

BREEDING FOR DISEASE RESISTANCE OF PENAEID SHRIMPS

James Cock, Thomas Gitterle, Marcela Salazar, Morten Rye-2008

Aquaculture 286(1-2): 1-11

Abstract:

Diseases are a major constraint on the intensive production of shrimps. Conditions in production ponds favour disease development, and epidemics of several previously unreported diseases have occurred and caused severe losses. When elimination, eradication or cultural control is difficult, selective breeding for host resistance to the pathogen may be an attractive option for disease control. However, host resistance is not a panacea and should only be considered when (a) the disease causes severe damage (b) there are no other existing simple cost effective control measures and (c) there is demonstrable genetic variation in resistance and this is not coupled with an excessive level of negative associations with other desirable characteristics. Shrimp have only recently been domesticated and breeding for resistance only began in the mid 1990s; there is limited experience with shrimp breeding in particular and crustaceans in general. Consequently, the principles and concepts behind breeding programmes are based largely on experiences with other species in both the plant and animal kingdoms. Commercial growers now seed ponds with shrimp populations selected for resistance to Taura Syndrome Virus with excellent results, whilst up to now development of White Spot Syndrome Virus resistant populations has been an elusive goal. The original TSV resistant populations were developed using simple mass selection techniques (Colombia). In later generations family based selection has been applied on populations, which initially had survival rates of about 30%, with care taken to reduce inbreeding and loss of genetic variation. This suggests that when the original populations have a reasonable level of resistance, and straightforward, effective selection protocols exist, it is relatively simple to breed for resistance. With catastrophic diseases, such as WSSV, which cause mortalities of 98% or more the frequency of resistance is low and it is suggested that for theoretical reasons single gene, rather than polygenic, resistance is likely to develop. The low frequency of resistance genes in breeding populations may cause genetic bottlenecks which will greatly reduce the genetic variation in the populations. In order to maintain the genetic variation the genes from the small numbers of survivors should be introgressed into populations with broader genetic variability. Furthermore, in order to minimize the probability of breakdown of resistance pyramiding of resistant genes on different loci would be advantageous.

Genetic variation in resistance may be encountered either in the initial base populations or may spontaneously arise due to mutations or new recombinants. With extremely prolific species such as shrimps, millions of animals can readily be screened for survival and hence resistant mutants or recombinants may be identified. Once genetic variation has been detected the most appropriate breeding methodology will depend on the nature of both the resistance and the disease or diseases that are of interest to the producers.

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HISTOLOGICAL EVALUATION OF THE ELIMINATION OF ARTEMIA NAUPLII FROM LARVAL REARING PROTOCOLS ON THE DIGESTIVE SYSTEM ONTOGENY OF SHI DRUM (UMBRINA CIRROSA L.)

Ioannis E. Papadakis, Mario M. Zaiss, Yiannos Kyriakou, Georgios Georgiou, Pascal Divanach, Constantinos C. Mylonas-2008

Aquaculture 286(1-2): 45-52

Abstract:

The influence of the absence of Artemia nauplii from larval diet protocols on growth and digestive system ontogeny was studied using histological techniques in the shi drum (*Umbrina cirrosa*). One group of larvae was reared using the standard intensive rearing protocol, which offers a combination of enriched rotifers (*Brachionus plicatilis*), Artemia spp. nauplii and artificial diet (Std-group). Another group was reared using the same protocol, but without the offering of Artemia nauplii (group

No-Artemia). The ontogenesis of the digestive system from hatching to metamorphosis was a very rapid process, and there were no differences between the two feeding regimes in the temporal appearance of the various components of the digestive system. The first organised presence of the hepatic and pancreatic tissue appeared at 2–3 d after hatching (dah), suggesting that these organs function from a very early developmental stage. In the No-Artemia larvae between 13 and 29 dah there was a reduction in the height of enterocytes in the intestinal mucosa, a progressive flattening of the primary intestinal folds in the anterior and posterior intestine and a decrease in lipid stores in the liver, suggesting a period of relative starvation. However, by the end of the study at 41 dah, there were no significant differences in body length, intestinal morphology or liver lipid stores between larvae reared under the two feeding regimes. The study suggests that the diet may influence the maturation and/or function, but not the ontogeny of the digestive system. Furthermore, the rapid differentiation of the digestive system in shi drum and the prompt recovery of the No-Artemia larvae from the symptoms of starvation by 29 dah, indicate a plasticity during ontogenesis and the ability of larvae to adapt to artificial diets at very early developmental stages.

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SURVIVAL AND QUALITY OF HALIBUT LARVAE (HIPPOGLOSSUS HIPPOGLOSSUS L.) IN INTENSIVE FARMING: POSSIBLE IMPACT OF THE INTESTINAL BACTERIAL COMMUNITY

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Aquaculture 286(1-2): 53-63

Abstract:

The high mortality commonly observed during the early life stages of intensively reared halibut (*Hippoglossus hippoglossus* L.) is believed to be caused by e.g. opportunistic bacteria. However, the impact of particular bacterial species is poorly defined and still remains disputable. The study describes the bacterial diversity in the gastrointestinal tract of halibut larvae in a large number of incubators at a commercial production site. The overall success of larvae was found to be highly variable and analysis of the gut microbiota revealed high variation of the cultivable part as well as the bacterial community of surface sterilised larvae analysed by denaturing gradient gel electrophoresis (DGGE) of PCR amplified 16s rDNA products. Analysis of the bacterial community of unfed yolk sac larvae revealed higher diversity than previously reported, with *Marinomonas*, *Marinobacter*, *Aeromonas* and *Shewanella* dominating the community structure. There are indications that *Marinomonas* is found only in the overall most successful first feeding larvae of the period where the *Vibrio* group dominated the bacterial community together with *Shewanella*. *Vibrio wodanis* was identified as a part of the bacterial community of feeding larvae that yielded the poorest overall success of the period. α -Proteobacteria, not previously reported in halibut, were also found as a part of the bacterial community of first feeding larvae. The diverse bacterial community was only partly reflected in the cultivable part which, however, may reflect the dominating bacterial groups of the highly heterogeneous bacterial community of larvae in the production system as a whole. The bacterial community of the *Artemia* was found to be highly variable in different samples collected through the period. Only a small part of the different groups observed in the bacterial community of surface sterilised larvae was reflected in the cultivable part which was dominated by highly variable groups in different samples of *Artemia*. Also, the numbers of cultivable bacteria were found to positively correlate with jaw deformation of unfed yolk sac larvae as well as incomplete metamorphosis of feeding larvae.

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THE EFFECT OF SPERM TO EGG RATIO AND GAMETE CONTACT TIME ON FERTILIZATION SUCCESS IN ATLANTIC COD GADUS MORHUA L.

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Aquaculture 286(1-2): 89-94

Abstract:

Currently, Atlantic cod (*Gadus morhua*) is the primary finfish species being developed for aquaculture in North Atlantic waters. Despite the importance of this species, no research has been conducted to assess the effects of sperm density and gamete contact time on egg fertilization. In two separate experiments male and female gametes were crossed using nested factorial designs. For each male–female combination we tested sperm to egg ratios ranging from $1 \times 10^3:1$ to $5 \times 10^6:1$. We also tested two gamete contact times where sperm and eggs were held in contact with each other for 5 or 30 min. Mixed-model ANOVAs indicated that sperm density and gamete contact time had a significant effect on fertilization success. Below a sperm to egg ratio of $1 \times 10^5:1$ fertilization success significantly decreased. Therefore, a standard sperm to egg ratio of 1×10^5 sperm per egg is recommended for fertilization in Atlantic cod. At the $1 \times 10^3:1$, $5 \times 10^3:1$, and $1 \times 10^4:1$ sperm to egg ratios maximum fertilization occurred after 30 min sperm to egg contact time. Gamete contact time was not significant at sperm to egg ratios of $1 \times 10^5:1$ and $1 \times 10^6:1$. Both the maternal and paternal variance components were significant for fertilization success. This information has important implications for optimizing family production in selective breeding programs, conserving sperm from superior pedigree in genome banks, maximizing the use of available gametes in hatchery or research facilities, and understanding mating success in the wild.

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EXAMINATION OF A PRACTICAL METHOD FOR ZINC ENRICHMENT OF EURYHALINE ROTIFERS (*BRACHIONUS PLICATILIS*)

Seiji Matsumoto, Shuichi Satoh, Tomonari Kotani, Hiroshi Fushimi-2008

Aquaculture 286(1-2): 113-120

Abstract:

Larval growth and survival of marine finfish in mass seed production are affected by the nutritional value of live feeds such as rotifers and *Artemia*. Thus far, many studies have been conducted to develop effective methods for the enrichment of live feeds with essential fatty acids and vitamins. In this study, a practical method for enrichment of rotifers with zinc was investigated. Changes in the concentrations of other minerals when zinc was added to the rotifer-enrichment tanks were also studied. The mineral composition of rotifers and *Chlorella* after zinc enrichment revealed that the direct addition of zinc to the culture media was not effective because rotifers cannot efficiently accumulate waterborne zinc. The ability of *Chlorella* to absorb waterborne zinc is much higher than that of rotifers, and hence, zinc was pre-accumulated in *Chlorella*, which was then fed to the rotifers. The maximum zinc content of the rotifers was $585.0 \mu\text{g g}^{-1}$ (dry matter) when the rotifers were enriched with zinc alone. This zinc concentration is comparable to that found in natural zooplankton. In rotifers simultaneously enriched with zinc and n-3 highly unsaturated fatty acids (HUFAs), the zinc content increased, but the n-3 HUFA content did not. Therefore, separate enrichment with zinc and fatty acids was adopted. The zinc content of rotifers fed zinc-enriched *Chlorella* was significantly higher than that of rotifers fed unenriched *Chlorella*. After zinc enrichment, rotifers were enriched with fatty acids, and the docosahexaenoic acid (DHA) and n-3 HUFA levels in rotifers were higher than the levels obtained after simultaneous enrichment with zinc and fatty acids. With regard to the concentration of other minerals in rotifers after zinc enrichment, the manganese content tended to decrease when the zinc content increased.

The results of this study demonstrated that zinc enrichment of rotifers was successfully performed by using microalgae that had accumulated zinc, and the enrichment of rotifers with fatty acids was also achieved after the completion of zinc enrichment and before feeding the larvae. This method could be utilized for the enrichment of zooplankton with other minerals as well.

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LARVAL ORGANOGENESIS OF FLATFISH BRILL *SCOPHTHALMUS RHOMBUS* L: HISTOLOGICAL AND HISTOCHEMICAL ASPECTS

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Aquaculture 286(1-2): 138-149

Abstract:

The present tabular overview of organogenesis in larvae of the flatfish brill *Scophthalmus rhombus* (L.) provides valuable information on its structural status during ontogeny, and it can be useful for establishing the functional systemic capabilities and physiological requirements of larvae for optimal welfare and growth. The organogenesis of the brill larvae was studied during the first month of larval life by means of zootechnical, histological, and histochemical approaches. Based upon its feeding mode, and analysing the main morphohistological characteristics of the organs and systems, larval development in brill was divided into four stages from hatching: Stage 1: 0–1 days after hatching (DAH); Stage 2: 2–9 DAH; Stage 3: 10–22 DAH and Stage 4 from 23 DAH onwards. As in most marine fish species, brill larvae at hatching had an undifferentiated digestive tract composed of monostratified epithelium cells, each containing a basal nucleus and evident apical microvilli. At the beginning of stage 2, both the mouth and the anus opened in conjunction with the differentiation of the digestive tract. During stages 2 and 3, the digestive tract differentiated and buccopharyngeal cavity, oesophagus, incipient stomach, and anterior, mid- and posterior intestine became distinguishable. At this time, prey capture began and the digestive processes continued to develop (e.g. gut mucosa folds, lipid infranuclear vesicles and protein supranuclear inclusions). The first taste buds appeared at the onset of the exotrophic stage, at 8 DAH, while the first pharyngeal and mandibular teeth were apparent at 4 DAH. Histological and histochemical observations suggest that digestive tract development of the brill larvae, involving the presence of functional liver (glycogen lipids), exocrine pancreas (proteins) and gall bladder, enabled early larvae (from 4 DAH) to ingest, digest, and assimilate the first exogenous food, even before endogenous reserves were completely resorbed. The appearance of the digestive mucous cells (from 3 DAH), containing neutral and/or acid mucosubstances, and the functional gastric gland differentiation (23 DAH) resembled such developments in other flatfish species. The most critical ontogenetic events occurred at stage 2. Primordial gills were detected at 2 DAH while gill filament, vascular structures, and lamellae differentiated from 6 DAH. At the beginning of stage 2, four defined cardiac cavities (sinus venosus, atrium, ventricle, and bulbus arteriosus) were discernible. The primordial swim bladder was differentiated from the dorsal wall of the digestive tube at 2 DAH and at this time renal tubules between interstitial kidney tissue were detected. Endocrine elements (Langerhans islets and thyroid follicles) were also evident at stage 2. From stage 3 onwards, most organs essentially exhibited an increase in tissue structure number and size.

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CHANGES IN FATTY ACID AND STEROL COMPOSITION DURING OOGENESIS IN THE PEARL OYSTER *PINCTADA MARGARITIFERA*

Isabelle Vahirua-Lechat, Frédéric Laure, Jean René LeCoz, Jean Pierre Bianchini, Mereani Bellais, Gilles Le Moullac-2008

Aquaculture Research 39(16): 1739 – 1746

Abstract:

The fatty acid and sterol composition of the oyster *Pinctada margaritifera* during oogenesis and in eggs was analysed. No major differences were observed during oogenesis, but the egg composition was significantly different from that of gonads. The amount of saturated fatty acids was the highest in eggs and the C16:0 predominant ($P < 5\%$); by contrast, the amount of 22:6(n-3) was significantly lower ($P < 5\%$) than in gonads. No major differences were observed for the polar lipid (PL) composition during oogenesis. The main free sterols in gonads and eggs were cholesterol and brassicasterol. Among free sterols, the proportion of cholesterol diminished continuously from the beginning to the end of gonad maturation, and this decrease persisted in eggs after spawning. Cholesterol represented 40% to 55% of the sterol ester encountered in gonad and eggs. This study allowed us to investigate

the fatty acid and sterol composition during oogenesis of the pearl oyster *P. margaritifera*, leading to a clearer understanding of the nutritional requirements of pearl oyster during the reproduction process. (Laboratoire des Substances Naturelles, Institut Louis Malardé, Tahiti, French Polynesia; email of G. L. Moullac: Gilles.Le.Moullac@ifremer.fr)

GROWTH, MORTALITY AND REPRODUCTION OF THE TRANSPLANTED MANILA CLAM (RUDITAPES PHILIPPINARUM ADAMS & REEVE 1850) IN JIAOZHOU BAY

Yiping Ren, Binduo Xu, Yonglu Guo, Ming Yang, Jianwei Yang-2008

Aquaculture Research 39(16): 1759 – 1768

Abstract:

Samples of Manila clam (*Ruditapes philippinarum* Adams & Reeve 1850) were collected from May 2004 to April 2005 monthly, and plankton net trawling of planktonic larvae and bottom sediment sampling surveys were further conducted from May to October 2006 in Jiaozhou Bay. Based on the data collected, growth, mortality and reproduction of the transplanted Manila clam and the environmental effects were examined. The results showed that the enhanced clams grew well and showed a growth trend similar to the local wild ones. The main growth periods lasted from April to September, with the water temperature being the main factor affecting the growth, which was the same as that of the wild clams. There were also two reproduction cycles for the farmed Manila clams each year in Jiaozhou Bay and the main breeding period was from May to June. The phenomenon of delayed metamorphosis was quite common through larval development. The farmed clams could spawn when they reached sexual maturity, but they could not perform effective recruitment as many planktonic larvae died during metamorphosis and settlement. A preliminary study indicated that sediment perturbation and marine environment pollution were the main factors causing the death of larvae in the development process.

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REAL-TIME MEASUREMENT OF PROTEIN LEACHING FROM MICRO-PARTICULATE LARVAL FISH FEEDS

Peter M. Nicklason, Donald B. Johnson-2008

Aquaculture Research 39(16): 1793 – 1798

Abstract:

The small size and high surface area to volume ratio of larval fish feed presents challenges for nutrient retention in micro-particulate diets. A method for the accurate and rapid measurement of nutrient retention or loss from micro-particulate feed in water is needed to help develop micro-particulate feeds with good nutrient retention characteristics. The present study developed and validated an instrument method using fibre optic technology that measures protein leaching in real time. Larval fish feed particles of different sizes (100–500 μm) and formulations were measured. Under consistent experimental conditions, a feed could be assayed for the rate of mass loss and the half-life or time of 50% total soluble mass loss. The results closely approximated natural decay models with coefficients of determination (r^2) > 0.95. The end result is a fast and accurate method to quantify and provide solid reference data for a feed formulation or particle size. Using this method allows different feeds to be compared and conclusions drawn for relative performance.

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HYPOXIA INHIBITS FISH SPAWNING VIA LH-DEPENDENT FINAL OOCYTE MATURATION

Shuhong Wang, Sylvia S.F. Yuen, David J. Randall, Ching Yee Hung, Tommy K.N. Tsui, Wing Lin Poon, Jimmy C.C. Lai, Yong Zhang, Haoran Lin-2008

Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 148(4): 363-369

Abstract:

To evaluate the effects of long term hypoxia exposure on fish spawning, mature common carp, *Cyprinus carpio carpio* (Linnaeus) were subjected to either normoxia ($7.4 \pm 0.2 \text{ mgO}_2\text{mg O}_2 \text{ L}^{-1}$) or hypoxia ($1.0 \pm 0.2 \text{ mgO}_2\text{O}_2 \text{ L}^{-1}$) for more than two months. Gonadosomatic index (GSI), and concentrations of serum luteinizing hormone (LH), testosterone (T), and estradiol (E2) were measured and gonad histology examined. Hypoxia inhibits fish spawning even though the gonad and oocytes developed under hypoxia exposure. LH levels of female carp were significantly decreased upon chronic exposure to hypoxia, and the final oocyte maturation in hypoxic females was significantly retarded. The results indicated that hypoxia may inhibit fish spawning through LH-dependent final oocyte maturation. In addition, no courtship was observed in hypoxic males. In conclusion, hypoxia impairs fish ovulation and, therefore, spawning and reproduction. LH levels were reduced leading to a failure of oocyte maturation. This, along with a lack of courtship by males may be the major mechanisms involved in hypoxic inhibition of reproduction in carp.

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EFFECTS OF SALINITY STRESS ON ANTIOXIDANT ENZYMES OF *PENAEUS MONODON* OF TWO DIFFERENT LIFE STAGES

Wei-Na Wang, Juan Wu, Shi-Juan Su-2008

Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 148(4): 466-

Abstract:

Face to environmental stress, each type of organisms, and for any species has a capacity of adaptation, based on regulating processes. Oxidative stress is a state of unbalanced tissue oxidation, involving enhanced intra- and extracellular ROS production, peroxidation of lipids, proteins, and often causes a general disturbance of the cellular redox balance, i.e. The antioxidant enzymes: Superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase(GPx) and glutathione S-transferase(GST) that play a very important role in the protection against oxidative damage in living cells, We investigated the effects of salinity stress on O_2^- production and antioxidant enzymes in muscle of *Penaeus monodon* of two different life stages.

Groups of 100 Juvenile and 200 early larval stage shrimps were selected and kept in 20 L tank at various salinity sea water maintained at 2‰, 7‰, 20‰, 30‰, 40‰ in laboratory (three replicate groups each). The water was recirculated by an airlift and kept at $25 \pm 1 \text{ }^\circ\text{C}$, $\text{pH}7.47 \pm 0.12$, ammonia-nitrogen free. Prawns were freeze clamped in liquid nitrogen after 24 and 48 h respectively and the O_2^- production and activity of antioxidant enzymes (CAT, GPX, SOD, GST, and GR) in muscle were tested.

The results indicated that the O_2^- production and activity of antioxidant enzymes of *P. monodon* in two different life stages was affected by salinity stress, suggesting the salinity stress could induced oxidative stress. The SOD and CAT activities in juvenile show significant difference in response to time of stress, whereas show no significance changes in early larval stage. However, the changes of GR and GST activities in early larval stage response to time of stress were remarkably but changed little in juvenile exposed to different stress time. The compensatory function of between CAT and GPX was also found in this study. When exposed to 2‰, the CAT activity increased significantly ($p < 0.05$) but the GPX activity dropped markedly ($p < 0.05$) after 24 h in the muscle of juvenile. After both 24 and 48 h the CAT activity increased significantly ($p < 0.05$) but the GPX activity dropped markedly ($p < 0.05$) in the muscle of early larval stage of shrimp in 20‰.

In conclusion the regulation of antioxidant system in early larval stage of *P. monodon* is different from the Juvenile when exposed to salinity stress. There are compensation mechanisms of between CAT and GPX for *P. monodon* response to stress.

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A NEW VIEW EXPLAINING HOW CADMIUM-TREATED PARENTS HAVE HIGHER CD-RESISTANT OFFSPRING: THE CASE OF TILAPIA LARVAE (*OREOCHROMIS MOSSAMBICUS*)

Su Mei Wu, Wan-Ling Yang-2008

Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 148(4): 469-
Abstract:

Cd-treated parents breed higher-Cd-resistant larvae than those that were never exposed. The purpose of this study was to identify the major factor enhancing the Cd resistance as assessed by the metallothionein (MT) expression in tilapia larvae. We compared the MT contents and Cd concentrations in larvae bred for different times, which were collected from parents that were either pretreated with Cd, pretreated with saline, or given no pretreatment. Changes in MT and Cd levels in the ovaries were determined upon an intraperitoneal (i.p.) or subcutaneous (s.c.) injection with Cd in female tilapia. Twelve weeks after an i.p. Cd injection, the MT contents and Cd concentrations in hepatic tissues of female tilapia were detected. Results showed that (1) Cd-pretreated females were more effective in transferring enhanced Cd tolerance to offspring than were males; (2) the higher Cd resistance, Cd contents, and MT expression of offspring were limited to larvae produced from breeding within 4 weeks of the injection; and (3) at 12 weeks after the i.p. Cd injection, females contained higher MT contents and Cd accumulation in hepatic tissue than those that had received an s.c. injection. In summary, we suggest that higher Cd resistance of larvae from the egg stage occurred after contamination of the female by Cd, as evidenced by an increase in MT expression induced in tilapia larvae.

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COMPARISON OF THE DIAMETER OF THE FRONTAL KNOBS IN ARTEMIA URMIANA GÜNTHER, 1899 (ANOSTRACA)

De Los Ríos, Patricio, Asem, Alireza-2008

Crustaceana 81(11):1281-1288

Abstract:

In four samples of *Artemia urmiana* Günther, 1899 from Urmia Lake, Iran, the following parameters were compared: the diameter of the frontal knobs (right and left), and the respective ratios of that diameter with total body length and head width. The results do not denote significant differences in frontal knob diameter that would agree with similar results observed in frontal knob diameter in populations of *A. franciscana* Kellogg, 1906 and *A. persimilis* Piccinelli & Prosdocimi, 1968. However, we report differences in the other parameters studied for each of the populations studied, that could be explained by the environmental heterogeneity of the studied site. Ecological, biogeographical, and systematic issues related with the results are discussed.

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RE-EMERGENCE OF VIBRIO TUBIASHII IN BIVALVE SHELLFISH AQUACULTURE: SEVERITY, ENVIRONMENTAL DRIVERS, GEOGRAPHIC EXTENT AND MANAGEMENT

Ralph A. Elston, Hiroaki Hasegawa, Karen L. Humphrey, Ildiko K. Polyak, Claudia C. Häse-2008

Diseases of Aquatic Organisms 82(2): 119-134

Abstract:

During 2006 and 2007, we documented the re-emergence of severe episodes of vibriosis caused by *Vibrio tubiashii* in shellfish hatcheries on the west coast of North America. Lost larval and juvenile production included 3 previously undescribed hosts, Pacific (*Crassostrea gigas*) and Kumamoto (*C. sikamea*) oysters and geoduck clams *Panope abrupta*, with a 2007 decline in larval oyster production of ~59% in one hatchery. Losses of larval and juvenile bivalves were linked to *V. tubiashii* blooms in the coastal environment, which were associated with the apparent mixing of unusually warm surface seawater and intermittently upwelled cooler, nutrient- and *Vibrio* spp.-enriched seawater. The ocean temperature elevation anomaly in 2007 was not clearly linked to an El Niño event, as was a similar

episode in 1998. Concentrations of the dominant shellfish-pathogenic vibrios were as high as 1.6×10^5 cfu ml⁻¹ in the cold, upwelled water. The bacteria possessed the genes coding for a protease and hemolysin described for *V. tubiashii*, and pathogenic isolates secreted these peptides. Lesions resulting from a classic invasive disease and a toxigenic noninvasive disease occurred in oyster and geoduck clam larvae. Management and prevention require reduction of incoming concentrations of the bacteria, reduction of contamination in water and air supplies and in stock chemical solutions, removal of bacterial toxins, and interruption of the cycle of bacterial amplification in the hatchery and in microalgal food supplies.

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DEVELOPMENT OF SENSORY ORGANS IN LARVAE OF AFRICAN CATFISH CLARIAS GARIEPINUS

Y. Mukai, A. D. Tuzan, L. S. Lim, N. Wahid, M. S. Sitti Raehanah, S. Senoo-2008

Journal of Fish Biology 73(7): 1648 – 1661

Abstract:

African catfish *Clarias gariepinus* hatched with morphologically immature features; however, sensory organs developed rapidly with fish growth. Although the eyes of newly hatched larvae were immature without pigment, in 2 day-old larvae, the retina of the eyes had already developed except for the rod cells. No free neuromasts were observed in newly hatched larvae. In 1 day-old larvae, however, free neuromasts were observed on the head and trunk. Free neuromasts increased with larval growth. Newly hatched larvae had simple round-shaped otic vesicles; however, all sensory epithelia of the inner ear were observed until the larvae were 3 days old. Two day-old larvae swam horizontally, had sharp teeth, commenced ingesting rotifers and also artificial feed (small-size pellets) under both light and dark conditions; by then the larvae already had many taste buds. Three day-old larvae showed negative phototaxis and cannibalism by eating their conspecifics. Most of the free neuromasts observed in this study had the peculiar feature of many microvilli around the sensory cells on the apical surface. Detected free neuromasts as ordinary type lateral-line organs were not observed in previous reports in teleosts. In 10 day-old larvae, there were two lines of free neuromasts on the flank and lower edge of the trunk; presumptive canal neuromasts were oval shaped and had begun to sink under the skin. The direction of maximum sensitivity of the neuromasts was parallel with the longitudinal axis of their elliptical apical surface.

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EVALUATION OF PCR METHODS FOR FIXED BIVALVE LARVAE

Hideki Sawada, Hajime Saito, Masatomi Hosoi, Haruhiko Toyohara-2008

Journal of the Marine Biological Association of the United Kingdom 88: 1441-1449

Abstract:

Investigating the spatio-temporal patterns of planktonic larvae is fundamental to studies regarding stock assessment and larval dispersal of commercial and non-commercial, i.e. invasive or rare marine invertebrates. Because of the difficulty involved in morphological identification of marine invertebrate larvae, various molecular methods based on PCR have been used to enhance taxonomic resolution. In previous studies, different methods for the preservation or pretreatment of larvae were applied in each case. However, no comparative studies have been conducted to determine the optimal method for PCR testing for bivalve larvae, and no information is available regarding the selection of an appropriate method.

This study compared the PCR success rate of 6 pretreatment methods for larvae of the Mediterranean blue mussel, which was preserved using different fixatives (70% ethanol, 100% ethanol, 70% acetone and 10% formalin). The results revealed that the success rate of PCR was different for each pretreatment; moreover, the use of ammonium sulphate and Tween 20 buffer with proteinase K digestion was found to be the most effective method. Some pretreatments showed lower success rates

for long-fixed larvae than for short-fixed larvae for formalin-fixed larvae; however, the success rate of PCR amplification for ethanol-fixed larvae pretreated by this method did not decrease through 1-year fixation. In addition, this pretreatment showed a high success rate for different fixation periods. These findings suggest that the selection of the pretreatment method is critically important for successfully amplifying larval DNA and that the pretreatment involving the use of ammonium sulphate prior to PCR amplification enables the use of fixatives for preserving bivalve larvae. This method will be utilized in various field studies and molecular genetic studies.

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EFFECTS OF PHOTOPERIOD ON SURVIVAL, GROWTH AND FEEDING OF LONGTOOTH GROUPER EPINEPHELUS BRUNEUS LARVAE

Kazuhisa Teruya, Kenzo Yoseda, Aya Fujii, Yuko Kurokawa, Shinichiro Kawai, Masakazu Oka, Toyohiro Nishioka, Syoji Nakano, Koh-Ichiro Mori, Takuma Sugaya, Katsuyuki Hamasaki-2008
Nippon Suisan Gakkaishi 74 (6): 1009-1016

Abstract:

The larvae of longtooth grouper *Epinephelus bruneus* were reared in a 150 kL tank until ten days post hatching under a natural photoperiod condition and diurnal rhythms of feeding and tryptic activity of larvae were examined. Further, effects of different photoperiods (24L: 0D, 12L: 12D, 6L: 6D: 6L: 6D, and 0L: 24D) on larval survival, growth and feeding were examined using 500 L tanks until 11 days post hatching. The longtooth grouper larvae fed on rotifers during light periods and tryptic activity was highest at 7 p.m. Larval gut contents were quite few during dark periods and larvae did not grow and survive in tanks with 0L: 24D photoperiod. The number of rotifers in larval guts was low in tanks with 24L: 0D photoperiod though larvae fed on rotifers slightly at night. Total length and survival rates of larvae reared in tanks under 24L: 0D and 6L: 6D: 6L: 6D conditions showed similar low values, and best growth and survival rates were observed in tanks under 12L: 12D condition.

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INFLUENCE OF PRESOAKING CONDITIONS OF SPAT COLLECTORS IN SEAWATER CONTAINING ADULT PACIFIC OYSTERS (*CRASSOSTREA GIGAS*) ON THEIR LARVAL SETTLEMENT

Yasushi Hirata, Asushi Hirata, Yoshinobu Tamura, Kazuya Nagasawa-2008

Nippon Suisan Gakkaishi 74 (6): 1017-1023

Abstract:

The presoaking of spat collectors in seawater containing adult Pacific oysters *Crassostrea gigas* is known to induce their larval settlement. In this study, we experimentally examined how presoaking conditions affect variations in success of larval oysters, using scallop *Patinopecten yessoensis* shell chips as spat collectors. The rate of larval settlement was enhanced by increase in water temperature, number of adult oysters, and length of presoaking period, while it was not affected by feeding to adult oysters. The ammonia-N concentration in the presoaked water was also closely related to the success of larval settlement. We herein propose a new indicator, termed condition index, which is the product, expressed in mg/L · hours, of the ammonia-N concentration multiplied by presoaked hours, and suggest that larval settlement is very successfully induced at about 20 mg/L · hours.

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EFFECTS OF HERBAL ENRICHED ARTEMIA SUPPLEMENTATION OVER THE REPRODUCTIVE PERFORMANCE AND LARVAL QUALITY IN SPENT SPAWNERS OF THE TIGER SHRIMP (*PENAEUS MONODON*)

M. Michael Babu, V. Sivaram, G. Immanuel, T. Citarasu, S.M.J. Punitha-2008

Abstract:

The effects of methanolic extracts of two terrestrial herbs *Withania somnifera* (WS) and *Mucuna pruriens* (MP) enriched *Artemia* supplementation (5% of the spawner's body weight/day) over the reproductive performance, biochemical parameters and larval quality indices in the spent spawners of *Penaeus monodon* were investigated. Among the three groups, MP fed group registered higher values in the reproductive performance like fecundity and frequency of spawning than the control group ($P<0.05$). The offspring quality in terms of egg size, fertilization rate, cumulative metamorphosis, cumulative larval survival and reduced larval abnormalities were significantly influenced by the WS supplementation when compared to the control ($P<0.05$). Total Protein and lipid levels were significantly higher in the WS fed group values than the control ($P<0.05$). WS supplementation also significantly influenced the hatching percentage, reduced larval abnormalities, cumulative metamorphosis, cumulative larval survival, haemolymph protein and lipid level than the control group ($P<0.05$). Both MP and WS have the potential to improve the performance of fecundity and larval quality in multiple spawning during the forced reproduction in the tiger shrimp.

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EARLY DEVELOPMENT OF CEPHALIC SKELETON IN HATCHERY-REARED GILTHEAD SEABREAM, *SPARUS AURATA*

Şahin Saka, Deniz Çoban, H. Okan Kamaçı, Cüneyt Süzer, Kürşat Fırat-2008

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Abstract:

The development of the cephalic skeleton in gilthead seabream, *Sparus aurata*, (L. 1758), larvae was examined from 1 to 41 days post-hatching during the early ontogeny under intensive culture conditions. At hatching, *S. aurata* larvae had a 2.68 mm total length (TL) and were devoid of any cephalic elements. At 2.85 mm TL, the trabecular bars and the ethmoid plate appeared. Development of jaws started during the yolk-sac stage. Teeth first appeared at larval stage (7.78 mm TL) and gill filaments formed at size of between 8.05 and 8.9 mm TL. Finally, the dentary, maxillary and Meckel's cartilage began to ossify at 9.20 mm TL. It is concluded that cartilaginous elements and structures could be related with vital functions in *S. aurata* larvae. Also, onset time of skull was found differently although same developmental pattern of skull has been described for most Sparidae species to date.

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