The "immune capacity" of fish larvae is apparently not fully developed until relatively late post hatching. Until then larvae probably rely on non-specific defenses, and may be influenced by their indigenous microbial consortia.

The study of how mutualistic relationships (symbiotic or commensal) are established between a microbe and its mammalian host represents an emerging field. One obstacle to defining the molecular foundations of mutualistic relationships has been the complexities of the ecosystems where such relationships are negotiated. This is particularly true in the intestine, where identifying a 'language' involving one or more of its resident commensals is difficult, because a multitude of signals are occurring at the same time and because we do not yet know what to look for. These models permit experimental analysis of how both host and microbe actively collaborate in shaping and manipulating the gut's nutrient foundation, and the approaches used, and the results obtained, are likely to be applicable to other animal ecosystems [8].

Marine models that have contributed significantly to the understranding of host-pathogen interactions are scarce. However, symbiosis is considered a higly evolved and sophisticated form of host-microbe interaction, and marine habitats have abundant examples of symbiotic relationships. One particularly elegant and ground-breaking model is the study of the interaction between a species of marine squid (*Euprymna scolopes*) and its bacterial symbiont (*Vibrio fischeri*). This study was among the first to reveal that bacteria can induce morphological phenotypes in their animal partners, and demonstrated that bacteria can play a crucial inductive role in the normal development of animal organs by effecting fundamental developmental processes, such as cell death and differentiation [13]. The developmental consequences of the squid–Vibrio interaction share some similarities with the microbial impact on mammalian gut development. The squid–Vibrio partnership is founded on mutualistic benefit. The bacteria inhabit the light organ of the squid and produce the light that illuminates the lower surface of the animal, providing camouflage. Thus, both partners benefit from the association: the bacteria are offered a protected nutrient-rich niche, whereas the host is somewhat protected from predators [13]. Squid that are not exposed to *V. fischeri* remain in an arrested state of morphogenesis, and thus the microorganism actively promotes its association with its host.

Comparison of germfree versurs conventional zebrafish (*Danio rerio)* provided an opportunity to investigate the molecular mechanisms underlying such interactions through genetic and chemical means during larval and juvenile stages germ-free and conventional zebrafish [14]. Using a DNA-microarray technology, this work demonstrated that 212 genes were differentially expressed in germ-free fish in respect to conventionalised fish. In addition it was demonstrated that some genes were specifically expressed in response to certain micro-organisms, indicating that the early colonisation of the GI tract by a particular microorganism can be responsible for changed metabolism in the fish larvae. The results with zebrafish demonstrated that microorganisms may stimulate the intestinal epithetial development and affect the enterocyte morphology also in fish. The findings also provided an argument for using gnotobiotic wild-type and or genetically manipulated zebrafish as a model organism for deciphering the molecular foundations of symbiotic commensal host–bacterial relationships in the vertebrate digestive tract.

In aquatic ecosystems the intimate contact between microorganisms and other biota, and the constant flow of water through the digestive tract of fish and invertebrates affect host-microflora relationships. A diverse and natural indigenous microflora, but it is not yet known to what extent the natural microflora of fish may protect against pathogen colonization, for discussion see [2, 3]. There is increasing evidence that microflora manipulation, or addition of microorganisms that are antagonistic or probiotic, may improve health conditions and survival of larvae in intensive rearing. The term probiotic is mostly used for "living cells that exerts beneficial health effects on the host by improving the microbial balance or properties of the indigenous microflora" [15]. The use of probiotic microorganisms has proven advantageous in domestic animal production, and is one of the most significant technologies that have evolved in response to disease control problems. Such "bacterial management" approaches are applied at production level in poultry farming. Considering the recent successes of these alternative approaches, the Food and Agriculture Organization of the United Nations defined the development of affordable and efficient vaccines, the use of immunostimulants and nonspecific immune enhancers, and the use of probiotics and bioaugmentation for the improvement of aquatic environmental quality as major areas for further research in disease control in aquaculture [16]. The characteristics may

include antagonism or colonization prevention towards pathogens, stimulation of immunity or innate defenses or health benefits from released factors. The use of probiotics in aquaculture has been reviewed [6, 17].

Table 1. Some effects of probiotic bacteria, modes of action, aquaculture systems where they have been used, and selection criteria.

Probiotic effect	**Modes of action**
Growth promotion	Produce inhibitory compounds
Effects on other infections	Compete for chemicals or energy
Alleviation of lactose intolerance	Compete for iron
Anticarcinogenic effect	Compete for adhesion sites
Enhancement of immune response	Interaction with phytoplankton
Increase production in aquaculture	
Improvement of water quality	
Aquaculture system used	**Selection criteria for probiotics**
Fish eggs and larvae	Acquisition of putative probiotics
Fish juveniles and adults	Screening and preselection
Crustaceans and Penaid shrimps	In vitro antagonism tests.
Crabs	Colonization and adhesion
Bivalve molluscs	Small-scale tests
Live food	Evaluation of pathogenicity of strains
- Unicellular algae	*In vivo* evaluation of probiotic effects
- Rotifers	Mode of application of putative probiotic
- Artemia	Experimental infections
Microbially matured water	*In vivo* antagonism tests.
Interaction with nutritional effects	

In aquaculture severe microbial problems may start at the egg stage, and consequently manipulation of the egg epiflora would appear reasonable. We tried to manipulate the egg epiflora by incubating aseptically dissected, bacteria-free eggs with defined cultures of antibiotic producing bacteria [4]. However, the antibiotic-producing isolates failed to prevent colonization by a natural seawater microflora. This may suggest that egg-surface receptors that accounts for a colonization of a diverse natural microflora may furnish protection against domination by particular strains [4].

It has been observed that *Artemia* and rotifer cultures may transfect bacteria that are harmful to the fish larvae during start feeding, and various attempts at microbial control of the startfeeding cultures the live food organisms (*Artemia* and rotifers) used for larvae. Microbial control of *Artemia* juveniles has been achieved by pre-emptive colonization by selected bacterial strains [18], and the resulting changes in the *Artemia* microflora appeared stable. In contrast, preincubation in bacterial suspensions may be aimed at suppressing opportunistic bacteria in *Brachionus* or *Artemia* cultures [19] - an ecosystems approach to suppress opportunistic bacteria. Successful attempts at controlling the microflora by manipulating of the ecosystem has been reported, using microbially matured water in a system that competitively selects against opportunistic and potentially pathogenic bacteria [20]. It thus appears that improved microbial control of food organisms used for larvae is feasible through microflora or ecosystem manipulation. However, stable transfection of microorganisms between hosts, or from food organisms to hosts, is difficult and still has to be tested for long-term effects.

In intensive invertebrate culture diseases often prevail as a result of the build-up of organic pollution. Promising results have been obtained in shrimp pond aquaculture in China, by adding immobilized bacteria that effectively reduce sediment organic pollution, an example of probiotic bioremediation [21].

Lactic acid bacteria have been isolated from the intestinal mucosa of a variety of fish species, and may produce growth-inhibiting factors that could inhibit various *Vibrio* spp., especially *V. anguillarum*. Lactic acid bacteria are widely used as probiotics, and the use of a *Carnobacterium* sp. as a probiotic for Atlantic salmon and rainbow trout has been reported [22], and the potential of lactic acid bacteria as probiotics in aquaculture has been reviewed [23].

Vibrios are natural members of the indigenous microflora of healthy fish, and also dominate in the intestinal tract of marine fish, reviewed in [3]. Fish pathogenic vibrios may be present on wild fish or healthy fish in aquaculture, and commensal vibrios with inhibitory activity against pathogens may be isolated from mucosal surfaces of healthy fish. It has been assumed that commensal, apathogenic strains that may help to confer protection against related pathogenic strains, reviewed in [3]. We observed, in agreement with this concept, that survival of halibut (*H. hippoglossus*) larvae could be affected by incubation with indigenous bacteria isolated from fish [24]. Thus, introducing a strain that may compete with the pathogen may increase survival. However, introduction of apathogenic strains may not be unequivocally beneficial. We also demonstrated increase in the number of epidermal saccular mucous cells of halibut larvae following incubation of the larvae in seawater with increased numbers of bacteria and following addition of apathogenic bacteria to the incubators [12], suggesting that changes in the microflora may induce non-specific defenses of the larvae.

Many bacteria that cause diseases of humans and animals are highly motile. The role of motility and chemotaxis in the host-parasite relationship of pathogenic bacteria has been reviewed [25], arguing that for many pathogens, motility is essential in some phases of their life cycle and that virulence and motility are often intimately linked by complex regulatory networks. Possibilities to exploit bacterial motility as a specific therapeutic antibacterial target to cure or prevent disease are discussed. Also for some fish pathogens motility appears to play a dominant role during the infection process. Using a range of motility-mutants, we demonstrated that motility per se was required for the association of *V. anguillarum* O1 with mucus, and that no adhesin-receptor interactions could be demonstrated to play a role in this association (G.K. Knudsen and J. A. Olafsen, unpublished). The motile mutants included one with full motility but no chemotaxis, and three with reduced motility due to a truncated flagellum. The non-motile mutants included four with no flagellum and one with a paralysed flagellum.

Perspectives

The basic mechanisms of microbial interactions during production of fish larvae in aquaculture are not well understood. To achieve improved microbial control in larval rearing systems we still need information about bacterial colonization factors, host regulation of the adherent microflora as well as interaction with egg surfaces and food organisms. Also, we still lack information about development of tolerance to the commensal microflora.

Most marine bacteria adhere well to fish mucus, but we also lack information about microbial adhesion factors as well as non-specific defence systems in mucus. Despite the fact that many fish pathogenic strains are relatively host specific, little is yet known about receptors for bacterial adhesion. Moreover, for marine bacteria there is a conspicuous lack of information about invasion strategies like antigen shift or phase variation, mechanisms that are known to be key factors in microbial pathogenicity.

The use of probiotics, or microbial manipulation, in intensive rearing of marine organisms may have a profound potential in health management. However, it is not likely that improved microbial control may be achieved by finding the “ideal probiotic solution”. Various stages and situations may call for different approaches, such as antagonism, competition, bacteriocin production, immune stimulation, health promotion and bioremediation. Thus it is likely that the use of a selected mixture of beneficial strains, or different approaches at different stages may prove more effective in different situations - and more stable over time.

A feasible approach in aquaculture will be the use of controlled bacterial communities at various critical stages of larval rearing. Such “multifunctional bacterial communities” will have several advantages over single-strain probiotics. Introduction of such measures will require a better basic understanding of various basic aspects of host-microbe interactions in the marine environment and a set of protocols for practice of bacterial management in fish hatcheries for the industry to take advantage of collected experience.

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Current status of live food culture in Japan

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Abstract

Euryhaline rotifer *Brachionus plicatilis* complex has been used as a first live food for marine fish larvae. Even under similar environmental conditions, genetically close rotifer strains have variable size, thus, selection of appropriate size of rotifer strains is useful for feeding fish larvae whose mouth sizes differ among species and growth stages. Rotifer life history parameters such as reproductive characteristics and lorica size can be regulated artificially using chemicals such as neurotransmitters and hormones. In vivo enzyme activity test is useful for detecting instability of rotifer cultures.

Culturists generally feed rotifers several times a day. In this condition, however, rotifers suffer periodical starvation, which results in low quality rotifers that live long and spawn less. Recent development of continuous culture system for rotifer mass production has enabled the aquaculturists in Japanese public hatcheries to carry out stable rotifer production of higher quality live food. This system utilizes large tanks (e.g. 25 m^3) used in conventional rotifer cultures. With continuous culture water inflow, feeding and harvest, L-type *B. plicatilis* can be produced at 0.2-1x10^8 rotifers/day/m^3 for 40-220 days. Rotifer population growth rates of this system are higher than those of conventional batch culture or semi-continuous culture methods, while the production costs are the same. Live rotifers can be stored under low temperature such as 4-12^{o}C. Resting eggs are appropriate for long-term preservation of rotifers.

Keywords: live food; rotifer; practical mass culture; basic research

Introduction

Before 1960, euryhaline rotifer *Brachionus plicatilis* was considered a noxious animal, which caused huge damages in traditional eel cultures conducted in outdoor pond in Japan. Because of their rapid reproductive rate, rotifers completely consume phytoplanktons and completely dominate ponds resulting to a change in water color from green phytoplankton to brown rotifers. This phenomenon is called "Mizukawari" (change of water color). From 1950's, Takashi Ito conducted a series of studies on this phenomenon (e.g. Ito, 1958, 1960, 1971), and revealed the difficulty of eliminating rotifers using chemical treatments, while maintaining good water quality necessary for keeping eels alive. Although ponds are dried off during off season, rotifers still survive since they are capable of producing resting eggs, which can thrive in dried pond sediments. Resting eggs are highly tolerant to toxicants and environmental stressors, thereby posing greater difficulty in removing them from pond sediments.

In the same period, research has been focused to develop techniques for artificial breeding as well as mass rearing of producing juveniles of marine fishes. Culturists however, have encountered difficulty in obtaining sufficient amount of live food for fish larvae (Hirano and Ooshima, 1963). The success of acclimating brackish *B. plicatilis* to seawater (Ito, 1960) enabled its use as diet for rearing larvae of marine fishes. Studies were conducted to establish *Nannochloropsis* and baker's yeast as rotifer food (reviewed by Hirata, 1980), as well as to detect nutritional problems of mass cultured rotifers as live feed and improvement of nutritional value by the fortification of n-3 HUFA (reviewed by Watanabe et al., 1983). The development of marine fish larval rearing technique as well as of rotifer mass culture technology have been reviewed by several authors (Fujita, 1973; Fukusho, 1983). During late 1980's, phytoplankton industry has developed products such as paste of condensed microalgae that enabled them to cultivate high density rotifer of more than 1x10^3 ind./ml (Yoshimura et al., 1996).

Recently, Hagiwara et al. (2001b) reviewed the development of technique on rotifer culture, as well as the progress of laboratory studies, which can be used as a basis for rotifer culture practices. In this paper, we attempt to summarize some progress obtained thereafter.

B. plicatilis strains are quite variable in size and shape. Based on the results of morphometric and genetic (allozyme and karyotype analysis) studies with *B. plicatilis* (reviewed by Hagiwara et al., 1995, 2001b), Segers (1995) reclassified large morphotype into *B. plicatilis* and small morphotype into *Brachionus rotundiformis*. Recent works on rotifer gene analysis provide the possibility of several species boundaries among geographically isolated *Brachionus* strains. Ciros-Pérez et al. (2001) suggested the use of *B. ibericus* as the S-type strain found in Torreblanca marsh in Spain. Based on these facts, we use the term *B. plicatilis* complex with this group, and when further description is necessary, we describe them as one of three morphotypes L, S and SS, according to Hagiwara et al. (2001b).

Diet for Rotifers

The use of baker's yeast as rotifer diet is still beneficial to aquaculturists since it allows rotifer production at a cheaper price. But the culture is less stable due to the decline of water quality. Moreover, the produced rotifers need further nutritional enrichment before feeding to fish larvae. Phytoplanktons are still the ideal food for rotifer cultures, because it results to higher stability of culture, but its low productivity poses a problem in ensuring enough amount of food for rotifer production.

Chlorella Industry Co Ltd. pioneered the production of commercial microalgal products. They started on producing freshwater *Chlorella. Chlorella* has genetically high productivity (e.g. doubling time) compared to other marine microalgae, and they can be mass cultured in organic medium at the same growth rate without light (Maruyama and Hirayama, 1993). Hirayama et al. (1989) succeeded in incorporating Vitamin B_{12}, an essential vitamin for rotifers inside the *Chlorella* cells. In 1997, Hayashi & Maruyama (in prep) developed a technique to introduce n-3 HUFA inside Chlorella cells and such product is already commercially available. Other *Chlorella* products also contain large amount of βcarotein, that make fish larvae to have higher tolerance against viral disease (Tachibana et al., 1997). Frozen paste of marine alga *Nannochloropsis oculata* is also a popular product mainly because of its higher EPA content.

Recent laboratory studies in this area provided basic genetic information that are important for future technology development. A *Chlorella* strain acclimatized at 3°C can survive even after they are frozen, and this mechanism involves the appearance of genes for n-3 fatty acid desaturase (Suga et al., 2002). Through treatments of mutagenesis, Chaturvedi et al. (2004) obtained a *N. oculata* strain that contain higher amount of eicosapentaenoic acid with higher temperature optima for growth.

Rotifer mass culture

Before the introduction of phytoplankton products as major food for rotifer culture system, baker's yeast was widely used for rotifers mass cultured by batch or thinning-out (semi-continuous) methods. Despite its cheaper cost, the overfeeding of yeast tend to bring a decline in water quality and result to instability of rotifer culture. With this system, the amount of food is insufficient to the physiological demand of rotifers, thus, rotifers are likely starved. Yoshinaga et al. (2000) reported however, that periodical starvation of maternal *B. plicatilis* has a positive effect because it can induce higher starvation tolerance in offspring, although it reduces fecundity on the exposed animals.

Due to the commercial availability of microalgal diets, it became feasible to provide ample amount of diet for rotifer population without causing water quality to decline. Thus, continuous feeding of phytoplankton diet would result in higher productivity of rotifers compared to traditional culture system. Based on this concept, high density batch-culture technique of rotifers have been developed (reviewed by Yoshimura et al., 1996, 1997), and recent modification of this technique achieved the culture density exceeding $1x10^5$ ind./ml (Yoshimura et al., 2003). The development of this technique allows small hatcheries to produce sufficient amount of rotifers even in small tanks. It is generally recognized, however, that the physiological condition of rotifers at higher culture density may deteriorate either due to ammonia accumulation, bacteria/protozoa influence, food shortage or oxygen decline. One arising question is, whether rotifers produced at ultra high density would have equal quality as diet for fish larvae than those produced using traditional method.

Recent study on red seabream *Pagrus major* larvae (Tomoda et al., 2004) revealed that nutritional quality of rotifers varies depending on the population growth stage. Furthermore, fish larvae fed on those rotifers have different body chemical composition. In this experiment, batch cultured rotifers were harvested at four different growth stages: lag, exponential growth, late-exponential and stationary phases. Table 1 summarizes these results.

Table 1. Rotifer density, mortality, and egg ratio enriched at different culture days and red seabream *Pagrus major* growth and chemical composition fed enriched rotifers (after Tomoda et al., 2004).

	A	B	C	D
Rotifer				
Density at harvest (ind/ml)	$0.25 \pm 0.1 \times 10^3$	$0.65 \pm 0.1 \times 10^3$	$1.2 \pm 0.2 \times 10^3$	$1.8 \pm 0.4 \times 10^3$
Mortality 6 hrs after enrichment[a]	1.7 ± 0.7^{a}	1.8 ± 1.2^{ab}	2.9 ± 1.1^{ab}	6.2 ± 3.2^{c}
Egg ratio 6 hrs after enrichment[a]	46.4 ± 8.3^{a}	31.5 ± 7.1^{b}	38.1 ± 8.6^{ab}	35.7 ± 4.8^{b}
Fish Larvae				
Total length (mm)[b]	6.35 ± 0.5^{a}	6.30 ± 0.5^{ab}	6.25 ± 0.4^{ab}	6.0 ± 0.5^{c}
EPA content (g/100g)	0.60 ± 0.02	0.58 ± 0.03	0.5 ± 0.05	0.4 ± 0.01
DHA/EPA	5.02 ± 0.33	5.45 ± 0.59	6.24 ± 0.53	7.31 ± 0.80

[a] Values are means and standard deviation of 10 replicates per treatment. Values with common letter are not significantly different (Scheffe's test) at 95% confidence level.

[b] Values are means and standard deviation of 150 replicates per treatment. Values with common letter are not significantly different (Tukey-Kramer test) at 95% confidence level.

A,B,C and D are days 2,4,6, and 8 of *Chlorella* enrichment, respectively.

Although the rotifers received the same nutritional enrichment, rotifers in the late-exponential and stationary phases showed poor quality, such as lower egg ratio (number of carried parthenogenetic eggs per female) and lower survival. Larvae fed rotifers at the stationary phase also showed poor performance in growth and nutritional condition; total length was smaller on day 20 posthatch, and had less quantity of eicosapentaenoic acid (EPA) in larval body and higher content of DHA/EPA.

As discussed in Hagiwara et al. (2001b), the continuous culture system is more advantageous to maintain good water quality for rotifer culture (James and Razeq, 1986, 1989; Walz, 1993), and a possible solution to avoid production of rotifers with low quality. It appears that such system has greater potential to aquaculturists. A high density rotifer culture was developed using continuous culture system in 1-2 m^3 small culture tank. With this system, the culture density of SS type was maintained at 3 to $6x10^3$ ind./ml (Fu et al., 1997). This technique, however, can be effectively applied to S and SS-type *B. plicatilis*, while culture practices with L-type have been less common. But high-density continuous culture of rotifers showed less productivity in L-type *B. plicatilis* cultures and the entire equipment is costly for private hatcheries (Fu et al., 1997).

To date, Kuwada's group in Fisheries Research Agency (former Japan Sea Farming Association) developed an extensive continuous culture system for rotifers utilizing large rotifer culture tanks (20-50 m^3). This system has been common in public hatcheries in Japan (reviewed by Anon. 2000), because it does not require extra cost. The system is composed of one tank each for rotifer culture and harvest, which receives continuous inflow of filtered seawater and freshwater to adjust the salinity to 20 ppt, as well as continuous feeding of refrigerated condensed *Chlorella*. To further reduce the cost, a part of *Chlorella* can be replaced by cheaper diet such as baker's yeast (Snell and Hoff, 1985). The rotifer culture was continuously harvested by siphoning (Figure 1).

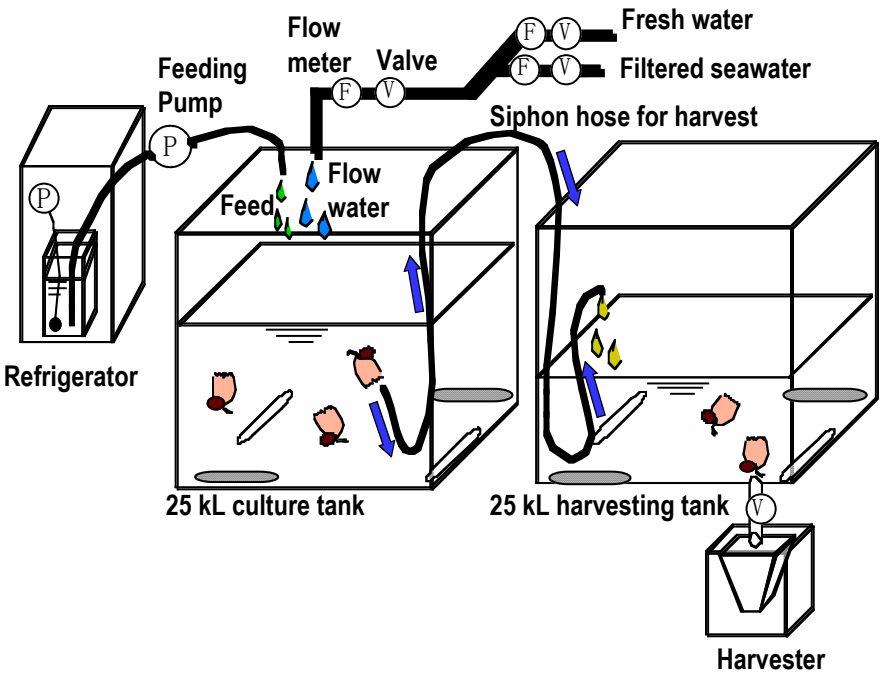

Figure 1. Schematic overview of extensive continuous culture.

This system is advantageous to provide rotifers a stable environment that enables them to maintain stable population growth. This system also utilizes a chemostat system, which maintains the amount of food at food limitation level, and maintains the dilution rate constant. In the steady state condition, therefore, dilution rate equals to rotifer specific growth rate, thus allows the culturists to harvest rotifers automatically, which are decided by spontaneous performance of rotifers based on given amount of diet and environment. With this system, culturists do not need to regulate the amount of feed to attain maximum growth of rotifer population. For example, about 1 x 10^{10} L-type *B. plicatilis* can be harvested daily in a 25 m^3 tank for 40 continuous days at 25°C with daily feeding amount of approximately 2.6 kg dry weight diet composed of *Chlorella* and baker's yeast. In our records of different culture trials, rotifer density was generally maintained between 100-500 ind./ml with population growth ranging between 70 and 100% per day. Culture period can reach to more than 200 days using the same culture unit. This system can also be applied to traditional thinning-out cultures, by regulating the water inflow and constant and continuous feeding, even though rotifer harvest is conducted once a day only. Another advantage of this system is that, culturists can conduct rotifer mass cultures at desired temperatures. In Figure 2, rotifer population growth rates were compared among different methods, and extensive continuous culture system showed a wide spectrum of temperature range, and gave high rotifer production and harvest. Especially at lower temperature between 10 and 20°C, this method produced more rotifers, compared to other methods.

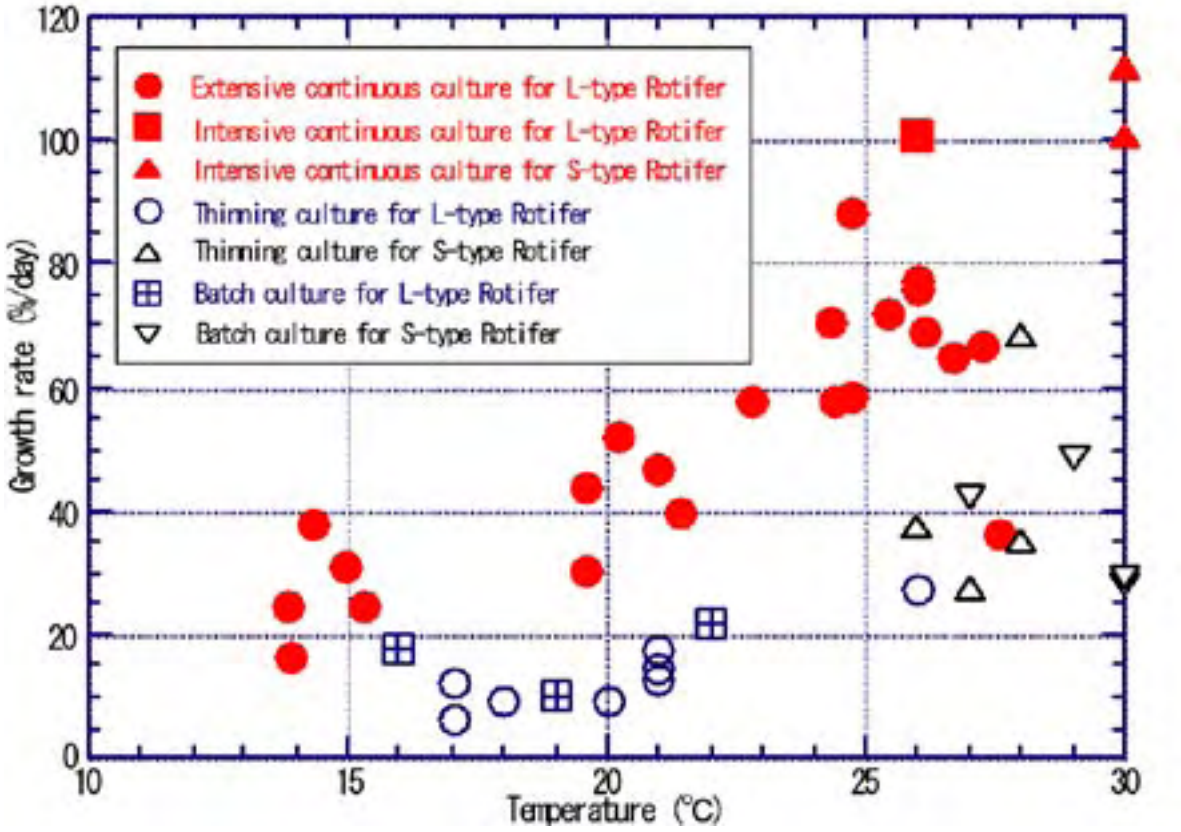

Figure 2. Population growth (%/day) of L- and S-type rotifers in different culture system.

Fundamental rotifer study and future application

Regulation of rotifer size

Size is one of the important biological parameters of *B. plicatilis*, since size-dependent food selectivity with *B. plicatilis* is found in marine fish larvae (Oozeki et al., 1992; Olsen et al., 2000). Some fish species including groupers can ingest only small sized diet of less than 100-150 µm, and, thus, the use of tropical SS-type *B. plicatilis* strains is important.

Figure 3 shows the lorica length and width of 70 rotifer strains of *B. plicatilis* including L-, S- and SS-type. These strains were collected from

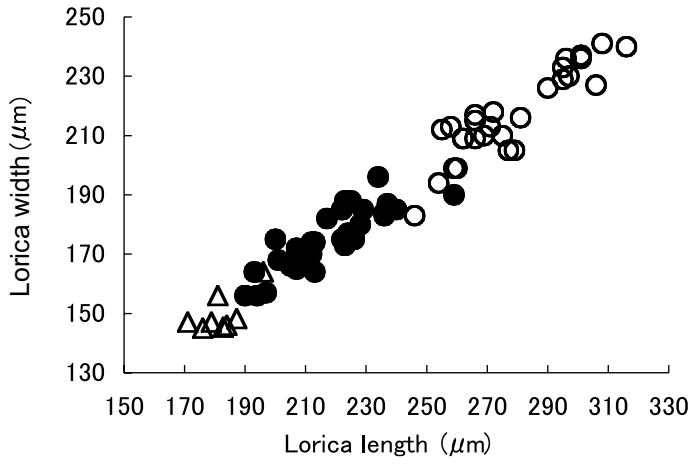

Figure 3. Relationship between lorica length and width of *Brachionus plicatilis*. Open circle, closed circle and triangle indicate data of L-, S- and SS-type *B. plicatilis*, respectively.

different sites in the world (Fu et al., 1991; Hagiwara et al., 1995), cloned and cultured in the laboratory at 23-25°C, maintained at 20-22 ppt salinity, fed *Nannochloropsis oculata*. It is known that the shape of lorica also differs among three rotifer types; S and SS-type lorica is more round than L-type. Rotifer size at sexual maturity (first spawning of parthenogenetic egg) was compared. A large variation in size was detected among rotifer strains within the same type and size distribution overlaps with other rotifer types was also observed. Although the lorica shape is variable, this does not affect the measurement of its length and width, because the plots fit in a linear pattern. It should be noted that the plots on Figure 3 shows only one aspect of rotifer size distribution. Rotifers are known to grow faster during the initial days from hatching; 30-40% growth of lorica length is observed during the first 48 hours from hatching at 25°C. A large plasticity in size is observed in rotifers under variable environment. For example, the lorica length of SS-type ranges between 170-195 µm at 25°C (Figure 3), while it reduces to less than 150 µm at 30°C. An L-type strain with the lorica length of about 280 µm at 25°C can grow to up to 330 um at 15°C (Hagiwara et al. unpublished). Moreover, feeding *B. plicatilis* with *Tetraselmis* results to 12.9% larger (lorica length) rotifers, compared when given *Nannochloropsis* (Okauchi and Fukusho, 1984).

Chemical treatment can also regulate the rotifer size (Table 2). When gamma-aminobutyric acid (GABA), 20-hydroxyecdysone and juvenile hormone were added to rotifer batch culture, a 4.4 to 9.6% increase in lorica length was observed. The mechanism of action of these chemicals in rotiers, however, has not been clarified.

Table 2. Lorica length and width of rotifer *B. plicatilis* after addition of chemicals (after Gallardo et al., 1997).

Chemical used (concentration)	Lorica length	Lorica width
γ-aminobutyric acid (5mg/L)	4.4% larger	n.s.
20-hydroxyecdysone (0.05 mg/L)	3.9 % smaller	4.8 % lower
Juvenile hormone (0.05 mg/L)	9.5% larger	8.9 % higher
Prostaglandin (0.05 mg/L)	5.8% larger	n.s.

Larger and smaller means % increase and decrease relative to the control (without chemical treatment), respectively.
n.s. means no observable difference compared to the control.

Culture diagnosis and treatment

The physiological processes involved in rotifer reproduction are still ambiguous. The effects of addition of some vertebrate hormones and neurotransmitters to the rotifer culture have been investigated in Hagiwara's laboratory. Changes in life history parameters were thoroughly monitored and analyzed. Tables 2 and 3 show the list of these chemicals and their effects on rotifer size, sexual and asexual reproduction. Rotifer sexual reproduction involves a series of processes such as the appearance

Table 3. Increase in population growth and mixis induction in rotifer *B. plicatilis* after addition of chemicals (after Gallardo et al., 1997).

Chemical used (concentration)	Population growth*	Mixis induction**
γ-aminobutyric acid (50mg/L)	2.0 times (on day 4,6,8)	n.s.
Porcine growth hormone (0.025 I.U./mL)	1.7 times (on day 8)	n.s.
Human chorionic gonadotropin (2.5 I.U./mL)	2.4 times (on day 6)	n.s.
Juvenile hormone (0.05 mg/L)	n.s.	1.9 times
Serotonin (5mg/L)	1.5 times (on day 6,8)	1.7 times

*values are compared with the control (without chemical treatment) on daily basis
**values are total increase on the final day of culture
n.s. means no observable difference compared with the control

of mictic females that conduct meiosis, parthenogenetic production of haploid males by unfertilized mictic females, mating, copulation and fertilization, and resting egg formation by fertilized mictic females. Among the chemicals tested, GABA and HCG (human chorionic gonadotropin) doubled the rotifer population growth (Table 3; Gallardo et al., 1997). Addition of GABA is especially effective when rotifers are exposed to stress such as shortage of food and increase of unionized ammonia (Gallardo et al., 1999).

Studies have been conducted to establish techniques to assess the physiological status of cultured rotifers, as well as to predict culture collapse. Table 4 listed possible methods to detect physiological condition of rotifers. Egg ratio means the number of parthenogenetic eggs per female, and has been regarded as an important parameter to assess the status of rotifer culture. This practice started in late 1960's. The flaw of this parameter is that, it takes 24 hours at 25°C until change in egg ratio happens after rotifers are exposed to unfavorable environment. Environmental change rapidly affects swimming speed and ingestion rate of rotifers, but it takes more than 1-2 hours to accomplish the measurements even using computer motion analysis system. In vivo enzyme activity test is also known to be effective in assessing the rotifer culture status, but again it takes 1 to 2 hours to conduct the analysis, and this method needs further refinement before it can be applied in rotifer culture practice.

Table 4. Index used to assess rotifer culture and their characteristics.

Index Used	Features	Reference
Egg ratio	easy; measurement could be done within 30 minutes; takes 1-2 days to reflect egg ratio	Snell et al., 1987
Ingestion rate	easy; takes 2-3 hours to measure; rapid response against environmental change; large variation in data	Ferrando et al., 1993 Juchelka and Snell, 1994
Swimming speed	easy; takes 1-2 hours to measure; rapid response against environmental change	Snell et al., 1987 Jassen et al., 1994
Enzyme activity	simple and easy, needs special instrument such as computer, flourometer, and image analyzer; takes 1-2 hours to measure; rapid response against environmental change	Moffat and Snell, 1995 Araujo et al., 2000

Changes of ingestion rate due to environmental stress provide us an insight of other aspects of rotifer physiology. Mass cultured rotifers need nutritional enrichment, which usually contain fatty acids that could degenerate the water quality. However, nutritional enrichment could not be avoided because it is paramount for fish larvae. It is plausible that culture collapse in mass cultured rotifer is related to the decrease of feeding activity of rotifers. Araujo and Hagiwara (2004) found that addition of GABA can improve the health condition of rotifers when they are exposed to stressful environment (e.g. increase of unionized ammonia, protozoa contamination). Addition of GABA during nutritional enrichment culture could improved survival and swimming activity of rotifers (Gallardo et al., 2001).

Despite the progress of techniques in rotifer mass culture, diagnosis of culture status and treatment for culture for recovery, it is important to have a means of rotifer preservation. To date, techniques have been developed to store live rotifers under low temperature such as 4-12°C (Assavaaree et al., 2001; reviewed by Hagiwara et al., 2001). Resting eggs are appropriate for long-term preservation of rotifers. The advantages are reviewed in several publications (Hagiwara and Hirayama 1993, Hagiwara 1994, Hagiwara et al., 1997)

Rotifers as larval diet

Dietary values of rotifers should not be limited to its nutritional quality. As fish larvae grow, the amount of food they need increases and they prefer to eat larger-sized prey (Ivlev, 1965). It has been generally accepted that the optimal prey size for fish larvae is determined by their mouth size. Feeding regime of fish larvae is

designed primarily based on mouth size data. This has resulted in successful improvement of larval survival and growth in many fish species. Adjustment of the amount of feed depending on larval developmental stage also resulted in better growth and survival. (Kitajima, 1978). Limited information is available, however, on rotifer size selectivity of fish larvae. Some of these, are studies on developmental change of food selectivity in striped mullet (Oozeki, 1992) and Atlantic halibut (Olsen et al., 2000) larvae. In Hagiwara's laboratory, a similar study was done on threeline grunt *Parapristipoma trilineatum* larvae for 15 days posthatch. During the initial 7 days, the larvae grew better by feeding S-type rotifer (90-210 µm in lorica length) than by feeding L-type (160-320 µm). The feeding of L-type rotifer resulted in slower growth during the initial 7 days, but showed better growth than that of S-type after 7 days posthatch. Olsen et al. (2002) studied the food selectivity of larvae when L (>174 µm in lorica length), S (137-174 µm) and SS-type rotifers (<137 µm) were mixed and fed. The 3, 4 days posthatch larvae showed high selectivity with SS-type, 6-9 days posthatch with S-type and 9-16 days posthatch with L-type. On day 17 posthatch, larvae showed highest selectivity with *Artemia* nauplii (ca. 800 µm) (Olsen et al., 2002). Comparison of food selectivity among fish species was also conducted with yellowtail *Seriola quinqueradiata*, spotted halibut *Verasper variegatus* and flathead *Platycephalus* sp. from day 0 to 15-20 days posthatch. The result indicates that among the three species, flathead larvae showed the highest selectivity to larger rotifers despite their mouth size being the smallest (mouth sizes at the onset of feeding were 280, 510 and 260 µm for yellowtail, spotted halibut and flathead, respectively). These results indicate that food selectivity of fish larvae does not only depend on larval mouth size, but also on species-specific characteristics. (Hagiwara et al., 2001a). It is therefore important to establish a rotifer culture stock comprised of many strains of variable sizes in order to have rotifers with sizes appropriate for different species and developmental stages of fish larvae.

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European Aquaculture: Recent Developments in Marine Hatchery Technologies

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Introduction

The most recent review of the state of world aquaculture from the Fisheries and Agriculture Organization (FAO) 2003 and the FAO Fishstat Plus statistics from 1950-2001 highlights the continuing growth of aquaculture in contributing to the total fisheries catch (Figure 1).

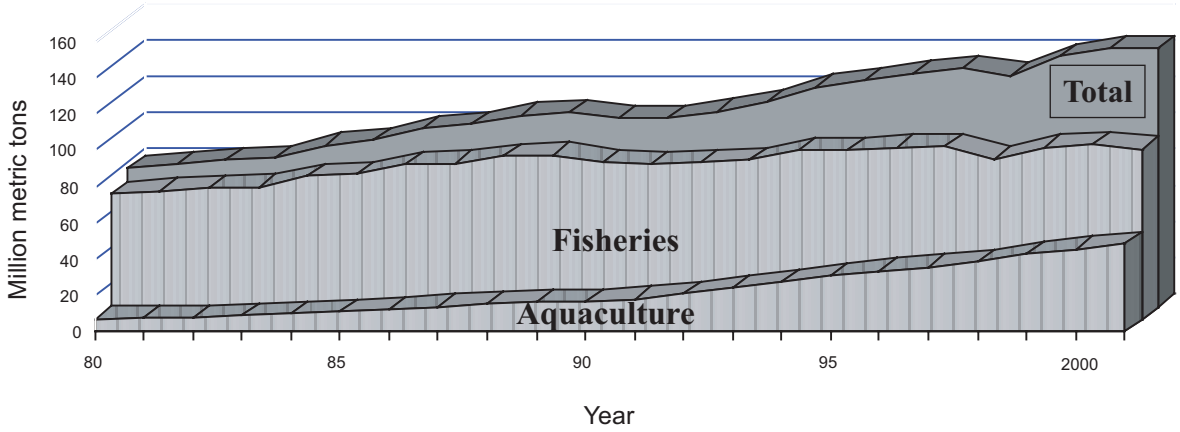

Figure 1. World fisheries and aquaculture production (FAO 2003)

Aquaculture represented 5.3% of the total fisheries landings in 1970 and this had increased to 34% in 2001, i.e. 48.4 million metric tonnes (mmt) of the total fisheries landings of 142.1 mmt. The value of world aquaculture production is now estimated at 61.5 billion US$. Globally the sector has shown an average annual compounded growth rate (APR) of 8.9% pa since 1970 compared to 1.4% for capture fisheries and 2.8% for terrestrial farmed meat production.

Finfish production at 24.4 mmt represents 50% of the aquaculture production and over 130 major finfish species are cultured world-wide. These statistics alone emphasize the scale, complexity, rate of development and diversification of the global finfish aquaculture sector.

European aquaculture

Europe has a total aquaculture production of over 2 mmt, and of the 210 aquaculture species cultured world wide 60 are cultured in Europe with a value of 4.6 billion US$. This means that while Europe contributes 4.4% to global production, it represents 8.2% of its total value. Recently growth from aquaculture has slowed from 7.8% p.a. in the period 1980 to 1990 to 2.3% p.a. in the period 1990 to 2000.

In 2001, 1.34 mmt of Europe's 2 mmt was attributed to finfish production and the break down of production between fresh, brackish and marine environments is shown in figure 2. Salmon, trouts, sea bass and sea bream account for over 1 mmt or 3 billion US$ in value. Salmon is by far the largest activity accounting for 647 thousand metric tonnes (tmt) and 1.86 billion US$. The marine and diadromous finfish species are valued at nearly 3 times the price of the fresh water species according to global FAO statistics and it is primarily in this area that European aquaculture has focused and developed. (Tacon, 2003).

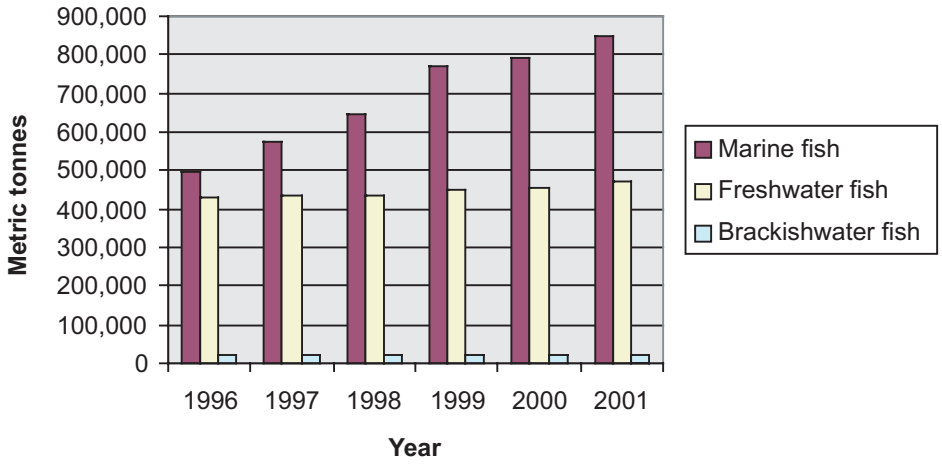

Figure 2. European finfish production by environment (1996-2001)

Table 1 shows the breakdown of the finfish species cultured in Europe and it can be seen that the largest growth is in mariculture with salmon, sea bream, sea bass and turbot. Currently the 25 countries of the European Union produce over 50 % of the finfish total.

Table 1. European finfish production by species (1996-2001) in metric tonnes.

	1996	1997	1998	1999	2000	2001
Freshwater species						
Tilapias	320	200	200	246	180	200
Sturgeon	1,285	1,471	2,022	2,441	3,083	3,087
Eels	8,614	8,696	9,792	10,536	10,713	10,187
Catfish/Perch/pike	19,321	16,389	15,437	19,903	16,583	14,868
Carps and Cyprinids	175,910	172,620	180,015	191,236	197,405	210,667
Trout	279,060	293,142	304,485	301,753	301,371	331,805
TOTAL	**484,510**	**492,518**	**511,951**	**526,115**	**529,335**	**570,814**
Marine species						
Sole	31	25	22	19	23	37
Halibut		2	8	13	34	93
Cod	191	304	199	157	169	763
Turbot	2,663	3,041	3,107	4,113	4,789	4,959
Amberjack & Tunas	78	1	1,959	3,246	3,686	4,453
Misc Marine	740	1,205	2,153	2,733	3,031	2,275
Other Breams & mullets	4,086	3,946	4,051	4,016	4,190	4,137
Sea bass	19,325	24,079	30,168	38,215	41,870	42,600
Sea Bream	23,304	29,139	37626	49,601	58,163	63,370
Salmonids	416,358	473,159	510,059	611,671	617,898	647,043
TOTAL	**466,766**	**534,901**	**589,352**	**713,784**	**733,853**	**769,730**
Total European Production	**951,286**	**1,027,419**	**1,101,303**	**1,239,899**	**1,263,188**	**1,340,544**

Salmon price: a controlling factor

Salmon prices have decreased since 2000 and in 2003 reached their lowest point ever at under 2 € /kg , with a recent average price of approximately 2.5 €/kg in early 2004.

This for many farms is at or below their production costs. Due to the importance of this species in the overall framework of the European industry the repercussions that this has had on the industry, as a whole has been significant. During the same period sea bass and sea bream juvenile production increased from approximately 500 million to 650 million juveniles while sea bream prices decreased from 4.58 to 3.73 €/kg. Many farms

producing only portion-sized sea bream faced sales prices well below their production costs. Overall in the EU their has been a 6.5% APR in production growth but the overall price trend has been negative (-0.5% APR) versus a positive global development (FEAP).

Share prices have fallen sharply, particularly in the salmon industry, investment confidence in aquaculture has been shaken and pressure has been placed on the industry to further improve efficiency and productivity. Acquisitions and mergers have been the order of the day and groups are consolidating in order to benefit financially.

Significantly with salmon being the power house of the European industry diversification into other marine finfish species has been slowed due to the reluctance of financial institutions to invest in a variant of this troubled sector. Further this financial pressure comes at a time when consumer awareness is focusing upon product quality, ease of product use, food safety, and traceability. This is also occurring during a period when the image of aquaculture has suffered by what the industry feels is often unfair treatment in the press.

The industry while weakened has responded with a positive approach. At every level the aquaculture sector is promoting transparency, cooperation and dialogue. The Federation of European Aquaculture Producers (FEAP) initiated the "Aquamedia" project and this is now providing factual, truthful and interesting information about European aquaculture to the public.

Evolution of European finfish culture

The marine and diadromus finfish sector is of vital importance to the European industry and consists primarily of high valued species requiring considerable technological and managerial sophistication to culture in a sustainable manner. The critical developments that were responsible for the commercialization of these species and their subsequent diversification are outlined below. Further areas of future developments and improvements required, to ensure the continued development of the industry, are considered in light of the rapidly evolving requirements of modern day Europe and the consumer.

While the latest FAO/ FEAP statistics described above provide a fascinating insight to the development of the aquaculture industry they obviously lack the benefit of real time information and the regional focus of development promoted by the European Union, National Governments and the industrial sector. This has been achieved through supporting research and development, financial support for the establishment of the industry and by addressing marketing and consumer needs. These driving forces have provided a basis for development and diversification.

Criteria for species selection

The selection of a species suitable for European aquaculture depends primarily on its market value, an understanding of its biology and the ability to produce juveniles in significant numbers for commercial production to take place. Given these characteristics and suitable site availability with the correct environmental conditions for culture the evolution to maturity of a specific species industry can be summarized in the following stages: -

- Identification and selection of high value species together with the R&D necessary to provide an understanding of the species biology and nutrition.
- The development of reproductive technologies to close the life cycle of the species considered together with the acquisition, domestication and manipulation of broodstock to produce eggs year round.
- The industrialization of technologies required for the commercial production of juveniles and their ongrowing.
- Improvement in productivity through economies of scale and the reduction of costs through vertical integration.
- Improvement in productivity through biotechnological solutions such as genetic advancement and nutritional engineering.

- Increased market activity through commercial pressure with the development of quality labeling, processing and other value added activities.
- Streamlining of the industry due to increased production, reduced profits resulting in grouping, mergers and consolidation to remain commercially competitive.
- The maturation of the industry and the species loosing its status of a high value item and becoming a commodity product.
- Species diversification in search of additional profits through a recycling of the above procedure.

Transfer of technology between species.

The transition from salmon to other marine species is of particular interest and importance as it involved the transfer of ongrowing technologies from Norway and Scotland to the Mediterranean for the sea bass and sea bream industry in the 1980's to 1990's. This, however, in itself was not sufficient as the reproductive technologies, zootechnical, nutritional and environmental conditions required for the culture of marine finfish juveniles were significantly different from those of salmon.

A swim up salmon fry (150mg wet weight) is many times larger in body mass than that of a sea bream (0.35mg wet weight) and while the salmon fry is capable, at first feeding, of ingesting and digesting inert particulate feeds this is not the case for many marine species. Due to the rather underdeveloped digestive system of these larvae and their behaviour a sophisticated live food chain was required. Researchers and industry concentrated efforts to develop a species specific nutritional strategy and identify the correct environmental conditions enabling these very small and sensitive larvae to be successfully reared and develop sufficiently to be weaned on to inert diets before traditional on-growing technologies could then be used.

Since the 80's, larval rearing technologies have been continually developing for the sea bass, sea bream and turbot industries and today they are well understood. Survival rates for these species have increased from less than 1% to between 20 and 40% in 10 years. In the last few years these warm water larval rearing technologies have been adapted and introduced to Norway and Scotland enabling the mass production of a marine cold water species the cod (*Gadus morhua*). While production of cod is expected to reach only 12,000 tonnes in 2005, this species, which occupies the same environmental conditions as salmon, offers an alternative or addition to the troubled salmon industry.

Economically the present viability of these two species in the UK can be summarized as shown in table 2. (R. Prickett, Personal communication) and it should be noted that as technology and food conversion rates improve for cod, so should profits. History indicates however that as volume increases so market prices will reduce.

Table 2: Comparative profitability of salmon and cod in the UK in 2003.

	Salmon	Cod
Cost per kg (round)	€ 2.04	€ 2.820
Cost of harvesting/gutting etc	€ 0.423	€ 0.423
Yield (head on gutted)	90%	86%
Cost per kg (head on gutted)	€ 2.735	€ 3.765
Market price (head on gutted)	€ 2.735	€ 4.935
Profit/kg	0	€ 1.170 (31%)
Cost of filleting	€ 0.141	€ 0.141
Yield after filleting (head on gutted)	68%	55%
Cost per kilo (fillet/skin on)	€ 4.23	€ 9.23

It is developments such as these that offer Europe the potential to continue expanding and diversifying its existing aquaculture industry by looking forward to new and more profitable species through the adaptation and development of existing biotechnologies and management strategies.

Reproductive technologies

It has been estimated that less than 3% of the total world aquaculture production is based on genetically improved stocks. Norway started to work on the selection of salmon in the 1960's and it was found that there was a large genetic variability for important production traits between wild and culture stocks offering potential for improvement. The best strains were then used as a base population for a national genetic improvement programme. This has lead to the development of salmon strains that show a genetic gain of around 100% for growth rate as well as other important characteristics such as late maturing individuals, resulting in improved production characteristics and reduced production costs. A similar breeding programme carried out using tilapia in the Philippines has shown more than a 100 % improvement in growth rate over 10 generations.

In both species, selection for high growth-rate can result in a gain of over 10% per generation. Only in the last 10 years has European Union research focussed on broodstock genetics and selection programmes for the sea bass and sea bream industry in the Mediterranean. Prior to this production was still based on wild broodstock and selection from F1 cage production, with little monitoring to avoid inbreeding.

Traditionally, preventing inbreeding has been the largest problem in fish breeding programmes, due to large full sibling groups and the lack of individual identification methods. Today the identification of individual broodstock fish using microchip tagging is commonplace. The cost of microsatelite DNA and AFLP's (amplified fragment length polymorphisms) labelling has significantly decreased enabling the tracking of pedigrees and providing linkage maps to identify quantitative trait loci, such as growth and disease resistance, that have commercial importance (Agresti et. al., 2000). These technologies have enabled individual producers to carry out selection methods and apply them in a practical manner in the industrial environment.

Growth rate is generally the first characteristic of importance for fish farmers as it is relatively easy to select and quantify. Other traits, thought to be genetically dependant, for example disease resistance and flesh quality (muscular lipid content, fat deposition), are difficult to measure and require a more complex approach often beyond the abilities of the individual farmer. Various thematic R&D programmes within the European Union are now addressing many of these issues.

Many species still prove difficult to spawn in captivity particularly in intensive production systems, an example being Sole (*Solea senigalensis*). Triggers for male and female maturation and ovulation and still not well understood for this and other species such as the groupers and some tuna species. Endocrine regulation of reproduction has been effectively applied in some species and hormonal implants are readily available. (Zohar and Mylonas, 2001) More investigation of the environmental and nutritional requirements of many species is required as the production of viable eggs is a prerequisite to the culture of any species.

Egg quality has a significant impact on the viability, survival and growth of marine larvae. The enrichment of broodstock diets with essential fatty acids (HUFA's) and other vitamins and minerals have been shown to relate directly with the levels of these substances in marine eggs and larvae (Watanabe, 1993; Cedra et. al., 1994; Harel et. al., 1994).

Marine larval rearing technologies

The very small and sensitive larvae require the establishment of a reliable chain of live food production consisting of unicellular marine algae, the rotifer *Branchionus plicatilis* and the brine shrimp *Artemia salina*. This is common to both the cold water developments namely cod and halibut and the warm water species such as sea bass, sea breams, turbot and sole.

The most commonly cultured algal species in Europe belong to the 5 taxonomic groups (Table 3) and the choice of species depends on the fish species being cultured.

Table 3: Commonly cultured unicellular planktonic algae.

Bacillariophycaea	Chaetoceros calcitrans
(Diatoms)	Skeletonema costatum
Chlorophycaeae – green algae	Dunaliella tertiolecta Chlorella sp. Nannochloris atomus
Chrysophyceae	Tetraselmis suecica Tetraselmis chuii
Eustigmatophyceae	Nannochloropsis oculata Nannochloropsis gaditana
Haptophyceae	Isochrysis galbana Isochrysis sp. (Tahititan strain) Pavlova lutheri

The use of algae in the larval rearing tanks, the "green water technique" is not limited to the purely nutritional side of rotifer enrichment but has other practical applications:-

- Algae can act as an antibacterial agent. (Austin and Day,1990; Cooper et. al. 1983). In addition specific polysaccharides in the algae cell wall are thought to stimulate a non-specific immune response in young larvae.
- Algae has been reported to act as an in situ biological filter removing potentially harmful metabolites from the water by stripping off nitrogenous substances. It also produces oxygen through photosynthesis.
- Algae acts as a light filter and diffuser facilitating an even distribution of live food and larvae within the tank system.
- It acts as a promoter and background for the location of prey organisms hence playing a particularly important role in the critical first feeding stage of larvae.
- Algae has been shown to stimulate the enzymatic synthesis and onset of feeding in young larvae.

In recent years photo-bioreactor systems have provided an efficient alternative to traditional sack culture systems for the production of unicellular algae. These are labour saving, automated and cost effective. Productivity using these systems can be up to 10 times greater than that achievable with traditional culture methods. Undoubtedly the success of such systems is dependent upon light availability and the Mediterranean climate is particularly suitable.

The search for additional algal species continues and isolates of local species are being investigated in Norway in an attempt to replace some of the traditional non-indigenous species. In addition commercial companies are marketing concentrated algal pastes, delivered either alive with a limited shelf life or cryopreserved, which offer a back up and an alternative to traditional algal production.

The second link of the live food chain is the rotifer. The duration and quantity of rotifers required varies with the species, cod, for example require up to 4 times the number of rotifers per animal produced when compared to the sea bream. Some species such as the sea bass may avoid this stage altogether first feeding directly on Artemia nauplii, the last link in this chain.

Rotifer production methodologies have improved over the years from an algae and yeast based diet giving poor productivity and unpredictable results to improved culture diets. The new generation of diets enables culture densities of 2000 rotifers per ml or more to be achieved over a 4 day batch cycle. Further developments have resulted in improvements, 5000 rotifers per ml can be achieved using concentrated fresh water chlorella algae and automatic dosing pumps and re-circulation systems using protein skimmers, in association with novel

filters enable rotifer cultures to reach and be maintained at densities over 5000 per ml for prolonged periods of time. These developments has been shown to both provide significant economic benefit and importantly improve the microflora of the culture by reducing the incidence of Vibrio sp. (Suantika et.al., 2003).

Ongoing European Union projects have revealed that there is considerable genetic diversity in the rotifer populations in European hatcheries showing considerable difference in performance. It is not yet clear whether it will be necessary to work with selected genotypes cultured over a limited number of generations or how these cultures will be susceptible to changing culture conditions (Sorgeloos, 2004).

A better understanding of the nutritional requirements of the fish species cultured has lead to the development of number of commercially available cultivation and emulsion type enrichment diets for both rotifers and artemia. Dietary research has indicated the importance of the (n-3) highly unsaturated fatty acids mainly eicosapentaenoic acid (20:5(n-3), EPA), docosahexaenoic acid (22:6(n-3), DHA) and more recently the long chain n-6 HUFA arachidonic acid (20:4n-6, ARA) has been implicated as an essential fatty acid for a variety of developing marine species (Estevez et. al., 1999). It is the bio-encapsulation of these and other essential nutrients through the live feed chain in the ratios required by the species concerned that has led to the alleviation of several problems such as pigmentation and some deformity issues in larval rearing as well as improving survival.

These enrichment products today are available together with products, that for Artemia, are capable of altering the microbiological characteristics of the hatching and enrichment environments by reducing Vibrio levels to 30% or less of non-treated environments. This development both provides the farmer with custom nutritional packages and helps to reduce the possibility of disease that may be introduced through the live food chain.

Larval nutrition and feed technologies

Larval rearing technologies are today highly intensive with up to 250 larvae stocked per litre. Until recently the live food chain was entirely responsible for the nutrition of these larvae until weaning commenced at approximately 30 days post hatch. The role of the live food chain is still vitally important for many species but the development of a new generation of sophisticated inert co-feeding and replacement diets have enabled the further intensification of the larval rearing process, and considerably reduced reliance upon the live food chain simplifying production methodologies.

Nutrition, health and performance are concepts that are intimately linked and the industry is placing increasing importance and effort to optimize formulations and improve ingredient quality including the sourcing of fresh raw materials. A cold extrusion spherizer agglomeration system has been used to produce diets that are now aimed at the complete replacement of the live food chain and they focus on high digestibility. Skretting use the above technology together with a patented phospolipid content of 12%. This has been reported to play an important role in the reduction of juvenile deformation and improved growth performance.

It is hoped that further developments of micronised replacement diets will both simplify and standardize future marine fish larval rearing and enable a greater number of species to be commercialized. Possibly given the restriction of the very small mouth sizes of some marine larvae, such as the groupers, and the difficulty in maintaining extremely small strains of rotifers for first feeding highly digestible diets of this type may provide an alternative strategy for the industry.

Consumer confidence and food safety issues are important factors in aquaculture and following the BSE outbreaks, the European Union has banned the inclusion of ingredients derived from terrestrial animal by products. This has stopped the incorporation of hemoglobin, blood meals, meat, bone and feather meals amongst other ingredients. In addition to this many sales outlets and the large supermarket chains require European feeds to be certified GMO free as part of their drive to satisfy consumer demand and perception.

The newly established European Food Safety Authority (EFSA) runs risk analysis and risk management and promotes an integrated approach to the responsibility of feed manufactures, farmers and food operators on the traceability of feeds, food and their ingredients.

These actions while essential to regulate the industry and address consumer and public health issues severely restrict the formulations available for aquaculture. This in turn places a heavy load on the limited resource of fish meal as a protein source and adds to the cost of feed at a time when the industry is striving to reduce production costs by all means possible. Fish meal and oil substitutes are emerging now as viable, economic partial alternatives. The inclusion of digestibility enhancers and organic chelated trace minerals provide better bio-availability of important nutrients.

Health and disease issues

Parasitic, bacterial and viral diseases cause considerable financial loss to the European industry. Disease problems in the marine sector originate from a diverse range of infectious agents which have been reviewed by Le Breton, (1996) and Rodgers and Furones,(1998) a list that has rapidly developed and is continually expanding.

The development of molecular techniques for the identification and screening of pathogens offers the potential to improve disease prevention and control. The sensitivity of nucleic acid probes now enables the detection of sub-clinical carriers of some infections and this is an important development and tool for the establishment of specific pathogen free broodstocks.

Hatchery production units try to avoid the introduction of opportunistic pathogens through treatment of the incoming water supplies using a variety of filtration methods. Sterilization, either by UV and /or ozone, is common practice in both freshwater and marine environments. Production scheduling now includes specific periods where either units of / or the whole hatchery are shut down cleaned and sterilized and all in all out batch production provides regular sanitary control.

Areas within the hatchery environment are kept as discrete as possible with the minimum of interaction by working personnel and equipment from one area to another. Isolated quarantine facilities are employed to prevent the introduction of disease.

In larval rearing various strategies have been proposed for controlling the microflora of this environment. Mature water and the addition of probiotics either through the live food chain or directly in the larval rearing tank have been used. The larval pre-feeding and first feeding stages are critical to the establishment of the microflora of the gut. (Bergh et. al., 1994; Munro et.al., 1993) and the presence of opportunistic pathogens at this stage have been shown to lead to disease (Grisez et. al., 1996).

Fish transfer from the hatchery to the pre-ongrowing or ongrowing facilities necessitates the transfer of fish from a protected to an unprotected environment in which they might come into contact with a variety of different pathogens It is possible to protect against certain disease with vaccines and a limited number are available for commercial use. Vaccination takes places prior to the transfer from the hatchery facilities to the ongrowing but due to the limited duration of protection offered to fish further vaccination may be necessary during the ongrowing cycle.

With the complexity of vaccine licensing and the restricted use of antibiotics and some other therapeutic agents the industry is turning to other methods of prophylaxis and control to improve the fish health status. The concept of nutritional supplementation, the use and blending of selected nutrients, immunostimulants and immunomodulators are rapidly being considered by the aquaculture industry as it learns of their effectiveness in terrestrial animal culture.

Conclusion

European aquaculture is expected to show growth in the marine sector and the success of individual operations will depends on the successful application of a variety of multi disciplinarial activities. Economic viability must be linked to better marketing strategies and food safety, transparency, traceability, quality and sustainability issues are at the forefront of European concerns and actions. Technological improvements are expected to continue to improve cost efficiency and stimulate further species diversification at a time when fisheries production is stagnant and in certain sectors in decline. Simplified legislation and licensing procedures have been called for and continued and coherent policies for research and development are essential.

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Nutrition, digestion and development in marine fish larvae

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Introduction

Until recently, the feeding of marine fish larvae during the first weeks of life depended on live preys whose cost is expensive and which are less and less abundant. The substitution for these preys by a compound diet is now possible as early as mouth opening. The formulation of a compound diet makes possible to study the influence of nutrients on marine fish larvae development in order to adjust each nutrient to the need of the larvae. It is well known that nutrients influence larvae development; growth, survival and malformation rate of larvae. In this paper, we will pay particular attention to peptides, lipids and vitamin A.

Larval development

Marine fish larvae undergo major morphological and functional changes during the first month of life to acquire their adult features. These changes include the maturation of the gastrointestinal tract (Zambonino & Cahu, 2001) which is not mature at hatching. The development of the stomach is nearly complete around day 15 in sea bass and the gastric glands appears approximately at day 25. The exocrine pancreas also progressively develops, its secretory functions become efficient after the third week of life. A global decrease of amylase activity and an increase of the activities of other pancreatic enzymes (trypsin, lipase, phospholipase A2…) are characteristic of this maturation process. The intestine cells, the enterocytes, have two kinds of enzymes: the cytosolic ones (mainly peptidases) localized in the cytoplasm and the brush border membrane enzymes (alkaline phosphatase, aminopeptidase N, maltase…). Around the fourth week of life, the cytosolic activities decrease while membranous activities increase. This reflects the maturation of enterocytes and the establishment of an efficient brush border enzyme digestion represents the adult mode of digestion.

The morphological changes mainly concern the shape of the larvae and the differentiation of the fins. At day 3, European sea bass larvae elongate. At day 5, eyes start to be pigmented and the pectoral fins appear (Barnabé et al., 1976). At day 15, the vitellus is totally resorbed and the larvae only have an exogenous feeding. The vertebrae are visible at day 20. At day 35, the caudal, anal and dorsal fins are visible and around day 43, the pelvis fins start their differentiation. At this stage of development, the larvae present all the adult features. All these differentiation processes are under genetic control involving several families of genes such as the BMPs, IGFs (Solheim, 1998) and Hox genes (Krumlauf, 1994).

Larval nutrition

The formulation of a compound diet for sea bass larvae includes 50% of proteins, 15 to 20% of lipids, minerals and vitamins. The main source of proteins is the fish meal and it has been demonstrated that the molecular form of proteins is determining in larval nutrition. Lipid requirements have been also intensively studied, especially phospholipids and highly unsaturated fatty acids (HUFA) requirements. The main phospholipid source used in compound diets is soybean lecithin.

Peptides

It has been demonstrated for a long time that protein hydrolysates were beneficial to larvae growth. In 1997, it has been shown (Zambonino et al.) that a 20% replacement of fish meal by tripeptides in the diet improved the growth, survival and skeletal formation in sea bass larvae. This can be explained by the presence of specific transmembrane transporters and a high cytosolic peptidase activities in these larvae. Moreover, it also has been shown that the activity of a pancreatic protease (chymotrypsin) was enhanced by short peptides. In juveniles,

the incorporation of protein hydrolysates in formula does not represent an advantage mainly because of the decrease of the cytosolic peptidase activities.

Phospholipids and HUFA

Phospholipids (PL) are the major component of cell membrane and even though larvae can synthesize them de novo, their incorporation in diets improves the growth and the survival rate (Cahu & Zambonino, 2001). It has been suggested that larvae were unable to synthesize enough PL to cover their requirements during a period of intense cell multiplication (Kanasawa, 1993). It has been recently reported that a diet containing 19% lipids with almost 9% phospholipids induced a good growth in European sea bass first feeding larvae (Cahu et al., 2003). These authors suggested that marine fish larvae use phospholipids more efficiently than neutral lipids, since lipase transcription in response to dietary neutral lipid amount was poorly regulated while phospholipase A2 transcription followed dietary phospholipid content in a gradual manner with a greater modulation range in expression.

The n-3 HUFA are essential dietary components for marine fish because these fish can not synthesize them. Two fatty acids, the eicosapentaenoic (EPA = C20:5n-3) and the docosahexaenoic acids (DHA = C22:6n-3) are particularly important as they are present in large amounts in fish cell membranes and are involved in physiological processes. The optimal dietary level of EPA + DHA is 2.7% of dry matter for marine fish larvae and it is 1.5% when EPA and DHA are in PL (Cahu et al., 2003). Actually, these HUFA are more efficiently used when they are brought by the PL fraction (vs neutral fraction).

Vitamin A

The compound diets for marine larvae include in their formulation a vitamin complex containing all the vitamins thought to be essential. As larvae quantitative requirements in vitamin are unknown, a vitamin mixture is commonly added in excess. Our studies paid a particular attention has been paid to vitamin A because it acts on morphogenesis through nuclear receptors. These receptors regulate the expression level of genes, such as Hox (Ross et al., 2000). Recently, we demonstrated in sea bass that these receptors were expressed in the body areas affected by malformations and that their action was modulated by exogenous nutrition after hatching (Villeneuve et al., unpublished data). So, it seems really important to evaluate the optimal dietary level of vitamin A. The only available data was obtained in Atlantic halibut juveniles and was 2.5 mg retinyl esters/kg dry matter (Moren et al., 2004).

Conclusion

All the nutrients described in this paper act directly or indirectly on larvae morphogenesis and they also probably interact together to allow a harmonious development. In order to improve diet formulations for marine fish larvae, it would be very pertinent to study the pathways modulated by nutrition and involved in these morphogenetic processes. Some of these nutritional pathways are common to several nutrients and their study would allow, on one hand, to understand how nutrients influence genes of development and, on the other hand, to evaluate how nutrients interact together.

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Technical Session







Live and formulated feeds: challenges, capabilities and research at the Australian Institute of Marine Science (AIMS)

Mike Hall, David McKinnon, Michael Horne, Paul Southgate (JCU), Samantha Duggan, Alby Steffens, Matthew Salmon and Matt Kenway

The larval phase continues to represents one of the major challenges in the consistent and successful production of many finfish and crustacea aquaculture species as well as in the development of new candidates for captive reared production. Whereas it has proved possible to successfully rear the larvae of many species there is often significant variability between batch runs while in other cases high levels of larval attrition results in poor survival to the juvenile stage. Some aspects of these problems may be due to inadequacies in larval nutrition. As part of several aquaculture projects at AIMS and JCU on crustacea, finfish and molluscs, research has been undertaken on the development of live and formulated feeds. As knowledge is gained in larval rearing technologies of specific species, some of the most likely bottlenecks are tentatively identified. As a result, AIMS and JCU has invested into live and formulated larval feed production capability and equipment allowing a comprehensive approach to aspects of the larval rearing of a range of aquaculture species.

Live feeds are the larval diet of choice in the original development of the hatchery phase of aquaculture candidates and continue to form the mainstay feeds for the major penaeid prawn and finfish hatcheries. Typically, live feeds can be fed less frequently and at lower rates, as feeding to excess does not lead to as rapid deterioration in water quality. In some cases, sensory cues, such as movement, odour and electro-chemical properties, are necessary for acceptance by the larvae and live feeds meet these criteria. However, due to the cost of production of many live feeds there continues to be research and development effort towards the production of formulated larval feeds which are i) economical to produce, ii) have consistent nutritional profiles, iii) are readily digestible at various stages of larval physiological development and iv) have the capacity for medium to long term storage without spoilage. The choice of either live or formulated feeds also determines aspects of tank design, water exchange and flow, and the method and frequency of feeding.

Live Feeds

Microalgae

Microalgae are produced at AIMS in a newly constructed continuous algal production system capable of producing 1,000 litres per day. Microalgae cultures are kept under controlled microbial conditions using 0.22 µm to 1.0 µm filtered and pasteurised seawater and are supplemented with food grade carbon dioxide to increase productivity. As summer temperatures and cloud cover are variable in the Townsville region the unit is indoors as extreme weather bouts can occur which cause microalgae production to temporarily fail. Experience has demonstrated these may occur at critical periods in larval rearing and therefore the unit presently uses artificial lighting and environmental control. Continuous microalgae production is based on a few species chosen from several families held in small-scale stock cultures including *Chaetoceros* and *Skeletonema* (Bacillariophyceae), *Cryptomonas* (Crytophyceae), *Tetraselmis* (Prasinophyceae), *Isochysis* (Haptophyceae), *Pavlova* (Prymnesiophyceae), *Nannochloropsis* (Eustigmatophyceae) and *Heterocapsa* (Dinophyceae), all sourced from either the CSIRO Microalgae Research Centre, Darwin Aquaculture Centre or other collaborators. The microalgae are either used for conditioning of larval tank water, as feeds for *Artemia* and copepods or as live feeds themselves.

Rotifers

Although original considered as a pest due to their rapid proliferation causing deterioration of environmental water quality, rotifers are an important larval feed for marine finfish. As the AIMS Tropical Aquaculture Facility (TAF) concentrates on the larval rearing of various crustacea, rotifers are not regularly cultured.

Artemia

Artemia are primarily cultured as live feeds for prawn and rock lobster larval rearing. Protocols for decapsulation, hatching and enrichment are based on those developed by Inve. A recent change is the use of Virkon S (a strong oxidising agent availably commercially) to disinfect water in hatching tanks prior to the addition of cysts. Ongrown *Artemia*, fed a diet of mixed microalgae species, are also produced for rock lobster larvae. A key objective in *Artemia* production is the management and control of the entire bacterial community, including culturable and the unculturable majority, to minimize the risk of inadvertent transfer of potential pathogens into larval rearing tanks.

Copepods

The brine shrimp *Artemia* (Anostraca) is the mainstay for live feeds for many established aquaculture species. However, as *Artemia* are predominantly found in hypersaline environments they are a surrogate for the natural live prey of the aquaculture larvae. The natural prey of the majority of finfish larvae are copepods, and *Artemia* are a poor nutritional source for the larvae of most aquaculture species. Consequently, *Artemia* require enrichment diets to optimise their nutritionally profile to match the needs of specific aquaculture species. In contrast, copepods typically have a nutritional profile that matches the requirements of marine finfish larvae in particular. For example, the copepod, *Diaptomus (Leptodiaptomus) connexus* (sold freeze-dried under the commercial name of Cyclop-eeze) is considered to be an excellent larval finfish feed. There are, however, quarantine issues with the import of such material from offshore. If the desired prey size is greater than that of the *Artemia* nauplius, *Artemia* may be grown out to the desired size. However, with a massive biodiversity of copepod species, there is a much greater range in size classes that may be selected. Nevertheless, several challenges remain to successfully produce copepods on a continuous commercial scale, including consistent production rates and maximisation of growth.

Alternative Live Feeds

Whereas microalgae and copepods are the dominant form of plankton in the wild, and form the basis of the larval diet for many marine organisms, they are not the only prey of larvae. Gelatinous zooplankton, represented by Hydromedusae, Siphonophora, Scyphomedusae, Ctenophora, Heteropoda, Pteropoda, Thalicea and Appendicularia, typically exceeding >1 mm in size, can form a major proportion of the planktonic community and be larval feeds for some marine organisms. Other soft-body larval feeds include nematodes and various annelids. Various culturing methods have been published for these, some of which have been trialled, and continue to be an area of interest as larval feeds for some aquaculture species.

Formulated Feeds

The primary characteristics of formulated feeds includes very small size of the finished particle, typically in the tens to hundreds of microns, and, as a consequence a very high surface-to-volume ratio and hence a high leaching rate, high palatability and digestibility optimised to the various physiological competencies throughout larval development. Production methods can be generally categorised into microbound, microencapsulated and complex particles.

On-size feeds

Microbound particles are the most widespread and produced as a flake or crumble, typically by fracturing a larger pellet. If a small variance in particle size distribution is required, on-sized feeds are the method of choice but require specialised equipment, most of which are adapted from the pharmaceutical industry. The two-step microextrusion marumerization (MEM) process involves the production of a finely ground damp/wet mash, the production of thin noodles through a cold single screw extruder, followed by forming them into small diameter spheres by marumerization (spheronization) (Barrows F. T. 2000 Global Aquaculture Advocate 3(1):61-63).

Milling

If a small compositionally complex particle of 10-500 micron is required, then the individual components must be significantly smaller than the final pellet diameter. Typical fish meals are several hundred microns in

diameter and hence require further milling to a smaller size. Many milling methods, such as hammer mills, depend on high energy impact with the generation of significant amounts of heat. If the material being milled, such as fish meal, has a high protein (40-70%), oil (>10%) and moisture content, the particle tends to form a paste as it is milled. The most appropriate mill for the production of particles in the 1-100 µm range, is an air classified or air pulverizer mill. As part of the research program on larval feed production, AIMS has acquired an air pulverizer mill capable of reducing particles of fish meals from 572.5 ± 304 µm fish meal particles to 4.22 ± 1.02 µm, processing 0.5 to 7 kg of feed per hour. The greatly reduced particle size allows the incorporation of several hundred particles into a 100 µm microbound spherical particle.

Microextruded diets

AIMS has acquired a lab dome granulator with a range of screens to produce a variety of extruded pellet diameters, including dome (300 to 1,500 microns), radial (600 to 1,200 micron) and frontal (2,000 to 5,000 micron) dies/plates. Marumerization is accomplished with marume plates of groove pitches of 2, 3, 4, 6 and 8 mm, allowing the production of an extensive range of spherical particle size feeds and, more importantly, the ability to produce experimental diets with complex formulations. A modification of the MEM process is the particle assisted rotational agglomeration (PARA) method (U.S. Patent 5,851,574) which produces a wider particle size range of pellets, including a proportion that are smaller than that produced by the MEM method.

Encapsulation

An additional on-size feed production method includes encapsulation by forming a continuous coating around solid particles or droplets of liquids. A common form of encapsulation are spray beadlets which requires a gelling agent, typically with hydrocolloids such as alginates, carregeenans, agar, gum arabic, pectin, and gelatin as well as binders such as starch, cellulosics, chitosan and transglutaminase. A modification of the original technique of Villamar and Langdon (1993 Mar. Biol. 115:635-642) has been used in an attempt to make formulated feeds for the phyllosoma larvae of tropical rock lobsters (see case histories).

Functional properties of diet ingredients

A significant problem in the production of formulated larval feeds is the ability of early stage larvae to efficiently utilise the nutritional content of them, especially as the digestive physiology of marine larvae only develops gradually with time. One partial solution to this problem is adjusting the functionality of proteins in the feed by preconditioning such feed ingredients as fish meals by enzymatic hydrolysis. The large molecular weights of proteins in fish meals can be reduced to peptide or single amino acids sizes which are potentially more readily assimilated by the larvae. AIMS has recently commissioned an enzymatic reactor with pH-Stat titrator to control proteolytic enzyme activity to produce digests of fish meals with known molecular weights. This line of research is directed towards optimising the appropriate molecular weight class of proteins to give to crustacean larvae of specific ages.

An additional significant problem in the production of on-size larval feeds with dimensions in the tens to hundreds of microns is the rapid leaching rate of low molecular weight hydrophilic molecules, such as amino acids and vitamins. A plethora of methods have been developed within the pharmaceutical industry for controlled release technologies. AIMS has trailed the use of interdigital micro mixers which are capable of forming microspheres of immiscible fluids into spheres within the range of 25-500 µm that can be incorporated into microbound and complex particles. This approach holds some promise as one way to incorporate highly hydrophilic compounds of low molecular weight into microcapsules with minimal leaching rates.

Case Histories

Live Feeds

Copepods as live feeds in aquaculture

AIMS has a long-standing collaboration with the Queensland Department of Primary Industries Northern Fisheries laboratory in Cairns. QDPI's reef fish aquaculture program has a significant live feeds component, to meet the requirement to present appropriate live feeds to the first-feeding larvae of grouper species such

as the estuary cod, *Epinephelus coioides*. These fish require sub-100µm live feeds, and rotifers have proved inadequate. Best results have been obtained by the use of nauplii of the copepod *Acartia sinjiensis*, a locally obtained representative of a very widespread genus which has gained currency in aquaculture applications worldwide. However, some aspects of the biology of *Acartia* hamper its development at hatchery scale, and work conducted at AIMS has focused on the culture of small copepods of the family Paracalanidae (*Bestiolina similis* and *Parvocalanus crassirostris* – see McKinnon et al. 2003., *Aquaculture* 223:89-106.). These animals are important natural diets for fish larvae. They are smaller than *Acartia*, and can reach higher densities in culture systems. Coincidentally, the same species have proved to be ideal larval foods for the rearing of tropical snappers in Hawaii, and offer a lot of promise for large scale commercial culture. We are satisfied with the performance of these copepod species as live feeds, but recognise the need to develop reliable low-maintenance culture systems. Consequently, we are now establishing a flow-through continuous copepod culture system to operate in series with the continuous algal culture system.

Formulated Feeds

Spray beadlets as phyllosoma feeds

Efforts towards the development of an aquaculture sector for rock lobsters is primarily being supported by the Rock Lobster Propagation and Enhancement Subprogram (RLEAS) through the FRDC. Successful larval rearing through the entire larval phase of the phyllosomas of rock lobsters has only been reported with the use of Artemia in the first half of the extended larval period (extending over several months and typically 11 larval developmental stages) with fresh minced feeds being used in the latter stages of larval development. Although the natural diet of phyllosomas is not fully known, available evidence suggests that gelatinous zooplankton are the primary prey. Based on such tentative evidence some effort has been devoted to the development of a formulated gelatinous feed for phyllosomas through a MSc thesis by Michael Horne (JCU), co-supervised through AIMS and JCU. The research focussed on the production of microbound and complex feeds and in the first instance examined suitable production methods for spray beadlets of specific dimensions (Fig. 1).

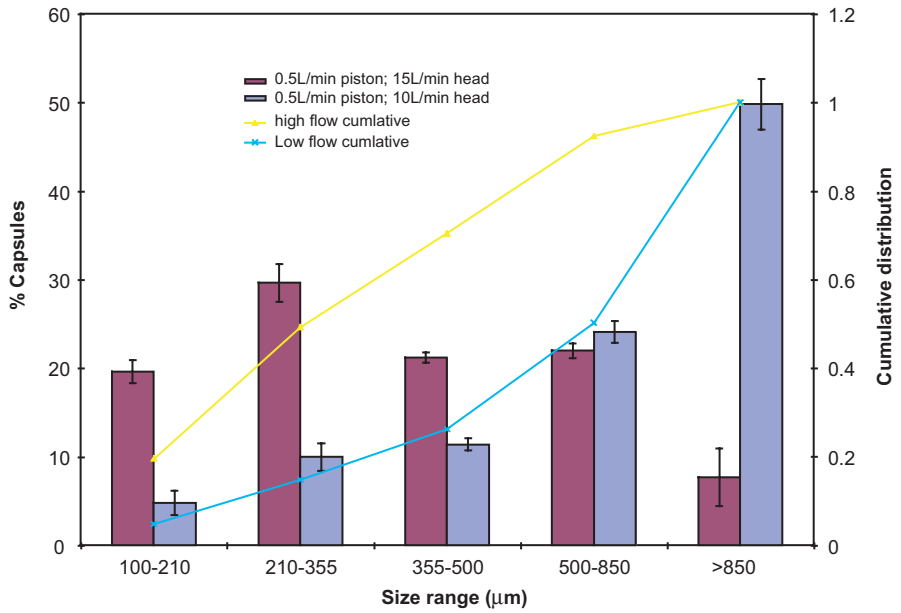

Figure 1 Size distribution of spray beadlets produced under specific flow rates and feeder rates of atomisation nitrogen gas.

Microencapsulated feeds of either homogenised Artemia or pipis (*Donax* spp.) were produced using sodium alginate as the binder (Figure 2). Whereas phyllosomas readily consume homogenized pippies, they were more reluctant to consume them when bound within an alginate capsule. Further work is necessary to identify a more suitable hydrocolloid binder.

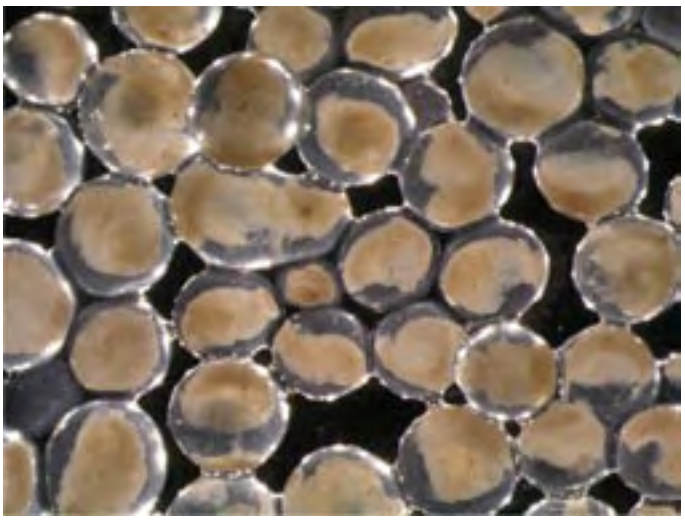

Figure 2. Microencapsulated spray beadlets containing homogenised pipis (*Donax* spp.) surrounded by a solidified sodium alginate coating.

To obtain further data on the ingestion rate of phyllosoma diets of alginate spray beadlets, Artemia where radiolablled by feeding them on carbon-14 enriched algae for set periods. The Artemia were dried and ground to a fine powder and incorporated into alginate spray beadlets. Ingestion of C^{14} alginate beads were tested in Stage 1, 2, 3 and 4 phyllosomas, followed by a 'cold chase' of non-radioactive Artemia (Fig. 3).

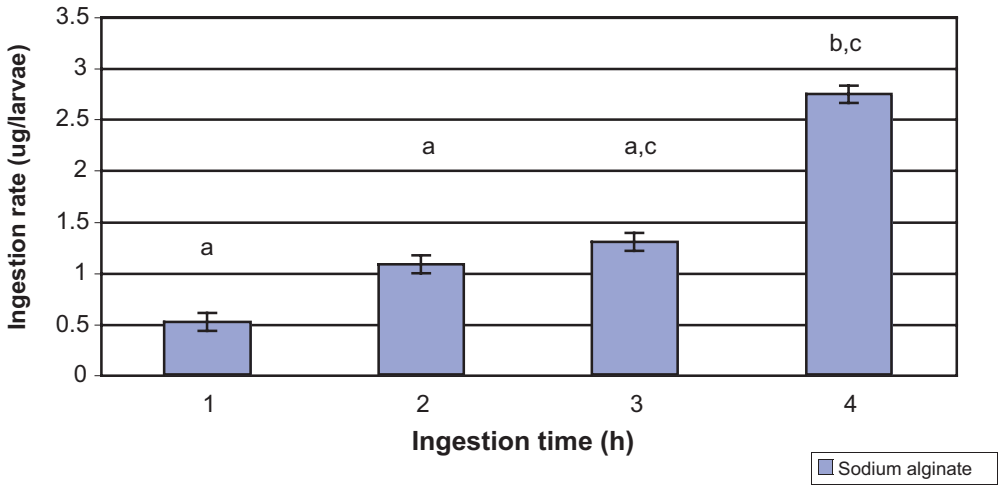

Figure 3. Mean ingestion rate (µg of biet per mg larval dry weight) of stage 4 phyllosomas fed radioactive labelled alginate-Artemia spray beadlets. Means for ingestion rate sharing the same letter were not statistically different (P>0.05).

As with the microencapsulated pipis the encapsulated Artemia were eaten but the phyllosomas appeared to be reluctant to consume the alginate beadlets. Further work continues on digestive physiology of phyllosomas and diets within an ARC-Linkage Postgraduate project of Matthew Johnston and Danielle Johnston (UWA).

Hatchery Capability at NIWA Aquaculture

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Introduction to NIWA

NIWA, National Institute of Water and Atmospheric Research Ltd, established in 1992 as one of nine New Zealand Crown Research Institutes (CRI's), NIWA's mission is to provide a scientific basis for the sustainable management and development of New Zealand's atmospheric, marine and freshwater systems and associated resources. As a CRI, NIWA operates as a stand-alone company with its own board of directors and its shares held by the Crown.

The company has a staff of around 650 and annual revenue of more than $75 million derived from competition-based research grants and commercial enterprise.

Spread throughout New Zealand, NIWA has its corporate headquarters in Auckland, main research campuses in Auckland, Hamilton, Wellington, Nelson, Christchurch and Lauder, and field offices in the smaller centres. Research vessels are maintained in Hamilton, Wellington and Christchurch. Subsidiary companies include NIWA Vessel Management Ltd (in Wellington), NIWA Australia Pty Ltd (in Brisbane), NIWA (USA) Inc and NIWA Environmental Research Institute (also in the USA).

NIWA collaborates in the operation of the Institute of Aquatic and Atmospheric Sciences with Auckland University and Centres of Excellence with Otago University, Canterbury University and Victoria University of Wellington.

The core business for the company falls into 5 main areas; Marine (oceanography, ecology, geomorphology etc), Freshwater (lakes and rivers), Fisheries (stock assessment, modelling and management), Atmosphere and Climate, and finally Aquaculture.

NIWA Aquaculture

NIWA staff have been at the forefront of aquaculture development in New Zealand since the inception and NIWA has invested heavily in facilities and expertise to underpin the commercial success of aquaculture in New Zealand. NIWA's latest development has been the construction in 2002 of a dedicated warm-temperate water marine aquaculture production and research facility, Bream Bay Aquaculture Park in New Zealand's North Island. In addition to this new facility NIWA has two additional established aquaculture facilities; Mahanga Bay, Wellington (cold water marine; urchin roe enhancement and seahorse) and Silverstream, Christchurch.

Figure 1. Location of NIWA aquaculture facilities in New Zealand.

NIWA Bream Bay Aquaculture Park has exceeded expectations and continues to attract investors keen to develop new species for aquaculture. The close association with industry allows NIWA to be very commercially focussed and target specific production issue for the development of new ventures.

Currently, NIWA has 4 companies on site working in association with NIWA developing the farming techniques for such species as paua (abalone), eel, mussel spat, Yellowbelly flounder, rock lobster and our own business of kingfish.

Figure 2. NIWA Bream Bay Aquaculture Park.

Kingfish

NIWA currently produce 100,000 kingfish fingerlings per annum with future contracts to produce 250,000 per annum for a commercial client in Northland. Currently there is no R&D on kingfish larvae but efforts are being expended on expanding the broodstock and manipulating photoperiod the enable the spread of the current production season to satisfy current and future customers.

Figure 3. Kingfish broodstock.

Broodstock are maintained in a 70,000l tank and naturally spawn (Figure 3). Fertilised eggs are collected using a surface skimmer drawing water across the surface of the tank by means of an airlift. Collected eggs are incubated under constant artificial light on ambient seawater in flow through systems using conical based 300l incubators[1].

Soon after hatch the larvae are transferred to 10,000l round tanks held under static water conditions using a mixture of *Nanochloropsis* and *C. mulleri* illuminated with natural light. The algae are cultured in bulk in outside tanks with the mature cultures pumped from the external algae culture tanks to the appropriate system within the hatchery (Figure 4).

Figure 4. External bulk algae culture tanks at NIWA Bream Bay.

The hatchery protocol uses the standard green water culture techniques beginning with enriched rotifers and transitioning swiftly through enriched *Artemia* on to artificial diets supplied by INVE.

Rotifers are produced under standard batch culture techniques from sterile stock cultures maintained at NIWA's site at Bream Bay Aquaculture Park (Figure 5).

[1] *All culture water for all hatchery activities 5micron filtered, UV treated seawater*

Figure 5. Stock cultures maintained at NIWA Bream Bay.

The culture diet used is another INVE product, Culture Selco 3000 mixed with "instant algae" which allows the culture of rotifers through several cycles before a new stock culture needs to be used. Standard practice at NIWA is to take the rotifers through 5-6 cycles before renewal (Figure 6).

Artemia are first decapsulated and incubated for hatch standard protocols employing the use of "hatch controller" (INVE). The *Artemia* are enriched using DHA Selco.

Enriched rotifers are introduced to the 10,000l larval tanks just prior to first feeding. Once feeding is established a slow ambient water flow is switched on. Rotifers are fed for a total of 26days post first feeding. *Artemia* are brought in and rotifers fazed out at day 18 post-first feeding. Artificial diets are first introduced at day 25-28 (dependant on the batch) and *Artemia* fazed out completely at day 40 post first-feeding. The first diet the larvae are weaned onto is Proton. Survival of animals once weaned is consistently greater than 95%.

Figure 6. Rotifer culture room at NIWA Bream Bay.

Scope for Improvement

Broodstock and Egg Quality

Much like any other hatchery NIWA seeks to improve the reliability of supply and the quality of eggs produced by the broodstock. In a bid to assess and improve the performance of broodstock NIWA embarked on a programme of DNA testing of all broodstock and egg batches resulting in weaned larvae. Through this process NIWA hopes to weed out poor performing broodstock. Currently all broodstock are from wild stock however, a proportion of all production each year are held and on-grown on site to establish an F1 broodstock with beneficial domesticated traits

First-Feeding Larvae

Total Kingfish larval abnormalities are now down below 10%. Typically, at NIWA we encounter shortened opercula, lack of swim bladder and jaw deformities which manifest themselves as a turned down or crisscrossed jaw. In previous years the instance of larval abnormalities was considerably higher but the improvements achieved were brought about by improvements in the broodstock diet and enhanced live feed enrichment procedures.

Weaning

One of the areas where NIWA feels greatest improvements can be made will be during the transition from live to inert feeds. Hence, significant effort will be directed towards the duration of transition and early inception of the whole process.

Flounder

Initial attempts are being made by NIWA to culture Yellowbelly flounder (*Rhombosolea leporina*) at Bream Bay for a commercial client. The attraction for NIWA is the winter spawning habit of this of this species ensuring the year round use of facilities at Bream Bay.

Broodstock are wild caught and have so far always induced to spawn through injection of Ovaprim (Figure 7).

Eggs produced have fertilised well but are only 0.62mm in diameter (Figure 8).

The larvae at hatch are on average 2.2mm long and have no functional mouth (Figure 9).

Figure 7. Yellowbelly flounder female broodstock at NIWA Bream Bay.

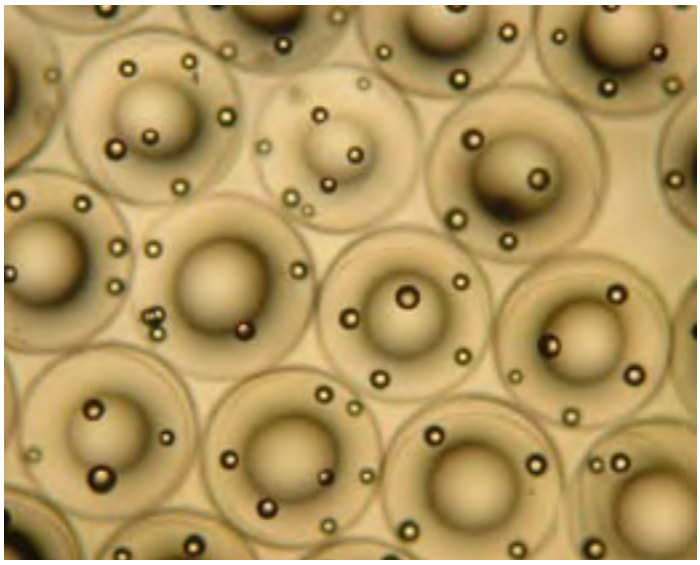

Figure 8. Flounder eggs 24 hours post fertilization.

Figure 9. Yellowbelly flounder yolk-sac larvae.

Larval Rearing

NIWA has found through these early attempts at larval rearing that the process becomes problematic at first-feeding when the size of the first feeding larvae becomes an issue. The main problem is the small size of the mouth. L-strain rotifers are too large. To get round the problem NIWA is trialing the use of mussel trochophores which can be easily produced on demand. In addition this year attempts will be made to initiate effective first-feeding through the use of various temperature regimes to provide a suitable start-feeding stimulus.

Conclusions

Kingfish culture is now well established in New Zealand with NIWA as the sole supplier. The emphasis for future development is to improve and spread the supply of seed and optimise the weaning period.

Yellowbelly flounder aquaculture is still in its infancy and the feasibility of commercial production is still to be determined.

CSIRO Marine Research Capabilities and Research in Larval Feeds

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Introduction to CSIRO Marine Research and its Aquaculture Research

CSIRO, through its Division of Marine Research (CMR; website: www.marine.csiro.au) provides research support across a range of coastal and oceanic industries and clients, including aquaculture. CMR has over 300 staff across three sites (Hobart, Tas; Cleveland Brisbane, Qld; and Floreat Perth, WA). CMR aquaculture research is delivered primarily through the Integrated Sustainable Aquaculture Research Group, which has ≈ 20 staff at the Hobart and Cleveland sites. The major research focus areas are: the genetics, nutrition and production of prawns and salmon, and to a lesser extent abalone, oysters and tuna. We also undertake research in microalgal feeds where there is a direct link to these key aquaculture species.

CSIRO Marine Research's Laboratories at Hobart (left) and Cleveland (right)

Research History in Larval Feeds

CSIRO's initial research and industry support in larval feeds commenced during the mid 1980's, with a microalgal supply service specifically set up to supply algal starter cultures to hatcheries, funded by FRDC. Alongside the supply service we established an R&D project to examine the biochemical properties (and hence likely nutritional value) of microalgae being used, or of potential use for Australian hatcheries. We have examined over 60 strains of microalgae, including many new Australian isolates, and provided detailed biochemical profiles (gross composition, fatty acids, amino acids, vitamin, sugars and sterols) under a range of different culture environments (Publications: Live Algal Feeds).

As an extension of our basic research on microalgal composition, we subsequently assessed the microalgae in feeding experiments and identified species with high nutritional value, especially for oysters (see Publications: Bivalve feeding and diet assessment). Our studies also included a range of alternatives to live microalgae either isolated or developed by CSIRO and our colleagues, that are potentially more cost-effective, i.e. algal concentrates, yeast, bacteria and thraustochytrids (Publications: Alternatives to Live Diets).

The lack of aquarium facilities at our Hobart Laboratories has meant that our larval feed research on temperate species has relied on strong research collaboration with industry (especially Shellfish Culture Ltd) and the Tasmanian Aquaculture and Fisheries Institute (TAFI). CMR has had a strong and productive collaboration with TAFI in projects on the larval production of striped trumpeter and rock lobster (see Publications; and later section on capability), where CSIRO has provided analytical capability, and expertise in live feed enrichment and design of nutritional experiments. At our Cleveland Laboratories, the nutritional requirements of prawn larvae have been assessed using a range of microalgal diets.

Current and Ongoing Research Interests in Larval Feeds

During the last 5 years, CMR has established international collaboration in algal feeds, specifically with development of algal concentrates (IFREMER, France) and high-biomass solutions for algal culture using photobioreactor systems (University of Florence, Italy). This work is now being written up into several manuscripts.

Currently, CSIRO is undertaking a survey to assess novel marine protein and oil sources as potential replacers of fish meal and/or for high value products. The survey will include single-cell protein and zooplankton commonly used as larval feeds. Based on a positive assessment, a fuller proposal may be developed later this year, to be considered as a project within one of the new CSIRO Flagship Programs, i.e. Wealth from Oceans (http://www.csiro.au/index.asp?type=blank&id=FlagshipPrograms).

CMR has had a major role in providing nutritional and analytical expertise to the TAFI-led, FRDC/Aquafin CRC projects (see more in next section) on larval striped trumpeter propagation. The current project is due to end this year, and during this time we will be writing publications from the research with our TAFI colleagues.

CMR has recently undergone a major restructure within its aquaculture projects. As a consequence, research in larval feeds has been scaled down. Nevertheless, despite the current status we plan to maintain our core capability to take up new co-investment opportunities as they arise through future industry needs.

Capabilities and Equipment

CMR houses the CSIRO Collection of Living Microalgae (website: www.marine.csiro.au/microalgae/collection.html), one of the largest collections in the world, with over 750 strains including representatives from all classes of marine microalgae, some freshwater microalgae, and unusual marine heterotrophic microorganisms (e.g. thraustochytrids). The culture collection specialises in the Australian region, with microalgae from tropical to polar waters, although microalgae from around the world are also maintained. The Collection (through its Microalgal Supply Service) plays a pivotal role in supporting Australian aquaculture, by supplying ≈ 80% of Australian hatcheries with starter cultures of feed microalgae. Specialised skills and capabilities of CMR Collection staff include culture maintenance, species isolation and axenisation, media preparation and knowledge of the specific environmental requirements and growth properties of the different species.

Cultures of microalgae from CSIRO's collection

In addition to the infrastructure for the laboratory scale culture of algae, CMR has 6 x 50 L photobioreactor units for assessing high-biomass cultivation Through our past and current research we have developed expertise in the culture of some key aquaculture strains (e.g. *Nannochloropsis, Skeletonema*) within these systems including optimisation of biomass and omega-3 long chain polyunsaturated fatty acids (PUFA). In the search for cheaper biomass and new sources of PUFA, we

15 L photobioreactor

have also developed expertise in the isolation and culture of thraustochytrids – heterotrophic micro-organisms with high concentrations of PUFA.

During the past two decades, CMR has built up an international reputation of expertise in marine lipids and more recently, in analyses of amino acids and vitamins. We have developed and validated microanalytical techniques to measure the concentrations of fatty acids, sterols, lipid classes, amino acids (free and total), sugars, vitamins (B-group, C and E) and proximate analysis. The Division maintains state-of-the-art laboratories equipped with gas chromatographs (GC), HPLC, GC-mass spectrometers as well as other general infrastructure (eg. freeze-drier, centrifuge, extraction hoods etc.) required for these analyses. This capability has been applied to recent TAFI-led projects on the production of rock lobster phyllosoma and striped trumpeter larvae, where CMR has taken a lead role in the nutritional component of the projects. Specifically, both projects have had a focus on lipid and vitamin nutrition, and their analyses have been undertaken at CMR. CMR has worked closely with TAFI on the design of nutrition-based larval feeding experiment, and also in the interpretation and write-up of the research (see Publications on rock lobster and striped trumpeter).

Based on our lipid chemistry expertise, CMR has also developed protocols for the development of experimental emulsions used and validated for enriching live-feeds in tocopherol and PUFA. These protocols have been applied within the TAFI-CMR striped trumpeter project collaboration. Staff also have expertise in microencapsulation technology, e.g. binding of water-soluble nutrients within abalone diets, and crystalline amino acid encapsulation within prawn diets.

Our Cleveland laboratory has the capability to run multiple treatment x replicate experiments with larvae through the use of its “larvatron” (see Publication: Technology). The larvatron is a computer controlled larval and zooplankton culturing apparatus developed at CSIRO which is capable of maintaining up to 100 x 1 L culture vessels simultaneously, allowing a wide range of experimental conditions (eg. temperature, salinity, food type and concentration, animal stocking density) to be controlled, measured and replicated. The larvatron has been used for experiments with prawn larvae, but could also be used in experiment with other larvae, or to assess the enrichment of live feeds.

LARVATRON at Cleveland Labs

HPLC analysis of vitamins

Summary

CMR has a well-established track record in larval feeds research, especially in microalgae applications for aquaculture. We have a strong capability (staff expertise, state-of-the-art equipment) for the microanalysis of key nutrients in larvae and larval feeds, e.g. fatty acids, vitamins, amino acids. We have developed strong collaborative linkages with local industry, TAFI and overseas institutions such as IFREMER and University of Florence. We have currently reduced our research effort in larval feeds, though we are maintaining our key infrastructure and staff skills, which will enable new co-investment opportunities to be pursued.

CSIRO Publications in Larval Feeds and Biochemistry

*(Descending chronological order within category; Lead CSIRO author indicated by *)*

Live algal feeds

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Early weaning of Barramundi, Lates calcarifer (Bloch), in a commercial, intensive, semi-automated, recirculated larval rearing system

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Introduction

The production of live food for larvae of barramundi (Asian sea bass), *Lates calcarifer*, like for most species of marine fish, constitutes a major operational cost and bottleneck in a commercial hatchery.

On one hand, mass culture of rotifers and live microalgae involves high costs and labour requirements, as well as having highly variable nutritional value while also being unreliable. On the other hand, *Artemia* production is also associated with high operational costs and the weaning of the cannibalistic barramundi larvae onto *Artemia* can be highly detrimental to the growth and to the size disparity (variation) of a commercial batch.

We present a commercial system that tackles both issues, with great results, by using two of the latest products available in larval culture methods.

We have developed an intensive, greenwater, recirculated larval system and a continuous, high density, recirculated rotifer system using the HUFA enriched chlorella paste (DHA-enriched Super fresh V12 Chlorella) produced by Pacific Trading Co. Ltd, Japan.

The other major improvement to the system is the use of the micro-diet, Gemma Micro (GM), from Skretting for early weaning. *Artemia* have effectively been replaced in the larval diet by GM making the larval culture process more efficient and economic and producing higher quality weaned juveniles.

Darwin Aquaculture Centre

The Darwin Aquaculture Centre (DAC) is situated on Channel Island in Darwin Harbour some 50km from the city centre. This research and development facility was designed by the aquaculture staff and completed in September 1998 at a cost of $2.3M. This DAC is a showcase of practical design in a tropical environment and currently accommodates 16 staff, and up until recently, 2 post graduate students.

The centre has specific areas dedicated to fish, crustacean, algae, live feeds and environmental control work, in addition to a large dry laboratory, office, workshop and store. A bank of self-cleaning sand filters maintains a supply of suitable sea water all year, a rare commodity from a tropical estuarine environment.

In 2001 the DAC was expanded with the addition of a commercial barramundi fingerling production facility capable of producing up to 2 million advanced fingerlings per year.

Apart from commercial fish production, current projects include: developing barramundi nodavirus control techniques and strategies, refinement of methods for larviculture of mud crabs and a joint venture arrangement with industry for the production of juvenile sea cucumbers for ocean ranching and pond culture. Development and application of techniques for aquaculture in remote indigenous communities is also a key focus area of the DAC and the NT Government.

Some of the finfish broodstock tanks at the DAC.

A modern barramundi nursery was completed in 2001.

The DAC is designed for research and development of tropical marine aquaculture.

Materials and Methods

Rotifer system

The rotifer system has two 1,000 l tanks with foam fractionator, floc traps (sediment filter), fluidised-bed biological filter and one UV unit for new water disinfection (Figure 1).

The chlorella paste is fed automatically and continuously to the two rotifer tanks 24 hours a day from a standard fridge via two peristaltic pumps (one per tank).

The algal paste offers tremendous advantages, particularly because the rotifers can be produced at high density in small volume cultures. A base rotifer density of less than 100/ml is maintained between larval batches. When rotifers are required the systems are run at a density of 1,000 –1,500/ml with a lead time of 7 days. The algal paste is already enriched with essential fatty acids and no further enrichment is required. The un-consumed rotifers are kept in optimal nutritional value in the larval tanks by maintaining a low algal paste cell density.

The culture system is clean, with low levels of ciliates (free-swimming and attached e.g. *vorticella*) and based on simple technology. Bacteriological studies showed that the recirculating system carries a more stable bacterial population than batch systems and there is an almost total absence of harmful *Vibrio* spp. The rotifers are then continuously and automatically pumped directly (no rinsing) from the rotifer tanks to the larval tanks via two standard diaphragm pumps. Forty to sixty percent of the rotifer cultures are harvested per day (over 1 billion rotifers).

The use of the chlorella paste has been costed at A$ 0.17 per million rotifers produced. The daily labour requirement to maintain the system is less than 1 hour and the construction of the system only requires small tanks, standard pumps and PVC and plastic welding.

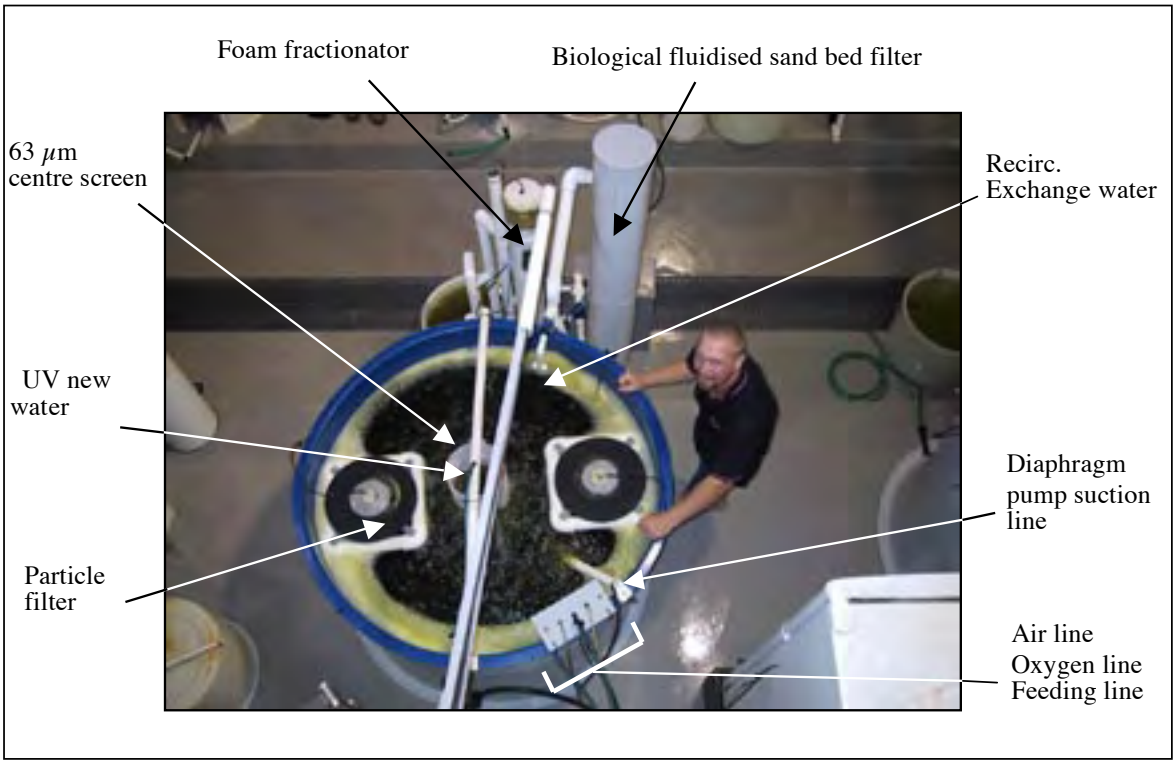


Figure 1. One of the two, 1,000 litre, high-density, rotifer culture tanks.

Larval rearing system

The larval system consists of two 6,000 l tanks in parallel (Figure 2), a rapid sand-filter, foam fractionator, fluidised-bed biological filter, degassing column, UV disinfection and a temperature control unit.

The larval tanks are stocked at 100 larvae per litre (a total of 1.2 million larvae) and the larval rearing regime is described in Figure 1. As per the rotifers system the chlorella is continuously and automatically fed to the larval tanks to keep a constant low algal density. The rotifer density is adjusted by changing the size of the outlet self-cleaning screens (63 to 500 µm) and/or by adjusting the pumping rate to the larval tanks.

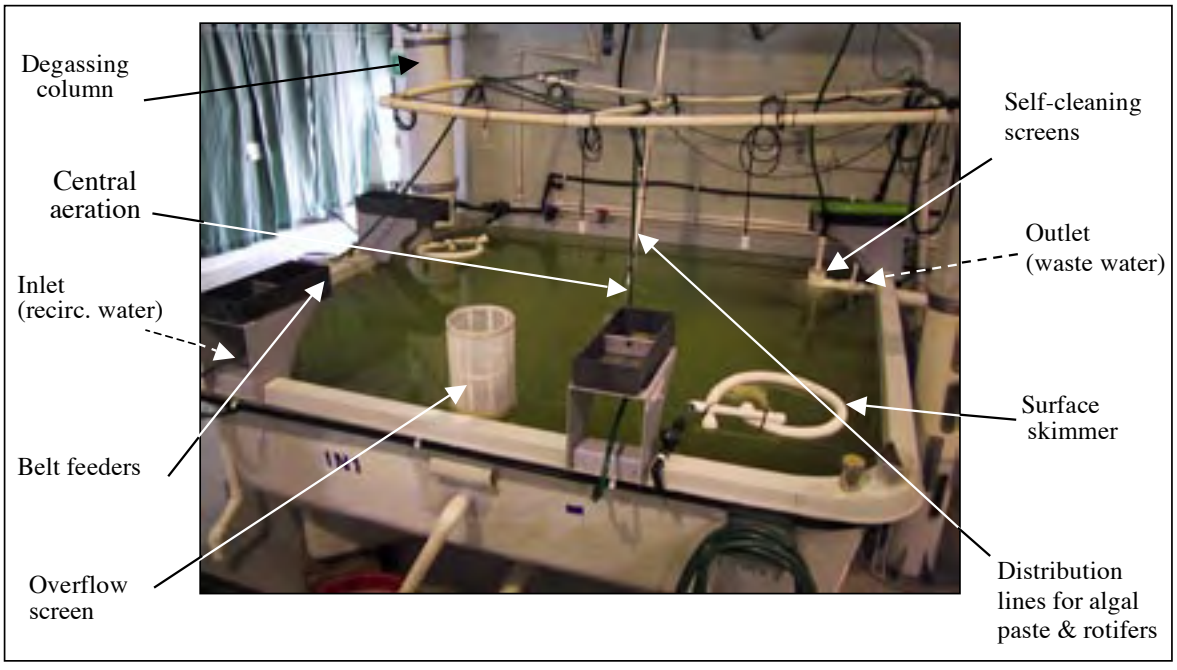


Figure 2. One of the two 6,000 litre intensive larval rearing tanks.

The other major improvement to the system was the use of the microdiet, Gemma Micro, from Skretting (Nutreco) for early weaning. The latest batch of barramundi was produced without the use of any *Artemia*. The larval tanks were stocked at 100 larvae per litre, fed only on rotifers for 6 days and then co-fed with GM for 7 days and were weaned by D16 with only 5% weaning mortality. Over 700,000 fry were produced from this batch with a survival of 60%. In the previous batch less than 2 kg of *Artemia* per million weaned fry (Day 18-20) were used with less than 0.5% weaning mortality. This represented a 95% reduction in *Artemia* use compared to previous batches (Table1). For economic reasons, once the larvae are weaned, Gemma Micro is slowly replaced by the microdiet, Proton (INVE), before moving on to the nursery feed, Gemma (Skretting) (Figure 3).

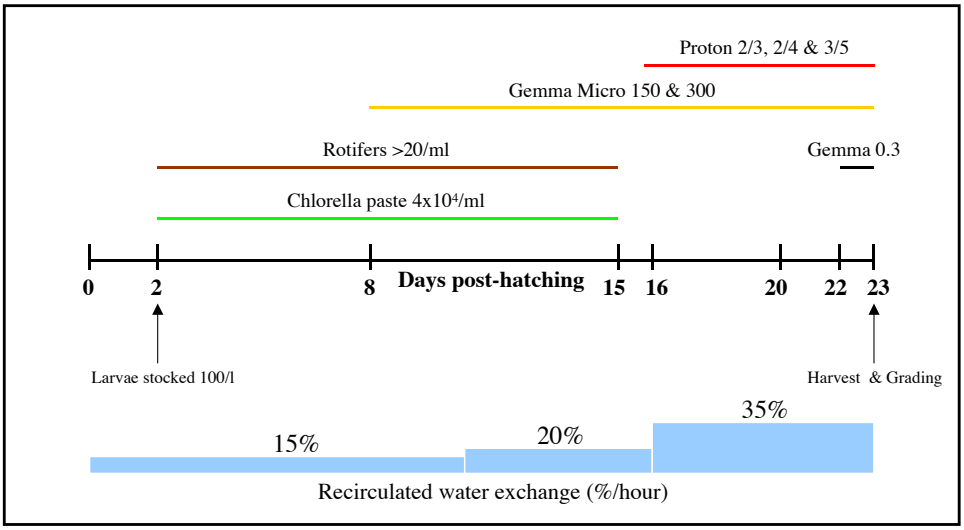


Figure 3. Larval rearing regime for barramundi, *Lates calcarifer*, in the intensive recirculated system from first feeding larvae to metamorphosis. Artificial diets: GM: Gemma Micro (Skretting); Proton (INVE); Gemma (Skretting).

Results and Discussion

Using this system we have routinely achieved a survival rate of 44-58% from 2 day old larvae through to 23 day old weaned fry (15 mm). The deformity rate of the fingerlings assessed at 100 mm was less than 1% (mainly jaw deformity and missing opercula) and was not greater than previous batches reared using *Artemia* (Table 1).

The latest batches of larvae weaned quickly with Gemma Micro and grew to 100 mm 15-25% faster than previous batches (9-10 weeks compared to 11-12 weeks from hatching). There is also less size variation, a great advantage since barramundi are very cannibalistic and need frequent grading on reaching 3-4 weeks old. Once transferred to the nursery, the fish are much healthier and less susceptible to stress (handling, grading…).

The total cost of weaning and post-weaning feeds (including labor) has been summarized in Table 1 and is 20% cheaper, using GM and minimal *Artemia,*, than the standard method. The labour requirement (and operational cost) has been significantly reduced to one person dealing with both the larval system and the live feed system (because of quarantine issues 2 x 0.5 technicians are running both systems). The capital cost of constructing the system was also minimal as the system was mainly built in-house using PVC and assembled using plastic welding.

Table 1. History and production parameters of the intensive larval system and cost of weaning and post-weaning feeds (including labour) per million of barramundi weaned fry produced (15 mm).

	Batch Number					
	03-03	**08-03**	**10-03**	**12-03**	**02-04**	**04-04**
Larval stocking density (/ ml)	54	70	84	100	103	106
Fry produced at D 25 (15 mm)	278,000	450,000	448,000	612,000	577,000	734,000
Survival (%)	44	54	45	51	47	58
End of weaning (Dph)	25-35	20-26	22-24	22-24	18-20	16-18
Weaning mortality (%)	1-2	1-2	0	1-2	0	5
Deformity rate (% assessed @ 100 mm)	< 0.1	<0.8	< 0.1	< 0.2	< 1.0	< 0.5
Artemia (kg) / 10^6 weaned fry	20.5	6.6	12.5	10.1	1.2	0
Cost of enriched *Artemia* (inc. labour) ($A)	5,700	2,500	3,900	3,300	700	0
Gemma Micro (kg) / 10^6 weaned fry	0	4.6	20.5	14.2	12.1	13.2
Cost of Gemma Micro ($A)	0	1,400	6,500	4,500	3,800	4,200
Proton (kg) / 10^6 weaned fry	11.5	6.2	5.8	10.9	10.2	11.8
Cost of Proton ($A)	500	200	200	400	400	500
Total cost in $A per million weaned fry	**6,200**	**4,100**	**10,600**	**8,200**	**4,900**	**4,700**

More than three million barramundi fry have been produced using this system at the Darwin Aquaculture Centre (DAC) over the last 12 months (5 batches of 600,000+).

We are now doing collaborative work with Dr. Sagiv Kolkovski, Department of Fisheries of Western Australia, to assess microdiets and co-feeding management to further improve the weaning process and to maximise survival and growth rates of barramundi larvae during the larval rearing and early nursery phases.

For the past two years we have also assessed grading and feeding strategies in the nursery stage from 15 mm fry to 100 mm fingerlings. Asian, European and Australian nursery feeds have been compared in these experimental trials.

We will be producing technical and scientific papers early in 2005 describing the larval system and presenting the nursery trials.

Queensland Government
Department of Primary Industries and Fisheries

Richard Knuckey and Elizabeth Cox

The Aquaculture and Stock Enhancement Facility at Northern Fisheries Centre, Cairns was commissioned in early 2002. This purpose built, marine fisheries facility was designed to enhance Queenslands development of tropical aquaculture. It contains dedicated broodstock, larviculture and live feed areas that support a variety of fish aquaculture.

Broodstock facilities for finfish include 7 x 30T and 6 x 60T tanks. These operate with recirculation through dedicated biofilters. Currently, 5 x 30T and 2 x 60T tanks are equipped with photoperiod and temperature control.

Five marine finfish species are maintained in this system. For the role of stock enhancement, Barramundi (*Lates calcarifer*) and Mangrove Jack (*Lutjanus argentimaculatus*) are held. Barramundi broodstock are spawned throughout the year with periodic replacement of fish. Mangrove Jack are F1 fish spawned in ponds at DPI&F Oonoonba, Townsville and on grown in Cairns from ~2 cm. These fish are now approaching sexual maturity where they may be used as broodstock for captive spawning. Another major project "Tropical Marine Finfish Aquaculture" is focused on the development of aquaculture of reef fish and in particular groupers. Three grouper species are held for this project:

Barramundi cod (*Cromileptes altivelis*), Flowery cod (*Epinephelus fuscoguttatus*) and Estuary cod (*Epinephelus coioides*).

Beside finfish the facility also supports research in the aquaculture of the tropical lobster (*Panulirus ornatus*) and sandfish (*Holothuria scabra*).

The live prey facility supplies food to all areas that require it as well as acting as a supply service to the aquaculture industry. It also has a research component to refine existing culture methods and develop new live food species.

Around 10 microalgal species are routinely held in the collection and grown up to a volume of 2000 L. Microalgae are used to feed rotifers, copepods and to on-grow *Artemia* as well as for "green-water" culture and the conditioning of plates for sandfish larvae. The SS-strain rotifer *Brachionus rotundiformis* and the calanoid copepod *Acartia sinjiensis* are grown for marine finfish larvae. *Artemia* are hatched and enriched for finfish larvae or on-grown in algae for lobster larvae.

Broodstock research

The increased broodstock facilities, particularly the increase in the number of tanks with photo-thermal control has enhanced the scope of research that can now be undertaken. Flowery cod and estuary cod research is focussed on assessing the use of photo-thermal control and the application of short-term stimuli to both control and extend reproductive development and to induce spawning. Research into captive populations of barramundi cod is targeting a range of issues including poor spawning performance and spontaneous sex reversal of male broodstock in captive culture systems. The target for all three grouper species is to develop protocols that will allow for extended and predictable spawning of captive breeding populations.

Larviculture research

Increased spawning success as a result of the increased scope of broodstock research has enhanced our ability to address bottlenecks within the larval rearing phase for these species. Poor and inconsistent survival remains an ongoing issue during the early larval rearing phases for many grouper species. The primary larval rearing objective has therefore focussed on identifying the basic physical parameters required to obtain consistent survival during the first feeding stages for each species.

The impact of light intensity on survival, growth and feeding incidence of both estuary cod and flowery cod has been assessed across multiple cohorts. Results of light intensity trials have indicated that pre- and early

feeding stages of larval grouper have a preference for lower light ranges. Morphological data suggest that energy expenditure is used for growth rather than increased swimming activity at the lower light levels tested, however the variability between cohorts require that this data is interpreted with caution until replicated further. In addition, larval survival and condition during more extensive feeding trials, must be undertaken in order to fully assess larval feeding ability in response to light intensity.

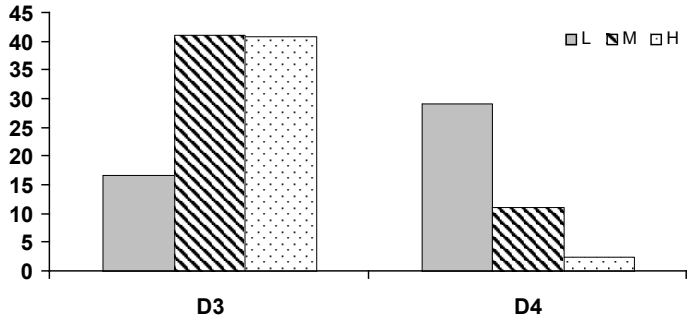


Figure 1. Feeding incidence of larval flowery cod exposed to three light intensities (low 0.05 - 0.085, medium 1.2 - 1.75 and high 6 - 10 µmol.s^{-1}.m^{-2}).

Live prey research

Research in Live Prey production is focused to supply suitable food for the fish species cultured in the facility. Marine finfish such as groupers have a small mouth gape at first feeding. This has necessitated the development of live prey species small enough to be consumed by these larvae. Recent research has focused on:

- Improved nutritional value of rotifers
- Reduced scale-up time and increased production of rotifers
- Increased production of copepods

Outcomes

Improve nutritional value of rotifers

A diet with a high DHA:EPA ratio is preferable for marine finfish larvae. Because of this, the use of Algamac-2000 for routine enrichment of SS-strain rotifers cultured at NFC has been adopted. Rotifers enriched with Algamac 2000 have a higher DHA:EPA (4:1) ratio and a more consistent fatty acid profile than those enriched with DHA Protein Selco. Although the Algamac product states that higher levels of fatty acids are possible if rotifers are enriched for 2 x 12 h periods, we recommend only a single 12 h enrichment. This is because, low rotifer fecundity present after a single 12 h enrichment would likely result in significant mortality during a second enrichment period. To maximise rotifer survival, the use of multiple aeration and additional O_2 is recommended for all rotifer enrichment.

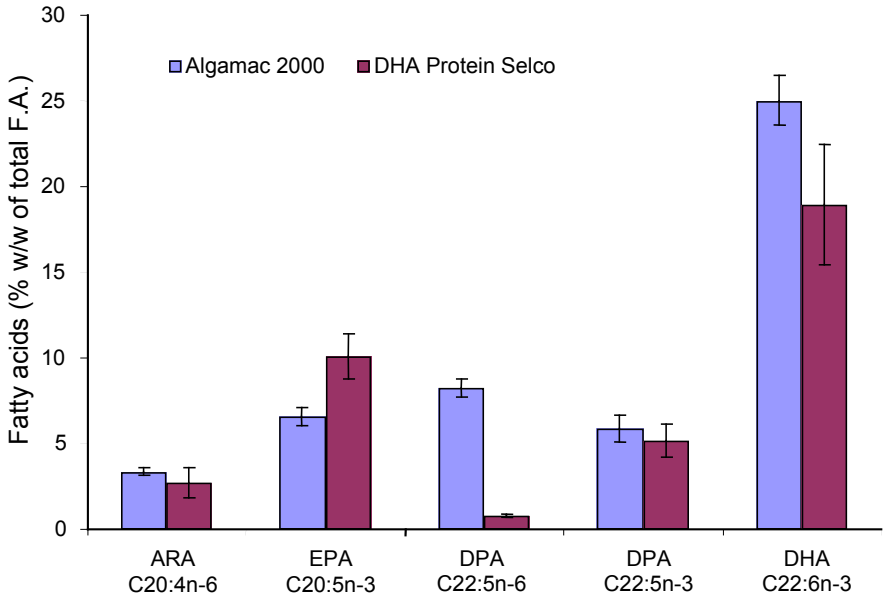
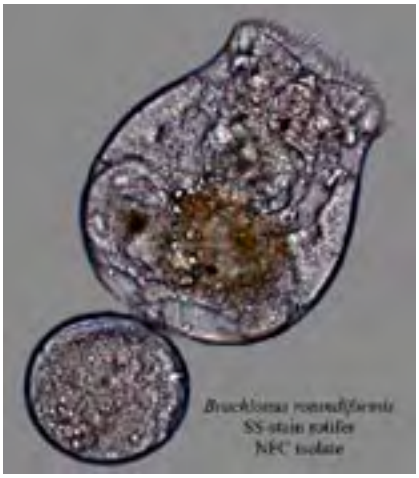


Figure 2. HUFA (% w/w of total fatty acids) content of rotifers enriched with Algamac 2000 and DHA Protein Selco. Error bars are ± SD, n = 3.

Reduce scale-up time and increase production of rotifers



The medium-density, Culture Selco 3000 system is being adopted as a method to increase the capacity of the rotifer culture facility at NFC while reducing the labour required for their production. This system was demonstrated to produce at least as many rotifers as the product and its method stated. Rotifer densities of 1500 to 1800/mL were achieved after 3 to 4 days of culture. Adoption of the method requires strict adherence to the instructions detailed for the product. In particular, the initial rotifer population must to be of a high quality with a fecundity of at least 20% and minimal contamination by protozoa. It is possible that feed rates recommended for the product could be refined and this would reduce the risk of protozoa blooming from excess food. The use of Culture Selco 3000 will reduce the reliance on algal cultures and the time required to scale-up rotifer cultures during spawning events.

Increase production of copepods

When fed on an equal ration (ash free dry weight), Cryptomonad CS-412 is as nutritious as *Rhodomonas* for *A. sinjiensis*. Because of its ease of culture relative to *Rhodomonas*, Cryptomonad is recommended to replace *Rhodomonas* in the diet for *A. sinjiensis* cultured at NFC. A daily diet ration of 1.13 µg AFDW/mL was found to support 99% maximal growth. It is recommended that the current daily feed rate of 1.34 µg AFDW/mL remain, this will ensure satiation with a low impact on water quality.

Increasing copepod production by scaling up of existing methods used in 400 L tanks to 1200 L tanks resulted in 83% of the production per unit volume. With modifications to harvesting equipment, this percentage is likely to approach par. To achieve a production rate to supply the hatchery with 2 nauplii/mL (12 × 280 L larval rearing tanks), it is recommended to increase the copepod culture system to 2 x 5000 L tanks. This would require approximately 150 L of mature Cryptomonad culture per day as feed. The capacity to produce algal species required for the copepod diet, in particular Cryptomonad sp. CS-412 and *Isochrysis* sp. (T. ISO) exists within the current facility.

Hatching of *A. sinjiensis* eggs was effectively (at least 90%) inhibited for 72 h at storage temperatures ≤ 10°C. Eggs and nauplii (N1) could be cold-stored at 10°C for 72 h with no development or loss of viability. Storage at

cooler temperatures (4°C) resulted in the rapid loss of viability of eggs. It is recommended to incorporate cold-storage techniques into the management of the upgraded copepod production (2 x 5000 L) facility. With an expected production of 5 x 10^6 nauplii/day/tank and the adoption of cold storage, up to 20 x 10^6 nauplii per 5000 L tank could be available for the crucial first feeding stage of finfish larvae.

Results will be discussed in relation to the above points and will cover selection of rotifer enrichment product, use of commercial artificial rotifer diets, methods to increase copepod production through scale-up of existing methods and the development of cold-storage techniques for copepod eggs and nauplii.

Bottlenecks still exist in the supply of suitable numbers of very small live prey items for first and early larval stages of marine finfish. An outline of future work aimed at addressing this issue and selection of potential new live prey species will also be presented.

Tropical Rock Lobster

Hatchery research of the tropical rock lobster *Panulirus ornatus* is also a significant project at the Northern Fisheries Centre, Cairns. The project is primarily funded by the FRDC within the Rock Lobster Enhancement and Aquaculture Subprogram, and has as its collaborators AIMS and MG Kailis Pty Ltd.

Management of *P. ornatus* broodstock, for captive breeding has proven to be quite straightforward. The species is amenable to a captive environment, and will readily breed throughout summer. Environmental manipulation of breeding has been applied to achieve out-of-season breeding. Photoperiod is the primary cue, although temperature and social factors have a significant influence on breeding success.

Larval culture is particularly difficult, primarily because of the protracted larval life which extends to over 6 months in the wild. Too date, the longest lived larvae in our system has persisted to 211 days of age, and stage IX of eleven stages. Recent larval runs with a focus on improved survival of early stage larvae (to stage V) have been increasingly successful. The premise of current research is to achieve consistent survival of larvae to stage V, before realigning our efforts to mid-stage larvae. Hygiene and nutrition are the two most important factors, and research is accordingly focussed in these two areas.

James Cook University

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Species cultured

Molluscs: Pearl oyster (*Pinctada* spp. *and Pteria* spp.), tropical abalone (*Haliotis asinina*), tropical rock oysters (*Saccostrea* spp.) and other tropical species (e.g. *Tridacna* spp. and *Pinna* spp. and *Pectinidae*)

Crustaceans: Mud crab, *Scylla serrata,* sand crab (blue swimmer), *Portunus pelagicus*; ornamental species including the cleaner shrimps (*Lysmata amboinensis* and *L. debelius*) and the dancing shrimp (*Rhynchocinetes durbanensis*).

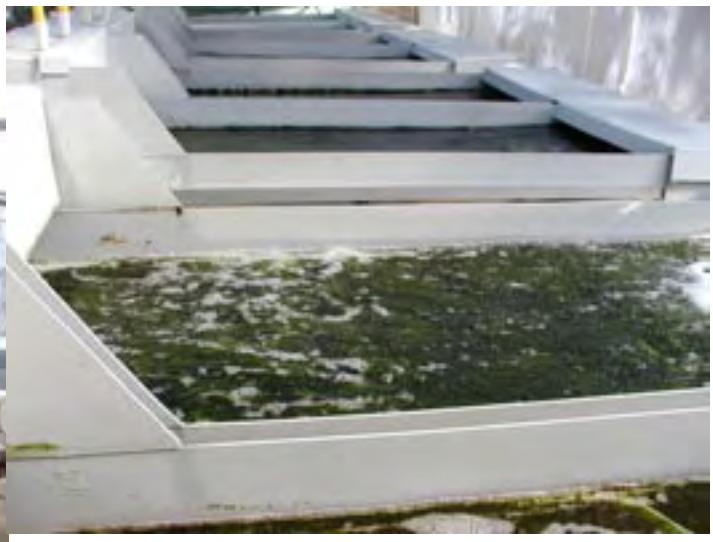
Finfish: barramundi, *Lates calcarifer*; damselfish *Acanthochromis polyacanthus*; clownfish, *Premnas biaculeatus*, *Amphiprion melanopus*, *A. akindynos* and *A. percula*; Coral trout, *Plectropomus leopardus* (new target species for 2005).

Systems

The JCU *Marine and Aquaculture Research Facility Unit* (MARFU) is a facility of the Faculty of Science Engineering and Information Technology and is available to all Schools within the Faculty. All seawater is transported to MARFU from the Australian Institute of Marine Science (AIMS) and MARFU technical staff maintain two seawater systems: primary treated 'new' water from AIMS, (un-chlorinated, UV sterilized, 1µm filtered at salinity of approximately 34‰) and secondary treated 'waste' water (treated using algal scrubbers, UV sterilization, foam fractionation, bag filtration to 20µm at salinity of approximately 31‰). Primary treated water is used for hatchery production of crabs and bivalves whereas secondary treated water is adequate for fish production and crustacean broodstock. A range of indoor, temperature-controlled rooms and outdoor shade-covered aquarium/tank space is available for use. Live feed production is now a MARFU service provided by the centre staff.

Figure 1. Main aquaculture facility area.

Figure 2. Primary water treatment for "new" incoming water including bag filtration, UV sterilizers, heat exchanger



▲ **Figure 4. Algal scrubbers are part of the secondary water treatment system.**

◀ **Figure 3. Bank of foam fractionaters for secondary water treatment.**

Orpheus Island Research Station (OIRS) is another facility of the Faculty of Science Engineering and Information Technology at JCU. Accommodation and research facilities have recently been refurbished and the station now has increased capacity to support broodstock, hatchery and growout research activities.

Molluscs

Bivalve conditioning is conducted in 200 L shallow tanks with either partial flow-through or static water conditions. Small-scale experiments with larvae are conducted in replicate 10 L aquaria. Water is either partially or totally replaced on a daily basis (using a flow-through system incorporating banjo screens), or is totally replaced every 2 days in static systems.

Larger scale bivalve larval culture is conducted in 500 L tanks and 18 tanks allow good replication. Tanks can be run as static water changed every 2 days or flow-through systems. Larger scale bivalve larval culture in conducted in replicate 3000 L tanks at OIRS.

Oyster broodstock are held on long-lines at either White Lady Bay, Magnetic Island, or at OIRS. Broodstock capacity at Magnetic Island is approximately 600-800 and at OIRS is 1200. Nursery and grow-out culture research with bivalves is also carried out at these sites.

Crustaceans

Mud and sand crab larvae are cultured in circular, flat-bottom, 400 -1000 L tanks, using green water (*Nannochloropsis oculata*) and supplied with UV sterilized, primary treated seawater (1 µm) with partial water exchange as required. There is holding capacity for 15-20 broodstock mud crabs at any one time and between 25 and 30 crabs are used for larval production annually. Holding capacity for sand crab broodstock is similar to that of mud crabs.

Culture of ornamental shrimp species is at an early stage of development, currently using small aquaria (20-50 L), and

Figure 5. Mud crab crablet.

recirculated, secondary treated seawater with partial water exchange as required. Production for each larval run is from single female broodstock.

Finfish

Barramundi larval culture is conducted in cylindro-concial tanks in sizes ranging from 250 L to 2,000 L, using green water (*Nannochloropsis oculata*), and supplied with recirculated secondary treated seawater with partial water exchange as required. No barramundi broodstock are held at JCU; larvae 2 days post-hatching are purchased from commercial hatcheries with some provided by QDPI, Northern Fisheries Centre. Both upwelling and downwelling tanks have been used for larval culture.

Clownfish and damsel fish larvae are produced in flat-bottom, cylindrical, 100 L tanks with a collar around the tank perimeter to reduce incident light. Water supply is flow-through secondary treated water with multiple inlet points around the tank perimeter and a single banjo screen at the bottom of the tank for water discharge.

Production capacity

Molluscs

We have the capacity for commercial-scale hatchery production of bivalves, but most is experimental. The production season is September to March but can be extended to June. On average 3-4 experimental runs with pearl oysters are conducted per year producing approximately 40,000 spat each. We do about 2 experimental runs with *Saccostrea* sp., annually each producing approximately 20,000 spat.

Crustaceans

Mud crab broodstock are mostly wild-caught (several hatchery produced mud crabs have reached sexual maturation and started spawning) and are maintained in oval, 2,000 L flat-bottom tanks until spawning. They are fed mussels, squid and prawns every day. After spawning berried females are transferred into indoor 400 L tanks with recirculated, primary treated seawater (1 µm). Larval production can be at any time of the year although it is more common between May and Jan. Production runs are driven by research need and between 10,000 to 50,000 juveniles are produced annually.

Broodstock sand crabs are maintained under similar conditions to mud crabs although berried sand crabs are often collected directly from the field. They are fed mussels, squid and prawns. There is capacity for year-around production; however, this is driven by research need. Currently up to 500,000 juveniles can be produced annually.

Excess production of mud crabs and sand crabs services teaching requirements and the remainder is currently culled. There is capacity for scaling up production of both depending upon demand.

Ornamental shrimp production has just recently been attempted. These species generally spawn twice per month, producing hundreds to more than a thousand eggs per spawn. They appear to have the capacity to produce eggs year-around under controlled environmental conditions. Larva durations are long, often 100 days or longer; however, production trials routinely only achieve survival to between 50 and 80 days. Given the high retail prices achieved for these shrimp species, culture protocols will continue to be a research priority.

Finfish

Ambient production of barramundi occurs from approximately October to February. Blue Water Barramundi have the capacity for photo-thermal manipulation of broodstock and have provided 2 day old larvae from August to March. Production runs are driven by research need and between 10,000-200,000 late-stage larvae or juveniles are produced annually. Excess production is culled or used for teaching.

There is almost year round capacity for damsel fish and clownfish production which occurs as required to meet the immediate demands of research and teaching. Broodstock are maintained on a diet of minced trash finfish and commercial flake. There is capacity for scaling up production depending upon demand.

Live foods used

Currently only S-S-strain rotifers (*Brachionus rotundiformis)* are produced to support crustacean and fish larval culture. Rotifers are produced in outdoor tanks 1000-2000 L in semi-continuous batch cultures using *Nannochloropsis oculata,* without addition of yeast. No additional enrichment products are currently used for rotifers besides micro-algae.

Currently only newly hatched *Artemia* and Artemia instar II are used. A variety of approaches including decapsulation, non-decapsulation and A1 Selco products for enrichment are used.

Figure 6. Hands-on area for undergraduate teaching and outdoor large scale rotifer area.

Crustaceans

Mud crab and sand crab: Early larvae are fed rotifers (Zoea-I to Zoea-II). Larvae are switched to newly hatched *Artemia* at later stages. Depending on the experiment design and particular protocols being undertaken, mixed feeding of rotifers and *Artemia* may occur at Zoea-II to Zoea-IV (mud crab) or Zoea-III (sand crab) stages.

Finfish

Barramundi: D 2 - 12, S-S-strain rotifers; D 8 - 10, newly hatched *Artemia;* D 10 – until weaning, enriched *Artemia* instar II. Fish are weaned starting any time from D 18 - 30, depending upon the particular experiment protocol. Inert diets are sometimes used along with live feeds during the *Artemia* feeding stage. Rotifers are added twice daily at 20-50 ml^{-1} and *Artemia* are added twice daily at 0.05 - 3 ml^{-1}.

Damsel fish: Live feeds, *Artemia* and rotifers, are provided but larvae will accept a commercial flake from hatching.

Clownfish: D1-8, rotifers, gradually reduced from 20 rotifers ml^{-1}; D 5-10, *Artemia,* gradually increased from 1.5 to 2 *Artemia* .ml^{-1}; weaning starting from D 6 or 7.

Inert feeds used

Mud crab: Microbound diets have been developed at JCU that are readily accepted by mud crab larvae and support at least equal development rate and survival as *Artemia* for the megalopa stage. Particle sizes of the microbound diets for Zoea 1, Zoea III, Zoea V and Megalopa are <150 µm, 150-250 µm, 250-400 µm and 400-600 µm, respectively.

Barramundi: 100 µm inert diet if used during the *Artemia* feeding stage; 300 µm starting from D 18. Commercially available microparticulate feeds have been assessed for barramundi larvae and a prototype experimental microparticulate feed has been developed in collaboration with WA Fisheries during a recent FRDC funded project.

Algae

For bivalves: There is a focus on culture of tropical species. Standard species for bivalve culture include: *Isochrysis* sp. (T-iso), *Chaetoceros muellerei*, *Pavlova salina*. Others species used more experimentally include: *Pavlova* spp. (CS63, CS50, CSIRO Catalogue codes) and *Chaetoceros* spp. (CS257, CS256). Axenic stock cultures are maintained continuously in 250 ml flasks under 50-80 µmoles photons PAR m^{-2} s^{-1} at 12:12 light: dark cycle. Most micro-algae production for bivalves is conducted in 20 L carboys with capacity for culture in

100 L plastic tubes and 200 L bags. Autoclaved f/2 medium (0.5 µm filtered, UV sterilized seawater) is used for algae production. Production capacity depends upon experiments being undertaken. Standing capacity of algae for bivalve culture is approximately 2,000 L.

For fish: Large scale production of *Nannochloropsis oculata* for rotifer culture and green water culture of fish and crab larvae occurs outdoors and is produced using a commercially manufactured liquid plant fertiliser and natural sunlight.

Hygiene

Foot baths are provided at the entrance to hatcheries and algal culture rooms; gum boots, and use of ethanol sprays and hand washing are required for entry into all indoor production facilities.

Relevant R&D Activity at JCU (researcher) [projected outcomes]

- Pearl Resource Development in Pacific Islands; *ACIAR* 1993-2005 (Southgate)[Towards cultured pearl industries in Pacific Islands]
- Replacement of *Artemia* with artificial diets; *FRDC* - 2004 (Southgate)[reduce reliance on live feeds]
- Development of pearl resources in Tanzania; *WWF* - 2004 (Southgate)[develop technology for pearl oyster culture]
- JCU mud crab project; *ACIAR* 2004-2005 (Zeng)[reduce rates of cannibalism]
- Mud crab mass larval culture techniques and applying biotechnology to improve production; *China 863 [National High-Tech R&D]* 2004-2005 (Zeng)[development of protocols for commercial scale production]
- Selection of pearl oyster broodstock; *JCU internal grant* - 2004 (Southgate)[improving pearl quality through broodstock selection]
- Triploidy induction in pearl oysters and mud crabs; *JCU internal grant* - 2004 (Southgate, Zeng & Jerry)[improved growth rates and pearl quality}
- Metamorphosis cues in mud crabs; *JCU internal grant* -2004 (de Nys & Zeng)[accelerate, synchronize and enhance settlement]
- DNA pedigreeing *P. monodon*; *JCU internal grant* -2004 (Jerry)[improved selection of broodstock lines]
- Feed technology for temperate fish species; Aquafin CRC PhD project supervision 2005-2008 (Pankhurst and Southgate in conjunction with NSW Department of Primary Industries , Port Stephens Fisheries Centre)[improvement of intensive rearing protocols of larval and juvenile temperate fish species; Mulloway *(Argyrosomus japonicus)* and yellowtail kingfish *(Seriola lalandi lalandi)]*
- Improving growth and survival of cultured marine larvae striped trumpeter: a test case for Tasmania; Aquafin CRC PhD project supervision 2005 (Pankhurst in conjunction with TAFI, Marine Research Laboratories)[optimizing prey consumption through manipulation of the culture environment (green water, light, turbulence)]

Ned and Tish Pankhurst joined the staff at JCU in February 2004, bringing expertise in the areas of finfish larval culture (Tish) and broodstock manipulation (Ned), diversifying the current significant research strength in relation to bivalve and crustacean hatchery production and technologies.

Specific problems and issues

1. **Ornamental shrimp:** prolonged larval duration, undefined mortality at approximately 80 days.
2. **Mud crab and sand crab:** high cannibalism rate from the megalopa stage onwards.

3. **Mud crab:** significant variations in larval quality upon hatching; '*Moult Death Syndrome*' at later larval stages and still need to use antibiotics for the first day of stocking.

Collaboration

JCU hatchery researchers have close links to industry and other researchers both nationally and internationally: TAFI-University of Tasmania, Marine Research Laboratories (Dr Stephen Battaglene) and School of Aquaculture (Dr Marianne Watts, Dr Mark Porter); NSW Department of Primary Industries, Port Stephens Research Centre (Dr Stewart Fielder, Dr Geoff Allan); Fisheries Western Australia (Dr Sagiv Kolkovski); Salmon Enterprises of Tasmania [SALTAS] (Dr Harry King); Blue water Barramundi (Mr David Borgelt); Good Fortune Bay Fisheries (Dr Trevor Anderson); AIMS (Mr Matthew Kenway); QDPI, Northern Fisheries Centre (Dr Mike Rimmer, Ms Liz Cox); Secretariat of the Pacific Community, Noumea (SPC) (Mr Ben Ponia); Xiamen University, China (Prof. LI and Wang); Sematan Fisheries Centre, Sarawa, Malaysia (Dr. Abdullah); Links with Fisheries Divisions in Pacific islands (Kiribati, Fiji, Tonga, Solomon Islands) with CIBNOR and UABCS in Mexico, with SEAFDEC (Phillipines) and Worldfish (Noumea/Penang).

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Applied research and training – the Aquaculture Development Unit approach

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Abstract

The Aquaculture Development Unit (ADU) of the WA Maritime Training Centre (WAMTC), Challenger TAFE is a world-class facility that undertakes applied aquaculture R&D as well as VET aquaculture training activities for marine finfish and other species. The ADU was established in 1992 to stimulate the development of marine aquaculture in Western Australia. A large-scale marine hatchery was established; species selected for commercial aquaculture; grant funding secured to undertake the applied research leading to the development of production technology for the selected species and technology transferred from the researchers to industry.

Projects that the ADU have completed or are currently running include:

Applied research

- Development of commercial production techniques for the culture of snapper and black bream;
- Development of production techniques for the culture of WA dhufish (FRDC) and King George whiting (WA Minister of Fisheries funded);
- Restocking of black bream into the Swan River (1995, 1997) and Blackwood River Estuary (2000 – 2004). Funded by the FRDC;
- Growout of snapper in a recirculation system (2001-current). Industry funded;
- Development of spawning and culture techniques for the abalone *Haliotis scallaris*. Funded by the Aquaculture Development Council (ADC) and industry;
- Demonstration of nutrient stripping from aquaculture wastewater using the sea-lettuce and feeding to abalone. Funded by the Natural Heritage Trust;
- Applied research into fish species and aquaculture systems suitable for use in inland saline water in the WA Wheatbelt (TAFEWA, FRDC, NAC, AusIndustry, AFFA and funded).

Industry Extension

- Publication of a hatchery manual for production of black bream (1999);
- Publication of a hatchery manual in text and CDROM formats for snapper and black bream (2003). Funded by the FRDC;
- Numerous Seminars and Workshops on aquaculture topics;

Training

- Development of a marine prawn culture Unit of Competency under the National Seafood Training Package (2000). Funded by the WA DOT's Science and Technology Innovation Strategy;
- Development of a Unit of Competency for the Environmental Management of Marine Finfish Growout Operations under the National Seafood Training Package (2003). Funded by the WA DOT's Science and Technology Innovation Strategy;

- Provision of a short course for the culture of temperate marine finfish that provides training for industry and research personnel in 'best-practice' culture methods for hatchery operations.
- A Graduate Diploma in Marine Fish Hatchery Management. This course is similar to a medical 'internship' where students qualified in the theory of aquaculture will further develop and extend their practical skills and knowledge. This course can only be effectively taught in a commercial-scale marine finfish hatchery such as at the ADU where VET can be combined with industry scale activities.

The ADU is a unique 'experiment' in the VET field: a successful combination of applied research, development, extension and VET training for the WA industry. This paper details some of these activities; and highlights some of the problems that could be expected in the VET sector in transferring new technologies to industry based on our experiences over the past ten years.

Key words: finfish, aquaculture, training, Western AustraliaBackground

The Aquaculture Development Unit (ADU) of the Western Australian Maritime Training Centre (WAMTC), Challenger TAFE is a world-class facility undertaking applied aquaculture R&D associated with VET aquaculture activities for marine finfish and other species. The ADU was established in 1992 with the charter to:

- 'Conduct applied research and development projects to stimulate development of marine aquaculture industries in WA'; and
- 'Transfer developed technologies, knowledge and skills to industry.'

During 1992, the then Chief Executive Officer of the WA Department of Training began implementing a policy of associating colleges closer to industry. This included the provision of facilities to encourage industry groups to participate in applied research projects and joint ventures. Aquaculture, as the fastest growing primary industry sector in Australia, was identified as an area of need and in 1993, funding was allocated to formally establish the Aquaculture Development Unit (ADU) at WAMTC. It should be noted that the WAMTC has been conducting aquaculture courses in Fremantle since 1989.

The WAMTC is located within the Fremantle Port Authority area on the foreshore between the entrance of the Swan River to the north and Bathers Beach to the south (See Figure 1). The majority of the buildings in which it is located were constructed during the Second World War for Navy support purposes. The WAMTC moved into the buildings in 1988 with a 20-year lease on the premises. The WAMTC has been providing aquaculture courses to Western Australia since 1987 and is the Australian VET pioneer in this area of training.

Figure 1. Aerial photograph showing the WA Maritime Training Centre, Fremantle.

A capital works program of over $2 million was initiated in 1993 to upgrade facilities to provide for the establishment of the Aquaculture Development Unit. An ocean intake, capable of supplying up to five million litres of filtered seawater per day, was installed and commissioned in September 1993. An enclosed area for aquaculture R&D was developed and reticulated with air and seawater. During 1996 two seawater bores were commissioned, supplying filtered seawater to the hatchery. The ocean intake was abandoned at this time, having been technically and operationally problematic and expensive to run and maintain.

The substantial ADU marine hatchery was established and key staff involved themselves in relevant industry associations. The ADU operates with a range of facilities (See Figure 2) and equipment which includes:

- 2000 square metres of enclosed area reticulated with air and ocean water
- 2 saltwater bores supplying 20 litres per second of seawater each
- Hatchery laboratories, aquarium and live food culture rooms
- Controlled environment (photo therm) rooms
- Experimental feed mill facility and testing unit in collaboration with Fisheries
- Quarantine facility

Figure 2. A sample of ADU facilities.

Other specialised equipment at the WA Maritime Training Centre – Fremantle include:

- 7 boats, including the Maritime Image, a purpose built 18.5 metre training vessel;
- Radar and Automatic Radar Plotting Aid Simulator;
- Global Maritime Distress and Safety System simulator;
- Stability Laboratory;

- Global Positioning Systems survival and fire fighting facilities;
- Well appointed air-conditioned classrooms including a computer laboratory;
- Standardised electrical/electronic and hydraulics workshops and laboratories;
- Dedicated maritime library;
- Access to international vessels to assist training;
- Multi media training programs at the Submarine Systems Centre at HMAS Stirling on Garden Island.

Species were selected for commercial aquaculture in conjunction with industry; grant funding was secured to undertake the identified applied research; production technology was developed for the selected species; and specialised training courses were developed to transfer technology from the researchers to industry.

The ADU's research and development work involves it with industry and other research groups, including universities, in a range of projects that benefit industry and the community. The ADU has strong cross-sectorial collaborative arrangements and has a history of successful joint aquaculture projects with Murdoch University, the WA Department of Fisheries and the WA Department of Agriculture as well as with private Western Australian companies. Additionally the ADU provides access to its facilities for these agencies in collaborative projects. Several University students have used the ADU facilities for their post-graduate studies, including for PhD studies in fish genetics, nutrition and molecular biology. Co-location of other government agencies is another strategy the ADU has successfully adopted, ensuring that expensive facilities, equipment and other resources are not duplicated. The Department of Fisheries mariculture section is co-located on the ADU premises and the Murdoch University Centre for Fish Research is co-located on the Fremantle campus. The ADU is an excellent example of leading edge research and development being achieved through collaborative partnerships between education and training, other government agencies and the private sector.

Formal feedback from the participants of the ADU short courses and seminars, as well as from the general industry, demonstrates the effectiveness of the ADU. The ADU rapidly developed a reputation for relevance, effectiveness and efficiency among its clientele. This clientele includes school and University students.

The ADU runs as a business unit and is responsible for generating its own operating budget each year to fund its activities on behalf of industry. A Department of Training grant provides funds towards the ADU staffing and Challenger TAFE provides the facility and power costs. The ADU must generate funds for its activities and additional operational costs. These funds are generated through a diverse range of activities including competitive grants, contracts with private companies, fee-for-service training activities and sale of by-products of training such as fish, live feeds, and publications. The need for the ADU to generate substantial funds each year to provide for its activities ensures that it remains closely aligned with the industry and its needs, and operates in an efficient manner. The business unit is recognised in Australia as the leading organisation extending results of marine finfish research to industry through hands-on training. Its close collaboration with industry and joint approaches to research and other projects ensures the highest standards of customer service.

The history for Challenger TAFE's entry into applied aquaculture research could be seen as 'filling the gap' in the existing market. But, as a sector, VET has much to offer in applied research and should be involved in models of this kind, and not only in areas where no-one else has put in a claim. There are sure to be many models, including Centres of Excellence and Special Research Centres where TAFE could play a legitimate role.

The Systems, Capacity, Species and Projects

The ADU have 5 dedicated broodstock holding tanks, two of 40 tonne capacity with phototherm manipulation capability, one of 40 tonne without phototherm capability, and two of 10 tonne capacity, both with photoperiod capability and one with and one without thermal manipulation capability. Other tanks of sizes ranging from 10 to 25 tonne capacity are utilised for occasional broodstock purposes as required.

The ADU utilises three separate larviculture systems of marine fish. The first is comprised of 9 x 5,000L tanks,

the second of 6 x 1,000 L and the third (which is a joint WA Department of Fisheries and ADU unit) of 24 X 100 L tanks. All systems contain cylindroconical fibreglass tanks. The first two systems will be described here. Dr Sagiv Kolkovski of the WA Department of Fisheries will describe the joint larviculture facility and its capacity in a separate paper.

Figure 3. ADU 5 tonne larval tanks.

The ADU has continuous production of algae and rotifers throughout the year, with a capability of producing a maximum of 0.5 billion rotifers per day utilising the current production systems. This quantity of rotifers will support 4 of the 5 tonne larval tanks at any one time.

The ADU operates it's arrays of 5 tonne and 1 tonne larviculture tanks on a semi – intensive green-water basis (See Figure 3). This entails static green-water culture for the rotifer stage followed by flow-through for the *Artemia* feeding stage and beyond.

The ADU operates the hatchery on a high volume flow-through basis. The ADU draws its water supply from the two salt-water bores which are located at 18 metres depth and approximately 10 metres inland from the Bathers Beach side of the South Mole (see Figure 4). This water supply is free of contaminants and sediments as it is substrate-filtered to approximately 10 microns. Despite an intake water pH of approximately 6.8 due to the limestone substrate, the water is of a sufficiently high standard to raise the larval stages of marine fish and abalone.

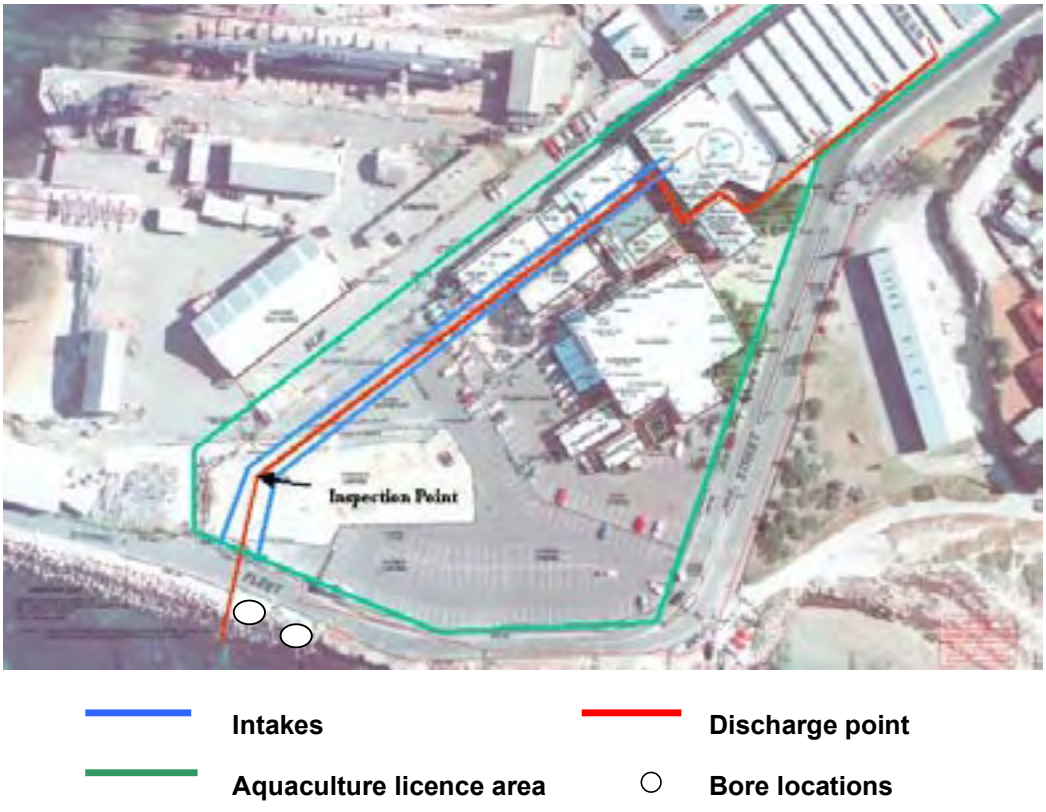

Figure 4. Plan showing location of the ADU, two seawater bores and discharge point at Bathers Beach, Fremantle, WA.

The 5 tonne larval tanks are contained within rooms comprised of black/white 'hydroponics' plastic sheeting (Figure 3). Each of the 5 tonne tanks has lighting above comprised of dual 36 Watt fluorescent (cool white) lights and single 400 Watt Metal Halide lights. The 1 tonne tanks have single 36 Watt fluorescent lights. Lighting systems have an auto-dimmer function. Banjo outflow screens retain the larvae.

The maximum manageable stocking rates in these tanks is 100 newly hatched larvae per litre. This stocking density enables minimal input during the rotifer feeding stages and results in excellent survival. The larviculture techniques are fully described in Partridge et. al. 2003 (See Figure 5).

The production capacity of these tanks is defined by the capability of the ADU staff to maximise the survival rates of the species under culture. Typical survival rates from newly hatched larvae to weaned juveniles are 80-90 % for black bream and 30-55 % for snapper. There have been insufficient opportunities to culture the yellowtail kingfish and therefore survival rates have not been ascertained in these two systems.

During weaning, and as the juveniles grow, additional space is required to maximise survival. Numerous tanks of sizes to 25 tonne capacity (6) are available for this purpose.

The ADU is permitted within its Act to function as a 'pilot' commercial hatchery for a discreet period of time if required by industry. However, one of the limitations of the Fremantle site for commercial production is a restriction placed on the ADU's Department of Environmental Protection licence to hold no more than 5 tonnes of fish at any one time. This certainly limits the facilities capacity as a growout site, but does not limit it's capacity to be a short-term hatchery for a commercial industry with juveniles exiting at 5 grams. This restriction, in combination with two major (or more smaller) larval runs per annum would enable the facility to produce a theoretical maximum of 1.8 million 5 gram juveniles per annum. This capacity however, would require a regular egg supply and an upgraded ADU rotifer production, something that could be achieved relatively easily with the advanced rotifer culture systems currently available.

Research is currently underway or completed at the ADU for a range of species for aquaculture in Western Australia including the following:

Snapper (Pagrus auratus)

The ADU and NSW Fisheries are the pioneers for the culture of snapper in Australia. The ADU recently published the 'Hatchery Manual for the Production of Snapper (*Pagrus auratus*)' for the Australian aquaculture industry. This manual and its accompanying CD-ROM multimedia presentation is the first in Australia to describe the techniques required for the culture of this marine fish species and follows on from the very successful publication in 1999 of the ADU manual for black bream. The project was undertaken in collaboration with

NSW Fisheries and the CRC for Aquaculture.

Black bream (Acanthopagrus butcheri)

The ADU are the recognised Australian experts for the culture and restocking of the estuarine Australian species the black bream. The ADU (in collaboration with Murdoch University and the Molloy Island Residents Group) are currently undertaking a program to restock the Blackwood River Estuary in the South West of WA with black bream. The Blackwood River has a well-documented stock decline of black bream and the project has so far been very successful. Over 200,000 fish have been restocked with very good survival. The fish will be monitored until 2005.

WA Dhufish (Glaucosoma hebraicum)

The ADU pioneered the techniques required to culture this deepwater fish species. Although the WA Dhufish has a single haemoglobin type that restricts its commercial aquaculture potential, the techniques developed are suitable for future restocking projects for the species.

Yellowtail Kingfish (Seriola lalandi)

The yellowtail kingfish is a fast growing marine species that is popular in the eastern states of Australia and

overseas. The ADU has captured specimens of this species and is growing them for brood stock purposes in collaboration with the WA Department of Fisheries.

Mulloway (Argyrosomus japonicus)

The mulloway is a fish that tolerates a wide range of salinities and grows rapidly. This species is targeted by the ADU for inland saline aquaculture for both commercial and recreational opportunities. The ADU has broodstock and is testing various attributes of the juveniles for their commercial culture prospects in inland saline areas.

Roes and Staircase Abalone (Haliotis roei and H scalaris)

These are temperate abalone species with aquaculture potential in the warmer waters of the west coast of Western Australia. Research at the ADU has developed culture techniques for the Staircase abalone and highlights its potential for commercial development.

Projects that the ADU have completed or are currently running include:

Training

Curriculum Development

- Development of a marine prawn culture Unit of Competency under the National Seafood Training Package (2000). Funded by the WA Department of Training's Science and Technology Innovation Strategy, this project developed a best-practice VET training course for marine prawn aquaculture in Australia, an industry worth over $50 million dollars in 2001. The project was also successful in establishing prawn VET training infrastructure in WA (at Kimberley TAFE) and in growing the first marine prawn juveniles in WA with the assistance of VET students (Jenkins 2001).

- Development of a Unit of Competency for the 'Environmental Management of Marine Finfish Growout Operations' under the National Seafood Training Package (2003). Funded by the WA Department of Training's Science and Technology Innovation Strategy; this project developed a VET course for the environmental management of the growout of marine finfish in WA and is supported by the MG Kailis Group of Companies, the Conservation Council of WA and the Esperance Marine Institute. The project involved identifying and documenting environmentally sustainable national and international aquaculture best practices for marine fish farming. The project also established guidelines in conjunction with industry, conservation groups and the community and set the standards for the future development of the industry in Western Australia in an ecologically sustainable manner (Jenkins 2003).

Training Delivery

- Provision of a short course for the culture of temperate marine finfish providing training for industry and research personnel in culture methods for hatchery operations. This is a direct transfer of knowledge and skills from the ADU staff to industry. The one-week course can be delivered on demand as the ADU have spawning fish available throughout the year.

- A Graduate Diploma in Marine Fish Hatchery Management. This course is unique in Australia, as it combines research and coursework at a Graduate Diploma Level AQF VII, in a workplace environment, providing hands-on, practical training in a fully functioning marine hatchery. This course provides students with skills and knowledge required for hands-on managers in the marine hatchery field. Prerequisites for this course are a University Degree in aquaculture or closely related science or a Diploma of Aquaculture with substantial industry experience (or skills recognition).

Industry Training and Extension

- Publication of a Hatchery Manual for production of black bream (Jenkins et al 1999);

- Publication of a 'Hatchery Manual for the Production of Snapper (*Pagrus auratus*) and Black Bream (*Acanthopagrus butcheri*)' for the Australian aquaculture industry. This manual and accompanying CD-ROM multimedia presentation is the first in Australia to describe the techniques required for the culture of this marine fish species and follows on from the very successful publication in 1999 of the ADU Hatchery Manual for Black Bream. The project was undertaken in collaboration with NSW Fisheries and the CRC for Aquaculture with funding support from the FRDC (Partridge et al, 2003). See Figure 5.
- Completion of an AusIndustry project with McRobert Aquaculture Systems (MAS) to develop a novel new tank system for the culture of fish. The company was successful in winning a R&D Start grant from AusIndustry during the latter part of 2000 and subcontracted the ADU to trial the grow-out of snapper in the MAS system. MAS have subsequently constructed a $400,000 system in the USA where significant interest exists in this novel Australian technology.
- The ADU has been undertaking a marketing trial for aquaculture snapper for the hospitality industry over the past 24 months. This project involves growing and selling snapper to a local restaurant chain to test the interest in premium aquaculture fish. These fish have been well received and this trial is to continue into 2003.
- The ADU has several areas available for business start-up opportunities by private companies. There are currently several projects being undertaken by private companies at the ADU including the culture of marine aquarium fish and the trial culture of new abalone species for aquaculture.

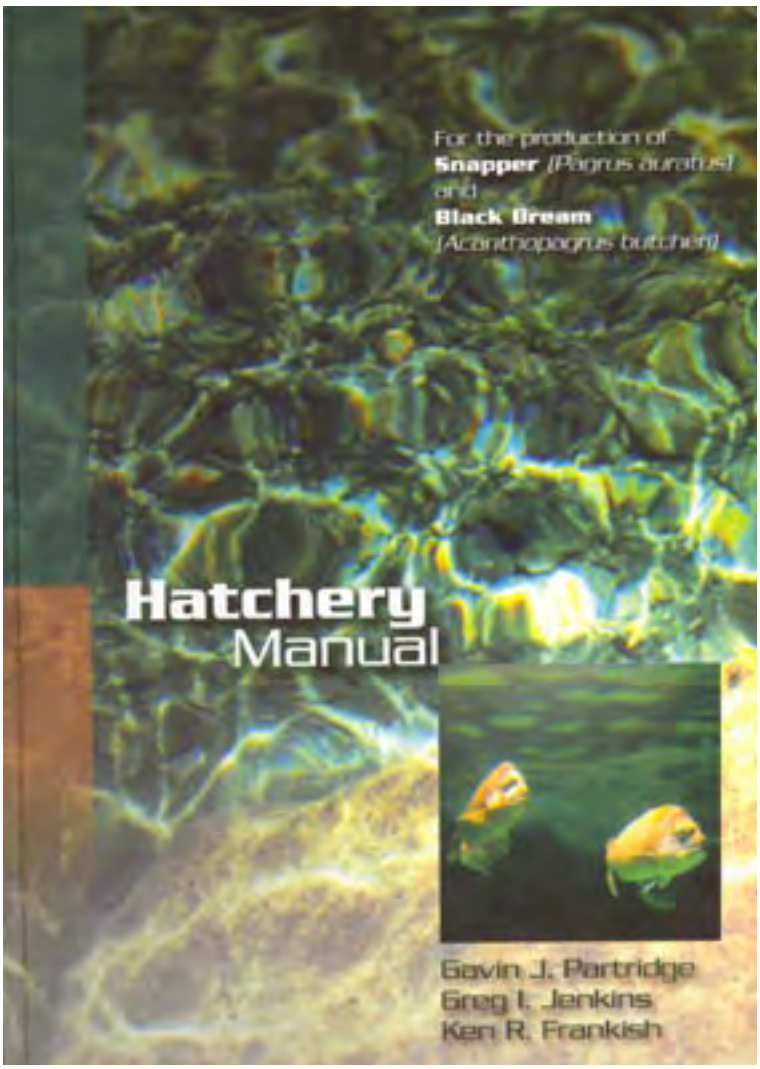

Figure 5. ADU Hatchery Manual 2003

- Numerous user-pay courses and seminars have been run by the ADU in recent years. These included the ADU short course for marine fish culture, a Murray cod aquaculture seminar, hosting a Victorian Fisheries researcher presenting the most recent interstate data for the industry, and two Recirculation Aquaculture Technology Workshops (2000 and 2003) run by Professor Thomas Losordo, a recognised US world expert.
- Key ADU staff were also instrumental during 2002 in the organisation and running of the Marine Fishfarmers Association's 'Sustainable Development of Marine Fishfarming Workshop' that was held on October 29, 2002. This project was related to the ADU's Science and Technology course development project and was successful in securing the world renown conservationist, **Dr David Suzuki** as the key-note speaker at the event. The event was hailed as very successful by industry, government and environmental groups and Dr Suzuki was very complimentary of the aims of the Workshop. (Jenkins 2003). See Figure 6.

herald earthcare

Seeking common ground

EARTHCARE
with ADRIAN KWINTOWSKI

ONE of the world's best-known environmental campaigners, David Suzuki, paid a brief visit to the new maritime museum in Freo last Tuesday to speak at a sustainable fish farming forum.

The event was organised by the marine fish farmers association and the WA conservation council in an effort to nut out parameters for a sustainable commercial fish farming industry.

Organisers also hoped to build trust between the industry and conservation groups.

• David Suzuki reflects on sustainable marine farming in Freo.

Before the talkfest got underway, Dr Suzuki told the *Herald* the concept was a refreshing approach in an industry which, in other parts of the world, had often put profits before environment safeguards.

"I can't emphasise enough how wise this is," Dr Suzuki said. He said in Europe the industry took on a "gold rush mentality" and only became concerned when depleting stocks and increase in disease began to impact upon business.

But he said it wasn't just the business sector that had a duty of care.

"What astounds me is that the consumer is so uncritical," Dr Suzuki said.

WA had a unique opportunity to set up a sustainable marine fish farming culture before mismanagement, spurred by greed, resulted in fish losses in farms and in the wild.

The seasoned environmental campaigner of 40 years says achieving a common ground was better than winning the war with greedy developers.

"In all these battles there's always going to be a loser," he said.

"We can't afford it now. We've got to stop demonising and work together."

His aim is "the development of trust" between the aquaculture industry and conservationists.

Other speakers at the forum included WA fisheries department chief Peter Rogers and Notre Dame University management professor and MG Kailis director George Kailis.

Dr Suzuki dodged questions from the *Herald* on his thoughts about the Ningaloo Reef marina proposal, which last week was deemed inappropriate in its present form by the WA environmental protection authority.

"I don't know what's going on," he replied, stating he could not be an expert on everything.

Fremantle Herald, Saturday November 2, 2002

Figure 6. MFA Sustainable Aquaculture Workshop press

- Direct assistance was provided by the ADU to several local companies in WA during 2003/04, e.g. maintaining barramundi brood-stock for a private company and live abalone export trials from the ADU premises.
- The ADU Manager is Chair of the WA Sustainable Development of Marine Fishfarming Forum; Vice-Chair of the WA Fish Foundation; Secretary/Treasurer of the WA Marine Fish-farming Association; a Board Member of the WA Fisheries Research Advisory Board and the Aquaculture Council of WA and a member of the National Steering Committee for Inland Saline Aquaculture.

Applied Research

- Development of commercial production techniques for the culture of snapper and black bream (Partridge, & Jenkins, 2002 - a&b, Boarder, S. J & Partridge, G. J. in press);
- Development of experimental production techniques for the culture of WA dhufish (funded by the Fisheries Research and Development Corporation - FRDC) (Cleary and Jenkins 2003): and King George whiting (WA Minister of Fisheries funded).
- Growout of snapper in a recirculation system (2001-current). Industry funded; (Partridge - in press)
- Development of spawning and culture techniques for the abalone *Haliotis scalaris*. Funded jointly by the WA Aquaculture Development Council (ADC) and industry;
- Demonstration of nutrient stripping from aquaculture wastewater using the sea-lettuce and feeding to abalone. Funded by the Natural Heritage Trust; (Boarder 1998, 2000).
- Factors required for the successful aquaculture of black bream for recreational fishing in inland water bodies. This study investigated the requirements to grow-out black-bream, King George whiting and snapper in saline ponds at Northam, WA, and was run in collaboration with Murdoch University and a private company. (Sarre et al 2003), (Partridge et al in press).
- Genetic improvement of black bream. (Doupe et al, in press). Mr Doupe is completing his PhD studies from Murdoch University at the ADU facilities.
- Following on from an AusIndustry-funded study tour of inland saline aquaculture projects in the USA, the ADU and partner, CY O'Connor College of TAFE, was successful in securing a grant from the Science and Technology Innovation Strategy Fund to undertake an Inland Saline Aquaculture Demonstration Farm project in Northam. This project has been further supported by Commonwealth funding through the National Aquaculture Council and the FRDC. This project is utilizing new technology (Semi Intensive Floating Tank System – SIFTS)jointly developed by the ADU and a private company. A sharing agreement for the IP rights to this technology have been negotiated. See Figure 7.

Figure 7. Gavin Partridge of the ADU and Dr Gavin Sarre of CY O'Connor College of TAFE at the ISA Demonstration site.

- The ADU scientist, Mr Gavin Partridge won a Commonwealth Agriculture, Forestry and Fisheries Australia (AFFA) young scientist award during the latter part of 2002 associated with this work. A small grant accompanied this prestigious award and Gavin is investigating the suitability for aquaculture of a CALM pumping site near Narrogin. A related project is also being undertaken with the Dumbleyung Shire (Partridge & Furey, 2002). AusIndustry also provided funding to the ADU in 2002 to investigate the relevance of inland saline aquaculture projects in the USA to the WA Wheatbelt.

- The ADU (in collaboration with Murdoch University and the Augusta community) are leading a Fisheries Research and Development Corporation (FRDC) grant to restock the Blackwood River Estuary near Augusta in the South West of WA with black bream (Jenkins et. al. 2000). This three-year project has been extended for a further year by the FRDC due to the outstanding success of the restocking (due for completion in June 2005). The project has undertaken a stock assessment of black bream in the Blackwood River, has restocked 222,000 fish and is currently monitoring the survival and growth of the fish.
- Indications from the WA Dhufish broodstock capture program were that individuals of the species caught in over 20 metres water depth and released do not survive. Subsequent research by the ADU examined the effects of decompression sickness and focused on the immediate damage sustained by the fish upon capture. Since the majority of WA Dhufish caught by recreational and commercial fishermen are captured at depths of greater than 20 metres, this finding has major implications for the management of deepwater commercial and recreational fisheries.

The Highlights of Technology Transfer

To facilitate technology transfer to industry, the ADU has developed two very specific training courses, both of which are the only training programs of their type in Australia. The first is an intensive, marine finfish hatchery-training course. This one-week intensive course provides 'hands-on' training in all aspects of hatchery technology for temperate marine finfish. Student numbers are restricted to eight to ensure maximum benefit of the 'hands-on' nature of the training. The fish species of interest are the black bream (*Acanthopagrus butcheri*) and the pink snapper (*Pagrus auratus)*.

All students receive a CD-ROM copy of the ADU's hatchery manual for snapper and bream prior to arriving at the course to ensure that they have a basic understanding of the process of marine finfish culture. The number of formal lectures are limited to five during the week with the majority of each day spent in the hatchery where techniques for handling and culture of marine fin-fish are undertaken by each student in commercial-scale systems. By the completion of the course each student has gained skills and experience in the set-up and management of an aquaculture hatchery for temperate marine fish.

The second unique course offered by the ADU is the 'Graduate Diploma in Marine finfish Hatchery Management'. The Graduate Diploma has been designed to address specific training needs identified in the finfish industry and is aimed at students holding a Diploma or Bachelors degree in aquaculture or closely related fields. The course aims to turn theory into successful practice to benefit Australia's rapidly growing aquaculture industry. Similar to a medical internship where doctors spend a year in a public hospital, this course can only be effectively taught in a commercial-scale marine finfish hatchery such as at the ADU where VET can be combined with industry scale activities.

The Graduate Diploma is a highly targeted specialist 12 month long course that offers no more than four student places per year with two intakes per annum. On completion, these students are sought after by industry due in part, to the reputation of the ADU for R&D in conjunction with training.

The Problems of Technology Transfer

The ADU has undertaken many important projects to implement the research and development component of its charter. Advances are irrelevant however, if the new technology is not recognised by and integrated into the industry.

The successful transfer of technology from researchers to industry is a key component of industry development. Impediments to successful technology transfer from researchers to industry include:

- inadequate communication between government agencies and industry to develop effective extension services to the industry, ie to determine what form of training the industry requires;
- the focus of many researchers and agencies on the publication of papers and the consequent lack of experience in more direct technology transfer to industry; and

- the lack of recognition of expertise held in government or quasi-government agencies. This is linked with both the low regard of government employees held by some industry sectors and the often inaccurate perception, that the best expertise is always to be found interstate or overseas.

Collaboration and cooperation, rather than competition, between government agencies and between government and industry are required to ensure that effective technology transfer occurs. Collaboration between TAFE and other agencies is a very effective method of technology transfer to industry. TAFE is a traditional training provider in Australia and has strong links with industry. Challenger TAFE in Fremantle, Kimberley College of TAFE in Broome and Central West TAFE in Geraldton have extended marine aquaculture facilities. This enables effective collaborative applied aquaculture research to be undertaken in these locations. Other TAFE Colleges in WA have forged links with research organisations in order to act as conduits for up-to-date aquaculture technology transfer.

The ADU has developed collaborative research arrangements with Fisheries WA, NSW Fisheries, Agriculture WA, three WA Universities, the National Centre for Mariculture in Israel and numerous private companies. This collaboration has led to the development of synergies that have fast-tracked the applied research progress.

The ADU has also established effective working relationships with the Conservation Council of WA, and is working hand-in-hand to ensure sustainable practices are encouraged and that suitable training programs are available for the industry and the community.

While collaboration and cooperation are important, trust is the key value that is required between government and government and government and industry proponents to develop a new industry. Without trust then it is likely that working relationships will break down and the various proponents and service providers will become frustrated as the development process slows.

Summary

The ADU is a unique ‘experiment’ in the VET field: a successful combination of applied research, development, extension and VET training for the WA industry. The ground-breaking work of the ADU has been recognised by the industry and by government in recent years:

- In 1997 the ADU was awarded the Premiers Award in the category "Provision for the Future' for its restocking vision in WA;
- In 1999, the ADU was presented with the “Innovation and Development Award for the Community and Industry" as part of the Fisheries Reward and Recognition Program and for “….leadership in practical development of aquaculture species for the Australian market.”;
- In 2002, the ADU was a finalist in the WA Premiers Award for 'Environmental Sustainability';
- In 2003, Gavin Partridge, the Aquaculture Development Unit, Challenger TAFE aquaculture scientist, was presented with a Rural Industries Research and Development Corporation 'Science and Innovation Award' from the Department of Agriculture, Fisheries and Forestry – Australia (AFFA). Senator Hon. Ian Macdonald, Minister for Forestry and Conservation, presented the award to Gavin Partridge; and
- The recognition of the TAFE sectors role in Inland Saline Aquaculture Development with the FRDC/NAC funding of the ISA Demonstration Farm in Northam, WA.

The ADU enjoys the wide support and involvement from both the private sector and Government because it provides a focused and targeted approach to applied research and development that, with the direct involvement of industry, is delivering solutions and innovations that have real commercial potential in Western Australia. The fact that the ADU must generate all of its own expenditure ensures it operates in an extremely prudent manner financially and operationally with a keen focus on the future of the industry.

The key to the success of the ADU has been both the focus on the R&D needs of the industry and its associated training needs. The ongoing success of the ADU will continue to be determined by the industry with external funding becoming more important for its continuing operation.

The economic and employment potential of aquaculture in Western Australia is enormous and the ADU, through its training and research programs, is helping establish this emerging and new industry in WA. The future benefit to the aquaculture industry and the Western Australian economy will far out-weigh the investment in the ADU, making it both cost effective and value for money.

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Department of Fisheries
Fish for the future

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General

The Mariculture Research and Advisory Group (MRAG) is located at the maritime centre run by Challenger TAFE at Fremantle port. Both agencies, TAFE and the Department of Fisheries (DoFWA), have an agreement where many of the R&D activities are on a collaborative basis.

The MRAG includes three major activity centres: finfish, nutrition and abalone. The nutrition activity mainly involves fishmeal replacement (lupin) and environmental impacts of feeds and feeding. The abalone research focuses on improving hatchery and nursery production, and has two main objectives:

- To improve spawning success and 'seed' (settled larvae) performance when conditioned abalone broodstock are used
- To evaluate alternative feeds for the nursery phase when the abalone are between 5 and 15 mm in length.

The finfish group has three main areas of R&D:

1. Systems design and development,
2. *Artemia* replacements, i.e. microdiets development, co-feeding and weaning protocols, and
3. *Artemia* production.

The MRAG activities are strongly linked to industry and all R&D projects are jointly funded with industry.

Finfish Section

Systems

The group has strong technical background that helps with the design of rearing systems and associated equipment.

Larvae system

An experimental larval rearing system was developed to reduce variability amongst tanks (due to manual feeding and other parameters) and enhance control of environmental parameters while reducing the workload. The system includes twenty-four 270 l conical tanks with the option of either an upwelling or bottom-draining flowthrough water delivery system. The inlet water passes through a gas exchange column that saturates the water with dissolved oxygen and stabilizes the pH. The system

was originally designed for nutritional experiments using formulated feeds. The use of an upwelling water inlet method extends the suspension time of inert particles in the water column and also helps to suspend very small or passive swimming larvae. However, when the system is used to grow-on larvae or juvenile fish, it can easily be switched to bottom draining to provide self-cleaning water dynamics for high organic loads.

Although the system was built as an experimental system with relatively small volume tanks (270 l), it can easily produce commercial numbers of fingerlings and can be 'up-scaled' to increase production.

Outlet filter

A unique outlet filter was developed that eases the daily routine of replacing screens when enriched live food is used. This filter can be exchanged with a screened standpipe and outlet surface skimmer when the bottom draining flow characteristics are engaged.

Automatic Control

The system is fully controlled by a single programmable logic controller (PLC). The PLC controls the light intensity, photoperiod, dimming time, live food and algae pumping intervals, substantially reducing labour requirements.

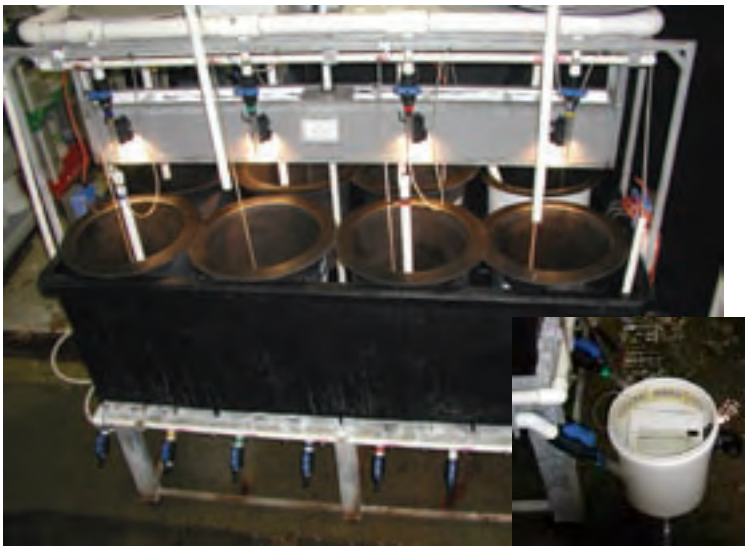
Automatic Feeding System

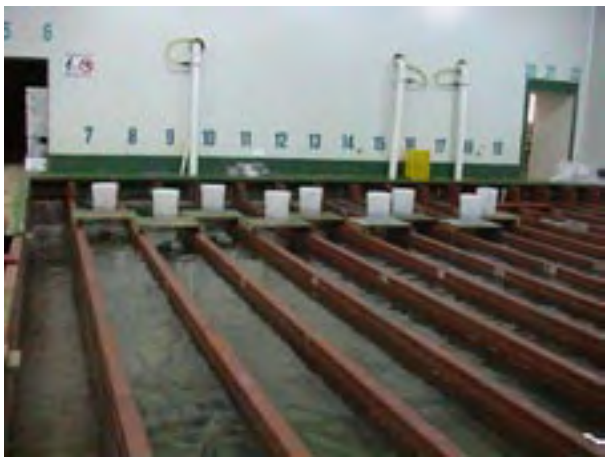
Automated Microdiet Dispensers (AMD) are installed in a 24 tank experimental system in order to feed up to 8 different experimental or commercial micro diets (MDs) for larval weaning and/or nutrition experiments. The feeders can be operated individually or simultaneously via multiple or single outputs from the Programmable Logic Controller (PLC). Specific PLC output programs are directed to operate particular AMDs within each feeding regime, via a network of 8 input – single output rotary switches. This then connects a 12 V DC 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.

The AMD is designed to periodically administer a small amount of microdiet to larvae culture tanks, in order to spread the allocation of the required daily amount of feed evenly across the whole day. The feeder can cope with a diet particle size range of 150 microns to 1.5 mm.

Live Food system

A simple, compact experimental system was developed in order to provide a reliable platform for nutritional, growth and other experiments involving live food organisms. The system was built as a compact, all-in-one system with eight 50 l conical tanks in a water bath. The system reduces variation between the replicates (tanks) resulting from individual heaters and aeration. It reduces the manpower time through simple procedures for harvesting, washing and refilling all of the tanks synchronously and allows automated addition of enrichments. The system has been





used for a variety of experiments, such as comparing commercial and experimental enrichments, bacterial monitoring and evaluation of different *Artemia* procedures. Enrichments can be added at predetermined times via an automated dosing system.

Other equipment and systems includes an automatic feeder for grow out fish (large particles, crumbled and/or extruded) using a PLC controller, a filter for *Artemia* ponds cysts, nauplii and adult *Artemia*, and large-scale *Artemia* harvesting devices.

Artemia replacements

Microdiets

Microdiet development comprises all the aspects involved in microdiet utilisation by the larvae, such as physical and chemical properties of particles including leaching and sinking rates, diet technology – microbound and microencapsulation methods, protein sources based on digestion index of the protein, and digestive system and enzyme development of the larvae.

Rearing and weaning protocols

Development of co-feeding and weaning protocols is aimed at reducing the dependency and use of *Artemia*. A variety of microdiets, which are commercially available in Australia, as well as experimental ones, are being tested and compared. Algae, *Nanochloropsis* or algae paste (*Chlorella*) are being used in a 'green water' method. The algae paste was found to be superior to the live algae used in the past both for rotifer enrichment and green water in the larvae tank. Some of the trials are done in collaboration with other R&D centres (Darwin Aquaculture Centre, NT) and commercial companies.

Artemia

Artemia enrichments

Hygiene of *Artemia* and *Artemia* processing (hatching, enriching, harvesting and growout) are being investigated. Commercial products as well as experimental ones are investigated in terms of bacteria loads and levels during the process.

As part of an FRDC project, 'tailor-made' enrichments and broodstock additives are produced for R&D institutes, commercial hatcheries and public aquariums. Lipids, essential fatty acids, vitamins, immune-stimulants and any other nutrient can be manipulated and adjusted to match required levels and ratios and be added to a stable, oil-based emulation. The 'tailor-made' products are produced in collaboration with a private company (Nutra-Kol, Western Australia).

Intensive culture

An experimental system that includes 18 x 165 l heated tanks has been used for the *Artemia* intensive culture. The system can be operated on a static or semi flow-through basis. The system is currently being used to compare inexpensive, locally available, inert feeds as well as algae and algae products (pastes and wastes from algae extraction). A series of experiments will be used to further determine the types and effects of bacteria associated with culture techniques (static or semi flow-through culture)

and feeds (micro-alga, inert diets). With current prices as high as AU$ 600 per kg of live *Artemia,* it is invisaged that these experiments and data collected from them will soon be applied to develop a commercial intensive system.

Large scale Artemia production

As part of an FRDC project, an initial assessment of commercial production of *Artemia* cysts was carried out in Western Australia. Hut Lagoon at Port Gregory, north of Geraldton in WA, is a natural saline lake where the microalga *Dunaliella salina* grows naturally. At high salinity the algae starts to produce beta-carotene. Besides *Dunaliella, Artemia* also grows in the lake. The combination of high salinity and an abundance of food is an ideal situation for *Artemia* production.

During the last two years the Department of Fisheries, WA and Cognis Australia, a company based at Port Gregory which harvests and processes the *Dunaliella* for the food and the pharmaceutical industry, have commenced an assessment of the potential for commercial scale production of *Artemia* cysts.

A pilot scale grow out experiment was carried out using six 22 m^3 plastic-lined ponds. A specific 'in-line' filtration system was developed to separate adult *Artemia*, nauplii and cysts for population control and cyst harvesting. *Dunalliela* harvested from the main algae ponds was used as a food source for the *Artemia*.

During the next three years, DoFWA together with Cognis will continue to develop the commercial production of *Artemia* cysts and biomass as part of a new FRDC project.

Fish species

The larvae system at Challenger TAFE can supply marine bore water at temperatures ranging between 19°C and 28°C using a heater-chiller to heat the water (ambient temperature is 20°C ± 1°C). Therefore, a variety of species from temperate to tropical can be reared. The main species that the group is working with includes, snapper *Pagrus auratus*, yellowtail kingfish *Seriola lalandi* and barramundi *Lates calcarifer*. Coral trout is now under investigation as a new species together with a private company.

Other marine organisms such as western rock lobster *Panulirus Cygnus* and shovel nose lobster are also under investigation. Both larvae stages (mainly nutritional requirements) and juveniles (grow-out) are being looked at. A black tiger prawn (P*enaeus monodon*) farming project is currently conducted using local broodstock in the north of WA (G. Maguire PI).

R&D Collaboration and Links

The marine finfish group at MRAG is strongly linked with industry partners. Currently, R&D collaboration is established through a joint project with Cognis Australia. The group is advising to other commercial companies such as M. G. Kailis and Marine Farms (mahi-mahi farm), and western rock lobster fishing/holding companies.

The group also established research collaborations and links with many of the research centres in Australia including, Darwin Aquaculture Centre, NT, TAFI, Tas, and James Cook University, Qld. Research links through current projects have also been established with overseas centres in Spain, Portugal, Mexico, Japan New Zealand and Malaysia.

Abalone Section

Improving spawning success and seed performance

Successful conditioning and spawning of farm-grown abalone broodstock are crucial for a selective breeding program in Australia. However, large variability in spawning success, hatchability of the eggs and survival of the larvae and juveniles has been observed between batches and hatcheries. It has been reported that farm-grown female abalone, that were fed with commercial formulated feed, spawn less readily and produce eggs of poorer quality than animals collected from the wild. Formulated diets used successfully for growout are not always an adequate diet to maintain captive broodstock that yields viable, high quality eggs and larvae. Our preliminary results indicated that eggs spawned from wild caught females differ from conditioned females that were fed formulated diets for several years, in their fatty acid composition. Eggs derived from wild caught broodstock feeding on mainly red seaweeds showed about twice as much arachidonic acid compared to eggs from conditioned broodstock, which are feeding on formulated feed low in arachidonic acid. Arachidonic acid is a major precursor of prostaglandins, which influence reproduction in molluscs. The question of whether arachidonic acid is essential to abalone reproduction needs to be examined. A broodstock conditioning trial is now underway with wild-caught greenlip abalone (*Haliotis laevigata*) to determine if low levels of a particular fatty acid (arachidonic acid- ARA) affect abalone reproduction. Two levels of ARA enrichment are being tested and compared to a formulated diet without the enrichment (negative control), and a diet of red seaweeds (positive control). All animals were spawned out and induced to spawn again after a conditioning period of 16 weeks. Egg size and colour, fatty acid composition of unfertilised eggs, fertilisation and hatch rate, larval survival, settlement success and post-larval survival are being determined and compared between batches (1 Female x 1 Male) using replicated groups of abalone.

New microalgae suitable as a food source for juvenile abalone

Currently commercial abalone nurseries rely on diatoms as a food source for post larvae and juveniles. When juveniles reach 5 mm in shell length, the volume or composition of the food on the nursery plate becomes inadequate. One new diatom species (*Cocconeis* sp.) has been isolated and maintained in culture. This isolate is about 3-4 times larger than other species previously kept in culture. Alternatively, and in contrast to *Cocconeis*, chain-forming diatoms offer a 3D structure compared to the 2D structure of non-chain forming, prostrate attaching species. This 3D structure may provide a more continuous food source for the juveniles through grazing of the top cells of the chain leaving the remainder of the chain to continue dividing. Only one chain forming species, *Delphineis* sp., showed ability to attach to substrate and only this species was tested further. Scale-up cultures have been successful and feeding trials are conducted comparing the growth of juvenile *H. laevigata* (4 mm in shell length) when feeding on new and old isolates.

New macroalgae suitable as a food source for juvenile abalone

Feeding macroalgal germlings, which can grow on the nursery plates, can provide more biomass and may be an alternative feed for the later stages of the nursery phase. We developed methods for the spore release, attachment and germination of the green alga *Ulva* sp. Ulva germlings are now successfully grown on PVC nursery plates. Feeding trials indicated that *Ulva* sp. germlings might be a suitable and practical additional food source for advanced juveniles in a commercial nursery. Further experiments are planned to investigate the potential of other macroalgal germlings (eg red seaweeds) as potential food sources.

Tropical molluscs

Enhancement of trochus fisheries project is on-going in the north of WA (Broome) using hatchery stock. A small-scale production of juvenile tropical abalone (*H. asinna*) is also carried out.

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Species cultured

Snapper	*Pagrus auratus*
Mulloway	*Agyrosomus japonicus*
King George whiting	*Sillaginodes punctata*
Yellowtail Kingfish	*Seriola lalandi*

Systems

SARDI R&D Hatchery facilities are located at the South Australian Aquatic Science Centre (SAASC), West Beach, SA. This building is 29 x 16m with 4.5m high work areas constructed of 75mm –100mm thick metal clad polystyrene panels within an external frame and roof. R&D and culture systems available include broodstock egg incubation, larval rearing, microalgae and live feed production areas together with an office and laboratory.

Figure 1. SARDI R&D Hatchery at the South Australian Aquatic Science Centre, West Beach

Broodstock

Finfish broodstock holding systems used to support SARDI R&D Hatchery operations include;

1. Outdoor ambient tanks (5 x 40,000L) to provide eggs during the natural spawning season.
2. Indoor tanks (4 x 10,000L and 2 x 22,000L, Figures 1a and 1b) are located within an insulated controlled

environment room to provide eggs at alternated spawning times during the year (Figure 1a and 1b). These broodstock tanks are supplied by recirculated seawater treatment systems that incorporate mechanical and biological filtration, foam fractionation and control of water temperature and light cycle control.

Figure 2 a) Four 10,000L broodstock holding tanks in a controlled environment room within the R&D Hatchery facility at the SAASC. b) Side view of the broodstock tanks, 2 x 10,000L front and centre and a 22,000L tank at rear.

Egg incubation

Eggs collected from tank spawning or manual stripping of broodstock are incubated in a room housing 8 x 220L tanks. Each tank is supplied with 5µm filtered seawater and aeration and has a central 300um screen with internal standpipe. Eggs are stocked at up to 1500 per litre and maintained until larvae can be assessed for quality as determined by hatch rate. Selected batches of larvae are transferred into larval rearing tanks in another room.

Figure 3. Eight 220L egg incubation tanks adjacent to the broodstock room in the SARDI R&D Hatchery.

Larval rearing system

Larval rearing facilities in the SARDI R&D Hatchery are installed as a system of 12 x 1800L fibreglass tanks (Figure 3) supported by 3 recirculated seawater treatments systems (Figure 4). These can be operated as separate systems each supplying 4 tanks, or joined together to provide common water to all tanks. Water treatment systems provide mechanical filtration and biological filtration, with additional foam fractionation and disinfection using ultraviolet irradiation on side streamed plumbing lines. Tanks have been allocated at random to each water treatment system and plumbed accordingly for water supply and return. All tanks are provided with independently switched overhead lighting.

Figure 4. Larval rearing tanks (12 x 1800L) in the SARDI R&D Hatchery.

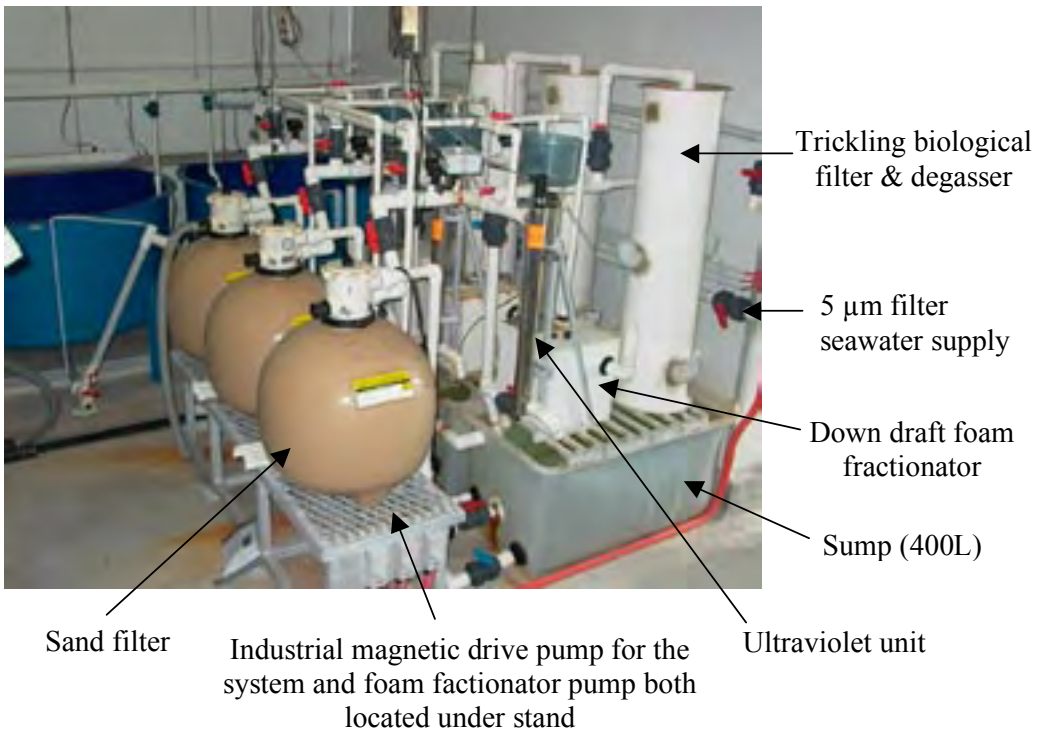

Figure 5. Components of the recirculated seawater treatment systems supplying the experimental larval rearing tanks in the SARDI R&D Hatchery.

Live foods used

Rotifers and *Artemia* are the only live prey organisms cultured for marine finfish larval rearing research or production in the SARDI R&D Hatchery. Generally only large L-strain rotifers (*Brachionus plicatilis*) are cultured, although small S-strain rotifers (*Brachionus rotundiformis*) have been used when culturing King George whiting. Rotifers are cultured at 24 – 28°C in 3 x 600L conical bottomed fibreglass tanks with heating and aeration, and an additional 3 x 2,000L polyethylene tubs if required (Figure 5). Rotifer production capacity can be increased to 200 million per day, but is typically in the order of 50 – 100 million per day during normal operations with cultures maintained at 200 – 400 rotifers/ml. During larval rearing rotifers are fed microalgae once per day with 2-3 additional feeds of yeast (*Saccaromyces cerevisae*) based upon the rate that feeds are being cleared from culture tanks. Future improvements to rotifer cultures that may be considered include use of specific rotifer culture diets, feed dosing systems and use of microalgal concentrates.

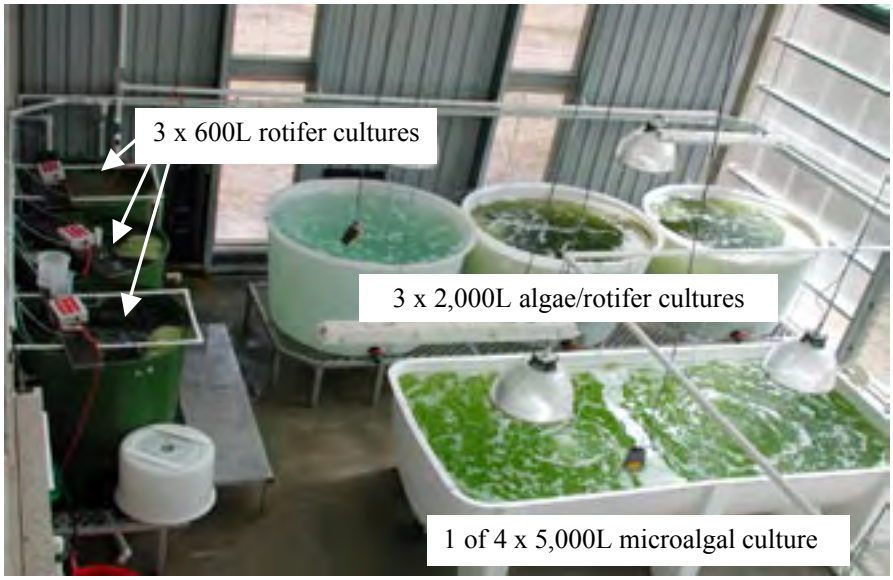

Figure 6. Rotifer and mass microalgal production tanks in the SARDI R&D Hatchery.

Artemia

Artemia production facilities consist of an insulated room adjacent to the larval rearing area that can be maintained at 30°C. Two rows of 3 x 240L white conical bottom tanks are used to hatch *Artemia* cysts at densities up to 2g/L. Each tank has an overhead light with separate switching (Figure 6). Two 450L tanks are used to provide extra capacity during overnight enrichment. When more than 1.5kg of cysts/day need to be hatched the rotifer culture tanks are used to provide capacity of up to 8.0kg/day. All seawater is chlorinated and dechlorinated before use and all cysts are decapsulated before addition to hatching tanks. Hatch controller (INVE) is used to minimise bacterial build up during hatching. Enrichment products used for *Artemia* include DC DHA Selco and DC Super Selco (INVE), although a range of other enrichment products are available for consideration.

Figure 7. Artemia room in SARDI R&D Hatchery.

The live feed use schedule for each species cultured (Figure 7) is varied according to the growth rate of the fish larvae and their ability to ingest and utilise different sizes of live feeds. Major influences on the outcome of hatchery production have mostly been attributed to the time that different size and quality of inert feed is introduced to the larvae.

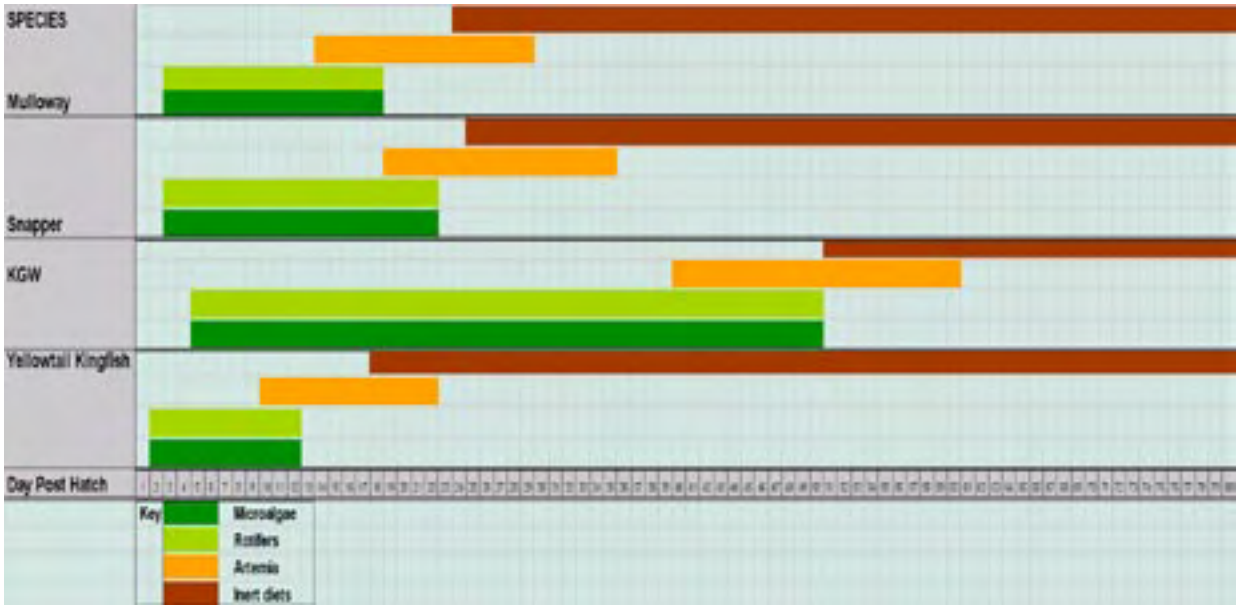

Figure 8. Standard feeding schedules used at the SARDI R&D Hatchery for different species of finfish larvae cultured.

Production capacity

The SARDI R&D Hatchery was designed to be able to produce batches of up to 100,000 weaned fingerlings for pilot scale stocking of commercial ongrowing systems. In the past, clients have been supplied with batches of up to 40,000 snapper and 440,000 mulloway fingerlings for R&D purposes, although in-house R&D has involved much smaller batches. This required temporary installation of extra seawater supply and oxygenation of 5,000L microalgal mass culture tanks that were converted for use as extra nursery tanks (Figure 8).

Inert foods

SARDI has not pursued larval nutrition research as a number of international feed companies (e.g. Kyowa feeds, Nippai ML range; Higashimaru – Minami; Nutreco – Gemma; INVE – Proton, Lansy and NRD; Dana – Larviva Start, Wean-Ex and Dan-Ex; Ewos – Promal and AgloNorse; Salt Creek – Progression) have dedicated research teams working on this area. These efforts are aligned to either large marine finfish culture industries for species such as European sea bream (*Sparus auratus*), sea bass (*Dicentrachus labrax*) or developing industries such as Atlantic cod (*Gadus morhua*). The expansion in weaning, Atremia replacement and nursery culture diets that are now available to finfish aquaculture suggests that Australian hatcheries can now take advantage of improvements in the production and application of these products in their facilities. It is expected that small variations in the timing for use and selection of product will be required as these are applied to the culture of Australian species (i.e. barramundi, snapper, yellowtail kingfish and mulloway). Typical inert feeds that have been used for culture of finfish species in the SARDI R&D Hatchery include (in order of use):

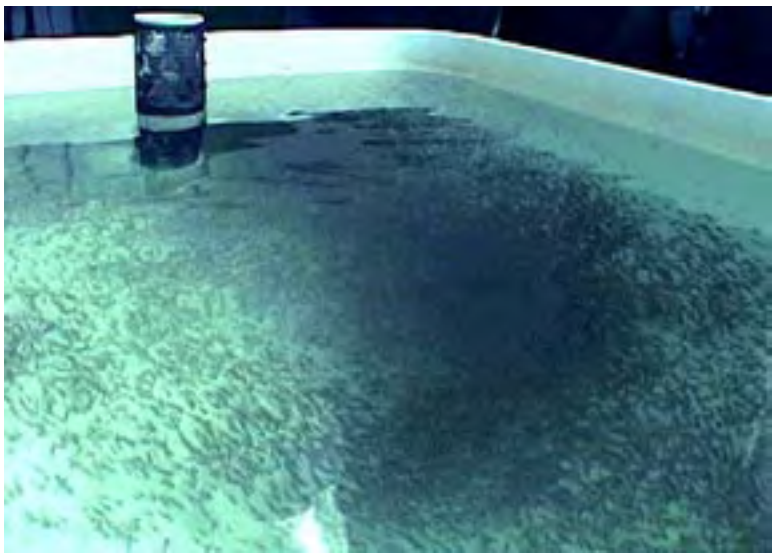

Figure 9. Typical density of weaned 1-2g mulloway fingerlings in 5,000L nursery tanks in the SARDI R&D Hatchery.

King George whiting	Proton 2, ML 300 Proton 3, ML 400 NRD 4/6 NRD 5/8, ML 800
Mulloway	Proton 4 (300 – 500 µm) NRD (500 – 800 µm) EPAC 800/1200
Snapper	Gemma Micro 150 Gemma Micro 300, Proton 3 (200/400), Lansy W3 Proton 4 (300/500 µm), Gemma 0.3 NRD (500 – 800 µm), Gemma 0.5, Gemma 0.75 EPAC 800/1200, Gemma 1.0, Gemma 1.2
Yellowtail kingfish	Proton 2 Proton 3 NRD 4/6 NRD 5/8 EPAC 800/1200

Microalgae

A range of microalgal stock cultures are maintained at the SAASC to support bivalve and finfish aquaculture research. Species used for marine finfish culture are restricted to *Nanochloropsis oculata*, *Pavlova lutheri*, *Isochrysis* Tahitian strain T. Iso and *Tetraselmis suecica.* Stock cultures are used to inoculate 250ml working cultures that are scaled up to aseptic 3L then 16L cultures in 20L polycarbonate carboys. Carboys are used to start 400L bags in a continuous harvest system of 10 bags (Figure 9). Alternatively carboys are transferred to the R&D Hatchery where they are used to start 2,000L (1 carboy per tank) or 5,000L (2-3 carboys) open

mass cultures. Mass microalgae culture facilities comprise 3 x 2,000L polyethylene tanks and 4 x 5,000L rectangular fibreglass tanks with parabolic ends. Open mass cultures and bag cultures are supplied with a carbon dioxide enriched air supply to increase cell density and maintain stable pH.

Microalgae are used in finfish culture operations for rotifer feeding (*N. oculata*, *T. suecica* and T. Iso), rotifer enrichment (T. Iso and *P. lutheri*) and to provide green/brown water (*N. oculata* T. Iso and *P. lutheri*) during the rotifer feeding phase of larval rearing.

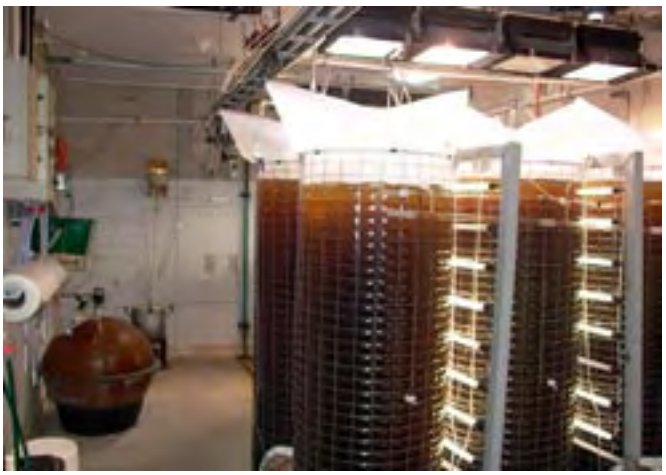

Figure 10. Continuous microalgal production system (10 x 400L bags) at SAASC.

Hygiene

A number of steps are followed to manage the level of micro-organisms during larval rearing operations. These include;

- Culture vessels and fluids that are cleaned and disinfected before use. Algal culture tanks are thoroughly cleaned, refilled with 5um filtered seawater then chlorinated and dechlorinated prior to inoculation.
- The use of batch production methods for rotifers and microalgae so that these live feeds do not accumulate high levels of micro-organisms over long periods. Each rotifer culture is used over 1-3 days depending on demand, then thoroughly rinsed and a proportion used to restart a new culture in a clean tank. Microalgal cultures are used completely over 1-3 days while in the exponential growth phase. Cultures are not restarted from old cultures, rather, new aseptic carboys are used to start each mass culture.
- Thorough rinsing of all rotifer and Artemia additions to larval rearing tanks.
- Daily siphoning of all larval rearing tanks and repeated spot siphoning as required.
- Recirculated seawater is UV irradiated within the water treatment system that supports each larval rearing tank.

R&D Activities

Initial marine finfish research undertaken by SARDI from 1992 – 1996 was done in conjunction with a group of regional small business people in the Whyalla and Port Augusta region. Research was conducted to assess the suitability of snapper for aquaculture in the hypersaline Upper Spencer Gulf. In addition to salinity trials SARDI provided small batches of up to 10,000 snapper fingerlings and assisted with grow-out assessments and transfer of hatchery technology required to provide the confidence needed for local investors to commit to a commercial hatchery at Port Augusta.

SARDI completed an FRDC funded project to evaluate the aquaculture potential of King George whiting during the period that the R&D Hatchery was under construction and for a period of 2 years after completion. This project identified a number of issues related to egg supply and quality, and difficulties with larval rearing. It was concluded that although King George whiting have an excellent market price and their propagation and rearing is possible, commercialisation of this species would require a significant commitment to further research upported and there would still remain an issue with the slow growth of this species to market size.

Mulloway aquaculture development in South Australia was initiated using the SARDI R&D Hatchery facilities in a project conduct for industry. Since the production of over 400,000 fingerlings by SARDI in 2000/01 the industry partner has been provided broodstock and conducted successful hatchery production of up to 750,000 mulloway fingerlings for stocking in commercial seacages at Arno Bay. In 2003 a batch of 20,000 mulloway was produced to stock inland saline aquaculture R&D systems at Cooke Plains.

Recent research has been confined to trials on *Artemia* replacement during snapper production as part of an Aquafin CRC project with NSW Fisheries. The R&D Hatchery also provides ongoing support for research conducted by Aquafin CRC PhD student Bennan Chen who is conducting research on the development of the digestive system in King George whiting and yellowtail kingfish.

Specific problems and issues

The most significant hatchery related issue in South Australia remains the amount of deformities that occur during culture of yellowtail kingfish, although the degree and significance of deformities varies between years and batches of fingerlings. This research problem may be complex but an understanding of the factors that contribute is constrained by the willingness of the commercial hatcheries to share information and work with researchers. While fingerling requirements from commercial hatcheries remains moderate (200,000 – 500,000 pa) it is expected that excess production in the early stages can largely compensate for the percentage of deformed fish discarded at the nursery stage prior to transfer into sea cages.

Both SA commercial hatcheries are continually varying different elements of hatchery operations (i.e. physical conditions, nutritional enhancement through enrichments and testing of modern weaning and *Artemia* replacement diets, etc). These ongoing evaluations provide incremental improvements to production through improved quality of fingerlings and cost reduction within the hatchery.

Mulloway can be produced in numbers in excess of grow-out capacity and the survival and performance of this species does not support any further investment of Hatchery R&D effort. Commercial snapper aquaculture in South Australia has ceased due to the developments that have occurred with yellowtail kingfish and mulloway, which are both faster growing.

Collaborations

Aquafin CRC, marine finfish companies in South Australia, the SA Marine Finfish Farmers Association and other National marine finfish researchers including, Dr S Fielder (NSW Fisheries) and Dr S Battaglene (TAFI).

Tasmanian Aquaculture and Fisheries Institute

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Species cultured

Fish: striped trumpeter (*Latris lineata*), pot-bellied seahorse (*Hippocampus abdominalis*), spotted pipefish (*Stigmatopora argus*), Barbour's seahorse (*Hippocampus barbouri*), White's seahorse (*Hippocampus whitei*), greenback flounder (*Rhombosolea tapirina*), damselfish (*Acanthochromis polyacanthus*), Tasmanian whitebait (*Lovettia sealii*), galaxids (*Galaxias maculatus*), blenny (*Parablennius tasmanianus*), black bream (*Acanthopagrus butcheri*)

Crustaceans: southern rock lobster (*Jasus edwardsii*).

Shellfish: Pacific oyster (*Crassostrea gigas*), blue mussel (*Mytilus edulis*).

Squid: southern dumpling squid *Euprymna tasmanica*, Pygmy squid *Idiosetius sp.*

Systems

Striped trumpeter eggs are incubated at 14 ºC in 250 L upwelling tanks. Yolk-sac larvae are reared at 5 to 50 per L. A temperature and photoperiod controlled system of 24 replicate 300 L black hemispherical tanks is available for experimentation at MRL. Each 300 L tank has a central screened outlet and is typically supplied with recirculating seawater and ozonated flow-through seawater. Production runs are conducted in 3000 L black conical-bottomed tanks using downwelling and upwelling at different stages in the production cycle. Early culture is better in greenwater (*Nannochloropsis* sp.) usually to at least D 20. Post-larvae are reared in clearwater and are weaned using automated feeders and modified light background environments.

Seahorse broodstock are held in 1 m^3 tanks in which courtship, egg transfer to the males, egg incubation and juvenile release occur. Juveniles are removed and reared in smaller 60 L conical based cylindrical tanks supported by recirculation systems and light- (12:12 LD) and temperature-control (18ºC). Experiments are undertaken in 18 x 3 L, 20 x 25 L or 12 x 60 L tank systems. Juveniles are fed enriched *Artemia* instar II metanauplii until they are weaned onto frozen mysids or amphipods.

Phyllosoma larvae of southern rock lobsters are cultured in 10 L circular tanks at 20-40 per L. They are reared at 18°C, at a photoperiod of 12 h light: 12 h dark with the light phase of low intensity to minimise stress. The culture water is flow-through which has been filtered to 1 µm and disinfected with ultraviolet irradiation. The culture tanks have four jets positioned near the base at the outer perimeter and another two jets at the base near a central cylinder to achieve circular, steady flow. A screen is fitted to the wall of each tank to allow escape of water while retaining live *Artemia* in the tank.

Shellfish broodstock conditioning is carried out in a 270 L recirculating system. Experimental larval runs are conducted in triplicate 200 L static tanks at around 10 per mL (decreasing to 1-2 per mL at set), with aeration and water changes at approx 2 day intervals. Around day 14, larvae are either set chemically and transferred to a 160 L recirculating spat upweller system, or set onto billets and on-grown for 2-3 weeks prior to movement to a farm-based nursery.

Production capacity

Reliable supplies of broodstock striped trumpeter, seahorses and southern rock lobsters are available for research in Tasmania. At MRL over 100 striped trumpeter broodstock are held in five 25 000 L tanks on photoperiod and temperature control to provide a consistent source of eggs from February to June and August to December. One 4000 L tank of greenback flounder broodstock provide eggs during the ambient season from June to September. F1 generation broodstock striped trumpeter are available and F3 generation greenback flounder. Broodstock are fed moist pellets incorporating commercially available ingredients, fresh fish and squid and vitamin supplements. At SOA around 50-100 seahorse F2-F3 broodstock are held in two 1 m^3 tanks. Although these fish spawn year-round, the SOA also acquires significant numbers of juveniles from Seahorse World at Beauty Point for replicated research trials. Broodstock are fed frozen (and live) mysids and amphipods.

Ovigerous female lobsters naturally hatch their phyllosoma larvae in late September for a period of 6-8 weeks. At MRL, wild-caught broodstock are also held at photoperiods and temperatures to hatch out-of-season, ensuring the production of larvae for up to 8 months each year (April-November). The lobster diet consists of mussels, squid and Kuruma prawn pellets, with each component fed on rotation on separate days of the week. They are housed in 12 x 4000 L tanks with concrete shelters and supplied with filtered, recirculated water.

Conditioned Pacific oyster broodstock are currently maintained from around August to February. Several small larval rearing runs of oysters (1-10 million larvae per run) and/or blue mussels are carried out during this period to support research projects and molluscan biology teaching.

Live foods used

Rotifers, L-strain rotifers *Brachionus plicatilis* and S-strain *B. rotundiformis* are available. At MRL, rotifers are intensively cultured using ozonated seawater at densities up to 1000 per mL on a diet of microalgae *Nannochloropsis* sp. and bakers yeast *Saccharomyces cerevisiae* in recirculation systems. Stock cultures are kept in test tubes and 20 L carboys. Intensive systems of 50, 250 and 1800 L are used and they are comprised of a reservoir tank, foam fractionator, circulation pump and dosing system for liquid algae. Larger production systems hold up to two billion rotifers and provide a harvest 200 million rotifers daily. Rotifers are enriched with vitamins, algae and commercial products depending on the nutritional needs of the target species. Algamac (Aquafauna Biomarine) enrichment is the standard hatchery practise at MRL with enrichment of 0.2 g per million rotifers over 12 h. Batch cultures or semi-continuous cultures of *B. rotundiformis* and *B. plicatilis* fed yeast and microalgae in 100 or 500 L containers is practised at SOA. Rotifers are normally enriched on both micro-algal and Selco (INVE) products.

Brine shrimp, *Artemia* (early stage nauplii through to adults) are an important component of most larval diets, and especially for striped trumpeter, sea horses and southern rock lobster. The newly hatched *Artemia* nauplii are reared using ozonated seawater from decapsulated cysts. Hatch Controller from INVE (Primo Aquaculture) reduces bacterial loads in 70 L conical tanks. Care is taken in selecting brands of *Artemia* with good nutritional profiles and in matching the appropriate sized *Artemia* to each development stage. There is a need for large-scale on-growing of *Artemia* to around 12 mm in total length for feeding to southern rock lobster phyllosoma larvae and sea horses. At MRL, *Artemia* are ongrown in 600 L tanks using flow-through seawater on a diet of rice pollard and algae (*C. muelleri* and T. *Iso*). Considerable effort is expended to reduce external and internal bacterial loads in on-grown *Artemia* using a series of algal purges incorporating formalin. At SOA, *Artemia* instar II and adults are mainly used to support seahorses and squid. *Artemia* instar II production uses a variety of approaches including decapsulation, non-decapsulation, A1 Selco products, microalgal enrichment, and a range of other commercial product enrichments.

Copepods (e.g., harpacticoid *Tisbe,* cyclopoid *Apocyclops*, and calanoid *Acartia*) have been used as a supplementary feed to rotifers and *Artemia* in the culture of "difficult" marine fish. However, mass-culture of cold-water copepods has been problematic. *Tisbe* has been cultured in Launceston using batch, semi-continuous and recirculation systems on diets of microalgae, fortified yeast, pelleted feeds, vegetables, macroalgae and associated microflora. Striped trumpeter have been reared successfully using harpacticoid copepods as a supplementary feed. Seahorse juveniles have been reared successfully on cultured copepods (*Tisbe* sp.) and a range of crustaceans (copepods, amphipods, caprellid shrimps) collected as net biofouling. Research during

these trials has documented the relationship between seahorse size and prey size, gut enzyme development and digestive tract development.

Amphipods have been used to feed sea horses and the endangered handfish *Brachionychthys hirsutus.* The techniques for mass-culture of amphipods are still being developed in collaboration with aquaculturists in the seahorse industry. Frozen mysids are used routinely in seahorse cultures starting at about 3 months of age while live mysids are fed to dumpling squid held for behavioural experiments. Whilst the culture of mysids is possible, at present it is more effective to collect them from the wild under licence.

Striped trumpeter: D 5 to D 20, L-strain rotifers; D 18 to D 50, 48 h brine shrimp; copepods as available to D 30. Rotifers are added twice daily at 5 to 10 per mL. *Artemia* at 0.1 to 2 per mL.

Greenback flounder: D 5 to D 20, L-strain rotifers; D 18 to D 35, 48 h brine shrimp.

Seahorses: *Artemia* instar II metanauplii are used on pot-bellied seahorses from day of birth onwards. Seahorses consume around 35-45% BW/day (wet weight basis) at the early juvenile stages. Current trials aim to identify a suitable replacement feeding regimen starting at copepods, followed by amphipods and mysids. In the smaller seahorse species (*H. barbouri* and *H. whitei*) and pipefish (*S. argus*) both *Artemia* and rotifers are added for the first 1-2 weeks. Copepods also are used as a supplementary feed.

For newly-hatched rock lobster phyllosoma larvae, *Artemia* ongrown to 1.5-2.0 mm are fed at 1.5 per mL. As phyllosoma grow, they are fed at a relatively lower rate with larger *Artemia*. For late stage larvae (≥ Stage 9), 6-9 mm *Artemia* are fed.

Inert feeds used

Striped trumpeter are fed formulated diets from as early as D 30. Recent weaning strategies have centred on the use of *Artemia* replacement diets like Gemma Micro (Skretting). Striped trumpeter are generally weaned by D 100. Japanese ML diets (Nippon) have also been used successfully. A recent experiment has shown that greenback flounder can be reared from first feeding on Gemma formulated diets with only one day of rotifer feeding. At this stage, trials using a range of commercially available and manufactured formulated diets on early seahorse juveniles have not proved successful with ingestion levels being sub-optimal. Such trials are continuing on other products.

Algae

At SOA Launceston, a wide range of marine and some freshwater axenic stock cultures are maintained in 250 mL Erlenmeyer flasks in autoclaved f/2 medium at 18°C under 50-80 µmoles photons PAR m^{-2} s^{-1} with a 12:12 light dark cycle. Aquaculture feeds species such as *Chaetoceros muelleri, Chaetoceros calcitrans, Isochrysis* sp. (T. *Iso*), *Pavlova pinguis, Pavlova lutheri, Tetraselmis suecica, Nannochloropsis oculata* are on-grown in f/2 medium (0.2 µm filtered seawater) in batch and semi-continuous cultures with CO_2/air injection, under metal halide lamps in 10 L carboys (axenic), 500 L poly bags, at high cell density in 10 L "mini-bags", or in 1000 L fibreglass tanks. Microalgae are batch cultured at MRL. Autoclaved 20 L carboys provide high quality axenic cultures and microfiltration (0.2 µm) and chlorination are used in batch culture in tanks up to 1000 L, using metal halide lamps, CO_2 and aeration injection. *N. oculata* is the main alga used in green water culture of marine fish. *C. muelleri, I. galbana,* and *P. pinguis* are used for enrichment and bacterial purging of *Artemia.* There is increasing reliance on liquid algae from Reed Mariculture (Proaqua), particularly liquid *N. oculata* as a rotifer feed and for greenwater culture.

Hygiene

Eggs of striped trumpeter are disinfected with ozone prior to hatching at 1 ppm for 1 min to control nodaviruses. Eggs are less sensitive to shock and ozonation after the embryo is well developed, around three days post-fertilisation. Seawater used in the fish and rock lobster hatcheries at MRL is filtered, foam fractionated, heated, ozonated, UV treated and charcoal filtered. Seawater is sterilised in the fish hatchery to remove parasites and disease organisms by ozonating at an ORP of over 850 for 10 min. Water baths are provided at the entrance to

both hatcheries, gum boots, laboratory coats, ethanol sprays and hand washing are required to enter the fish hatchery. Seawater for seahorse and live feed culture at SOA is filtered and UV treated. Seahorses occasionally display protozoan, annelid or anemone infestations of the skin and are treated by short freshwater bath or chemical bath.

R&D Activity

Projects include:

1. Aquafin CRC: Improving growth and survival of cultured marine larvae striped trumpeter: a test case for Tasmania
2. Aquafin CRC: Enhanced hatchery production of striped trumpeter in Tasmania through system design, microbial control and early weaning
3. Rock Lobster Enhancement and Aquaculture Subprogram: Advancing the hatchery propagation of rock lobsters
4. Rock Lobster Enhancement and Aquaculture Subprogram: Propagation of southern rock lobster (*Jasus edwardsii*) in Tasmania
5. TAFI: Manipulating genetic variation in Pacific oysters
6. TAFI: Optimising juvenile oyster growth and condition in spat upweller systems
7. TAFI: Novel microalgal feeds for bivalve culture
8. TAFI: Bacterial flora replacement and probiotic delivery via *Artemia* and other live feeds

There are three main planned outcomes from the research on striped trumpeter:

1. Australian aquaculture will be provided with a more systematic way to evaluate and match the nutritional profile of live feeds with the needs of new species of marine fish larvae leading to improvements in growth and survival of a wider range of cultured juveniles. For example, survival has increased from <1% to >10% for striped trumpeter over the last three years.
2. Australian aquaculture will be provided with more systematic ways to control microbial communities. Methods of water treatment, system design, and techniques to evaluate, identify and produce probionts for use in improving hatchery survival rates in finfish, especially in relation to the needs of new 'difficult' species of marine fish larvae, leading to improvements in growth and survival of a wider range of cultured juveniles.
3. There will be a greater choice of new marine fish species available for culture through the efficient technology transfer between research agencies and industry of new products and systems for culturing marine fish larvae.

The industries to benefit from the planned outcomes include the Atlantic salmon aquaculture industry and the emerging marine finfish culture industry throughout Australia. In particular, the development of novel techniques for water treatment using ozone, probiotics and enrichment of live feeds is of direct relevance to barramundi, dhufish, kingfish, snapper, whiting and grouper culture.

The successful outcome of the research on rock lobster larval rearing will be a strong foundation on which to close the life cycle and has application to temperate and tropical species. A reliable and consistent supply of high quality Stage 5 phyllosoma and the development of suitable culture protocols will assist the development of lobster aquaculture. Effective probiotic techniques will control the microbial environment of *Artemia* and larval cultures leading to a significant improvement in the survival of phyllosoma. It will also have wider application elsewhere due to the ubiquitous nature of *Artemia* as the live food of choice for many aquaculture species.

The seahorse culture program has five main aims in relation to live feeds:

1. Use of alternate live feeds to replace or partially replace *Artemia* in the production cycle. Trials currently show the use of copepods, mysids and net biofouling organisms to be promising replacements to reduce costs and avoid reliance on a product which can be difficult to obtain and expensive to use as a feed.

2. Trial greenwater culture on newborn juveniles to assess if there is any benefit over clear water culture. Preliminary experiments suggest that there is little or no benefit to *H. abdominalis* but possible benefit (in the absence of copepods) to other seahorse species.

3. Trial the use of formulated diets to further reduce the reliance on live feeds. Seahorses are visual feeders which places a heavy reliance on live prey so weaning is not easy. However, it is possible to wean fish from live to frozen diets and a focus on formulated diets soon after birth will be examined.

4. Nutritional needs of seahorses at various ages to be assessed. Regardless of whether *Artemia*, live, frozen or formulated diets are used there is a need to quantify the requirements of the fish to optimise growth and survival. Coupled with nutrition, feeding behaviour and activity patterns are also indicators of the success of various diets and feeding regimes.

5. Rearing protocols for new syngnathid species. The existing protocols for *H. abdominalis* will be transferred to other species and further refined.

Shellfish and microalgal research aims to improve larval nutrition and growth rates through improved nutritional profile of microalgal feeds and a better understanding of larval/juvenile raising technologies. Work on oyster genetic variation aims to develop reliable methods for producing genetically consistent shellfish broodstock. These broodstock lines can be used develop oyster lines with consistent growth rate and desirable shape, colour and flesh quality characteristics for the Australian and international market.

Specific problems and issues

Four key areas of research are being examined with striped trumpeter through the Aquafin CRC:

1. Improving the health of larvae through better control of disease and parasites. Control of myxosporeans and nodavirus will be undertaken in companion projects outside the Aquafin CRC.

2. Further development of live feed enrichments, weaning, and grow-out diets, leading to a better understanding of nutritional requirements and other factors.

3. Further improvements in tank design, management and operation to improve post-larval quality, particularly malformations possibly aggravated by “walling behaviour”.

4. Contingent on above, assessment of survival and growth of post-larvae and juveniles under semi-commercial conditions.

The overall objective of the research on the larval rearing of southern rock lobsters is to understand the essential requirements for culture of early-stage phyllosoma by:

1. Identifying and assessing broodstock conditioning protocols that permit routine production of high quality, viable larvae.

2. Developing pilot-scale larval rearing systems which result in high survival of large numbers of Stage 5 phyllosoma.

3. Better understanding the role of water quality and controlling microbial contamination as one of the key bottlenecks to survival and growth of phyllosoma.

4. Developing diets and feeding protocols which optimise the survival and growth potential of phyllosoma to Stage 5

The key areas to be researched in relation to seahorses are:

1. Optimising feeds and feed utilisation, including a better understanding of adult and juvenile feeding

behaviour and a cost analysis of replacement diets.

2. Gaining a better understanding of the source, prevention and treatment of seahorse diseases including a range of protozoan, bacterial, annelid and anemone infestations.

3. Although seahorses breed year-round some research is required on the manipulation of breeding cycles, including the effect on courtship behaviour, re-conditioning of broodstock and the social interactions of broodfish within rearing tanks.

4. Seahorses are used in the aquarium trade and while size is an important determinant of price so too is colour. Preliminary research suggests that colours can be changed in *H. abdominalis* using food and environment. Further research would better define how colour can be manipulated and which conditions can be used to optimise this character.

5. Tolerances of newborn seahorses to environmental conditions such as temperature, salinity, ammonia, nitrite and nitrate need to be quantified to assist with the development of management plans for high density culture in recirculation systems. Further, the viability of low density cage culture of large juveniles and adults needs to be explored.

Microalgal production research is focused on:

1. Optimising the use of 10 L hanging "mini-bags" high density algal cultures as a flexible and space-efficient alternative to 500 L bags. Preliminary work indicates a 10-fold improvement in algal biomass per litre of medium (3-4 x 10 L bags = 1 x 500 L bag) due to more efficient utilisation of light and nutrients in the smaller diameter 10 L bags.

2. Examining the nutritional value of small hardy dinoflagellates as supplemental feeds (10-20% of cell biomass) for larval bivalves. This group or microalgae are a major feed source for bivalves in natural systems that contain a range of potentially important long-chain fatty acids and novel sterols. They are currently not utilised in intensive aquaculture due to slow growth rates and poor growth in standard algal systems.

Collaboration

TAFI researchers have close links to industry and other researchers both nationally and internationally. The striped trumpeter and rock lobster teams have worked closely with the CSIRO Marine Research scientists including Drs M. Brown, P. Nichols and K. Williams on aquaculture nutrition. Collaborating departments of UTAS include, Fish Health Unit (Drs J. Carson and J. Handlinger), Agricultural Science (Dr J. Bowman) and School of Human Life Sciences. Other institutions include Curtin University (Professor Bruce Phillips), QDPI Cairns (Dr Clive Jones), James Cook University (Dr T. Pankhurst), the Ludwig Institute for Cancer Research (Dr A. Trotter), and Department of Fisheries Western Australia (Dr S. Kolkovski). Recent working groups and review teams have included leading scientists from SA (Mr W. Hutchinson, Mr S. Clarke), NSW (Dr S. Fielder) and WA (Dr S. Kolkovski). International links include collaborations with the United Kingdom on marine finfish culture, Stirling University (Dr G. Bell, Prof J. Sargent) and the Northern Atlantic Fisheries College (Dr L. McEvoy), and in Spain at the Centro de Acuicultura -IRTA (Dr A. Estevez). Other close associations include researchers working on probiotics in New Zealand, NIWA (Drs E. Maas and Dr M. Bruce) and on sea cucumbers in New Caledonia, Worldfish (Dr S. Purcell). Industry collaborations include M G Kailis Pty Ltd, major food manufacturer Skretting and the salmonid industry through the Tasmanian Salmonid Growers Association. The SOA has close industry links with Seahorse World (Beauty Point, Tasmania) who support general syngnathid research and PhD research. The School also has informal links with seahorse researchers in New Zealand, NIWA (Chris Woods) and Singapore, TMSI (Dr K. Reddy, Dr J. Walford, Dr B. Sivaloganathan).

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Hatchery Technology on the Breeding and Fry Production of Marine Finfish in Indonesia

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Interest in culturing the various species of marine finfish has always been around, fuelled by high on-farm price. In Indonesia current ex-farm price ranged from US$ 9-12 for the tiger grouper *(Epinephelus fuscoguttatus)* whereas prices for Barramundi cod *(Cromileptes altivelis)* reach US$ 30-38. The recent upward trend in production is attributed to advancement in hatchery seed production and improvements in nursery techniques. The interest in marine finfish farming has not only provided jobs for many coastal communities, but also established secondary servicing industries such as hatchery technology, feed manufacturing, product processing, transportation and marketing. This paper describes the present status of hatchery technology for the breeding and fry production of marine finfish in Indonesia, such as Tiger grouper *(Epinephelus fuscoguttatus),* Estuarine grouper *(Epinephelus coioides)*, Barramundi cod *(Cromileptes altivelis*), Coral trout *(Plectrophomus leopardus),* Napoleon wrasse *(Cheilinus undulatus*) and Red snappers *(Lutjanus sebae and L.argentimaculatus,* based primarily on the work have been carried out in Gondol Research Institute for Mariculture and Lampung Mariculture Development Center. Broodstock are maintained in tanks (100-150 MT capacity) on land and fertilized eggs are obtained by natural spawning. Broodstock are fed by mixed fresh squid and trash fish *(mainly Sardinella sp*.). For larval rearing, eggs instead of hatched larvae, are mainly stocked in the rearing tank. Feeding of super small (SS-type) rotifers for very early larvae and feeding of artificial diets for late larvae and juveniles improved larval survival. The recent advances are in the seed production of the Tiger grouper, estuarine grouper and Barramundi cod, hatchery breed grouper fry are well accepted by farmers. There are now 75 groupers hatcheries (55 small/backyard hatcheries, ten medium, nine large/complete and one intensive hatchery which hold broddstock. The total production of grouper fry in 2003 was 4.20 million juvenile for tiger grouper, 1.13 million for Barramundi cod and 350 thousands juveniles for others groupers. In addition to the groupers mentioned above, corral trout, napoleon wrasse and red snappers being targeted for development.

Introduction

Marine finfish culture is expanding in many areas of Indonesia. While there is lack of statistical data available on marine finfish culture in Indonesia, national statistic aquaculture shows cage culture growing at 16 per cent during the 1990s. The primary areas for grow-out culture are Aceh (Nias and Sibolga), Riau Islands, Bangka-Belitung Islands, Lampung Bay, Seribu Islands, Karimun Jawa Islands (Central Jawa), Teluk Pengambetan (North-West Bali), Teluk Saleh (West Nusa Tenggara), South Sulawesi, South-East Sulawesi, North Sulawesi and Gorontalo. Marine fish culture is generally characterized by the use of wild caught seed and use of trash fish for feed. There is limited use of hatchery reared seed, although this is growing especially for groupers.

There has been a good deal of research on hatchery production of groupers, snappers and other marine finfish. This has been stimulated by the development of a large number of private milkfish hatcheries by applying the technology that has been developed in GRIM.

Species Cultured

At Gondol Research Institute for Mariculture has been doing research and development on seed production of Milkfish *(Chanos-chanos)*, Shrimp (*Penaeus monodon, P.vannamei, P.stilyrostris)*, Mud Crab *(Scylla*

sp.), Swimming *Crab (Portunus sp)*, Yellow fin Tuna *(Thunnus albacores),* Tiger grouper *(Epinephelus fuscoguttatus),* Estuarine grouper *(Epinephelus coioides)*, Napoleon wrasse *(Cheilinus undulatus*), Red snappers *(Lutjanus sebae and L.argentimaculatus* Barramundi cod *(Cromileptes altivelis*) and Coral trout *(Plectrophomus leopardus),*. In this paper present only marine finfish seed production.

Systems

Broodstock

Broodstock tanks are used not only for rearing of broodstock but also for spawning. Based experiences in Gondol Research Institute for Mariculture (GRIM), ideal size of broodsock tanks is 60 MT for Barramundi cod *(Cromileptes altivelis)* and Coral trout *(Plectrophomus leopardus)* and tanks of 100-150 MT for other groupers *(Epinephelus fuscoguttatus, E. coioides and Plectrophomus* sp), Red snapper (*Lutjanus sebae and L. argentimaculatus)* and Napoleon wrasse (*Cheilinus undulatus*). Since broodstock swim around on the tank during spawning activity, it is recommended to use round shape tank with the depth of 2-2.5 m. Each tank is equipped with a water inlet and outlet and an aeration system. The source of water supply is direct from sea using sea water pomp. It had a flow through by changing 200-300 % of the tank water daily. An overflow pipe is connected with an egg collection tank where a net to collect spawned eggs is placed. The size of tank for collecting egg is 2x2x1 m depth Fig.1. The water temperature and salinity is ranged from 26.8-28.9°C and 33-34 ppt, respectively. The broodstock were usually fed to satiation once a day by mixed trash fish (mainly *Clupeidae* and *Scombridae*) with squid. The feed is supplemented with 1% of vitamin mix.

Figure 1. Broodstock tank for Marine finfish in GRIM .

Larval rearing tanks

The size of larval rearing tanks is approximately 10 MT with 1.2 m depth and equipped with aeration system, this size is enough to produce 10,000 juvenile of groupers and snapper seeds. Both circular and rectangular shape tanks can be used for larval rearing. For rectangular shape tank, corners should be rounded to avoid larval aggregation at the corners. The sea water used in the larval rearing tank is pre-treated using sand filters. The water temperature ranged from 27.8-30.0 °C and water salinity ranged from 34.0-34.5 ppt. Based on experiences, a light blue color is preferred for larval rearing in GRIM. Larval rearing tanks should be roofed to avoid direct sunlight and water rain, and to avoid fluctuation of water temperature. (Fig.2).

Figure. 2. Larval rearing tanks for marine finfish.

Larval rearing

Larval of groupers shows drastic changes in their morphology as they grow from the larval stage to juvenile stage, therefore, careful management is necessary. The factor contributing to larval mortality are floating death, sinking death, entanglement with the spine, nutritional deficiency , cannibalism and diseases. For larval rearing, eggs, instead of hatched larvae, are mainly stocked in the rearing tanks with initial stocking density

10 ind./L. The larva rearing is carried out in the same tank for 45-50 days. Live food for the larval rearing consisted of micro algae, *Nannocloropsis sp*, SS type rotifer (Size 80-120 micron), S-type rotifers 140-200 micron) and *Artemia* nauplii. Artificial diets were introduce prior to feeding *Artemia* nauplii. The larval raring protocol is shown in Fig.3.

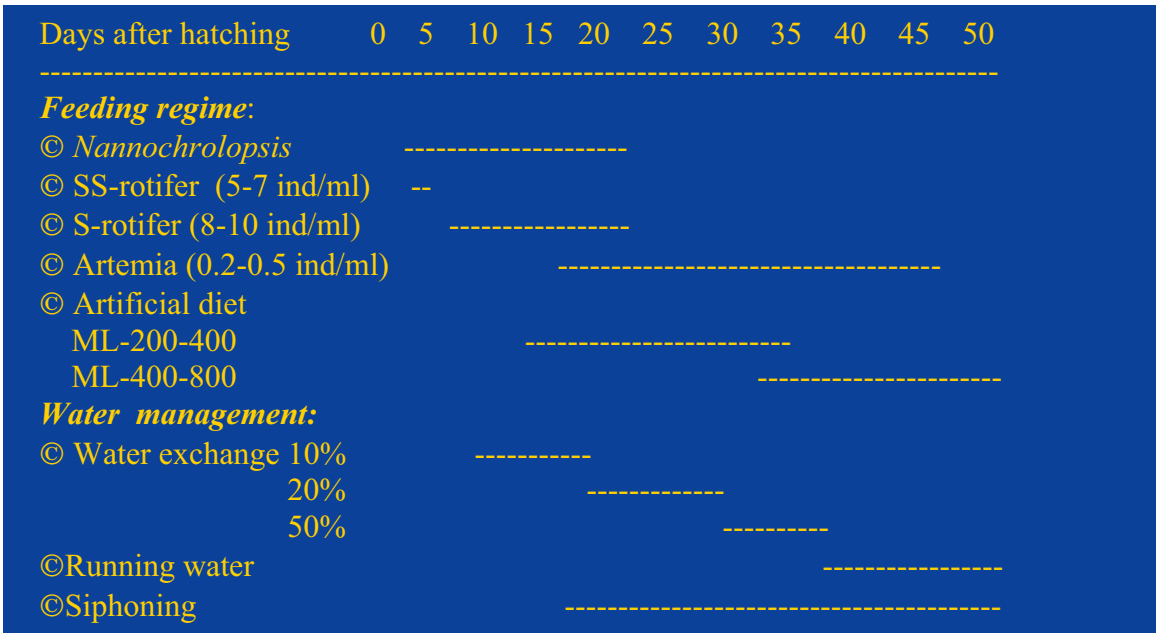

Figure. 3. Larval rearing protocol of groupers.

The *Nannochloropsis* was introduce in the larval rearing tanks after 24 hours of stocking the newly hatched larvae (1-DAH), the Nannochloropsis density was maintained at 300 thousand cell/ml. The SS-type rotifer, are introduce on day two when the larvae partly absorbed their yolk. The SS-type rotifer density is maintained at density 5-7 ind/ml during 2-5 DAH. The S-type rotifer with a density of 8-10 ind/ml was maintained during 6-20 DAH and the density gradually decreased as the rate of rotifer consumption by the larvae increased and eventually rotifer disappeared at day 25. From days fifteen onward, small size commercially formulated diet (ML Powdered) with a particle size of 200-400μwas used. The feed size was gradually increase from 400 t0 800μ from day 30 to day 50. From day seventeen onward, newly hatched *Artemia* were introduce with a density of 0.2-0.5ind/ml. Before introduction to the larval rearing tanks, one day old *Artemia* were treated or enriched with "Super Selco" to increase their nutritional value. One day after the fish were fed artificial diets and *Artemia*, 20-50% of the rearing water was changed once daily. At day 30, running water at an exchange rate of 100 % were done to avoid water quality problem. By applying to these protocol improved larval survival with survival rate ranged from 30-50 %.

Main Problem

The major cause mortalities are due to diseases especially Viral Nervous Necrosis (VNN). Once VNN break out during larval rearing, a high mortality occurs and some time total mortality was happen. Up to now, there is no effective treatment has been developed for VNN. Dead juveniles caused by VNN are shown in Fig.4

Figure. 4. Dead juveniles caused by Viral Nervous Necrosis.

Production

By applying the technology mentioned above, the total production of the tiger grouper in 2003 was 4,300,000 juveniles of Tiger grouper, *Epinephelus fuscoguttatus* (Size 5-8 cm in total length), 1,130,000 juveniles of Barramundi cod, *Cromileptes altivelis* and 350,000 juveniles of Estuarine grouper, *Epinephelus coioides*, Red snappers, *Lutjanus sebae and L. argentimaculatus*. Hatchery bred seeds are now well accepted by farmers, There are now 75 marine fish hatcheries (55 small/backyard hatcheries, 10 medium hatcheries, 9 large hatcheries which hold broodstock and 1 hatchery super intensive and several more under development. In addition to those fish other marine fish under propagation are Napoleon, *Cheilinus undulatus,* Coral trout , *Plectrophomus leopardus and* Yellowfin Tuna. *T albacore* In GRIM currently hold the brodstock of Tiger grouper 83 fish, Barramundi cod 127 fish, Coral trout 42 fish, Napoleon 23 fish, Red snapper 70 fish, Yellowfin Tuna 42 and Cobia 8 fish. With the production capacity 6-9 million seeds per year. GRIM has also run the supper intensive hatchery, this hatchery built by Denmark Government and now under observation weather can be use to produce grouper seed or not. The Super Intensive hatchery or Close system hatchery is shown in Fig. 5.

Figure 5. Super intensive hatchery at GRIM .

Research Collaboration

The Government of Indonesia has formulated several research programs that help address main problem faced the marine finfish farmer or industry. Research and development will focus on study related to broodstock genetics, feed formulation with low pollution, improve of seed production technology, production of free diseases broodstock and seeds, produce vigor seeds and vaccine. GRIM currently doing research collaboration with ACIAR-Australia on improvement of hatchery technology of grouper, especially Coral trout, *Plectrophomus leopardus*, Research collaboration also with OFCF Japan for yellow fin tuna propagation.

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Industry Perspectives

Commercial practices for the production of barramundi, *Lates calcarifer*, fingerlings: An industry summary

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Introduction

Barramundi fingerlings have been produced commercially in Australia for over 15 years. Early industry development was assisted by the activities of the Qld Department of Primary Industries who developed an extensive culture technique described essentially in Rutledge and Rimmer (1991). This technique is still used to some extent in commercial practice accounting for 25% of fingerling production.

Fingerlings produced in intensive systems were considered to be inferior to those produced in extensive systems being smaller for age, likely to show more frequent deformities and thought to be less robust. Recently, the establishment of hatcheries in regions outside of the natural range of barramundi in Australia and the development of improved live food production processes, enrichment media and weaning diets has led to increased use of intensive hatchery production systems.

In 2003/4, the majority of barramundi fingerlings produced in the 7 major hatcheries in the Australian commercial sector were produced in intensive systems. In 2003/4, approximately 12.5 million barramundi fingerlings were produced using intensive larval rearing methods compared with 4.5 million fingerlings using extensive methods. Intensive systems allow much more predictable survival of larvae through to fingerlings, more reliable quality of fingerlings and production out of the natural breeding season.

In view of the nature of this workshop, this presentation will be restricted to the methods and issues of intensive production of barramundi fingerlings. Most procedures used in the barramundi industry are not greatly different to those of other intensive commercial marine fingerling production systems. A significant modification of the traditional method developed recently is described by Bosmans *et al* (2004) in this Proceedings. Since that method varies in nature and timing from the traditional method and is described elsewhere, it will not generally be included in this discussion. This presentation is general in nature and does not describe the practice of any individual company and some of the methods used in commercial hatcheries are proprietary information and will not be discussed.

Production capacity

Capacity for production of barramundi fingerlings is not fully utilized in Australian hatcheries for a variety of reasons. These include the fact that some companies are in the development phase while others are unable to sell all of their production. It is estimated that approximately 85% of the capacity is utilized.

The production season is largely dictated by the capacity to subsequently rear fish through nursery culture. Fingerling production occurs throughout the year and most companies have temperature controlled hatcheries that allow them to produce fingerlings when required. However, as heated nursery facilities are limited, most northern hatcheries suspend production for some part of the winter period and take advantage of this time to perform maintenance and spend a period of time in dry out.

The number of broodstock held is in great excess to that actually required for spawning purposes. Generally, 50 to 100 broodstock (including both male and female) are held per hatchery. The major constraint on broodstock performance is poor fertilization of eggs. Poor fertilization results from insufficient numbers of developed males in the spawning tank, early onset of sex inversion with consequent loss of males from the spawning

population and a stress response to handling in male fish that results in regression of gonadal development. The relatively large number of broodstock allows for variable performance and some capacity for selection. Broodstock turnover is at a relatively high rate as hatcheries seek to respond to these challenges, particularly the effects of early sex inversion and as they seek to improve the genetic characteristics of their stock.

Larval feeding regime

Larval feeding regimes incorporate a mixture of rotifer (*Brachionus plicatilis*), Artemia and formulated feeds.

The larval feeding regime is shown in Figure 1. Rotifers are provided from Day 1 after hatch (ah) and may continue up to Day 14 ah if available in sufficient numbers. Artemia as newly hatched nauplii are introduced between Day 9 ah and Day 12 ah and continued until fish are fully weaned or are determined to be too slow to grow and are consequently sacrificed. Weaning diets are introduced at some time on or after Day 12 ah.

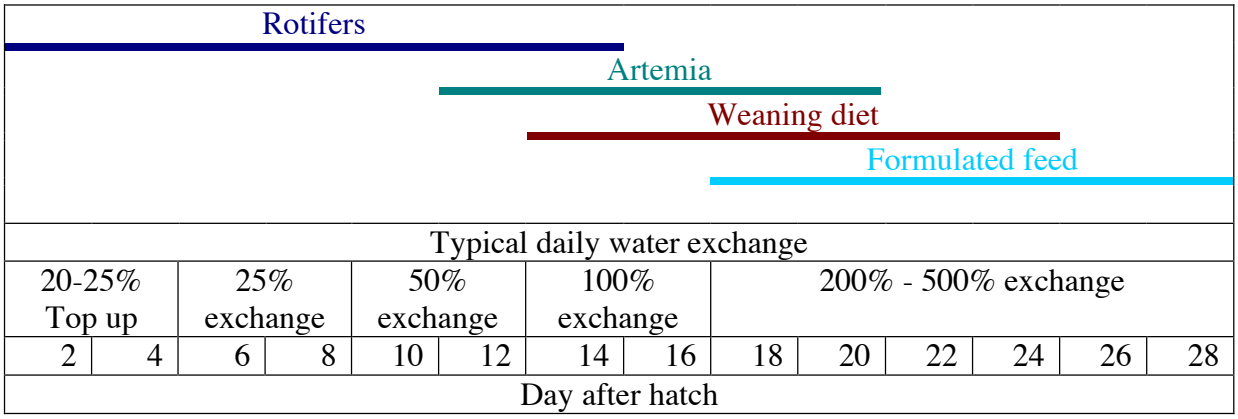

Figure 1. Diagrammatic representation of the typical feeding regime and water exchange used in barramundi larval rearing in Australian commercial hatcheries.

Traditionally, rotifers are produced using a culture of *Nanochloropsis* sp. supplemented by enrichment media and yeast. Relative levels of algae, enrichment media and yeast can be varied to achieve higher or lower densities of rotifers as required for the stage of the culture system. The INVE product *Culture Selco* appears to be the enrichment of preference for rotifer culture and densities of over 250 animals/ml can be easily achieved cost effectively using a combination of relatively low amounts of *Culture Selco*, yeast and *Nanochloropsis* in a batch culture. Some hatcheries in the past have used the published method of INVE involving super high levels of *Culture Selco* only but, as far as the author is aware, are currently not doing so.

Recently, some work has been undertaken by the Darwin Aquaculture Centre in the Northern Territory using an algal paste as the only production and enrichment ingredient for rotifers. Those authors (Bosmans *et al*., 2004) claim great success with that method, described elsewhere in this proceedings, and the use of algal paste in comparison to culturing algae has much economic merit.

Artemia production follows standard procedures. The enrichment product of choice for Artemia appears to be the INVE product *DC DHA Selco*.

Weaning diets vary according to availability and price. Proton (INVE) 3-5 is the most prevalent product used. Some hatcheries use Nippai although availability and price prevent more widespread use of this and Skrettings Gemma Micro is becoming more widely used. Weaning can be a period when large losses of fingerlings occur. Weaning techniques incorporate continuous delivery of feed using automatic feeders and careful monitoring of feed delivered to ensure enough feed is provided. A high degree of technical skill on the part of the person weaning the larvae is important in ensuring good survival through this phase.

Larval Culture System

Greenwater culture, usually utilising *Nanochloropsis*, is almost universally used for intensively rearing barramundi larvae. Water exchange varies from zero at the start of the culture to 100% by Day 14 ah. Water exchange may be driven to a large extent by the concentration of available algae but, by preference, it follows a pattern similar to that in Figure 1. Larvae are stocked at between 12.5 and 37.5 larvae/L. Stocking density used is dependent upon the number of larvae, the number of available rotifers, the number of fingerlings required and physical constraints such as water supply, tanks available and labour. Fish grow more quickly and show fewer deformities at lower stocking densities than at higher densities and risk management dictates that larvae are divided between a number of tanks.

Tank size used varies between 2000 L and 10000 L but a tank of 5000 L provides a good commercial combination of numbers produced/unit labour combined with adequate control of the biological and physical constraints.

Figure 2. Photograph of the GFB Fisheries Ltd hatchery showing typical tank design and operation of a greenwater culture.

Tank design usually incorporates a slight cone in the bottom to facilitate removal of waste during water exchange. Keeping the inside of the tank free of structure facilitates cleaning, sterilisation and limits surfaces for bacterial growth. Internal screens of 500□m to day 10 followed by 1 mm prevent egress of fish from the tank. Additional aeration is provided by normal aquarium air stones at a density determined by the surface area of water rather than volume. A tank of 2.5 m diameter would have 5 to 7 air stones providing aeration. Layout of a typical hatchery is shown in Figure 2.

Hygiene

Losses of larvae to disease occur from either infection by Viral Nervous Necrosis (VNN or Nodavirus) or by bacterial infection. While it is possible with excellent husbandry, low stocking densities and particular attention to hygiene to rear larvae infected with VNN, it is undesirable. The resulting fingerlings may infect other fish in the production system, may cause a residual infection in the farm particularly those farms where complete sterilisation is not possible and may not perform with regard to growth rate and food conversion as well as uninfected fish perform later in the growth cycle. Therefore, it is important to prevent infected larvae entering the hatchery. This is easier said than done, however. Determination of VNN infection in broodstock is unreliable with a reasonable probability of a false negative result. A prudent management regime involves testing broodstock for carrier status and removing those that are positive. As a secondary barrier to transfer, eggs are treated with an iodine bath which provides some protection in the event that the screening of broodstock failed to identify a carrier. All larvae in Queensland are tested for VNN using histological techniques according to State determined protocols prior to shipping interstate. Most hatcheries test all batches of fish in order to prevent distribution of infected fish through the industry.

Bacterial infections occur occasionally. Although it is difficult to determine the precise cause of these infections, a *Vibrio* species is the most likely agent. Prevention of bacterial infections involves using strict hygiene protocols. Tanks are cleaned using various protocols incorporating detergent, chlorine, acid and sterilized water. Incoming water to hatcheries is sterilized with chlorine subsequently neutralized by sodium thiosulphate or by ozone. Sterilised water is then usually filtered through 1 □m filters prior to distribution to larval tanks. Bacteria can be treated upon emergence of a disease or prophylactically with a fresh water bath at about Day 10 ah, or with the use of antibiotics. Most hatcheries operate using a minimum of antibiotics in order to prevent development of resistance.

Introduction of disease can also occur with introduction of algae or rotifers. As well as bacteria, it is possible to introduce ciliate infections by this route. Prevention of ciliate transfer is achieved by passing algal and rotifer cultures through filters but is limited by the need to use filtration that allows the food organism to pass.

Deformities

The prevalence of physical deformities in fingerlings, particularly spinal deformities, is known to be greatly affected by dietary vitamin C levels during early rearing (Fraser, 2002). Therefore, it is important that whatever rearing technique for rotifers is used ensures that the level of vitamin C delivered to the larvae is adequate to allow cartilage development and ossification to prevent deformities. Incorporation of sufficient levels of *Culture Selco* appears to have achieved this with spinal deformities in larvae being less than 1%. Swim bladder deformities determined by histological section may or may not impact on subsequent performance of the fish appear to be about 5% of larvae produced. Use of surface skimmers to prevent build-up of surface oils on the water up to Day 6 ah is important to optimize swim bladder inflation.

Constraints

While techniques for rearing barramundi larvae are reasonably well defined, industry constantly strives to improve survival and reduce costs. This must be done in an environment where changing infrastructure design can be very expensive, both in terms of capital cost and in terms lost production. Prevention of disease particularly VNN and providing alternatives to, or less expensive methods for, culturing algae and live feeds would provide opportunities to improve profitability in commercial hatcheries.

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Marine Farms Limited

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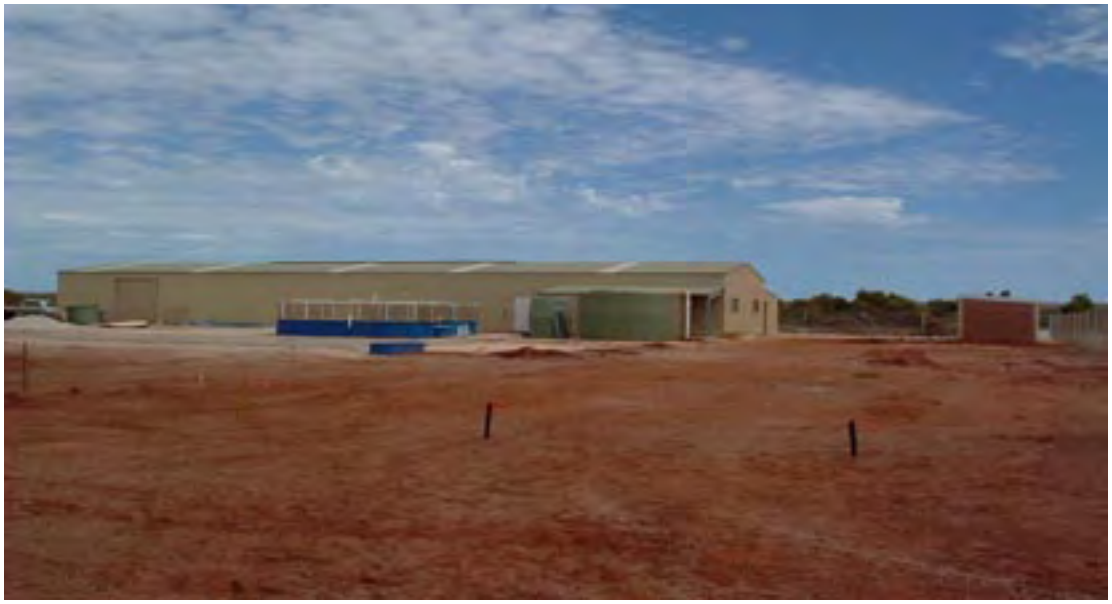
Company profile

Marine Farms Limited ("MFL") is an unlisted public company that was formed to develop a commercial mahi mahi farm. The company has well-established R&D and commercial credentials in its main area of expertise: the culture of marine fish.

Over the past three to four years, Marine Farms conducted an R&D programme at a facility it constructed in North Fremantle. The main purpose of this work was to develop the culture technology needed to proceed with the commercial development. The company is now in the process of constructing its first commercial aquaculture facility at a site it has secured near Exmouth, in the North-West Cape region of WA. Before selecting this WA site, the company identified and evaluated several other sites in New South Wales, Queensland and the northern Territory, as well as overseas in New Caledonia.



The commercial development is being staged through an initial pilot farm with a production capability of approximately 50 tonnes per year. Production will then be increased through successive increments and Marine Farms anticipates reaching an output of approximately 500 t/yr within three to four years. The ultimate yield from the farm has yet to be determined but will be governed by factors such as the availability of high-quality sea water and the market demand.

The mahi mahi farm is vertically integrated, in that it has its own dedicated hatchery to produce seed stock as well as growout facilities. The farm will use above-ground tanks for growing the fish to a market size of approximately 4.5 kg.

Initial production from the farm will be sold in domestic markets. The company has also identified and targeted several export markets for the fish.

Production capacity

The farm is currently under construction. The hatchery is operational but not quite completed. The first few growout tanks are being constructed. The growout tanks represent a new design for land-based marine aquaculture and are also under development.

The project will be developed in stages, the first of which will have a production capacity of 250 tonnes per year. Through ensuing development stages, we expect to continuously increase the production capability of the farm. The ultimate yield from the site will be governed by factors such as the quantity of sea water that can be extracted from the aquifer (we are using beach wells for sea water supply) and the ecology of the receiving environment. We are also focusing on developing a treatment system for used sea water. The project is being developed to be consistent with the principles of ecologically sustainable development.

We generally hold a group of five to six spawning stock, comprising one male and four to five females. The mature fish spawn perennially.

Live foods used

Artemia is the principal live food used.

Inert feeds used

We use inert diets starting from a particle size of 500 microns.

Systems

The project employs an intensive, land-based system using sea water pumped from an aquifer. There is no recirculation. Larvae are reared in cylindroconical tanks using clear water, flow-through techniques. After weaning and a pre-growout period, the fish are transferred to the growout farm.

The growout farm uses a series of above-ground tanks and a flow-through sea water supply system. The loading (kg fish per litre per minute water flow) varies according to the size of the fish. Some R&D is still being carried out in the growout area, but we anticipate growing fish to a market size of 4.5-5.0 kg.

Hygiene

The sea water supply is pumped from an aquifer, so is relatively clean and free of parasites and potential pathogens. The production system does not rely on the routine use of any chemical substances. Standard hatchery hygiene procedures are stringent.

Specific problems

It is very difficult catching wild broodstock and getting them back alive. Fortunately our F1 stocks are nearing maturity. We will have to introduce wild fish periodically. Larval rearing is at an acceptable level right now, but we plan to do additional R&D to improve survival. Weaning: similar comments to larviculture. We do get a little cannibalism and tail biting but nothing too serious.

There are many unknowns about the growout of the species in intensive land-based tanks. This is the main area on which we will be focusing our R&D efforts over the next two years.

MG Kailis Exmouth Hatchery

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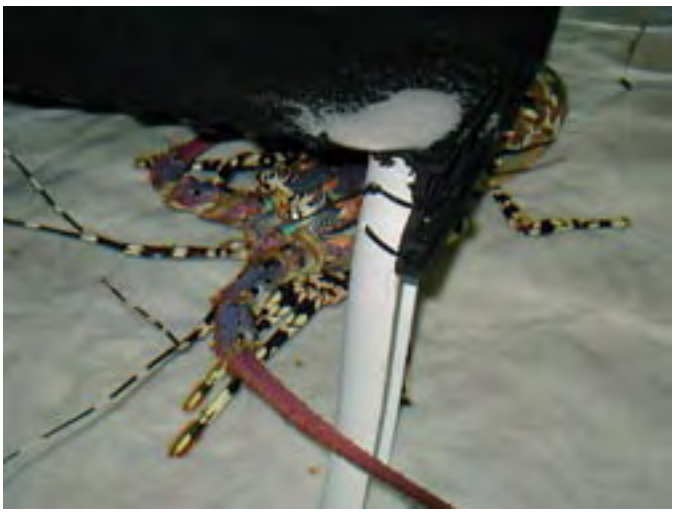
Background

The MG Kailis Group is involved in tuna farming in South Australia, lobster fishing in WA, tropical rock lobster fishing in the Torres Straits and Great Barrier Reef, prawn fishing in Exmouth and pearl oyster farming from Exmouth north to Darwin in the NT.

The Exmouth Gulf is just north of the Tropic of Capricorn on Australia's West Coast. The MG Kailis Group (MGK) has a long history in the Exmouth Gulf – from a time even before the town of Exmouth existed. Against the odds posed by a remote and inhospitable location, sometimes severe cyclone seasons and reports that commercial quantities of prawns did not exist, the Group's founder Michael Kailis pioneered the Exmouth Gulf Prawn Fishery in the early 1960's. MGK now owns and operates over 90% of the catching vessels in the Exmouth Gulf fishery.

When in the early 1990's the MGK group purchased some prawn fishing licences from NorWest Seafoods, they got a prawn processing facility thrown into the deal. Around the same time they bought out a pearling company's pearl quota and got a pearl oyster hatchery near Broome as part of that deal. As they already had their own prawn processing facility in Exmouth it was decided to convert the old NorWest Seafoods factory into a pearl oyster hatchery. The equipment from the Broome hatchery was trucked down to Exmouth and the MG Kailis Exmouth Hatchery started operating in 1995.

Initially solely pearl oyster spat were produced then later in 1999 the hatchery started to diversify and began trials into the propagation of tropical abalone (*H.asinina*). Next in mid-2000 a Prawn Fishery Enhancement project commenced as a joint venture with CSIRO and Fisheries WA. MGK built a super-intensive juvenile prawn raceway production system on a semi-commercial scale. During trials growing Brown Tiger prawns (*P.esculentus*) MGK achieved the highest prawn production densities anywhere in Australia at the time.



Due to problems encountered developing a DNA fingerprinting / tagging system to monitor recapture rates, high capital costs and a weakening prawn price, MGK put the prawn fishery enhancement project on hold. At this point the company decided to concentrate it's R & D efforts into lobster propagation. It had already funded the QDPI to do research into Tropical Rock Lobster (TRL) propagation and grow-out trials in Cairns for the previous three years. MGK now entered a three- year FRDC project as the commercial partner in a TRL propagation project, together with QDPI Cairns and AIMS Townsville.

Whilst doing R & D work with various species (currently lobsters) MGK Exmouth Hatchery remains predominantly a commercial pearl oyster hatchery.

Species cultured

Pearl Oysters (*P.maxima*), Prawns (*P.esculentus*) (project on hold at present), Lobsters (*P.ornatus, P.versicolor*)

Production Capacity

Pearl Oysters – 2 batches per year; 3-5 million spat; spawning October – March;

Prawns – 1 batch per year; 1 million juveniles (0.5-1.0g) spawning September; R & D - Fishery Enhancement

Lobsters – 6 batches per year; R & D – Lobster Propagation

Live Feeds used

Algae (*C.calcitran, T.isochrysis, P.lutheri, C.muelleri, T.suicica*), *Artemia*

Inert Feeds used

Prawn artificial feeds, 50-1000 micron. Various fresh feeds including squid, mussels and other molluscs

Systems and Rearing Methods

Pearl Oyster Larvae: 500 – 15,000 litre tanks - static, semi-continuous flow-through and continuous flow-through. Prawns: parabolic larval tanks 10,000 litre - semi-continuous flow-through; raceways 60,000 litre – continuous flow-through, re-circulation closed system.

Lobsters: tanks 100 – 5000 litre – re-circulation closed system. All species on clear-water / Galveston type system

Algae

All algae (*C.calcitran, T.isochrysis, P.lutheri, C.muelleri, T.suicica*) batch-fed for pearl oysters and for Artemia enrichment

Hygiene

Pearl Oysters – sterile seawater rinsing of eggs only. Prawns – iodine and seawater rinsing of eggs. *Artemia* – Hatch Controller DC, freshwater rinsing. Water (plus nutrients, fittings etc.) for algae – autoclaved. Water for animals – one micron filtered and UV treated (plus ozone on re-circ. system).

Specific Problems and Issues

Inadequate artificial diet for larval, grow-out and broodstock lobsters – fresh diets too variable in quality and expensive / labour intensive

Overview of lobster aquaculture research

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Introduction

Lobsters (family of Palinuridae and Scyllaridae) are highly prized seafood with a ready market. The Western rock lobster (*Panulirus cygnus*), the Southern rock lobster (*Jasus edwardsii*), the Eastern rock lobster (*Jasus verreauxi*), the Ornate rock lobster (*Panulirus ornatus*) and Slipper lobsters (eg., *Thenus* spp., *Ibacus* spp.) together comprise the highest value fisheries in Australia with over $460 million annual production value (ABARE, 2003). In most cases the wild stocks are highly utilised with management controls being applied to assure successful year-by-year recruitment. Aquaculture development is one of the keys to expanding production levels of these lobster species.

The major unsolved problem for developing aquaculture techniques of lobster species lies in the successful maintenance of the planktonic larval (phyllosoma) stages, where the phyllosoma stages are longer-lived (150 to 300 days) and oceanic in natural habitat. Although significant efforts for advancing rearing methods of rock lobster phyllosomas have been made in recent years, mass-culture of rock lobster phyllosomas is still not feasible at this stage. However successful results have been reported from a recent study of the slipper lobster species (*Thenus* spp.), where phyllosomas of *Thenus* spp. pass through only 25 to 30 days with high survival rate (>80%), and juveniles can grow to market size (250g) within 400 days in the growout phase (Mikami, 1995). This paper summarizes current rock lobster research and examines the potential to apply information and techniques obtained from larval rearing of *Thenus.*

Life cycle of lobster species

The families of Palinuridae (rock lobsters) and Scyllaridae (slipper lobsters) exhibit five major phases within the life cycle: adult, egg, phyllosoma, puerulus/nisto and juvenile. The male lobster deposits a spermatophoric mass (spermatophore) on the female's sternum during mating, and fertilisation of the eggs occurs when the female spawn the eggs onto the abdomen and pleopods. The time between mating and fertilisation of eggs differs depending on the species. For example, in *Panulirus japonicus* it is less than an hour, whereas *J. edwardsii* carry the spermatophore for months before fertilisation. Phyllosomas (derived from the Geek *phyllos* meaning leaf and *soma* meaning body) are planktonic larvae, dorsoventarally flattened with a transparent body and long appendages. In the case of palinurid lobsters, the phyllosoma period extends for a number of months (9 to 14 months) with many moulting stages, resulting in large late stage phyllosomas (often >35mm). After a number of months in the ocean, planktonic phyllosomas metamorphose to the briefer, non-feeding, free-swimming post-larval phase (puerulus for palinurid lobsters and nisto for scyllarid lobsters), the transaction stage between planktonic phyllosoma and benthic juvenile stages. The puerulus/nisto apparently navigates to the juvenile nurseries by complex receptor systems formed by the antennae and a pinnate setal system, and once in the nursery grounds, the puerulus/nisto settles into preferred habitats. The understanding of larval recruitment of many lobster species is fragmentary due to the complexity of the oceanic habitats of phyllosomas, and much information concerning the biology of oceanic phyllosomas, such as food, behaviour, predation and physical environmental requirements, is still missing.

Overview of phyllosoma rearing attempts

There have been a number of successful rearings of scyllarid lobsters phyllosomas (eg., *Scyllarus americanus* (Robertson, 1968); *Ibacus ciliatus and Ibacus novemdetatus*. (Takahashi & Saisho, 1978); *Scyllarus demani* (Ito & Lucas, 1990); *Ibacus perionii* (Marinovic et al., 1994); *Thenus* spp. (Mikami & Greenwood, 1997)) due to the relatively shorter phyllosoma periods (a month to a few months). On the other hand, the complete phyllosoma rearing of palinurid lobster species has been recorded by only four institutes, three from Japan and one from New Zealand (see table). However these successful rearings have been limited in scale, often using systems of less than 10l, and with less than 1% survival to the juvenile stage.

Successful records of palinurid lobster phyllosomas

Species	Duration	Temperature	Source
Southern rock lobster (*J edwardsii*)	300-324 days	10-20°C	Kittaka *et al*. (1988), Illingworth *et al.* (1997)
Cape rock lobster (J. lalandii)			Kittaka (1988)
European rock lobster (*P. elephas*)	132 days	17-19°C	Kittaka and Ikegami, (1988)
Japanese spiny lobster (*P. japonicus*); White-whiskered rock lobster (*P. longipes*); Double-spined rock lobster (*P. penicillatus*)	app. 200 days (shortest)	24-28°C	Matsuda (pers. comm.) Murakami (pers. comm.)
Scalloped spiny lobster (*P. homarus*)	app. 180 days	24-28°C	Murakami (pers. comm.)
Eastern rock lobster (*J. verreauxi*)	184-341 (av. 341) days	15-20°C	Kittaka (unpublished) Tong (pers. comm..)

To date (Sep 2004), the most advanced research groups are perhaps the National Centre for Stock Enhancement (NCSE, formerly known as JSFA) at Izu, Japan and the Mie Prefectural Science and Technology Promotion Centre, Fisheries Research Division (MPSTPC), at Hamajima, Japan. These groups are capable of producing up to a few hundred juveniles per year. The keys for their success may be the highly sterilised rearing environments and the use of antibiotics (bacterial control). However, their previous methods have been unsuitable for large-scale production (>1,000 juveniles) due to difficulties in expanding and maintaining sterilised facilities and the heavy use of antibiotics. In fact, though they achieved yearly production of a few hundreds juvenile several years ago, no further major achievement has been reported in last few years.

In Australia there are four institutes studying palinurid lobster phyllosoma rearing. Tasmanian Aquaculture and Fisheries Institute (TAFI) is rearing *J. edwardsii* (Southern rock lobster) phyllosomas, and the Queensland Department of Primary industries Northern Fisheries Centre (QDPI NFC), AIMS and MG Kaillis are rearing *Panulirus ornatas* (Tropic rock lobster) phyllosomas. So far, no successful mass rearing of these phyllosomas to the juvenile stage has been reported.

Three key issues have now been identified for the successful rearing of oceanic phyllosomas; 1) nutrition, 2) hygiene (bacteria/virus control) and 3) hydrodynamics (tank design). Although Artemia and mussel gonad have been used a food source, poor survival and growth rate records indicate that they may not be ideal in terms of quantity and quality, particularly for late stage phyllosomas. Zooplankton (eg., *Sagittas*, Copepod), hydromedusa and fish larvae have also been tested previously, but have not been successful due to difficulties in maintaining a continuous supply. The development of artificial diets is one solution for overcoming the nutritional problem, and NIWA and CSIRO Cleveland are currently working on this.

Bacterial/viral control is another key issue for the rearing of long-lived oceanic larvae under controlled environments. Traditionally, a number of antibiotics (eg. OTC, Streptomycin, Chloramphenicol) and chemicals (eg. formalin) have been heavily used for controlling bacterial colonies in the rearing water. Though antibiotics and chemicals are strong agents for minimising bacterial growth in the laboratory, they are not considered a long-term solution for the large scale rearing of lobster larvae. Alternative disinfection methods, such as UV and Ozone (O_3), should be considered.

Tank design with consideration of hydrodynamics is crucial for the maintenance of fragile phyllosomas. Because of the phyllosomas' unique morphology (flat body and long appendages), strong aeration can damage body segments. No water movement, or only gentle water movement, can be used in the tank. Movement of rearing water also needs to take into consideration the phyllosomas' behaviours (swimming, feeding and phototactic), food distribution and maintenance of tank system.

Development of Thenus aquaculture

Bay Lobsters (*Thenus* spp.), commonly known in Australia as Moreton Bay bugs, live on the sandy or muddy sea floor in coastal waters up to 30m deep. In Queensland, Bay lobsters represent an estimated 4% of the annual commercial catch by weight, however they are becoming increasingly sought after as a valuable seafood product, emphasizing the need for aquaculture to meet these demands. Females spawn as many as 60,000 eggs per individual during the summer. Phyllosomas hatched from eggs go through 4 moult stages, then metamorphose to the benthic nisto stage. After 19 moults, juveniles can reach a typical market size of about 250g. The time taken from eggs to market size depends on temperature and food supply, but generally ranges 400 to 450 days. Despite the advantage of a short growout phase, there has been no record of commercial production of *Thenus* anywhere in the world. The major hurdle in the commercialisation of *Thenus* aquaculture has been the difficulty in maintaining the phyllosoma stages. Recently however, the scientific riddle of growing Bay lobsters in a laboratory from eggs to juveniles was solved, with consistent survival of over 80%. A pilot system for growing Bay lobsters has been operating successfully, and the establishment of a commercial scale operation is now underway.

Pilot systems for larval rearing of Thenus (at BIARC)

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