

INTEGRATED PRODUCTION OF LONG CHAIN POLYUNSATURATED FATTY ACIDS (PUFA)-RICH SCHIZOCHYTRIUM BIOMASS USING A NUTRIENT SUPPLEMENTED MARINE AQUACULTURE WASTEWATER

I.S. Jung, R.W. Lovitt-2010

Aquacultural Engineering 43(2): 51-61

Abstract:

Schizochytrium limacinum SR21 was used to remove waste nutrients from a marine recirculating aquaculture wastewater while simultaneously producing a valuable biomass containing PUFA enriched with long chain fatty acids which may have potential as an aquafeed additive.

Clean seawater and marine aquaculture wastewater supplemented with yeast extract (1.0–10 g L⁻¹) and glycerol (10–90 g L⁻¹) were used for a comparative study of growth and PUFA production using *S. limacinum* in batch, continuous and in a packed bed reactor (pall rings, surface area 340 m²/m³) that allow surface growth and cell recycling for a high cell density culture. Good growth rates ($\mu = 0.013$ h⁻¹) were obtained in batch and continuous culture modes. Biomass of *S. limacinum* was increased from 4.0 g L⁻¹ as DCW in batch culture to 57.9 g L⁻¹ in retention culture ($D = 0.062$ h⁻¹). Retention culture of *S. limacinum* in a packed bed reactor also produced total lipids of 36.5 g L⁻¹ (53–65%, w/w as DCW) and long chain fatty acids (>C22) of 12.6 g L⁻¹ (21.8%, w/w as DCW) in 350 h. Long chain fatty acids of C22, C18 and C16, as the main fatty acids, were produced and production was enhanced when supplemented wastewater was used in growth medium. Changes to the relative concentrations of glycerol and yeast extract (C:N ratio) alter the amounts of oil produced and types of fatty acid produced.

S. limacinum in continuous culture mode ($D = 0.013$ h⁻¹) enabled significant reductions (<90%) in phosphorous, nitrogen and ammonia concentrations of marine aquaculture wastewater and efficiency of wastewater treatment was further improved in retention culture operating at $D = 0.062$ h⁻¹. This system would be well suited to treating nutrient concentrated effluent from sludge sedimentation/dewatering tanks in recirculating aquaculture systems (RAS). The treatment capacity of the reactor was considerable and in this current study, 420 g N, 190 g P and 10 g total NH₃ day⁻¹ m⁻³ reactor could be removed using the retention culture system.

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CHARACTERISTICS OF OXYGEN FLOW THROUGH FINE BUBBLE DIFFUSERS USED IN THE AQUACULTURE HAULING APPLICATIONS

John Colt, Eric Kroeger, Michael Rust-2010

Aquacultural Engineering 43(2): 62-70

Abstract:

The oxygen flow rate was studied for carbon stone and ceramic plate diffusers, two types of diffusers that are commonly used in hauling applications. New diffusers were used in all experiments. It was found that oxygen flow was largest for dry diffusers operated in air and reduced for diffusers started in air and then placed in water and further reduced for diffusers soaked in water prior to use. The coefficient of variation for the two types of diffusers ranged from 7 to 18% for new units. If individual flow adjustment is not provided for each diffuser in a manifold distribution system (one oxygen source, several diffusers), small differences in pressure losses between diffusers can result in significant differences in oxygen flows. The initial oxygen flow during the loading of fish may be depressed until accumulated water in the hose and diffuser is expelled. Under some conditions, the oxygen flow rate can increase by 2–4 times over a 6–8 h period. Decreases in oxygen flow due to prior history may make it difficult to achieve the needed operating flows in a timely manner without exceeding the diffuser's pressure rating. This problem is likely to become more important due to aging and clogging of the diffusers.

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EVALUATION OF NITRIFYING BACTERIA PRODUCT TO IMPROVE NITRIFICATION EFFICACY IN RECIRCULATING AQUACULTURE SYSTEMS

David D. Kuhn, David D. Drahos, Lori Marsh, George J. Flick Jr.-2010

Aquacultural Engineering 43(2): 78-82

Abstract:

Recirculating aquaculture systems (RASs) rely on nitrification to convert toxic ammonia and nitrite to less toxic nitrate. Nitrification is accomplished using biofilters with nitrifying bacteria and can be inefficient in biofilters that are new or that have been compromised due to stressors. Failure in a biofilter can result in very high levels of ammonia or nitrite, both of which are toxic to aquatic animals and can result in health issues, suppressed growth, and mortalities. For these reasons, a commercially available nitrifying bacterial product (Pond Protect-L®, Novozymes Biologicals Inc., Salem, VA, US) was tested in both a controlled pilot-scale experiment and within production-scale RAS that were experiencing elevated levels of nitrite. Juvenile Pacific white shrimp (*Litopenaeus vannamei*) were used as the aquatic species in both the pilot- and production-scale systems. In the pilot-scale experiment, three aquaria received no product (control) and three systems received bacterial product using dosing specification as recommended by the manufacturer. Control systems experienced significantly ($P < 0.05$) higher levels of ammonia and nitrite compared to the tanks that received bacterial product. Quantitative PCR was used as tool to verify the presence of nitrifying bacteria (*Nitrobacter winogradskyi* strain in the bacterial product) in the systems that received bacterial product. Meanwhile, none of these nitrifiers were detected in the control systems. Similar nitrification benefits were noted in the production systems that received bacterial product. More specifically, improved nitrite-oxidation was observed. Within 2 days of the addition of bacterial product, nitrite levels began to decrease in the production systems and after a steady decrease remained within safe limits for the entire shrimp culture period.

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EFFECT OF AQUAVAC™ VIBROMAX™ ON SIZE AND HEALTH OF POST LARVA STAGE OF PACIFIC WHITE SHRIMP *LITOPENAEUS VANNAMEI* AND BLACK TIGER SHRIMP *PENAEUS MONODON*

J. Wongtavatchai, M.V. López-Dóriga, M.J. Francis-2010

Aquaculture 308(3-4): 75-81

Abstract:

This study was conducted to evaluate the efficacy of AquaVac™ Vibromax™, an inactivated *Vibrio* bacterin product, to promote health and resistance to vibriosis in penaeid shrimp post larva (PL). Black Tiger shrimp (*Penaeus monodon*) and Pacific White shrimp (*Litopenaeus vannamei*) were fed *ad libitum* with Vibromax™-enriched *Artemia* for 10 consecutive days, PL5–PL14. *Artemia nauplii* were enriched by incubating 150 g *Artemia nauplii* in 1 L Vibromax™ for 1.5 h prior to feeding. Varying dosages; half, single and two times overdose were compared for their effectiveness. A control group was fed on normal (non-enriched) *Artemia*. Shrimp PLs were challenged with a single immersion bath of $1.0\text{--}1.4 \times 10^5$ CFU/ml *Vibrio parahaemolyticus* on day one (PL15) or day seven (PL21) following the 10 day feeding treatment to test their disease resistance, and the survival rates amongst groups were compared. Shrimp PLs were observed for their growth (body wet weight and length) and survival over the experiment until PL23. Histological preparation of the hepatopancreas and other visceral organs were examined for general health condition of the shrimp PLs.

The body wet weight and length of shrimp PLs following a 10 day feeding trial indicated that the use of Vibromax™ promoted growth in both Black Tiger shrimp (average length and weight in treated groups at PL23: 1.77 ± 0.08 cm and 26.84 ± 2.50 mg; length and weight in control group at PL23: 1.72 ± 0.19 cm and 22.11 ± 7.19 mg) and Pacific White shrimp PLs (average length and weight in

treated groups at PL23: 1.71 ± 0.01 cm and 31.82 ± 2.85 mg; length and weight in control group at PL23: 1.46 ± 0.37 cm and 23.08 ± 12.95 mg). The health promoting effect of Vibromax™ was also supported by the higher survival of PL23 in Black Tiger Shrimp from the treatment groups (average Relative Percent Survival: 52 %, 72 %, and 48 % for half dose, single dose, and two times overdose, respectively). Histological examination of hepatopancreatic tissue from the treated PLs did not demonstrate any adverse effect of the treatments. Although hepatopancreatic inclusion bodies, indicating pathological signs of monodon baculo virus (MBV) infection, were observed in certain populations of Pacific White shrimp PLs, the PLs that received the feeding treatment generally demonstrated better growth and survival than those of the control group.

Overall results from the present study support the health promoting effect of Vibromax™ on growth and survival of shrimp PLs. The efficiency varied according to the dosage of Vibromax™ administered and pre-existing health condition of the PL.

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PRESERVED COPEPODS AS A NEW TECHNOLOGY FOR THE MARINE ORNAMENTAL FISH AQUACULTURE: A FEEDING STUDY

I. Olivotto, N.E. Tokle, V. Nozzi, L. Cossignani, O. Carnevali-2010

Aquaculture 308(3-4) : 124-131

Abstract:

The aim of this study was to evaluate the potential use of preserved copepod as prey in *Amphiprion clarkii* larviculture. After hatching, *A. clarkii* larvae were divided in three experimental groups for feeding studies as follows: group A (control group) fed rotifers (*Brachionus plicatilis*) followed by *Artemia* nauplii; group B fed a mixed diet of rotifers-*Artemia salina* nauplii and preserved copepods and group C fed preserved copepods solely. In this study we observed a positive effect of feeding preserved copepods in *A. clarkii* larviculture as a supplement food to the traditional diet based on rotifers and *Artemia* nauplii. In group B larvae, fed a combination of rotifers/*Artemia* and copepods, a significant increase of insulin like growth factor I and II, peroxisome proliferator activated receptor α - β and thyroid receptor α and β gene expression together with a significant decrease of myostatin gene expression was evidenced by real time PCR compared to the other experimental groups. In this same group we also observed the best results in terms of growth (total length and weight) and survival. These preserved copepods may be considered a suitable food for marine fish larvae larviculture when used as a supplement to the traditional diet based on rotifers and *Artemia* nauplii.

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INFLUENCE OF DIETARY ARACHIDONIC ACID COMBINED WITH LIGHT INTENSITY AND TANK COLOUR ON PIGMENTATION OF COMMON SOLE (*SOLEA SOLEA* L.) LARVAE

Ivar Lund, Svend Jørgen Steinfeldt, Benni Winding Hansen-2010

Aquaculture 308(3-4): 159-165

Abstract:

Supplementation of dietary arachidonic acid (ARA) is known to cause hypopigmentation in common sole larvae (*Solea solea* L.). This study examined a possible link between dietary ARA supplementation — light intensity and tank colour on pigment defects in common sole larvae.

Larval tissue ARA and prostaglandin PGE₂ content increased significantly when fed *Artemia* enriched by a fish oil emulsion supplemented with 24% dietary ARA during premetamorphosis (until 11 days post hatch, dph) as compared to larvae fed on *Artemia* enriched by a fish oil based emulsion. More than 90% of all larvae in groups treated with the ARA supplemented emulsion during premetamorphosis showed partly or complete dorsal hypopigmentation. There were no significant effects of light intensity or tank background colour in combination with ARA on malpigmentation. Larval hypopigmentation was below 10% in the groups not treated with ARA supplemented diets. In these groups, however, the proportion of hypopigmented larvae increased significantly by a combination of a high visual light intensity (4000 lx) and transparent tanks. A high light intensity of 4000 lx increased growth as

compared to low intensity of 100 lx, suggested to be related to a higher feed intake. Early pigment cell (chromatophor) development until 11 dph (i.e. start of metamorphosis) was not significantly related to dietary treatment, but during metamorphosis (from 16 dph) total chromatophore concentration (cells larvae⁻¹) was significantly lower for larvae treated with ARA and a possible lack of pigment cell differentiation or degeneration/cytolysis continued for this group during post metamorphosis.

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EFFECT OF NUTRITION ON CRASSOSTREA GIGAS LARVAL DEVELOPMENT AND THE EVOLUTION OF PHYSIOLOGICAL INDICES: PART B: EFFECTS OF TEMPORARY FOOD DEPRIVATION

R. Ben Kheder, C. Quéré, J. Moal, R. Robert-2010

Aquaculture 308(3-4): 174-182

Abstract:

In the natural environment bivalve larvae are exposed to variable conditions and can therefore face periods of food shortage. To understand the resistance of early life stages of Pacific oyster (*Crassostrea gigas*), larvae were experimentally starved for 4 days at different periods of their development, and the resulting variations in their lipid reserves then analysed using image analysis and biochemical techniques. Faced with a temporary lack of food, the larvae halted their growth in size and weight and started to live off their lipid reserves; this effect was highly marked when the period of food deprivation was at the beginning of larval rearing, but was less marked when food deprivation was after day 14. The larvae conserved their developmental capacity despite these starvation treatments: when feeding was resumed, the larvae started to grow once more, rebuilt their reserves and showed a survival rate between 45 and 91% to the end of the experiment. Apart from the treatment where larvae were starved between days 6 and 10, high percentages became competent (56–85%) leading to a metamorphosis rate of 58–93%. Coloration of neutral lipids with Nile Red, followed by their quantification using image analysis to calculate lipid surface relative to total larval surface (OLI: overall lipid index) was particularly suited to studying the evolution of reserves through larval development. Results collected in this way supported those obtained in parallel using biochemical tests: triacylglycerols/sterols ratio (TAG/ST). The image analysis method offers the advantage of requiring less biological material to obtain a result; the correlation coefficient between OLI and TAG/ST ratio was 0.75.

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REMEDICATION OF AMMONIA ACCUMULATION DURING LIVE TRANSPORT OF JUVENILE COD, GADUS MORHUA L., AND THE EFFECTS OF FAST PERIOD ON AMMONIA LEVELS AND WATER QUALITY

J.W. Treasurer-2010

Aquaculture 30(3-4): 190-195

Abstract:

The stress and trauma associated with handling and movement of fish may have welfare implications and may also increase susceptibility to disease post-transfer. Juvenile salmonids have been transported live from hatchery to production grow out units for some time but little information is available on the live transport of marine finfish species such as Atlantic cod, *Gadus morhua* L. The present study examines the accumulation of un-ionised ammonia (UIA-N) in live transport of juvenile cod and examines the effectiveness of aeration, an ammonia absorbing agent and duration of fast period in controlling the level of UIA-N. UIA-N increased significantly with higher stocking densities in simulated transports of 24 h duration, from 0.08 µg l⁻¹ at 10 kg m⁻³ to 0.43 µg l⁻¹ at 30 kg m⁻³, and also with duration of the simulated transports, rising from 1.14 µg l⁻¹ after 3 h to 6.01 µg l⁻¹ at 24 h in a second trial. Average UIA-N was significantly higher when diffuse aeration with air as well as oxygenation was used, 9.03 µg l⁻¹ compared with 1.54 µg l⁻¹ with oxygenation only. Air diffusion had the effect of maintaining a higher pH which consequently increased the potential for ammonia toxicity.

Mean ammonia level was significantly less at 3.17 $\mu\text{g l}^{-1}$ in a simulated 24 h transport when an ammonia absorbing product was used compared with 6.01 $\mu\text{g l}^{-1}$ in control tanks. This additive would be beneficial during transports of marine finfish for the regulation of ammonia. The effects of varying fast periods and related duration of gut evacuation in juvenile cod on water chemistry including ammonia levels were also assessed. From this an optimum fast period of 36 h for live transport of cod at 12 °C is recommended. Stocking densities of up to 30 kg m^{-3} did not give rise to toxic levels of ammonia. Aeration in addition to oxygenation, while increasing pH, leads to elevated ammonia, but ammonia accumulation can be reduced by using an absorbing agent.

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GROWTH AND FATTY ACID COMPOSITION OF OCTOPUS VULGARIS PARALARVAE FED WITH ENRICHED ARTEMIA OR CO-FED WITH AN INERT DIET

Pedro Seixas, Ana Otero, Luísa M. P. Valente, Jorge Dias, Manuel Rey-Méndez-2010

Aquaculture International 18(6): 1121-1135

Abstract :

The rearing of *Octopus vulgaris* paralarvae during its planktonic life stage is a major challenge, as mortality is currently very high and unpredictable. In this study, we examined the survival and growth rates, as well as the fatty acid composition, of *O. vulgaris* paralarvae fed on three different dietary treatments: group ArDHA was offered juvenile Artemia enriched with a lipid emulsion (Easy DHA-Selco®); group ArMA was fed with juvenile Artemia enriched with a mixture of microalgae (*Rhodomonas lens* and *Isochrysis galbana*); and group ArMA+ID received the same Artemia as group ArMA complemented with an inert diet. Dietary treatments were tested in triplicate with homogenous groups of paralarvae (25 individuals ± 1) established in 50 l tanks, and the experiment was conducted for 15 days. The survival rate of 15-day post-hatch (-dph) paralarvae from groups ArMA (20 \pm 8%) and ArMA+ID (17 \pm 4%) tended to be higher than in group ArDHA (13 \pm 5%), though these differences were not statistically different. The dry weight (DW) of 15-dph paralarvae increased by almost 60% in groups ArMA and ArMA+ID, and nearly 40% in group ArDHA, with respect to hatchlings. The fatty acid (FA) composition of paralarvae revealed a remarkable drop of docosahexaenoic acid (22:6n-3, DHA) from hatchlings to 15-dph paralarvae of all groups ($P < 0.05$). However, paralarvae from group ArDHA contained higher levels of DHA than those from ArMA and ArMA+ID ($P < 0.05$). Despite Artemia enriched with DHA-Selco® contained three-times more DHA than Artemia enriched with microalgae, no beneficial effects of this dietary treatment were observed on the performance of paralarvae.

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DEVELOPMENT OF TESTIS AND DIGESTIVE TRACT IN LONGNOSE GAR (LEPISOSTEUS OSSEUS) AT THE ONSET OF EXOGENOUS FEEDING OF LARVAE AND IN JUVENILES

Marta Jaroszewska, Konrad Dabrowski, Gustavo Rodríguez-2010

Aquaculture Research 41(10): 1486–1497

Abstract:

The aim of this study was to describe the ontogenetic development of the testis and the alimentary tract in longnose gar (*Lepisosteus osseus*) related to fish size and age at the onset of exogenous feeding and late ontogenesis. Using light microscopy, testes were first detected histologically by the appearance of primordial germ cells 9 days after the first exogenous feeding [31–31.5 mm total body length (TL)] and presumptive seminiferous tubules (maleness characteristic) in fish of 107 mm TL. The present histological studies indicated that the alimentary tract of lepisosteids is completely functional at the beginning of exogenous feeding, several days before the completion of yolk absorption. Based on these results, we have concluded that garfish larvae/juveniles can be effectively trained to consume formulated diets at early stages, after an initial feeding of live food for 2–3 days (23.5 mm TL). Our

findings provide evidence of the first controlled rearing of longnose gar using live and formulated diets, providing the possibility of experimental work with this non-teleost fish.
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TYROSINE AND PHENYLALANINE SUPPLEMENTATION ON DIPODUS SARGUS LARVAE: EFFECT ON GROWTH AND QUALITY

Margarida Saavedra, Luis E. C. Conceição, Yoav Barr, Synnove Helland, Pedro Pousão-Ferreira, Manuel Yufera, MariaT. Dinis-2010
Aquaculture Research 41(10): 1523-1532

Abstract:

Phenylalanine is the precursor of tyrosine, which is involved in the synthesis of several molecules with key roles in the regulation of metabolism and growth, stress response and pigmentation. In this study, three experimental diets were tested: an amino acid (AA) balanced diet supplemented with phenylalanine, another supplemented with phenylalanine and tyrosine and a non-supplemented AA balanced diet. Rotifers were enriched with liposomes encapsulating free AA in order to obtain a balanced AA profile. The experimental diets resulted in similar larval survival, growth, enzyme activities of AA catabolism and nitrogen excretion in all treatments. High levels of skeletal deformities were registered and significant differences were found between the control and the phenylalanine treatment for the percentage of vertebral compressions in the trunk region of the vertebral column (30% in the control and 5% in the phenylalanine group). A significantly higher survival to a temperature stress test was found for larvae fed the diet supplemented with phenylalanine and tyrosine. The results suggest that supplementation of phenylalanine/tyrosine in fish diets may be useful in order to reduce skeletal deformities and mortalities caused by stress. The present study confirms that AA requirements may be sufficient for covering growth and survival but insufficient to cover other metabolic processes.

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SHORT COMMUNICATION

COMMERCIAL-SCALE CRYOPRESERVATION OF WELS CATFISH (SILURUS GLANIS) SEMEN

Zoltan Bokor, Bela Urbanyi, Laszlo Horvath, Akos Horvath-2010

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BARNACLE CULTURE: BACKGROUND, POTENTIAL AND CHALLENGES

Daniel A. López, Boris A. López, Christopher K. Pham, Eduardo J. Isidro, Mirko De Girolamo-2010

Aquaculture Research 41(10): e367–e375

Abstract:

There are approximately a dozen species of commercially interesting barnacles worldwide, some of which have been cultured on a semi-industrial scale. These species are listed and information is provided with regard to geographical distribution, landings and prices. Traditionally, ‘goose’ barnacles (four species) are considered to be the most important for consumption. World production already stands at 500 tonnes year⁻¹, but this species has not been cultured to date. Some ‘acorn’ barnacles are also consumed (seven species), with harvest levels per species that do not exceed 200 tonnes year⁻¹ and selling prices that can reach US\$17/kg. ‘Acorn’ barnacle culture on a world scale is still developing. Nevertheless, production has occurred on a semi-industrial scale; specifically, spat have been collected from the wild and grown in suspended systems. Farming trials have focused on two species of acorn barnacles: *Austromegabalanus psittacus* (Molina 1782) ‘picoroco’ in Chile and *Megabalanus azoricus* (Pilsbry 1916) ‘craca’ in Portugal. The large-scale production of these crustaceans will depend on the optimization of spat collection from the wild and/or the parallel

development of mass production technologies for larvae (hatcheries). In addition, further development will be achieved by opening up new markets for commercialization.

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FATTY ACID PROFILES OF SPINY LOBSTER (*PANULIRUS HOMARUS*) PHYLLOSOMA FED ENRICHED ARTEMIA

Kajal Chakraborty, Rekha Devi Chakraborty, E. V. Radhakrishnan, Koyadan Kizhakedath Vijaya-2010
Aquaculture Research 41(10): e393–e403

Abstract:

Three different life stages of spiny lobster larvae (phyllosoma) of *Panulirus homarus* were fed A1-Selco-enriched Artemia in two culture treatments, one with the microalgae *Nannochloropsis salina* (green water) and the other without the microalgae (clear water) to assess the ability to manipulate their fatty acid composition. Phyllosoma fed with 3-h A1-Selco-enriched Artemia salina attained Stage VIII (5.3 mm) and Stage V (3.4 mm) in 42 days in the green and clear water treatments respectively. The higher content of the essential fatty acids in *N. salina* (eicosapentaenoic acid, 25.8%; arachidonic acid, 9.5%; and docosahexaenoic acid, 4.2%) in the green water system increased the fatty acid content of the live food Artemia, and ultimately the phyllosoma. In spite of phyllosoma being fed with enriched Artemia in the clear water system, the total polyunsaturated fatty acid content of the early (Stages I–III) and mid stage (Stages IV–V) phyllosoma were significantly smaller (18.8% and 14.6% respectively) ($P < 0.05$) than in the green water system (25.3% and 21.2% respectively). These results indicate the positive role of the microalgae in boosting the essential fatty acid content of lobster larvae.

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CHARACTERIZATION AND EXPRESSION OF DIGESTIVE NEUTRAL LIPASES DURING ONTOGENY OF ATLANTIC COD (*GADUS MORHUA*)

Øystein Sæle, Andreas Nordgreen, Pål A. Ølsvik, Kristin Hamre-2010

Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 157(3): 252-259

Abstract:

The major neutral lipase excreted by the pancreas in fish, is bile activated lipase (BAL). Here we present evidence that cod have a functional BAL and a non-functional pancreatic lipase related protein (PLRP). The Atlantic cod genome does not seem to contain colipase which is essential for pancreatic lipase activity. During the larval stages, the gene expression of BAL was low until the point when pyloric caeca started to differentiate and develop (~ 20 mm standard length (SL)). Then the expression increased until ~ 50 mm SL. The PLRP gene was expressed but showed very little regulation. The activity of neutral lipase did not increase in parallel to gene expression. The mismatch between activity and gene expression measurements may be partly explained by the unspecific analytical method, when analysing lipase activity in larva whole body. There is neutral lipase activity in numerous tissues in the fish larvae and the lipase activity in the gut, relatively to activity in the whole body, decreased with age. Furthermore, neutral lipase activity in rotifers was ten times higher than in whole cod larvae with full guts. Activity originating from the live prey may therefore explain the high whole body lipase activity from 3 to 20 dph. The results also indicate that “adult type” digestion of neutral lipid develops late in the larval period (from 20 mm SL), while other mechanisms of lipid uptake are active at the early larval stage.

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COMPOSITION AND METABOLISM OF CARBOHYDRATES AND LIPIDS IN *SPARUS AURATA* SEMEN AND ITS RELATION TO VIABILITY EXPRESSED AS SPERM MOTILITY WHEN ACTIVATED

Franz Lahnsteiner, Nabil Mansour, Stefano Caberlotto-2010

Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 157(1): 39-45
Abstract:

The present study investigated aspects of lipid and carbohydrate metabolism in *Sparus aurata* semen and tested the effect of lipids, carbohydrates and related metabolites on sperm viability using *in vitro* incubation experiments. *Sparus aurata* semen contained enzyme systems to metabolize sugars and lipids. Also key enzymes of the tricarboxylic acid cycle and enzymes involved in ATP metabolism were detected. When spermatozoa were incubated in sperm motility inhibiting saline solution for 48 h phospholipid levels decreased constantly and triglycerides levels during the first 24 h of incubation indicating that spermatozoa utilize lipids as energy resources. After 24 h triglycerides levels started to re-increase indicating a change in sperm metabolism, in particular the onset of triglycerides synthesis by the fatty acid synthase complex. In the incubation period from 0 to 24 h glucose levels were constant, and decreased thereafter. Glycogen levels did not change at all. Semen contained also considerable amounts of sialic acid, glucuronic acid and hexosamines, components of mucopolysaccharides. To find out whether lipids, carbohydrates, and related metabolites had a positive effect on sperm functionality semen was incubated together with the described compounds in sperm motility inhibiting saline solution and motility when activated was determined. In the control $37.2 \pm 10.1\%$ of the spermatozoa were locally motile and $38.3 \pm 13.3\%$ motile after 24 h, $36.4 \pm 5.2\%$ were locally motile and $9.6 \pm 4.5\%$ were motile after 48 h. The swimming velocity was $89.0 \pm 13.1 \mu\text{m/s}$ after 24 h and $61.3 \pm 12.6\%$ after 48 h. Different types of lipids (arachidic acid, linoleic acid, and glycerol trimyristate) and metabolites acting as fuel for the tricarboxylic acid cycle (hydroxybutyrate, ketoglutarate, and pyruvate) had a positive effect on the sperm viability. Tested carbohydrates (fucose, galactose, glucosamine, glucose, glucoheptose, glycogen, and sialic acid) had no effect. Also lactate and fructose-6-phosphate had no effect on sperm viability while glucose-6-phosphate, oxalacetate, and phosphoglycerate had negative effects.

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BIOMARKERS AS A TOOL TO ASSESS EFFECTS OF CHROMIUM (VI): COMPARISON OF RESPONSES IN ZEBRAFISH EARLY LIFE STAGES AND ADULTS

Inês Domingues, Rhaul Oliveira, Joana Lourenço, Cesar Koppe Grisolia, Sónia Mendo, A.M.V.M. Soares-2010

Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 152(3): 338-345

Abstract:

The present work aims to compare the sensitivity of embryos and adult zebrafish to chromium (VI) (as potassium dichromate) focusing on biomarkers (cholinesterase, glutathione S-transferase and lactate dehydrogenase) as endpoints. Zebrafish eggs showed less sensitivity to Cr (VI) ($96 \text{ h-LC}_{50} = 145.7 \text{ mg/L}$) than adults ($96 \text{ h-LC}_{50} = 39.4 \text{ mg/L}$) probably due to the protective action of the chorion. However, biomarkers were much more responsive in larvae than in adults and gave clear indications about Cr (VI) mode of action: it seems to be neurotoxic (inhibited cholinesterase), to inhibit glutathione S-transferase activity and to interfere with cellular metabolic activity (changes in lactate dehydrogenase activity) in larvae. In adults, only glutathione S-transferase was responsive, showing a clear inhibition. The responsiveness of the analyzed biomarkers in larvae reinforces the idea of the usefulness of early life stage assays in the assessment of chemicals effects. Moreover, early life stage assays also contributed with relevant information regarding anomalies in larvae development and behavior. Further research should focus on the use of biomarkers to assess long term effects which are ecologically more relevant.

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A NULL MODEL TO EXPLAIN ZOOPLANKTON SPECIES ASSOCIATIONS IN SALINE LAKES OF THE SOUTH AMERICAN ALTIPLANO (14-27°S)

Los Ríos, Patricio De; Gajardo, Gonzalo-2010

Crustaceana, 83(7): 769-777

Abstract:

Zooplankton diversity in shallow salt lakes of the Andean countries in South America is low and distribution is highly dependent on salinity, which varies from moderate to high. At salinities lower than 90 g/l, the halophilic copepod *Boeckella poopoensis* (Marsh, 1906) predominates, whereas above that level the anostracan *Artemia franciscana* (Kellogg, 1912) is the exclusive component of the habitat. This constitutes, however, fragmentary information only. A review of the available literature for Andean saline lakes in Bolivia, Chile, and Peru, confirms that presence and distribution of both species is significantly driven by salinity levels. The results of a species co-occurrence null model analysis, indicates that species associations are not random, and these results are supported by the correlation analysis, which indicates a significant, inverse correlation between species number and salinity, and a significant direct relation of species number with surface of the habitat. The low species diversity characteristically seen in these habitats and their dependence on salinity changes effectuated by weather conditions, provide a good model-system for monitoring weather change.

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MORPHOGENESIS OF THE EYESTALK AND EXPRESSION OF MOULT-INHIBITING HORMONE DURING EMBRYONIC DEVELOPMENT OF THE FRESHWATER PRAWN, MACROBRACHIUM ROSENBERGII (DECAPODA, PALAEMONIDAE)

Yao Jun-Jie, Luo Wen, Zhao Yun-Long, He Deng-Ju, Zeng Cuo-2010

Crustaceana, 83(8): 903-913

Abstract:

The morphogenesis of the compound eyes and eyestalks during embryonic development of *Macrobrachium rosenbergii* was characterized by using histological methods. The expression of moult-inhibiting hormone (MIH) at different stages of embryonic development was determined by the reverse transcription polymerase chain reaction (RT-PCR). Histological studies showed that compound eye pigment began to assemble and the eyestalk Anlage appeared during the protozoa stage. During the zoea stage, the eyestalk appeared and the structure of the compound eye was similar to that of the adult. RT-PCR results showed that MIH was expressed during both the late protozoa and early zoea stages of development. However, MIH was not expressed in the fertilized egg until the early protozoa stages or during the late zoea stage. MIH expression was detected only after the appearance of the eyestalk. The expression of MIH correlates closely with the morphogenesis of the compound eye and eyestalk. The absence of MIH expression during the zoea stage demonstrates that it regulates the synthesis of ecdysteroids in preparation for the first moult after hatching.

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ACROSOME REACTION OF SPERMATOOZOA IN THE CHINESE MITTEN CRAB, ERIOCHEIR SINENSIS (DECAPODA, GRAPSIDAE): INDUCED BY ANTI-SPERMATOOZOAL MEMBRANE PROTEINS ANTISERUM

Li Genliang, Kang Xianjiang, Li, Yanqin, Mu Shumei, Guo Mingshen-2010

Crustaceana, 83(8): 915-926

Abstract:

Three kinds of spermatozoal proteins, i.e., spermatophore matrix proteins, spermatozoal membrane proteins (SMPs), and proteins released during the acrosome reaction (AR), were extracted from good quality fresh spermatozoa of the Chinese mitten crab, *Eriocheir sinensis* (Decapoda, Grapsidae) and used to immunize three groups of ICR (Institute of Cancer Research) female mice to yield the respective antisera. Incubations of spermatozoa with or without the antisera obtained, were carried out in order to evaluate their effects on the acrosomal status and AR percentage. The results show that the

anti-spermatophore matrix proteins antiserum, control serum, or Ca²⁺-free artificial seawater, all failed to induce AR, and the anti-proteins released during AR antiserum only just induced an AR of 4.7%. In contrast, there was a score of more than 90% AR in spermatozoa incubated with the anti-SMPs antiserum, which presented all four steps of AR in about 1.5 h, and an extremely significant difference in the AR percentages ($P < 0.01$) between the anti-SMPs antiserum and other media. These results demonstrate that, on the spermatozoal membrane, there is/are a/some protein(s) inhibiting AR. AR may occur when the protein(s) is/are combined and blocked by its/their antiserum. In addition, the discovery that the anti-SMPs antiserum activates spermatozoa represents a novel, efficient, and operational method for inducing AR in *E. sinensis*.

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A MORPHOLOGICAL AND MOLECULAR STUDY ON ARTEMIA FRANCISCANA (BRANCHIOPODA, ANOSTRACA) FROM BASRAH, IRAQ

Mohammed Dawood S., Salman Salman D., Ali Malik H.-2010

Crustaceana 83(8): 941-956

Abstract:

The present study makes use of both morphometric and molecular biological data to identify the bisexual population of *Artemia* in Basrah, as there was confusion about the nomenclature regarding the identity of the species as currently used in Iraq. Thus, *Artemia franciscana* is now confirmed to occur in Basrah. A parthenogenetic species of *Artemia* was also encountered in this region. A comparison of morphometric characters and DNA sequencing data was made for strains of the same species from various localities, and with populations or strains of different *Artemia* species from all over the world.

(Department of Marine Biology, Marine Science Centre, University of Basrah, Basrah, Iraq)

BEHAVIOURAL AND ANATOMICAL MEASURES OF VISUAL ACUITY IN FIRST-FEEDING YELLOWTAIL KINGFISH (*SERIOLA LALANDI*) LARVAE

Alexander G. Carton, Melanie R. Vaughan-2010

Environmental Biology of Fishes 89(1): 3-10

Abstract:

Ontogenetic change in the visual acuity of *Seriola lalandi* larvae was measured using both behavioural and anatomical techniques. Visual acuity improved over early development (day 4 to day 7 posthatch), although for all three larval ages examined estimates of anatomical acuity were consistently lower (higher acuity) than estimates of behavioural acuity. At hatching the eyes of larval kingfish were characterized by an undifferentiated retina surrounding a spherical lens, by day 4 post-hatch the eyes appeared to be functional, the retina was fully pigmented and the optic nerve had made contact with the optic tectum. Analysis of prey search behaviour indicated that larvae employ a saltatory type search behaviour in which brief stationary periods are interspersed with repositioning movements. The mean reactive angle increased between day 4 and day 7 post-hatch indicating that the horizontal visual field was expanding with development, thereby increasing the search area of larvae. Pre-strike distances of early larvae were substantially less than one body length, being constantly around a 1/3 of a body length for all larval ages examined.

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WEANING REQUIREMENTS OF LARVAL MULLOWAY, *ARGYRO SOMUS JAPONICUS*

Debra A. Ballagh, D. Stewart Fielder, Patricia M. Pankhurst-2010

Aquaculture Research 41(10): e493-e504

Abstract:

Mulloway (*Argyrosomus japonicus*) is an emerging aquaculture species in Australia, but there is a need to improve the production technology and lower costs, including those associated with larval rearing and live feeds. Three experiments were conducted to determine appropriate weaning strategies from live

feeds, rotifers (*Brachionus plicatilis*) and *Artemia*, to cheaper formulated pellet diets. Experiment 1 examined the effects of feeding *Artemia* at different levels [0%, 50% or 100% ration of *Artemia* fed from 18 days after hatching (dah); based on current hatchery protocols] and a pellet diet from two larval ages (14 or 23 dah). In addition, rotifers were supplied to larvae in all treatments for the duration of the experiment (14–29 dah), at which time all larvae were successfully weaned onto the pellet diet. No significant ($P > 0.05$) differences existed between the growth of fish fed a 50% and 100% ration of *Artemia*; however, fish fed a 0% ration of *Artemia* had significantly ($P < 0.05$) reduced growth. The time of pellet introduction had no significant ($P > 0.05$) effects on the growth of larvae. Experiments 2 and 3 were designed to determine the size [total length (TL), mm] at which mullet larvae selected *Artemia* equally or in preference to rotifers, and pellet (400 μm) equally or in preference to *Artemia* respectively. Each day, larvae were transferred from a holding tank to experimental vessels and provided with rotifers (2 mL⁻¹), *Artemia* (2 mL⁻¹) or a combination of rotifers (1 mL⁻¹) and *Artemia* (1 mL⁻¹) (Experiment 2), and *Artemia* (2 mL⁻¹), a pellet diet or a combination of *Artemia* (1 mL⁻¹) and a pellet diet that was broadcast every 15 min (Experiment 3). After 1 h, a sub-sample of larvae was randomly selected from each replicate vessel ($n=5$) and the gut contents were examined under a light microscope. Mullet larvae began selecting *Artemia* equally to rotifers at 5.2 ± 0.5 mm TL and selected pellets equally to *Artemia* at 10.6 ± 1.8 mm TL. Our results have led to the establishment of weaning protocols for larval mullet, which optimize larval growth while reducing feed cost by minimizing the amount of *Artemia* used during production.

(Industry & Investment NSW and Aquaçon Cooperative Research Centre, Port Stephens Fisheries Institute, Taylors Beach, NSW, Australia; email of Debra Ballagh: debra.ballagh@industry.nsw.gov.au)

EFFECT OF HOT WATER EXTRACTS OF BROWN SEAWEEDS *SARGASSUM* SPP. ON GROWTH AND RESISTANCE TO WHITE SPOT SYNDROME VIRUS IN SHRIMP *PENAEUS MONODON* POSTLARVAE

Grasian Immanuel, Madasamy Sivagnanavelmurugan, Varatharajan Balasubramanian, Arunachalam Palavesam-2010

Aquaculture Research 41(10): e545–e553

Abstract:

An experiment was conducted to evaluate the effect of a hot water extract of brown seaweeds *Sargassum duplicatum* and *Sargassum wightii* on the growth and white spot syndrome virus (WSSV) resistance in shrimp *Penaeus monodon* postlarvae (PL). *Artemia* nauplii (instar II) were enriched with both seaweed extracts at various concentrations (250, 500 and 750 mg L⁻¹) and fed to the respective *P. monodon* (PL15–35) group for 20 days. A control group was also maintained without seaweed extract supplementation. The weight gain of the experimental groups was significantly higher (0.274–0.323 g) than the control group (0.261 g). Similarly, the specific growth rate was also significantly higher (16.27–17.06%) in the experimental groups than in the control group (16.03%). After 20 days of the feeding experiment, the shrimp PL were challenged with WSSV for 21 days. During the challenge test, the control shrimp displayed 100% mortality within 8 days. In contrast, the mortality percentage of the highest concentration (750 mg L⁻¹) of seaweed extract enriched *Artemia* nauplii fed shrimp was 54–79%. Comparatively, low mortality was observed in *S. wightii* extract-enriched *Artemia* nauplii fed shrimp. The polymerase chain reaction analysis indicated the concentration-dependent infection of WSSV in *P. monodon* PL.

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AN INTENSIVE HATCHERY REARING PROTOCOL FOR LARVAE OF THE BULLSEYE PUFFER, *SPHOEROIDES ANNULATUS* (JENYNS)

Maria Isabel Abdo-de la Parra, Armando García-Ortega, Irma Martínez-Rodríguez, Blanca González-Rodríguez, Gabriela Velasco-Blanco, Crisantema Hernández, Neil Duncan-2010

Aquaculture Research 41(10): e554–e560

Abstract:

In this study bullseye puffer, *Sphoeroides annulatus* larvae were reared from hatching through to 1 or 2 months after weaning on an experimental scale in three replicate 600 L tanks and on three occasions during the spawning season (nine tanks in total). The rearing protocol used was green water (*Nannochloropsis oculata* and *Isochrysis* sp.) 100 000 cells mL⁻¹ from 0 to 11 days after hatch (DAH), 5–10 rotifers, *Brachionus rotundiformis* mL⁻¹ from 2 to 26 DAH, 0.1–1 *Artemia* mL⁻¹ from 21 to 34 DAH and weaning from 29 to 34 DAH. Survival to a month after weaning was 1%, a total of 3153 juveniles were produced with an average wet weight of 0.29 ± 0.07 g and a length of 27.5 ± 0.82 mm. (CIAD, A.C. Unidad Mazatlan, Sinaloa, Mexico; email of N. Duncan: neil.duncan@irta.cat)

INCREASED SPAWNING ACTIVITY OF FEMALE NILE TILAPIA (*OREOCHROMIS NILOTICUS*) (L.) AFTER STOCKING DENSITY AND PHOTOPERIOD MANIPULATION

Boris Adrien De Lapeyre, Andreas Muller-Belecke, Gabriele Horstgen-Schwark-2010

Aquaculture Research 41(10): e561–e567

Abstract:

This study was conducted to evaluate the effects of stocking density and photoperiod in increasing the reproductive performance of *Oreochromis niloticus*. In experiment 1, a change in stocking density (from 47.7 to 6.8 kg m⁻³) was performed, with groups of 48 females moved to single compartments. In experiment 2, 36 females experienced a 6L:18D photoperiod for 21 or 28 days (stocking density: 31.3 kg m⁻³) before being placed in individual compartments (stocking density of 6.9 kg m³, 12L:12D photoperiod). The spawning rates in experiment 1 (32.2%) and in experiment 2 (21 days: 65.2%, 28 days: 36.1%) were significantly higher than those in the control (17.7%). On the other hand, hatching and swim-up fry rates were significantly lower in experiment 2 – 21 days (41.3%, 40.4%) but not in experiment 1 (64.0%, 56.3%) compared with the control (67.0%, 62.3%). The spawning rate in experiment 2 – 21-day treatment group was the highest, while the number of eggs collected per female was significantly lower than that in the control. In experiments 1 (20.8%) and 2 (21 days: 44.4%, 28 days: 19.4%), the daily spawning rates were the highest 3 days after maintaining females in single compartments. The current experiment demonstrates how specific stocking density changes can be used to induce higher spawning rates in Nile tilapia.

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APPLICATION OF PROSPECTIVE PROBIOTANTS AT EARLY STAGES OF ATLANTIC COD (*GADUS MORHUA* L.) REARING

Hélène L. Lauzon, Bergljot Magnadóttir, Bjarnheidur K. Guðmundsdóttir, Agnar Steinarsson, Ivar Orn Arnason, Sigridur Guðmundsdóttir-2010

Aquaculture Research 41(10): e576–e586,

Abstract:

This work aimed at validating the use of two prospective probiotics (*Arthrobacter* sp. and *Enterococcus* sp.) at early stages of cod (*Gadus morhua* L.) rearing. Ova at late post-fertilized stage and larvae during their first 4 weeks of life were bathed with both probiotics, isolated previously from the cod-rearing environment. This treatment was compared with groups fed rotifers supplemented with a commercial probiotic (Remus®) and those untreated. Microbiological analyses (total viable counts, presumptive *Vibrio* and lactic acid bacteria) were performed in rearing systems and larval survival, growth and development were assessed. Larval development was evaluated by proteolytic activity of larval lysates and immunological analysis of important proteins: apolipoprotein A-I, haemoglobin, C-reactive protein, C3 and cod serum proteins. Bacterial bathing led to a significantly higher larval weight, length and culturable microbial load in larval gastrointestinal (GI) tract when compared with the control and Remus groups. Development occurred earlier in bathed larvae. However, their survival was negatively affected compared with the control group, but was significantly higher than for the Remus group. The non-pathogenicity of both probiotics was demonstrated by intraperitoneal injection of 13 g cod juveniles. The results suggest that *Arthrobacter* and *Enterococcus* probiotics affected the larval GI microbiota and contributed to growth, development and digestion, either directly or indirectly.

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ARTIFICIAL INCUBATION OF NOBLE CRAYFISH (ASTACUS ASTACUS) EGGS IN A PARTIALLY RECIRCULATING SYSTEM USING FORMALDEHYDE AS AN ANTIFUNGAL TREATMENT

Antonín Kouba, José Manuel Carral, Miloš Buřič, Jan Mráz, Tomáš Polícar, Pavel Kozák-2010
Aquaculture Research 41(10): e618–e623

Abstract:

There is little information on the long-term artificial incubation of noble crayfish eggs. The present study evaluated artificial incubation in a partially recirculating system using formaldehyde baths as antifungal prophylaxis. Crayfish eggs were treated three times a week in a formaldehyde bath of 3000 ppm for 15 and 20 min and in 2500 ppm for 15, 20 and 25 min. In all treated groups, high final survival rates (87.8–92.0%) to stage 2 juveniles were obtained after 83 days. Significantly lower survival was observed in the control group (45.4%). It was concluded that recirculating systems can be used successfully for artificial incubation of crayfish eggs in order to conserve water. Formalin was found as an effective fungicide but its use should be carefully considered because of its safety issues for farm staff and environmental pollution.

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