

**LARVI 2005 - PROCEEDINGS OF THE 4TH FISH AND SHELLFISH LARVICULTURE SYMPOSIUM, GENT, BELGIUM, SEPTEMBER 2005**

Volume 268, Issues 1-4, Pages 1-294 (22 August 2007)

Edited by Peter Bossier, S.J. Kaushik and Patrick Sorgeloos

**PREFACE**

Peter Bossier, Patrick Sorgeloos, Sachi Kaushik

This special volume is comprised of the selected invited papers presented at the plenary sessions of LARVI'05 held at the Ghent University, Belgium on September 5–8, 2005. The “LARVI” symposia (1991, 1995, 2001, 2005) are among the few international scientific symposia, which have been completely dedicated to larval fish and shellfish research. With the ever-increasing demand for aquatic products and the stagnating production from fisheries, the pressure is on the aquaculture industry to supply the world market with high quality products produced in a sustainable way. This can only be achieved through the support of multi-disciplinary research. This need is completely reflected in the ongoing trends in larval research. Sophisticated tools are being developed and improved to underpin a sound biological understanding of larval requirements. It is hoped that this special volume, which carries reviews and research papers, will contribute to a deepening of our knowledge and to the development of interesting research projects, supporting the profitability of the hatchery sector both for the industrial as well as the backyard type of hatchery.

The Symposium contributions were divided into sections on broodstock management; general larval biology, ecology and physiology; live food production; larval nutrition and feeding; larviculture zootechniques: microbiology and health management. The programme consisted of 44 oral and 127 poster presentations. Three workshop were held, namely Larviculture for restocking; Backyard hatcheries, Pre- and probiotics in larviculture. The Symposium was preceded by a one day symposium on contemporary evolutions in Artemia research and developments, commemorating the 25th anniversary of the Artemia Reference Center at the Ghent University and followed by a rotifer workshop. Conference details, including pdf files of most of the oral and poster presentations, can be found at <http://www.aquaculture.ugent.be//larvi/index.htm>.

LARVI'05 was co-organised by the Laboratory of Aquaculture & Artemia Reference Center of Ghent University (Belgium), Norwegian University of Science and Technology, Trondheim (Norway) and the Laboratory of Aquatic Ecology of the Catholic University of Leuven (Belgium). LARVI'05 was organised under the patronage of His Majesty Albert II, King of Belgium and was sponsored in part by the Flemish Interuniversity Council, the Research Council of Norway and the Flemish Science Foundation.

We are grateful to the following referees who assisted with the peer-reviewing and final selection of the papers submitted by the invited speakers: V. Alday, B. Austin, G. Bell, C. Boglione, C. Cahu, L. Conceição, L. D'Abramo, K. Dabrowski, J. Dhont, K. Dierckens, H. Fushimi, T. Hecht, J. Holt, K. Reitan, E. Kenchington, E. Kjorsvik, H. Komen, S. Kooijman, C. Langdon, A. Le Breton, E. Lubzens, P. Menasveta, E. Moksness, H. Nauwynck, F. Ollevier, I. Olsen, A.I. Olsen, J. Rainuzzo, K.I. Reitan, G. Rombaut, I. Ronnestad, J.F. Samain, A. Tandler, D. Tocher, O. Vadstein, F. Volckaert, M. Wille and D. Ziemann.

---

**BROODSTOCK CONDITION, EGG MORPHOLOGY AND LIPID CONTENT AND COMPOSITION DURING THE SPAWNING SEASON OF CAPTIVE STRIPED TRUMPETER, LATRIS LINEATA**

## Abstract:

The striped trumpeter, *Latris lineata*, is a pelagic marine fish found in southern Australia that is currently being developed as an aquaculture species. Striped trumpeters are multiple spawners with group synchronous oocyte development and spawn in the wild from late winter to early spring. By shifting light and temperature regimes, it is possible to manipulate broodstock to spawn in captivity outside of their normal ambient season. Broodstock condition, egg morphology and biochemistry were examined over a spawning season for three groups of broodstock held under ambient or photothermal-manipulated (PTM) conditions. Using a Torry Fish Fatmeter the muscle fat of male and female striped trumpeter broodstock held under ambient conditions was found to decrease over a season by 25% and 40%, respectively. Five general trends were observed in eggs spawned during both ambient and PTM seasons. First, overall egg volume remained unchanged. Second, the egg oil droplet volumes decreased to < 80% of those measured at the start of the spawning season. Third, there was a decrease in the triacylglycerol (TAG) fraction of egg total lipid and a concomitant increase in the proportion of polar lipid (PL). Fourth, the relative proportions of egg monounsaturated fatty acids (MUFA) decreased over a breeding season, probably as they were depleted because of the energy requirements of vitellogenesis. Fifth, proportions of the essential fatty acids (EFA), 20:4n-6 (arachidonic acid, ARA), 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA) was typical of marine fish eggs (1.8%, 10.1% and 20.9% of total fatty acids, respectively), and remained unchanged or were elevated in eggs over a season. Data suggest that holding broodstock under PTM or ambient conditions results in similar temporal changes in egg biochemistry, albeit more truncated during the PTM season. Despite ongoing feeding by striped trumpeter during spawning, the total energetic cost of vitellogenesis results in a depletion of lipid in the muscle that is in turn reflected in egg biochemistry. Finally fatty acid requirements for striped trumpeter larvae determined during the live feeding period are not too dissimilar from average egg fatty acid profiles, supporting the theory that egg values can provide a basis for estimating dietary requirements.

(Marine Research Laboratories, Tasmanian Aquaculture and Fisheries Institute and Aquafin Cooperative Research Centre, University of Tasmania, Private Bag 49, Hobart, Tasmania, 7001, Australia; email of S. Battaglene: [Stephen.Battaglene@utas.edu.au](mailto:Stephen.Battaglene@utas.edu.au))

## EFFECT OF PHOTOPERIOD MANIPULATION ON RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) EGG QUALITY: A GENOMIC STUDY

13-22

E. Bonnet, J. Montfort, D. Esquerre, K. Hugot, A. Fostier, J. Bobe

## Abstract:

The purpose of this study was to investigate the effect of photoperiod advanced spawning on egg quality and egg transcriptome. Experimentation was performed using 2 groups of rainbow trout (*Oncorhynchus mykiss*) from an autumn-spawning strain. In 2003, a first group of females was exposed, after their first natural spawning, to a long-short photoperiod regime beginning in January (photoperiod manipulated group, PM group, n = 17) resulting in a second reproduction in June-July. A second group of females achieved their first reproduction in the fall (2003) and was used as a control (n = 25). For each female, egg quality was assessed by monitoring survival at eyeing (E) and yolk-sac resorption (YSR) as well as the occurrence of malformations at YSR. We observed a significant decrease of egg quality in PM group. Survival at eyeing was  $49 \pm 18\%$  (means  $\pm 95\%$  CI) in PM group while a  $93 \pm 3\%$  survival was observed in control group. At YSR, the frequencies of alive alevins exhibiting no noticeable malformation were  $37 \pm 16\%$  in PM group and  $84 \pm 5\%$  in the control group. This increase of mortality was associated with a higher individual variability between females and an increase of malformations observed on alive alevins. These malformations were mostly characterized by defects of yolk-sac resorption (48% of malformations observed in PM group). Transcriptome analysis was performed using nylon microarrays displaying 9152 rainbow trout cDNAs spotted. Differential analysis allowed the identification of 6 genes significantly less abundant in PM eggs than in control eggs. These results suggest that photoperiod manipulation of spawning date can induce significant egg quality defects. Moreover, the transcriptome of unfertilized oocyte

appears to be influenced by environmental perturbations, such as photoperiod regime, and seems to be reflective of egg developmental capacities after fertilization.

(INRA, UR1037 SCRIBE, IFR 140, Ouest-Genopole, F-35000, Rennes, France ; email of J. Bobe : [Julien.Bobe@rennes.inra.fr](mailto:Julien.Bobe@rennes.inra.fr))

---

#### PROGRESS ON THE GENETICS OF REPRODUCTIVE PERFORMANCE IN PENAEID SHRIMP 23-43

Ana M. Ibarra, Ilie S. Racotta, Fabiola G. Arcos, Elena Palacios

Abstract:

A typical feature of penaeid shrimp larval production is that a small proportion of females with multiple spawns contribute to the production of the majority of nauplii. There is no evidence for deterioration in the condition of females and in offspring quality over consecutive spawning in a single generation. Multiple spawning capacity is genetically determined and can be a target in selection programs. Predictive phenotypic traits for selection of multiple spawners might be an important tool to increase larval production. Some of these traits already tested for their inheritance include latency to first spawn, number of spawns, fecundity, egg size, egg vitellin, egg acylglycerides, and egg proteins content, and body weight in mature females; and oocyte diameter and ovary maturity in subadult females. The present review focuses on the recent developments on the genetics of reproduction in shrimp, and on presenting what is known of some of the candidate genes involved in the multiple spawning capacities in shrimp: vitellogenin and sinus gland hormones (for which a peptide homology and phylogenetic analyses are included) and some of the enzymes involved in the biosynthetic pathway of non-peptide hormones. Finally, we present advances in the use of quantitative trait loci identification and gene expression technologies – microarrays – on the study of reproductive performance in other organisms, technologies that are expected to advance our understanding of shrimp reproduction in the future as denser genetic linkage maps and sufficient EST markers become available.

(Aquaculture Program, Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR), Mar Bermejo 195, Col. Playa Palo Sta. Rita, A.P. 128, La Paz B.C.S. 23000, Mexico; email of A. Ibarra: [aibarra@cibnor.mx](mailto:aibarra@cibnor.mx))

---

#### DEVELOPMENT OF ROTIFER STRAINS WITH USEFUL TRAITS FOR REARING FISH LARVAE

44-52

Atsushi Hagiwara, Koushiro Suga, Atsushi Akazawa, Tomonari Kotani, Yoshitaka Sakakura

Abstract:

The euryhaline rotifer *Brachionus plicatilis* is a species complex, which is commonly used for rearing marine fish larvae. Providing cultures with an appropriate size of rotifers facilitates size dependent selectivity of the feeding larvae and results in larvae with higher survival, growth and stability. It is also important to obtain rotifers with higher growth rate and tolerance against environmental stress and better nutritional quality after enrichment. This paper reviews the importance of feeding rotifers with appropriate size for fish larvae, as well as how much variation of rotifer traits can be expected from natural population of rotifers, as well as from artificial manipulation. These artificial manipulations include (1) manipulation of culture history to establish descendant rotifer clones with different reproductive traits, (2) manipulation of culture environmental conditions and chemical treatments on rotifer traits, such as size and reproduction, (3) cross-mating and a molecular approach for obtaining rotifer strains with useful traits in clones of their descendants. Recent progress on the production of rotifer strains with ideal characteristics in terms of size, population growth, tolerance against external conditions and resting egg formation is discussed.

(Graduate School of Science and Technology, Nagasaki University, 1-14 Bunkyo, Nagasaki 852-8521, Japan; email of A. Hagiwara: [hagiwara@net.nagasaki-u.ac.jp](mailto:hagiwara@net.nagasaki-u.ac.jp))

---

## THE ONSET OF EXOGENOUS FEEDING IN MARINE FISH LARVAE

53-63

M. Yúfera, M.J. Darias

Abstract:

The onset of exogenous feeding in fish larvae can be considered as the period from which the ingestion is possible up to the moment when larval growth is detected. The main characteristic of this phase is that the source of nutrient and energy necessary to continue the larval development changes from the yolk reserves to the ingested food. To achieve this transition with success it is necessary that all structures and organs related with food uptake, digestion and assimilation are ready in due time and that the appropriate food is available. This review will focus on the most relevant processes during this short period of early life history of marine fish: gut anatomy, digestive capacities, feeding behaviour and metabolism.

At the opening of the mouth and anus the yolk is completely or almost exhausted. The gut is a simple tube histologically differentiated in foregut, midgut and hindgut. The pancreas, liver and gall bladder are functional. From the first moment of feeding the larvae are able to ingest, digest and assimilate food particles. The digestion starts in an alkaline environment with the contribution of pancreatic enzymes as well as cytosolic enzymes. The main limitations at the beginning of exogenous feeding are mouth gape, restricting the particle size and larval length, restricting swimming capacity and hunting success. After the opening of the mouth, the organogenesis continues. A quick growth and differentiation of the digestive tract is necessary to reinforce digestion and nutrients absorption. A few days later the larval length and jaw size have increased enough to allow a more effective predation.

(Instituto de Ciencias Marinas de Andalucía (CSIC), Apartado Oficial, E-11510 Puerto Real, Cádiz, Spain; email of M. Yufera: [manuel.yufer@icman.csic.es](mailto:manuel.yufer@icman.csic.es))

---

## NUTRITIONAL PHYSIOLOGY DURING DEVELOPMENT OF SENEGALESE SOLE (SOLEA SENEGALENSIS)

64-81

Luís E.C. Conceição, Laura Ribeiro, Sofia Engrola, Cláudia Aragão, Sofia Morais, Marc Lacuisse, Florbela Soares, Maria Teresa Dinis

Abstract:

The Senegalese sole, a species with a complex metamorphosis, difficulties in weaning and with occasional problems of malpigmentation and skeletal deformities, is a good model species to study larval nutritional physiology. In addition, the early metamorphosis and acquisition of a peculiar non-proactive bottom-feeding behaviour make early weaning an important issue in sole hatcheries. The present work reviews recent findings in different aspects of nutritional physiology during the development of Senegalese sole, in an attempt to optimize the composition of sole diets and to understand what are the limiting factors for weaning sole. Both digestive enzymes activity and tracer studies using <sup>14</sup>C-Artemia show that sole larvae, even at young stages, have a high capacity for digesting live preys. This is reflected in a high growth potential and low mortality rates for this species during the larval stage compared to other marine fish species. Based on the observation of the digestive enzymes profile, early introduction of inert microdiets in co-feeding with Artemia does not seem to affect intestinal function. However, when co-feeding is not provided, intestinal activity may be depressed. Furthermore, early introduction of microdiets in co-feeding with Artemia may have a positive effect on survival rates, but at the expense of lower growth rates and higher size dispersal. This may reflect variation in the adaptation capacity of individual larvae to inert diets. High dietary neutral lipid (soybean oil) content results in reduced growth and accumulation of lipid droplets in the enterocytes and affects the capacity of Senegalese sole larvae to absorb and metabolise dietary fatty acids (FA) and amino acids (AA). Through tube feeding of different <sup>14</sup>C-lipids and free FA it has been shown that FA absorption efficiency increases with unsaturation and that sole larvae spare DHA from catabolism. In addition, it was demonstrated that absorption efficiency varies according to molecular form, being highest for free FA, lowest for triacylglycerols and intermediate for phospholipids. Live preys commonly used in larviculture do not seem to have a balanced AA profile for sole larvae. Furthermore, the ideal dietary AA composition probably changes during development.

Rotifers and *Artemia metanauplii* are apparently deficient in one or more of the following AA depending on the larval development stage: histidine, sulphur AA, lysine, aromatic AA, threonine and arginine. It has also been demonstrated that balancing the dietary AA profile with dipeptides in *Artemia*-fed larvae increases AA retention and reduces AA catabolism. When supplementing larval diets with limiting AA it should also be considered that sole larvae have different absorption, and retention efficiencies for individual AA, and that they have the capacity to spare indispensable AA. In addition, the absorption of free AA is faster and more efficient than that of complex proteins. Improvements in biochemical composition of inert microdiets for sole are likely to contribute to the reproducible weaning success of Senegalese sole.

(CCMAR-Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; email of Luís E.C. Conceição: [lconcei@ualg.pt](mailto:lconcei@ualg.pt))

---

#### DIGESTIVE PHYSIOLOGY OF MARINE FISH LARVAE: HORMONAL CONTROL AND PROCESSING CAPACITY FOR PROTEINS, PEPTIDES AND AMINO ACIDS

82-97

I. Rønnestad, Y. Kamisaka, L.E.C. Conceição, S. Morais, S.K. Tonheim

Abstract:

For the majority of marine fish larvae a fully developed digestive tract, including gastric digestion, is acquired weeks to months (depending on species) after onset of exogenous feeding. Still, the processing capacity (capability to degrade and absorb dietary nutrients) of the larval gut is sufficient to support fast larval growth by digesting prey naturally available in the sea. However, the physiological constraints of the gut with respect to digestion of cultivated live prey and particularly formulated starter feeds still remain to be elucidated. This paper reviews some recent findings in the areas of control and efficiency of digestive function of marine fish larvae. For studies on the hormonal control, the peptide hormone cholecystokinin (CCK) has been targeted, since it is believed to play an important role in controlling digestion in vertebrates. Recent work on the processing capacity include studies of the digestibility and transfer kinetics of macronutrients from live prey and experimental work on absorption of protein, peptides and free amino acids from the lumen of the digestive tract into the tissues of larval fish and how this changes during ontogeny. The molecular and in vitro characterization of transporters is currently being integrated with ongoing in vivo studies.

(Department of Biology, University of Bergen, Allégt 41, N5007, Bergen, Norway; email of I. Rønnestad: [ivar.ronnestad@bio.uib.no](mailto:ivar.ronnestad@bio.uib.no))

---

#### DIETARY MODULATION OF SOME DIGESTIVE ENZYMES AND METABOLIC PROCESSES IN DEVELOPING MARINE FISH: APPLICATIONS TO DIET FORMULATION

98-105

J.L. Zambonino Infante, C.L. Cahu

Abstract :

During these last 20 years, many studies have focussed on the development of the digestive tract in marine fish larvae. Most of the studies aimed at acquiring knowledge on the optimal form of dietary supply for different nutrients, in order to formulate a compound diet able to totally replace live preys in the fish larvae feeding sequence. Consequently, most of the studies aimed at describing the effect of dietary adaptation on digestive enzymes profile, the morphology of the main organs, while others aspects of the physiology of the larvae digestive tract (gut hormones, intestinal transport...) were poorly investigated.

This review reports the more recent data on dietary modulation of digestive enzymes with a particular emphasis, when possible, on the molecular and hormonal mechanisms controlling enzyme expression in larvae. We examined how the dietary modulation of pancreatic and intestinal enzymes involved in protein digestion can provide useful information concerning the nature and molecular form of a dietary protein supply that would be adequate for larval stages. In the same way, data on lipase and

phospholipase A2 paralleled with recent findings on lipid transport, strongly suggests that fish larvae handle phospholipids better than triglycerides.

A new field of research has been opened in fish larvae nutrition, with the study of the effects of some nutrients on the functioning of some metabolic pathways involved in development and their impact on larvae physiology and morphogenesis, as well as the further development of juveniles. These studies revealed cross-talks between some metabolic processes, particularly those concerning vitamin A and polyunsaturated fatty acids, during fish larvae development and unveil the necessity to design a global approach for determining the requirements of some nutrients.

(Nutrition, Aquaculture and Genomics of fish, UMR 1067, IFREMER, BP 70, 29280 Plouzané, France; email of J.L. Zambonino Infante: [jlzambon@ifremer.fr](mailto:jlzambon@ifremer.fr))

---

#### DIETARY NEUTRAL LIPID LEVEL AND SOURCE IN MARINE FISH LARVAE: EFFECTS ON DIGESTIVE PHYSIOLOGY AND FOOD INTAKE

106-122

S. Morais, L.E.C. Conceição, I. Rønnestad, W. Koven, C. Cahu, J.L. Zambonino Infante, M.T. Dinis

Abstract:

A growth depressing effect of high dietary neutral lipid levels in marine fish larvae has been reported. This may be a result of a decrease in the efficiency or activity of digestive enzymes, a reduction in absorption efficiency and/or a decrease in food intake. The present work reviews recent studies carried out on commercially valuable species (Atlantic herring, Senegalese sole, European seabass and gilthead seabream) that investigated the effects of neutral lipid level and lipid source (fatty acid composition) on some of these key factors influencing larval growth. The results seem to collectively indicate that lipid transport from the enterocytes into the body may be more problematic in larval stages dealing with high neutral lipid diets than lipolytic enzymatic capacity, although both factors are likely to intervene. In seabass, lipase activity was significantly affected by the source of dietary lipid but not by its dietary level. Lipid sources differing in chain length and degree of saturation of their fatty acids may thus affect the efficiency or synthesis of neutral lipase through effects on substrate specificity. Phospholipid digestion appears to be more efficient than that of neutral lipids, although neutral lipase synthesis might not be a limiting factor for larval growth. High neutral lipid larval diets result in the accumulation of large lipid droplets in the enterocytes, which may in turn reduce fatty acid absorption efficiency and ultimately larval growth. Nonetheless, not all fatty acids are equally affected and mechanisms of specific essential fatty acid absorption probably exist. Food intake in seabream larvae is not strictly regulated by total lipid content of the diet and lipid source may have an important role in controlling ingestion. Therefore, the neutral lipid level in diets for marine fish larvae has a significant impact in several factors influencing growth but clearly it cannot be dissociated of its fatty acid composition, which appears to play a central role on the nutritional and physiological effects of dietary lipid, at the ingestion, digestion and absorption levels.

(CCMAR, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal ; email of S. Morais : [smorais@ualg.pt](mailto:smorais@ualg.pt))

---

#### SALINITY STRESS TEST AND ITS RELATION TO FUTURE PERFORMANCE AND DIFFERENT PHYSIOLOGICAL RESPONSES IN SHRIMP POSTLARVAE

123-135

Elena Palacios, Ilie S. Racotta

Abstract:

This review evaluates the use of the salinity stress test (SST) as an index of postlarvae (PL) quality, either at the end of experimental treatments or to predict performance during stocking and growout. The SST is easy to apply and does not require specialized equipment, hence its popularity. However, predictive value of the test seems to be limited to short-term performance during future culture. To determine to what extent SST depends on osmoregulation, the mechanisms in crustaceans are briefly

reviewed, with particular emphasis on studies related to SST in PL penaeid shrimp. Two main physiological features of osmoregulation, active ion transport through the Na<sup>+</sup>/K<sup>+</sup>-ATPase pump and fatty acid composition of membranes affecting permeability to water and ions, cannot fully explain differential survival to an SST. This observation is derived from different experimental models, which include nutritional conditions or normal (genetic) variation. Other traits of the overall physiological condition appear to be involved and are not related to physiological regulation but rather are a result of a possible increased tolerance capacity.

(Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo 195, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23090, Mexico; email of E. Palacios: [epalacio@cibnor.mx](mailto:epalacio@cibnor.mx))

---

## THE EFFECT OF DIETARY INDISPENSABLE AMINO ACID IMBALANCES ON FEED INTAKE: IS THERE A SENSING OF DEFICIENCY AND NEURAL SIGNALING PRESENT IN FISH?

136-142

Konrad Dabrowski, Murat Arslan, b, Bendik F. Terjesen, Yongfang Zhanga

Abstract:

Most studies on amino acid imbalance and dietary selectivity in fish have been conducted using mammalian models. In rats, amino acid deficiencies cause a rapid, within minutes, decrease in food intake. In comparison, teleost fish have no aversion to indispensable amino acid-devoid diets, although possible taste aversion was not distinguished from neurological feedback. Therefore, it remains to be answered if amino acid attractant(s) can override neurological responses to amino acid deficiency in the diet. We report here for the first time the effects of dietary amino acid imbalances on the diet intake, and survival and growth response in a juvenile cichlid, midas (*Amphilophus citrinellum*). In the first experiment, a protein-based, a free amino acid-based diet (Free AA) and two indispensable amino acid (IDAA) devoid diets were tested. Fish responded initially by a decreased feed intake, but two weeks into the experiment, a significantly increased intake of the amino acid imbalanced diets was observed. In the second experiment, free amino acid imbalanced diets were offered to the fish using four different feeding strategy/treatments. These strategies were based on frequency of feeding imbalanced diets that changed from “different diet in every meal” to “different diet each other day”. However, every two days the amount of IAA provided to all groups was identical. Fish in all feeding treatments increased their body weight significantly, but their final weights decreased linearly with lower frequency of complimentary diet provision. There were significant differences in diet utilization when expressed as feed conversions. However, the most significant results demonstrated that weight gains calculated on the weekly basis improved significantly in all imbalanced/complimentary diet treatment groups. It is hypothesized that due to their high tolerance to amino acid imbalances, fish are the ideal model to re-examine metabolic responses to dietary disproportions of amino acids.

(The School of Environment and Natural Resources, The Ohio State University, Columbus, OH 43210, USA; email of K. Dabrowski: [dabrowski.1@osu.edu](mailto:dabrowski.1@osu.edu))

---

## COMPLEX MICROPARTICLES FOR DELIVERY OF LOW-MOLECULAR WEIGHT, WATER-SOLUBLE NUTRIENTS AND PHARMACEUTICALS TO MARINE FISH LARVAE

143-148

Chris Langdon, Brendan Clack, Umur Önal

Abstract:

Most currently available microparticulate artificial diets for rearing marine suspension feeders, including larval fish, are not effective in retaining low-molecular weight, water-soluble (LMWS) nutrients and other materials [Lopez-Alvarado, J., Langdon, C.J., Teshima, S., Kanazawa, A., 1994. Effects of coating and encapsulation of crystalline amino acids on leaching in larval feeds. *Aquaculture* 122, 335–346; Baskerville-Bridges, B., Kling, L.J., 2000. Development and evaluation of microparticulate diets for early weaning of Atlantic cod, *Gadus morhua*, larvae. *Aquac. Nutr.* 6, 171–182]. Rapid leaching losses occur as a result of high surface-to-volume ratios and the porosity of the

matrix or walls of the particles [Langdon, C.J., 2003. Microparticle types for delivery nutrients to marine fish larvae. *Aquaculture* 227, 259–275]. In order to address this problem of high leakage losses, a novel complex particle type was developed consisting of LMWS incorporated in lipid beads that are then bound, with other nutrients, in a matrix of the water-insoluble protein zein.

A spray method for preparing lipid beads was developed to increase the concentration of incorporated core material as well as to reduce losses due to leakage from beads suspended in water. It was possible to prepare lipid spray beads (LSB) that delivered 8.5% of their dry weight as glycine after 1-h suspension in water, resulting in free amino acid concentrations similar to those of commonly used live feeds for fish larvae, such as rotifers and *Artemia*; however, this delivery efficiency was reduced to 1.2% glycine for complex particles made up of 30% w/w glycine-containing LSB and other dietary nutrients. Further experiments are required to determine if complex particles can replace living feeds for the early larval stages of marine fish species.

(Coastal Oregon Experimental Station, Hatfield Marine Science Center, Oregon State University, Newport, OR 97365, USA; email of Chris Langdon: [chris.langdon@oregonstate.edu](mailto:chris.langdon@oregonstate.edu))

---

#### HOW INITIAL PREDATOR–PREY RATIO AFFECTS INTRA-COHORT CANNIBALISM AND GROWTH IN EURASIAN PERCH *PERCA FLUVIATILIS* L LARVAE AND JUVENILES UNDER CONTROLLED CONDITIONS

149-155

S.N.M. Mandiki, I. Babiak, J. Krol, J.F.R. Rasolo, P. Kestemont

Abstract:

Two experiments were conducted in order to study whether initial density of potential cannibals in a stock or their early life history inducing high initial size heterogeneity affects further quantitative characteristics in Eurasian perch larvae or young juveniles restocked under intensive culture. In experiment 1, populations of half-sibling larvae produced from three females were pooled and sorted out as small and large fish on Day 30 post-hatching. Then, five experimental variants were set with 0, 1, 2, 4 and 8% of the largest fish (considered as potential predators) in each triplicate group of 200-medium fish per 20 l. In experiment 2, half-sibling larvae from stock A were produced 8 days earlier than larvae from stock B. On Day 45 post-hatching, basic stocks were composed of small and/or medium fingerlings of stock B; and 8 variants were set with 0, 2 and 4% of the largest fish of stock B or medium fish of stock A, considered as potential cannibals. Survival and growth related parameters were measured after 39 (experiment 1) or 32 (experiment 2) days. Results from both experiments show that initial weights and their variation increased significantly with the initial density of potential cannibals in the stocks but this did not affect significantly further cannibalism rates whatever the size-class differences or the early life history of cannibals. Nevertheless, type II cannibalism tended to decrease at low initial density of predators and to increase at high proportion in experiment 1. At the end of each experiment, large-growing fish appeared in all groups and their density did not differ. Survival and growth parameters were not affected by the initial intensity of predators or their early life history, except for a slight decrease in growth rate at higher initial density of cannibals in experiment 1 in relation to high initial size heterogeneity.

Data from the present study indicate that cannibalism emergence is not consistently size dependent in Eurasian perch larvae or young juveniles, and that the re-establishment of this phenomenon at restocking is independent of the initial predator–prey relationship because size heterogeneity is negatively related to growth rate.

(Facultés Universitaires Notre-Dame de la Paix, URBO, 61 Rue de Bruxelles, B-5000 Namur, Belgium ; email of S. Mandiki : [robert.mandiki@fundp.ac.be](mailto:robert.mandiki@fundp.ac.be))

---

#### DEFORMITIES IN LARVAL GILTHEAD SEA BREAM (*SPARUS AURATA*): A QUALITATIVE AND QUANTITATIVE ANALYSIS USING GEOMETRIC MORPHOMETRICS



156-168

Yves Verhaegen, Dominique Adriaens, Tania De Wolf, Philippe Dhert and Patrick Sorgeloos

Abstract:

Deformities in commercially raised fish are a common source of downgrading of product value. During the intensive rearing of gilthead sea bream (*Sparus aurata*), opercular deformities are the most commonly observed type of deformation (affecting up to 80% of the fisheries stock), sometimes showing a severe inward folding of the operculum. They are non-lethal malformations that appear during the larval stage but affect growth rate and morphology, with a significant economic loss as a consequence. In order to exploratory quantify and qualify these deformities, geometric morphometric analyses were performed on the external morphology from larvae with an age ranging from 50 to 69 days post-hatching (DPH), and on the cranial skeleton of 110 DPH old juveniles. The results showed several osteological cranial shifts and a striking left–right independency associated with deoperculation. Even though a significant size difference was observed at 65 DPH between normal and deoperculated specimens, allometries during the examined growth stages still appear to be very similar in normal and deoperculated specimens. At 69 DPH deoperculated specimens differed significantly from the normal specimens in their external morphology based on its shape variables, but the results suggest that discrimination is possible from earlier stages. Further analyses are needed, but the usefulness of this approach towards developing an early detection tool could be demonstrated.

(Evolutionary Morphology of Vertebrates, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium; email of D. Adriaens: [dominique.adriaens@ugent.be](mailto:dominique.adriaens@ugent.be))

---

#### QUALITY OF HATCHERY-REARED JUVENILES FOR MARINE FISHERIES STOCK ENHANCEMENT

169-180

L. Le Vay, G.R. Carvalho, E.T. Quintio, J.H. Leбата, V.N. Ut, H. Fushimi

Abstract:

The potential for stock enhancement by release of hatchery-reared juveniles continues to be a topic of interest to researchers and fisheries managers. While, in many studies, the focus has tended to be on the technology for production of juveniles, the need for a more multidisciplinary approach is now becoming accepted. Ideally, this includes studies of population dynamics and recruitment-limitation of wild stocks, environment–stock interactions, habitat availability, genetic studies of wild and released stocks and integration with appropriate fisheries management. While it may be relatively straightforward to culture large numbers of seed animals, the quality of hatchery-reared juveniles may limit the effectiveness of any release programme. The quality of juveniles may be defined either by their ability to attain the age and size to recruit to a commercial fishery or their fitness to survive to contribute to the spawning stock. Many factors will inevitably influence batch–batch variability in the viability of hatchery-reared juveniles and their ability to recruit and compete in the wild. Some effects of nutrition and environment in the hatchery are well-known or at least recognised and their manipulation offers the potential for improvement of survivorship of juveniles post-release. The choice and utilisation of broodstock also represent a crucial stage in enhancement programmes, and considerations of bottleneck effects arising from reduced effective population size due to skewed parental and family contributions must be given careful consideration. A broodstock design that encompasses sufficient numbers of animals that reflect the genetic, and preferably ecological, identity of the stocks to be enhanced should be adopted. In addition, environmental conditions and husbandry practices within the hatchery as well as broodstock and larval nutrition can all influence the quality of offspring. Further conditioning and/or selection during nursery culture may also be critical in maximising the physiological and behavioural fitness of hatchery juveniles post-release. Although evaluation of long-term performance of individual batches of juveniles requires considerable effort or may be impossible in some cases, this type of quantification is likely to be an important component in the determination of the effectiveness of release programmes. This paper reviews the effects of hatchery and nursery practice on larval and juvenile fitness for stock enhancement and presents examples of comparisons of the quality of wild and hatchery-reared juveniles and the effect of pre-release conditioning on subsequent survival and growth.

(School of Ocean Sciences, University of Wales Bangor, Menai Bridge, Anglesey, Wales, LL59 5EY, UK; email of L. Levay: [l.levay@bangor.ac.uk](mailto:l.levay@bangor.ac.uk))

---

#### A REVIEW OF THE LARVICULTURE OF COBIA RACHYCENTRON CANADUM, A WARM WATER MARINE FISH

181-187

G. Joan Holt, Cynthia K. Faulk, Michael H. Schwarz

Abstract:

*Cobia* *Rachycentron canadum* is a marine finfish species with emerging global potential for mariculture. Positive culture attributes include capacity for natural and induced tank spawning, growth rates in excess of 6 kg/year, high disease resistance, high survival rates (post-larviculture stage) in tanks and net pens, adaptability to commercially available extruded diets, and high-quality fillets suitable for the sashimi as well as white tablecloth restaurant markets. Nonetheless, cobia production faces several bottlenecks limiting industrial expansion including limitations in fingerling production per unit volume. This paper will provide an overview of production limitations, and focus on recent spawning and larviculture research results and ongoing research initiatives.

(University of Texas at Austin Marine Science Institute, 750 Channel View Drive Port Aransas TX 78373 USA; email of G. Joan Holt: [joan@utmsi.utexas.edu](mailto:joan@utmsi.utexas.edu))

---

#### COD JUVENILE PRODUCTION: RESEARCH AND COMMERCIAL DEVELOPMENTS

188-194

Grethe Rosenlund, Ólafur Halldórsson

Abstract:

Atlantic cod (*Gadus morhua*) has been a new candidate for aquaculture for some decades in countries around the North Atlantic Ocean. Greatest interest in developing a cod farming industry is found in Norway, Canada and the United Kingdom as well as in Iceland and the USA (north east). Modern hatchery methods for cod using cultivated plankton such as rotifers and *Artemia* were first described in 1984, but it took more than 15 years of dedicated research and development before these efforts now are being translated into up scaled hatchery protocols. Protocols for keeping reproductive stocks of Atlantic cod under different photoperiods have been established resulting in year around production of fertilised eggs. Hatcheries commonly keep 3–5 different stocks to achieve this. The relationship between brood stock nutrition, external cues and reproductive performance is largely unknown and should be studied further. Cod larvae have a very high growth potential (> 20% day<sup>-1</sup>), they are aggressive feeders and can be cannibalistic if food availability is sub optimal. Both temperature and light are found to influence growth in larval cod. Research has addressed cod larval nutrition, both qualitative and quantitative requirements. Also, development of feeds and feeding strategies that would allow early weaning onto inert diets has been a primary objective in cod juvenile production research. Using a diet designed to support larval requirements, weaning can start from 15–20 days post hatch. This strategy has resulted in satisfactory growth and survival without use of *Artemia*, thus reducing the dependency on live feeds. Huge variation in survival and a high incidence of deformities have been reported in hatchery-reared cod juveniles. These observations have been ascribed partly to unfavourable environmental conditions (gas super-saturation) and the problem has to a large extent been solved. Methods to produce large numbers of cod juveniles have been implemented in several Atlantic countries in recent years, and present hatchery capacities can support an annual production of several hundred thousand tons of farmed cod. Common practices in cod hatcheries are presented and future perspectives on cod juvenile production are discussed.

(Skretting Aquaculture Research Centre AS, P.O.Box 48, N-4001 Stavanger, Norway; email of G. Rosenlund: [grethe.rosenlund@skretting.com](mailto:grethe.rosenlund@skretting.com))

---

#### ADVANCES IN THE CULTURE OF STRIPED TRUMPETER LARVAE: A REVIEW

195-208

S.C. Battaglione, J.M. Cobcroft

Abstract:

Striped trumpeter, *Latris lineata*, was chosen as the best new candidate for sea cage culture in Tasmania in the late 1980s. It has a complex and extended post-larval or 'paperfish' stage lasting up to 9 months and has historically proven difficult to culture. Excellent progress has been made in understanding and controlling reproduction and broodstock are spawned year-round through photothermal control. Problems with early larval rearing have been overcome and egg incubation and early larval rearing protocols have been established. A mortality peak associated with first feeding has been reduced using better live feed production techniques and improved water quality. Using antibiotics showed that high bacterial loads were an important factor in larval mortality. A new water filtration and ozonation system has removed the need for antibiotics. Larval nutrition research focused on the link between potential deficiencies or imbalances in the three essential PUFA in live feeds: docosahexaenoic acid, eicosapentaenoic acid and arachidonic acid. Novel experimental emulsions were applied with dose response experimental designs to identify the dietary requirement for selected PUFA and vitamins. Despite the advances in live feed enrichments, the live feeds, particularly *Artemia*, were found to have sub-optimal lipid profiles. Copepods were cultured, as a supplement to traditional live feeds, and improved larval rearing success. Costs to scale-up production and to control extensive cultures presently restrict the usefulness of copepods. Important breakthroughs have occurred in health with the detection and control of nodavirus, myxozoan and bacterial disease. Ozone disinfection of eggs and sterilisation of hatchery seawater have been important control measures. Another bottleneck to production has been mortality of larvae from notochord flexion to metamorphosis. System changes to reduce nocturnal movements and a better understanding of optimal live feed densities, and weaning onto formulated diets, have improved survival and growth. High rates of jaw malformation remain a challenge and no definitive cause has been established. Reduced rates of malformations have been associated with one or a combination of high feed rates, lower larval densities and temperatures, and reductions in 'walling' behaviour. Future research is aimed at finding ways to reduce malformations, develop probiotics and early weaning strategies, control parasites and scale-up production to assess performance of juveniles in sea cages.

(Marine Research Laboratories, Tasmanian Aquaculture and Fisheries Institute and Aquafin Cooperative Research Centre, University of Tasmania, Private Bag 49, Hobart, Tasmania, 7001, Australia; email of S. Battaglione: [stephen.battaglione@utas.edu.au](mailto:stephen.battaglione@utas.edu.au))

---

## FLOW FIELD CONTROL FOR LARVICULTURE OF THE SEVEN-BAND GROUPEP EPINEPHELUS SEPTEMFASCIATUS

209-215

Yoshitaka Sakakura, Shigeaki Shiotani, Hisashi Chuda, Atsushi Hagiwara

Abstract:

This paper reviews recent findings on establishing flow field control for larval rearing by hydrodynamics approach from case studies of the seven-band grouper (*Epinephelus septemfasciatus*). We chose this species because grouper larvae are known to be highly sensitive to physical stress, although it is expected for the new target species of aquaculture and stock enhancement in Japan. We quantified and visualized the flow field by aeration in the rearing tank with hydrodynamics approach. A series of systematic experiments was conducted to evaluate the optimal flow for the survival of larvae in the 1 m<sup>3</sup> rearing tanks. Aeration at 200 mL/min produced the highest survival and growth for grouper larvae and the flow field was identified and then a computing method for estimating the flow field was established. We expanded this approach to the mass-culture scale (100 m<sup>3</sup>) and controlled the flow field similar to the optimal flow field in the 1 m<sup>3</sup> rearing tank. It was quite successful resulting in 3 times higher survival than the former rearing methods. The results from these studies may be very useful for estimating the stationary flow in a rearing tank and for designing suitable tanks for rearing larvae. We also discussed the possibility of larviculture without aeration.

(Faculty of Fisheries, Nagasaki University, Nagasaki 852-8521, Japan; email of Yoshitaka Sakakura: [sakakura@nagasaki-u.ac.jp](mailto:sakakura@nagasaki-u.ac.jp))

---

ESTIMATING LARVAL DENSITY IN COD (*GADUS MORHUA*) FIRST FEEDING TANKS USING MEASUREMENTS OF FEED DENSITY AND LARVAL GROWTH RATES

216-226

Morten Omholt Alver, Jo Arve Alfredsen, Gunvor Øie

Abstract:

Due to unpredictable mortality in larval cod rearing, a reasonably accurate estimate of the larval biomass in rearing tanks is important for production management such as determination of feed doses and planning of live feed production. A good estimate of larval numbers can also give an early warning if a larval group is suffering high mortality. Because direct measurement of the larval density is difficult, a model based estimator is developed to estimate the larval density from parameters that are simpler to measure, such as feed density and larval size. The estimator is based on an extended Kalman filter using measurements to update a process model.

The estimator was tested by acquiring data on feed densities and larval growth rates in a first feeding experiment on cod. Cod larvae were reared from hatching up to day 16 post hatch in nine 160 l tanks at three different densities. The larvae were fed with rotifers (*Brachionus plicatilis*) from day 3 until the end of the experiment. Results show that the estimator is able to correctly detect differences in larval density.

(Department of Engineering Cybernetics, Norwegian University of Science and Technology, Odd Bragstads plass 2D, 7491 Trondheim, Norway; email of Morten Omholt Alver: [alver@itk.ntnu.no](mailto:alver@itk.ntnu.no))

---

GENETICALLY BASED RESISTANCE TO SUMMER MORTALITY IN THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*) AND ITS RELATIONSHIP WITH PHYSIOLOGICAL, IMMUNOLOGICAL CHARACTERISTICS AND INFECTION PROCESSES

227-243

J.F. Samain, L. Dégremont, P. Soletchnik, J. Haure, E. Bédier, M. Ropert, J. Moal, A. Huvet, H. Bacca, A. Van Wormhoudt, M. Delaporte, K. Costil, S. Pouvreau, C. Lambert, V. Boulo, P. Soudant, J.L. Nicolas, F. Le Roux, T. Renault, B. Gagnaire, F. Geret, I. Boutet, T. Burgeot, P. Boudry

Abstract:

Summer mortality of Pacific oysters is known in several countries. However no specific pathogen has been systematically associated with this phenomenon. A complex combination of environmental and biological parameters has been suggested as the cause and is now starting to be identified. A high genetic basis was found for survival in oysters when a first generation (G1) was tested in three sites during summer. This paper presents a synthesis on physiological characteristics of two selected groups ('R' and 'S', from families selected for resistance and susceptibility to summer mortality respectively), of the second and third generations. R and S showed improvement or reduction of survival compared with the control in both field and laboratory trials confirming the high heritability of survival of juveniles < 1 year old. Interestingly, no correlation was observed between growth and survival.

Comparison between the two selected groups showed that S oysters invested more energy in reproduction and stayed a longer time without spawning than R oysters which had high synchronous spawning. This was mainly shown with high rather than low dietary rations (respectively 12% and 4% DW algae/DW oyster) in a controlled experiment. Moreover, early partial spawning was detected in S oysters and not R ones in the high dietary ration. S showed a higher respiration rate and an earlier decrease in absorption efficiency than R during gametogenesis, but they were not significantly different in glycogen or ATP utilisation. Two months before a mortality episode, hemocytes from S oysters had a higher adhesive capacity than R hemocytes and significantly higher reactive oxygen species production capacity. One month before mortality, S oysters had the highest hyalinocyte concentration and their expression of genes coding for glucose metabolism enzymes (Hexokinase, GS, PGM, PEPCK) was significantly lower in the labial palps. After a thermal increase from 13 °C to

19 °C, during 8 days in normoxia, S oysters showed a large HSP70 increase under hypoxia contrary to R oysters, suggesting their high susceptibility to stress. Their catalase activity was lower than in R oysters and showed no further change to subsequent hypoxia and pesticide stresses, in contrast to R oysters.

These observations suggest possible links between higher reproductive effort in S oysters, their specific stress response to temperature and hypoxia, ROS production, partial spawning, hyalinocyte increase and the infection process. To compare R and S oysters in a more integrated way, a suppression subtractive hybridisation (SSH) library and a micro-array strategy are being undertaken. (UMR 100 Physiologie et Ecophysiologie des Mollusques Marins, Ifremer, Centre de Brest, B.P 70, 29280 Plouzané, France ; email of J.F. Samain : [jfsamain@ifremer.fr](mailto:jfsamain@ifremer.fr))

---

MATERNAL TRANSFER OF IMMUNITY AND ONTOGENY OF AUTOLOGOUS IMMUNOCOMPETENCE OF FISH: A MINIREVIEW  
244-250

Iván Mulero, Alfonsa García-Ayala, José Meseguer, Victoriano Mulero

Abstract:

The effectiveness of immunostimulation and vaccination of cultured fish heavily depends on the degree of maturity of their immune system. In this review, we summarise the recent data on the maternal transfer of humoral innate and adaptive immune parameters of fish larvae that seems to be responsible for the protection of embryos and larvae in the early stages of their development. We also look at the onset of fish immunocompetence in relation to the appearance of the different cellular components of both innate (macrophages and granulocytes) and adaptive (T and B lymphocytes) immunity. In addition, the advantages of using the powerful zebrafish model to study the development of teleost immunity are mentioned. Finally, all these data are taken into account before proposing future directions that are likely to shed light on the ontogeny of immunity in teleosts and to help in the development of appropriate vaccination regimens for either broodstock or larvae.

(Department of Cell Biology, Faculty of Biology, University of Murcia, 30100 Murcia, Spain; email of I. Mulero: [vmulero@um.es](mailto:vmulero@um.es))

---

BACTERIAL TRANSLOCATION AND PATHOGENESIS IN THE DIGESTIVE TRACT OF LARVAE AND FRY  
251-264

Einar Ringø, Reidar Myklebust, Terry M. Mayhew, Rolf Erik Olsen

Abstract:

The digestive tract of fish is essentially a muscular tube lined by a mucous membrane of columnar epithelial cells that exhibit regional variation in structure and function. In the last two decades, our understanding of translocation of bacteria in the fish gut has increased, and electron microscopy has contributed to this knowledge. Translocation is the movement of viable and non-viable bacteria and bacterial products (such as endotoxin) from the intestinal lumen through the epithelial mucosa to infect otherwise sterile tissues. This paper provides an overview of the gastrointestinal tract of larvae and fry from an electron microscopical perspective focussing on factors (mucus, interactions between probiotics and pathogens, and stress) affecting translocation of bacteria (transcellular and paracellular), cellular damage (specific attack on tight junctions and desmosomes caused by pathogenic bacteria) and pathogenesis. Furthermore, the paper will present information on the occurrence and translocation of pathogenic bacteria in the gastrointestinal tract of larvae, fry and adult fish.

Currently, it is generally accepted that the major routes of infection in fish are through the skin, gills and gastrointestinal tract. As the gastrointestinal tract is a potential port of entry for pathogens, the use of electron microscopy in studies on microbiota in fish digestive tract is a valuable method for increasing our understanding of the mechanisms involved in entry of the pathogens. Possible

mechanisms involved in translocation of bacteria in fish gastrointestinal tract, autochthonous (indigenous) and pathogenic, are discussed.

(Aquaculture Protein Centre, Section of Arctic Veterinary Medicine, Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, Tromsø, Norway; email of Einar Ringø: [Einar.Ringo@nfh.iut.no](mailto:Einar.Ringo@nfh.iut.no))

---

#### AN ORAL NERVOUS NECROSIS VIRUS VACCINE THAT INDUCES PROTECTIVE IMMUNITY IN LARVAE OF GROUPEL (EPINEPHELUS COIOIDES)

265-273

Chin-Chiu Lin, John Han-You Lin, Ming-Shyan Chen, Huey-Lang Yang

Abstract:

Nervous necrosis virus (NNV) is a major viral pathogen that infects grouper and other fish at their larval stage. This infection often causes mortality rates higher than 99% and leads to total losses in the hatchery; hence, it is important to develop a preventive vaccine. However, the onset of this disease at the larval stage, with fish small and sensitive to handling makes the vaccination by injection or immersion impossible.

In this report, we describe an oral NNV vaccine composed of Artemia-encapsulated recombinant *E. coli* expressing the NNV capsid protein gene. The NNV VP-containing Artemia were used to vaccinate grouper larvae. Immuno-histochemical analysis showed antigen to be delivered to, and absorbed in, the hindgut of grouper, and that it induced anti-NNV VP specific antibodies 7 days after vaccination, as assayed by ELISA. The vaccinated larvae showed a certain degree of protection after challenge with NNV achieving a Relative Percentage Survival of 64.2% and 69.5%. Oral NNV vaccine could effectively immunize grouper larvae. This method could be expanded to the development of other oral vaccines and for use in other fish species.

(Institute of Biotechnology and Research Center of Ocean Environment and Technology, National Cheng Kung University, Tainan, 701, Taiwan; email of Huey-Lang Yang: [hlyang@mail.ncku.edu.tw](mailto:hlyang@mail.ncku.edu.tw))

---

#### MICROBIOLOGICAL ASPECTS OF PHYLLOSOMA REARING OF THE ORNATE ROCK LOBSTER PANULIRUS ORNATUS

274-287

David Bourne, Lone Høj, Nicole Webster, Matthew Payne, Mette Skindersø, Michael Givskov, Mike Hall

Abstract:

Rock lobsters of the Palinuridae are the most valuable wild fisheries sector in Australia and are currently target aquaculture species. Significant challenges exist however to produce commercial scale quantities of post-larvae due to an extended larval phase which exacerbates a high rate of larval attrition caused by inadequate nutrition and a challenging microbial environment. Here we investigate a diverse and varied bacterial community in four compartments of the larval-rearing system: the water column, the biofilm, live feeds and the phyllosomas themselves. External fouling of phyllosoma by filamentous *Thiothrix* sp. was documented by scanning electron microscopy (SEM) and fluorescence in situ hybridisation (FISH). Internal proliferation of bacteria coinciding with mass mortality of phyllosoma was observed in histopathological analysis and identified as *Vibrio* sp. by specific labelling of sectioned hepatopancreas tissue using FISH. Of particular interest in relation to larval mortalities was a range of *Vibrio* species, isolated from the four rearing compartments, closely affiliated with *V. alginolyticus*, *V. parahaemolyticus*, and *V. harveyi*. The presence of bacterial quorum sensing signal molecules within the system was demonstrated in both biofilm and phyllosoma environments during a larval-rearing run. Interestingly, a large increase in quorum sensing signal molecules was detected in phyllosoma corresponding with mass mortality.

(Australian Institute of Marine Science, Townsville, Queensland, Australia; email of M. Hall: [m.hall@aims.gov.au](mailto:m.hall@aims.gov.au))

---

## BIOCONTROL OF PATHOGENS IN SHRIMP HATCHERIES USING BACTERIOPHAGES

288-292

Indrani Karunasagar, M.M. Shivu, S.K. Girisha, G. Krohne, Iddya Karunasagar

Abstract:

Microflora associated with larval stages of shrimp could affect the health and development of the larvae. Some bacteria such as luminous *Vibrio harveyi* cause serious mortalities. Consequent to the ban on use of most antibiotics in aquaculture, there is a need for alternate technologies for control of bacterial pathogens. Bacteriophages have a potential to control bacterial pathogens. This paper reports isolation of lytic bacteriophages against *V. harveyi*. Four bacteriophages were isolated, three from oyster tissue and one from shrimp hatchery water. The bacteriophages lysed 55–70% of the 100 *V. harveyi* isolates tested. Two bacteriophages subjected to further study were found to belong to family Siphoviridae and were effective in reducing *V. harveyi* population in biofilm formed on high density polyethylene (HDPE) surface. In hatchery trials, bacteriophage treatment at  $2 \times 10^6$  pfu ml<sup>-1</sup> level resulted in over 85% survival of *Penaeus monodon* larvae suggesting that bacteriophage therapy would be an effective alternative to antibiotics in shrimp hatcheries.

(Department of Fishery Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore 575002, India; email of I. Karunasagar: [mircen@sancharnet.in](mailto:mircen@sancharnet.in))

---