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NOTE

CHARACTERIZATION OF THE EARLY-STAGES OF THE WOLFFISH HYBRID ANARHICHAS MINOR ANARHICHAS LUPUS: CONSERVATION AND AQUACULTURE APPLICATIONS

Catherine M. Gaudreau, Nathalie R. Le François, Delphine Ditlecadet, Helge Tveiten, Pierre U. Blier-2009

Aquat. Living Resour. 22: 371-377

Abstract:

This is the first report of early-life developmental characteristics (coloration pattern, growth and survival) and genetic identification of the wolffish interspecific hybrid between *Anarhichas minor* and *A. lupus*, both endangered species in Canadian coastal water and of interest for cold-water aquaculture diversification. A first growth trial at 8 C featuring pure strains *A. minor* and the hybrid *A. minor A. lupus* in triplicates was conducted in 2006 during the period from 0 to 140 days post-hatch. A second growth trial was runned in 2007 featuring *A. minor*, *A. lupus* and the reciprocal hybrids *A. minor A. lupus* and *A. lupus A. minor*. Egg development indicators and early-hatching characteristics are reported.

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CONTROLLED BREEDING AND LARVAL REARING TECHNIQUES OF MARINE ORNAMENTAL FISHES

G. Gopakumar, B. Ignatius, I. Santhosi, N. Ramamoorthy-2009

Asian Fisheries Science 22(2): 359-855

Abstract:

International trade of marine ornamental fishes has been expanding rapidly in recent years, and the fact that nearly 98% of the species traded are collected from reef habitats is of vital concern for the

conservation of the fragile coral reef ecosystem. Hence, it is widely accepted that the ultimate answer to a long-term sustainable trade of marine ornamental fishes is only through the development of hatchery production technologies. The techniques for broodstock development, breeding and seed production of three species of damselfishes viz. the threespot damselfish, *Dascyllus trimaculatus*, the humbug damselfish, *Dascyllus aruanus* and the blue damselfish, *Pomacentrus caeruleus*, were developed and standardised, which can be scaled up for commercial level production. Broodstock development was done in one-tonne Fiber Reinforced Plastic (FRP) tanks with biological filter and by feeding with natural feeds. The size range of broodstock fish of *D. trimaculatus*, *D. aruanus* and *P. caeruleus* were 9-10, 7-8 and 7-9 cm, respectively. The number of eggs per spawning ranged from 5000 to 15000. The interval between two successive spawnings ranged from 3 to 14 days. The eggs were attached either on the sides of the broodstock tank or on the substratum provided in the broodstock tank. Parental care by the male was noted. Hatching occurred on the evening of the fourth day of incubation. The larvae were altricial type with no mouth opening at the time of hatching for *D. trimaculatus* and *D. aruanus*. The larvae of *P. caeruleus* were with mouth opening at the time of hatching. The length range of newly hatched larvae was 1.5-2.5 mm and the range of mouth opening was 150-200 μ .

Larviculture was done in five-tonne capacity FRP tank by employing greenwater technique. Copepod nauplii were used as the starter feed and after about two weeks when the mouth opening of the larvae had reached around 450 μ , newly hatched *Artemia* nauplii were supplemented. The metamorphosis period ranged from 20 to 40 days. Several batches of the three species were hatchery produced, and the technique can be scaled up for commercial level production for ornamental fish trade.

MASS MORTALITY ASSOCIATED WITH A FROG VIRUS 3- LIKE RANAVIRUS INFECTION IN FARMED TADPOLES *RANA CATESBEIANA* FROM BRAZIL

Rolando Mazzoni, Albenones José de Mesquita, Luiz Fernando F. Fleury, Wilia Marta Elsner Diederichsen de Brito, Iolanda A. Nunes, Jacques Robert, Heidi Morales, Alexandre Siqueira Guedes Coelho, Denise Leão Barthasson, Leonardo Galli, Marcia H. B. Catroxo-2009

Diseases of Aquatic Organisms 86:181-191

Abstract:

Ranaviruses (Iridoviridae) are increasingly associated with mortality events in amphibians, fish, and reptiles. They have been recently associated with mass mortality events in Brazilian farmed tadpoles of the American bullfrog *Rana catesbeiana* Shaw, 1802. The objectives of the present study were to further characterize the virus isolated from sick *R. catesbeiana* tadpoles and confirm the etiology in these outbreaks. Sick tadpoles were collected in 3 farms located in Goiás State, Brazil, from 2003 to 2005 and processed for virus isolation and characterization, microbiology, histopathology, and parasitology. The phylogenetic relationships of *Rana catesbeiana* ranavirus (RCV-BR) with other genus members was investigated by PCR with primers specific for the major capsid protein gene (MCP) and the RNA polymerase DNA-dependent gene (Pol II). Sequence analysis and multiple alignments for MCP products showed >99% amino acid identity with other ranaviruses, while Pol II products showed 100% identity. Further diagnostics of the pathology including histology and transmission electron microscopy confirmed the viral etiology of these mass deaths. As far as we know, this is the first report of a ranaviral infection affecting aquatic organisms in Brazil. Additionally, our results suggest that American bullfrogs may have served as a vector of transmission of this virus, which highlights the potential threat of amphibian translocation in the world distribution of pathogens.

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EFFECTS OF THE TIMING OF INITIAL FEEDING ON GROWTH AND SURVIVAL OF SPOTTED MANDARIN FISH *SINIPERCA SCHERZERI* LARVAE

L. Zhang, Y. J. Wang, M. H. Hu, Q. X. Fan, S. G. Chenung, P. K. S. Shin, H. Li, L. Cao-2009

Journal of Fish Biology 75(6): 1158 - 1172

Abstract:

The effects of delayed first feeding on growth and survival of spotted mandarin fish *Siniperca scherzeri* larvae were examined under controlled conditions. Morphometric characters [yolk-sac volume, oil globule volume, head depth (HD), body depth (BD), eye diameter (ED), musculature height (MH), mouth diameter (MD) and total length (LT)], body mass (M), specific growth rate (SGR) and survival were evaluated under different first-feeding time (2, 3, 4 and 5 days after hatching). Larvae began to feed exogenously at 2 days after hatching (DAH) and the point of no return (PNR) occurred between 5 and 6 DAH at 23° C, range $\pm 1.0^\circ$ C. The yolk volume of larvae first-fed at 2 days had a significant difference compared with that of larvae first-fed at 3, 4 and 5 days on 3 and 4 DAH. The larvae first-fed at 2 days achieved comparatively better growth performance than that of 3, 4 and 5 days. On 5 DAH, all morphometric characters had significant differences between 2 and 5 days and 2 and 4 days initial feeding, respectively. Total mortality was recorded on 9 DAH for the larvae first-fed at 5 days. On 12 DAH, significant differences were observed between 2 and 4 days and 3 and 4 days initial feeding for all morphometric characters. From 16 DAH to the end of experiment, all growth variables of the larvae first-fed at 2 days were significantly higher than those in other treatments. The SGR (2–9 DAH) first-fed at 2 and 3 days were significantly higher than 4 and 5 day treatments, and the SGR (9–16 DAH) first-fed at 2 days was significantly higher than 3 and 4 day treatments. There was no significant difference, however, of SGR (16–28 DAH) among treatments. Survival rate was significantly higher at 2 days initial feeding (27.42%) when compared with 3 (15.96%) and 4 days (7.92%) initial feeding at the end of experiment. The present study suggests that the first feeding of *S. scherzeri* larvae should be initiated at 2 days after hatching for achieving good growth and survival.

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EARLY DEVELOPMENT OF THE DIGESTIVE TRACT (PHARYNX AND GUT) IN THE EMBRYOS AND PRE-LARVAE OF THE EUROPEAN SEA BASS *DICENTRARCHUS LABRAX*

E. Sucré, M. Charmantier-Daures, E. Grousset, G. Charmantier, P. Cucchi-Mouillot-2009

Journal of Fish Biology 75(6): 1302 - 1322

Abstract:

The European sea bass *Dicentrarchus labrax* is a marine teleost important in Mediterranean aquaculture. The development of the entire digestive tract of *D. labrax*, including the pharynx, was investigated from early embryonic development to day 5 post hatching (dph), when the mouth opens. The digestive tract is initialized at stage 12 somites independently from two distinct infoldings of the endodermal sheet. In the pharyngeal region, the anterior infolding forms the pharynx and the first gill slits at stage 25 somites. The other three gill arches and slits are formed between 1 and 5 dph. Posteriorly, in the gut tube region, a posterior infolding forms the foregut, midgut and hindgut. The anus opens before hatching, at stage 28 somites. Associated organs (liver, pancreas and gall bladder) are all discernable from 3 dph. Some aspects of the development of the two independent initial infoldings seem original compared with data in the literature. These results are discussed and compared with embryonic and post-embryonic development patterns in other teleosts.

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EFFECTS OF CALANOID COPEPOD *SCHMACKERIA POPLESIA* AS A LIVE FOOD ON THE GROWTH, SURVIVAL AND FATTY ACID COMPOSITION OF LARVAE AND JUVENILES OF JAPANESE FLOUNDER, *PARALICHTHYS OLIVACEUS*

Guangxing Liu, Donghui Xu-2009

Journal of Ocean University of China 8(4): 359-365

Abstract:

Zooplankton constitutes a major part of the diet for fish larvae in the marine food web, and it is generally believed that copepods can meet the nutritional requirements of fish larvae. In this study, calanoid copepod *Schmackeria poplesia*, rotifer *Brachionus plicatilis* and anostraca crustacean *Artemia* sp. were analyzed for fatty acid contents, and were used as live food for culturing larval Japanese

flounder, *Paralichthys olivaceus*. The total content of three types of HUFAs (DHA, EPA and ARA) in *S. poplesia* was significantly higher than that in the other two live foods ($P < 0.01$). Three live organisms were used for raising larvae and juveniles of *Paralichthys olivaceus* respectively for 15 and 10 d. Then the growth, survival and fatty acid composition of the larvae and juveniles were investigated. The results showed that the larvae and juveniles fed with copepods (*S. poplesia*) had significantly higher growth rate than those fed with the other two organisms ($P < 0.01$). The survival of the flounder larvae fed with copepods was significantly higher than that of the others ($P < 0.01$), and the survival of the juvenile fish fed with copepods was higher than that fed with *Artemia* ($P < 0.05$). The contents of three types of HUFAs (DHA, EPA and ARA) and the ratio of DHA/EPA in larval and juvenile flounder *P. olivaceus* were analyzed. The results showed that the contents of DHA, EPA and ARA in the larvae and juveniles fed with *S. poplesia* were higher than those fed with a mixed diet or *Artemia* only, and the ratio of EPA/ARA in larvae and juveniles of *P. olivaceus* fed with *S. poplesia* was lower than that in the case of feeding with a mixed diet or *Artemia* only. The present data showed that copepod is the best choice for feeding the larvae and juveniles of fish considering its effects on the survival, growth and nutrition composition of the fish.

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CRYOPRESERVATION OF VELIGER LARVAE OF TRUMPET SHELL, *CHARONIA SAULIAE*: AN ESSENTIAL PREPARATION TO ARTIFICIAL PROPAGATION

Kyoung Ho Kang, Zhifeng Zhang, Zhenmin Bao, Mingyu Shao-2009

Journal of Ocean University of China 8(3): 265-269

Abstract:

Trumpet shell, *Charonia sauliae*, is an endangered and valuable species, but its artificial propagation protocol has not been successfully established. To estimate the possibility of cryopreservation for larvae of *C. sauliae*, which is a potential preparation for its artificial reproduction and further research, in this study a protocol for the cryopreservation of veliger larvae of trumpet shell was optimized. Through a two-step cryopreservation procedure, four kinds of cryoprotectants (ethylene glycol, 1, 2-propanediol, dimethyl sulfoxide and glycerol) were employed at three concentrations (1.0, 1.5 and 2.0 molL⁻¹) respectively and survival rates of larvae were determined after a storage of 1h. The larvae frozen with these four cryoprotectants after 1 h storage were cultured, and then survival rates were determined at 24, 72 and 120 h after thawing. Dimethyl sulfoxide at a concentration of 1.5 molL⁻¹ showed the best protective effect in all experiments ($p < 0.05$). And survival rates of larvae frozen with dimethyl sulfoxide were determined after 1, 7 and 15 d of storage. The survival rates of larvae frozen with 1.5 molL⁻¹ dimethyl sulfoxide after 1 h, 1 d, 7 d and 15 d of storage were 80.77% \pm 7.51%, 80.34% \pm 11.28%, 83.10% \pm 9.14% and 77.23% \pm 6.22% respectively. No significant differences in survival rates of larvae frozen with dimethyl sulfoxide were observed after various storage periods ($p > 0.05$).

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USE OF MODIFIED LIVE VACCINES IN AQUACULTURE

Craig A. Shoemaker, Phillip H. Klesius, Joyce J. Evans, Covadonga R. Arias-2009

Journal of the World Aquaculture Society 40(5): 573 - 585

Abstract:

Vaccination is an important disease management strategy used to maintain human and animal health worldwide. Vaccines developed for aquaculture have reduced antibiotic use in fish production. Original fish vaccines were bacterins (formalin-killed bacteria) delivered through immersion or injection that induced humoral (antibody) immunity. Next generation vaccines relied on multiple killed antigens delivered with an adjuvant to enhance vaccine effectiveness. Work in the 1990s showed the use of various strategies to develop modified live vaccines for use in fish. A modified live vaccine is a live pathogen that has been rendered non-pathogenic or avirulent by physical, chemical, or genetic engineering methods. The modified live vaccine typically retains its ability to infect the host which allows for effective presentation of protective antigens to generate cellular immunity (CD4 or CD8 T-

cell responses). Modified live vaccines are advantageous in that they can be easily delivered (i.e., by immersion to young fish) and stimulate both humoral and cellular immunity of long duration. Disadvantages include issues with modified live vaccine safety to the host and environment. A successful modified live vaccine for use in warm water aquaculture is used to highlight the live vaccine strategy.

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CULTIVATION OF THE PARACALANID COPEPOD, *BESTIOLINA SIMILIS* (CALANOIDA: CRUSTACEA)

Kyle VanderLugt, Michael J. Cooney, Anna Lechner, Petra H. Lenz-2009

Journal of the World Aquaculture Society 40(5): 616 - 628

Abstract:

First feed production continues to be a major barrier to the cultivation of many fish species. Although copepod nauplii are a suitable food, consistency and high production have been difficult. Temporal changes in production in batch cultures of the calanoid copepod, *Bestiolina similis*, were investigated to develop management strategies for the use of copepod nauplii as a live food. Population abundances and female egg production rates were measured, and recruitment and mortality rates were calculated. Relative expression levels of a molecular biomarker for stress, heat shock protein hsp70, were determined using real-time quantitative polymerase chain reaction. The population cycle included a period of rapid increase in abundances, followed by a steep decline and a period of stable but low population densities. Initially, egg production exceeded 25 eggs per female per day and low mortality rates prevailed. The population decline was preceded by upregulation of hsp70 and followed by an 80–90% decline in female fecundity and an increase in mortality rates. Egg production rates remained below four eggs per female per day even after new generations of females reached adulthood. The predictable population cycle provides opportunities to coordinate nauplius production rates with first feed needs of fish larvae.

(Pacific Biosciences Research Center, 1993 East – West Road, University of Hawaii at Manoa, Honolulu, Hawaii 96822)

ASSESSMENT OF EGG AND LARVAL QUALITY DURING HATCHERY PRODUCTION OF THE TEMPERATE SEA CUCUMBER, *AUSTRALOSTICHOPUS MOLLIS* (LEVIN)

Andrew David Morgan-2009

Journal of the World Aquaculture Society 40(5): 629 - 642

Abstract:

A greater understanding of the causes of variation in egg and larval quality during larval rearing of sea cucumbers will assist in the development of methods to further improve production in hatcheries. For *Australostichopus mollis* the techniques and methods used in other hatcheries during fertilization resulted in polyspermy occurring in 10–60% of spawned eggs. Despite this, hatch rates were usually greater than 90%. By Day 18 when fed the diatom *Chaetoceros muelleri* at 2000 cells/mL/day, survival in cultures varied between $35 \pm 26\%$ and $57 \pm 25\%$. Of remaining larvae, 10–12% remained in the water column for long periods and did not complete metamorphosis. Furthermore, observation of development in relation to growth and feeding rather than gut content was a better indicator of larval competence. Larvae that were between 700 and $1000 \pm 50 \mu\text{m}$ in length had between four and eight hyaline spheres in the folds of the ciliated band and were in various stages of metamorphosis and settlement. Assessment and comparison of current and previous methods enabled a greater understanding of sources of variation in egg and larval quality, impacts on the production cycle, and suggested improvements.

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PARENTAGE DETERMINATION AND EFFECTIVE POPULATION SIZE ESTIMATION IN MASS SPAWNING PACIFIC OYSTER, CRASSOSTREA GIGAS, BASED ON MICROSATELLITE ANALYSIS

Ronghua Li, Qi Li, Ruihai Yu-2009

Journal of the World Aquaculture Society 40(5): 667 - 677

Abstract:

Seven high polymorphic microsatellite loci were used to determine the pedigrees in a mass spawning of Pacific oyster, *Crassostrea gigas*, and to estimate the genetic variability between broodstock and offspring. Parental assignment was performed on a total of 155 individuals, including 141 offspring, 8 candidate mothers, and 6 candidate fathers. The assignment results of real offspring were generally in agreement with simulation with a success rate over 99% using only six of these loci. The allelic diversity and observed heterozygosity (H_o) exhibited similarity between parents and offspring populations, but the expected heterozygosity (H_e) had a significant decrease in offspring. Although all the males and females contributed to the next generation, the variances of reproductive success and unequal sex ratio resulted in a decline in effective population size ($N_e = 11.42$). The inbreeding rate of this small-scale, mass spawning population was estimated at approximately 16.5% per generation. This gave us an insight that when designing breeding programs based on mass spawning for future oyster cultivation generations, the higher inbreeding and lower effective population size should be considered. (Fisheries College, Ocean University of China, Qingdao 266003, China)

THE EFFECTS OF TANK COLOR AND LIGHT INTENSITY ON GROWTH, SURVIVAL, AND STRESS TOLERANCE OF WHITE SEABASS, *TRACTOSCION NOBILIS*, LARVAE

Journal of the World Aquaculture Society 40(5): 702 - 709

Dave Jirsa, Mark Drawbridge, Kevin Stuart-2009

(Hubbs-SeaWorld Research Institute, 2595 Ingraham Street, San Diego, California 92109, USA)

DISAPPEARANCE OF MALACHITE GREEN RESIDUES IN FRY OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AFTER TREATMENT OF EGGS AT THE HATCHING STAGE

Kirsi Niska, Tiina Korkea-aho, Erja Lindfors, Tapio Kiuru, Markku Tuomainen, Jouni Taskinen, Kimmo Peltonen-2009

Aquaculture 297(1-4): 25-30

Abstract:

The disappearance of malachite green (MG) residues was determined in fry of rainbow trout (*Oncorhynchus mykiss*) after six repeated treatments of the eggs at the hatching stage with MG oxalate at exposure levels of 1, 3 and 6 mg l⁻¹ for 30 min. Fry samples were taken from newly hatched fry (0 days post-hatch, d.p.h.) and at regular time intervals at 16, 31, 43, 57 and 96 d.p.h. The residues of MG and its major metabolite, leucomalachite green (LMG), were found to accumulate in the fry after MG treatments of eggs, with the highest residue levels being determined in the newly hatched fry. After exposures of 3 mg l⁻¹ MG, mean concentrations of 1170 ± 106 µg kg⁻¹ and 276 ± 38.6 µg kg⁻¹ (n = 3) were found in fry for LMG and MG, respectively. However, the disappearance of residues occurred rapidly in the fry, such that by 43 d.p.h. only low levels of LMG could be determined. To confirm the elimination of residues, determinations were made also in fry muscle at 96 d.p.h. but no residues were detected. The residues of MG in fry were determined by liquid chromatography-tandem mass spectrometric (LC-MS/MS) analysis with a limit of detection (LOD) of 0.5 µg kg⁻¹ and a limit of quantification (LOQ) of 1.0 µg kg⁻¹. The accumulation as well as the elimination of residues correlated well with the level of exposure. During the study, the fry increased their weight, such that at the end of the study, their mean body weight was about 150 times greater than the mean body weight of the newly hatched fry. As the disappearance of residues occurred in conjunction with the growth of fry, the present results indicate that no residues of MG will remain in the fish intended for human consumption, if MG treatment takes place at the hatching stage under controlled conditions.

(Finnish Food Safety Authority (Evira), Chemistry and Toxicology Unit, Mustialankatu 3, FI-00790 Helsinki, Finland; email of Erja Lindfors: erja.lindfors@evira.fi)

HERITABILITY OF VITELLOGENIN IN HEMOLYMPH, A PRE-SPAWNING SELECTABLE TRAIT IN *PENAEUS (LITOPENAEUS) VANNAMEI*, HAS A LARGE GENETIC CORRELATION WITH OVARY MATURITY MEASURED AS OOCYTES MEAN DIAMETER

Ana M. Ibarra, Thomas R. Famula, Fabiola G. Arcos-2009

Aquaculture 297(1-4): 64-69

Abstract:

Vitellogenin, when measured in shrimp hemolymph has been found to reflect the degree of female shrimp ovarian development before eyestalk ablation, and to be a useful predictor of ovarian development after ablation. The present study estimated the heritability and genetic correlations of vitellogenin in hemolymph, and the number and diameter of oocytes in gonads, as a measure of reproductive potential of Pacific white shrimp. Furthermore, we examined whether any of these characters was influenced by the action of a segregating locus of large effect through complex segregation analysis. Shrimps were grown at high density in a raceway, and evaluated at an average weight of 35 g. The heritability of vitellogenin in hemolymph (Vtg) and oocytes mean diameter were significant (0.29 ± 12 and 0.23 ± 11), however, the heritability for the number of oocytes ($0.11 \pm .09$) was not significant. A large genetic correlation was found between Vtg and oocytes mean diameter (0.90 ± 0.08), but not for Vtg and number of oocytes, or for oocytes mean diameter and numbers. Regardless of a significant bimodal distribution being present in the shrimp population for vitellogenin in hemolymph, complex segregation analyses could not detect a major segregating locus for this or any of the traits. Vtg is the first reproductive genetic trait found to predict reproductive capacity of shrimp before eyestalk ablation, and given its high heritability and genetic correlation with oocytes mean diameter, its utilization in selective breeding is expected to allow simultaneous improvement of growth and reproductive capacity in shrimp.

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GENETIC DIVERSITY OF DOMESTICATED STOCKS OF STRIPED CATFISH, *PANGASIANODON HYPOPHthalmus* (SAUVAGE 1878), IN THAILAND: RELEVANCE TO BROODSTOCK MANAGEMENT REGIMES

Uthairat Na-Nakorn, Thakkina Moeikum-2009

Aquaculture 297(1-4): 70-77

Abstract:

Thai stocks of striped catfish, *Pangasianodon hypophthalmus* (Sauvage 1878), have a relatively long domestication history (> 20 generations) that began with fish of Chaophraya River origin. The genetic diversity of this species was studied in six hatchery populations with two different broodstock management regimes (without introduction of either wild or domesticated stocks vs. with occasional introduction of either original or non-original populations) and in three wild populations (from the Chaophraya River and its tributary and from the Mekong River). The results, based on five microsatellite loci, revealed high allele diversity of the hatchery populations ($Ar = 6.53-8.06$) with the introduction of non-original stock (from the Mekong population) relative to those with the introduction of original stock (from the Chaophraya population) or without introduction ($Ar = 3.18-6.06$). Notably, heterozygosity was high in the majority of populations ($Ho = 0.633-0.763$; $He = 0.593-0.834$). Genetic introgression from the Mekong population was observed in every population, as revealed by Bayesian population assignment. The wild populations showed unexpectedly low allele diversity ($Ar = 4.89-5.98$) and were not genetically differentiated from the hatchery populations (as revealed by AMOVA).

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A FRESH-FOOD MATURATION DIET WITH AN ADEQUATE HUFA COMPOSITION FOR BROODSTOCK NUTRITION STUDIES IN BLACK TIGER SHRIMP *PENAEUS MONODON* (FABRICIUS, 1798)

Nguyen Duy Hoa, Roeland Wouters, Mathieu Wille, Vu Thanh, Tran Kim Dong, Nguyen Van Hoa, Patrick Sorgeloos-2009

Aquaculture 297(1-4): 116-121

Abstract:

Two fresh-food maturation diets were tested on wild *Penaeus monodon* broodstock during a period of 1 month in primary quarantine and 3 months in secondary quarantine; diet A was composed of 70.30% squid (*Photololigo* sp.), 7.66% marine worm (polychaetes), 7.94% oyster (*Crassostrea* sp.), and 14.10% pork liver and diet B was composed of 37.39% squid, 16.50% marine worm, 27.14% oyster, and 18.98% pork liver; all on a dry-weight basis. The formulation of diet B was to resemble the ratios of ARA/EPA, DHA/EPA, and n-3/n-6 fatty acids of mature ovaries of wild *P. monodon*. At the start of primary quarantine, the average weight of the shrimps allocated to the two diets was not significantly different (129.71 ± 2.96 g for females and 87.88 ± 2.49 g for males in the group of diet A and 131.74 ± 1.75 g for females and 88.95 ± 2.05 g for males in the group of diet B).

After secondary quarantine, the growth rate of the female shrimps receiving diet B was significantly greater ($P < 0.05$) than the female shrimps fed diet A ($24.44b \pm 4.98\%$ compared to $12.89a \pm 3.24\%$, respectively). Shrimp fed diet B performed better than shrimp fed diet A in terms of spawning frequency (85% versus 57%) and fecundity ($458,796a \pm 35,658$ and $245,718b \pm 34,736$ eggs/spawn, respectively), but number of spawns, hatching rate, fertilization rate and metamorphosis rate of the nauplii into zoea did not differ between the treatments ($P > 0.05$). The success of diet B in terms of spawning frequency, fecundity, fertilization rate and hatching rate indicates the importance of the ARA/EPA and DHA/EPA ratios in broodstock nutrition of black tiger shrimp. This study also confirmed the success of natural mating of *P. monodon* in small tanks (1.25 m² bottom area). The two-step biosecure quarantine procedure was applicable for producing SPF shrimp.

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ARTEMIA REPLACEMENT IN CO-FEEDING REGIMES FOR MYDIA AND POSTLARVAL STAGES OF *LITOPENAEUS VANNAMEI*: NUTRITIONAL CONTRIBUTION OF INERT DIETS TO TISSUE GROWTH AS INDICATED BY NATURAL CARBON STABLE ISOTOPES

Julián Gamboa-Delgado, Lewis Le Vay-2009

Aquaculture 297(1-4): 128-135

Abstract:

The nutritional contribution from co-fed *Artemia* nauplii and inert diets to growth in mysis and early postlarval shrimp *Litopenaeus vannamei* was assessed by analyzing the carbon stable isotope ratios ($\delta^{13}C$) in diets and shrimp tissue. *Artemia* nauplii and inert diets showed significantly different $\delta^{13}C$ values but similar carbon contents and were supplied as single diets and also in three co-feeding regimes in which 25, 50 and 75% of the *Artemia* was replaced by inert diet on a dry weight basis, so that all feeding regimes provided similar amounts of dietary carbon. Shrimp $\delta^{13}C$ values were significantly influenced by the different feeding regimes and reached isotopic equilibrium with their respective diets as soon as 5 days. Survival was significantly higher in co-fed animals than in those fed either *Artemia* or inert diet alone. There was no significant difference in growth between shrimp fed on *Artemia* only and those co-fed *Artemia* with inert diet, although the variability was high. Growth and survival were very low in shrimp fed only inert diet. Results from an isotope mixing model suggest that observed nutrient contributions from *Artemia* nauplii were significantly higher than expected contributions indicated by proportions established in the co-feeding regimes. From mysis 3 to postlarvae 5, nutrient contributions to growth in the dietary regime providing equal carbon amounts of each diet type ranged from 73 to 87% for *Artemia* and from 13 to 27% for inert diet. Shrimp fed the 25% *Artemia* replacement regime exhibited a significantly higher retention of dietary carbon from the inert diet than those fed inert diet alone. This may have resulted from greater ingestion and/or assimilation of the inert diet in the presence of *Artemia*, combined with the higher growth rate supported by co-feeding. The results demonstrate the effectiveness of up to 50% replacement of *Artemia* with inert diet for *L. vannamei* mysis and early postlarval stages, indicating also that the inert diet may provide specific nutrients that promote higher survival, while digestibility may limit its

contribution to tissue growth. The isotopic influence of maternal nutrients and dietary sources on earlier larval stages was also described.

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EFFECTS OF DIETARY PHOSPHORUS AND CALCIUM LEVEL ON GROWTH AND SKELETAL DEVELOPMENT IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FRY

Stéphanie Fontagné, Nadia Silva, Didier Bazin, Angela Ramos, Peyo Aguirre, Anne Surget, Antonio Abrantes, Sadasivam J. Kaushik, Deborah M. Power-2009

Aquaculture 297(1-4): 141-150

Abstract:

The effects of dietary levels of phosphorus (P) and calcium (Ca) on skeletal development and mineral deposition in rainbow trout (*Oncorhynchus mykiss*) fry were studied. Six semi-purified diets were formulated with graded levels of P and Ca. The basal diet A contained only P supplied by casein at 0.5% of dry matter. Other diets B, C, D and E were supplemented with 0.4, 0.8, 1.2 and 1.6% P supplied as a 1:1 mixture of NaH₂PO₄/KH₂PO₄ resulting in 0.8, 1.2, 1.6 and 2.2% total P, respectively. These five diets were supplemented with 1% Ca supplied as CaCO₃ whereas another diet F, supplemented with 0.8% P, was Ca-free. Each diet was distributed to 3 replicate tanks of 600 swim-up fry (initial mean weight: 0.1 g) at a water temperature of 17 °C over a 12-week growth trial. Fish were hand-fed 6 times a day to visual satiety.

There was no significant effect of dietary P (0 to 1.6%) or Ca (0 or 1%) supplementation on growth (final mean weight: 4.0 ± 1.2 g). Survival of fish fed with diet E containing a high level of P was significantly lower compared to other groups (10% vs. 65%, respectively). Fish fed with diet A displayed lower whole-body ash and P content compared to other groups (1.98 vs. 2.49% and 0.35 vs. 0.46% wet weight, respectively). Phosphorus retention decreased with increasing dietary P level (from 100% in group A to about 20% in group E). The quantitative image analysis of 28-day-old fry double stained with Alcian blue and Alizarin red S revealed that individuals from group A were less ossified compared to individuals from other dietary groups. Quantitative analysis of skeletal formation using cumulative counts of endochondral and dermal structures revealed a significant delay in ossification of endochondral structures at day 11 in fish fed with mineral rich or poor diets (diets A, E and F). However, at day 28, no significant difference in ontogeny of endochondral or dermal structures was found between the trout fed with the control diet and those fed with a high P diet (E) or a low Ca diet (F), suggesting recovery is possible in rainbow trout.

In conclusion, we found that both dietary deficiency and excess of P are detrimental to rainbow trout fry development: excess P affects survival, while P deficiency affects bone calcification. Calcium deficiency appears to exert some delay in ossification processes without affecting final bone mineralization.

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EGG PRODUCTION, EGG HATCHING SUCCESS AND POPULATION INCREASE OF THE TROPICAL PARACALANID COPEPOD, *BESTIOLINA SIMILIS* (CALANOIDA: PARACALANIDAE) FED DIFFERENT MICROALGAL DIETS

Thomas Camus, Chaoshu Zeng, A. David McKinnon-2009

Aquaculture 297(1-4): 169-175

Abstract:

A series of experiments was conducted to evaluate the suitability of ten microalgal diets, including 4 monoalgal, 5 binary and 1 tri-algal diet, for the culture of the tropical paracalanid copepod *Bestiolina similis*. The four monoalgal diets were the Tahitian strain of *Isochrysis* sp. (T-Iso), Pavlova 50 (Pav), *Tetraselmis chuii* (Tet) and the diatom *Chaetoceros muelleri* (Chaet), the 5 binary diets were Tet + T-Iso, Tet + Pav, T-Iso + Pav, Chaet + Tet and Chaet + Pav while the tri-algal diet was T-Iso + Tet + Pav.

After feeding *B. similis* with 10 algal diets for 3 days, 24 h egg production rate (EPR, eggs female⁻¹ day⁻¹) was obtained for each diet treatment by averaging the egg output of 6 individual females (replaced daily) for 4 consecutive days. The tri-algal diet T-Iso + Tet + Pav produced the highest EPR (44.1 eggs female⁻¹ day⁻¹), which was significantly higher than any other diets tested ($p < 0.05$). Highest 48 h and 96 h egg hatching rates (EHR) were also found from the eggs produced by *B. similis* fed with T-Iso + Tet + Pav (48 and 96 h EHR = 91.0% and 96.3% respectively) and significant differences in 48 h EHR were detected for 5 out of the 9 other diets tested, while for 96 h EHR, only for 2 diets. Population increase was determined over a 12 day culture period for 10 initial *B. similis* adults (7 females and 3 males) and the result showed that the tri-algal diet T-Iso + Tet + Pav produced significantly higher population number by the end of 12 days than any of the mono-algal or binary diets tested ($p < 0.05$). When all developmental stages were included (including eggs), the tri-algal diet produced a population increase from 10 to 887 over the 12 days. The binary diet Tet + T-Iso was the second most productive diet, providing a total population increase from 10 to 541, which was significantly higher than the rest of the diets, except that of Pav + T-Iso (479), T-Iso (334) and Chaet + Pav (285) ($p > 0.05$).

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COMPARISON OF REPRODUCTIVE PERFORMANCE AND OFFSPRING QUALITY OF GIANT FRESHWATER PRAWN (*MACROBRACHIUM ROSENBERGII*) BROODSTOCK FROM DIFFERENT REGIONS

Dinh The Nhan, Mathieu Wille, Le Thanh Hung, Patrick Sorgeloos-2009

Aquaculture 298(1-2): 36-42

Abstract:

An experiment was conducted to compare the reproductive performance and offspring quality of *Macrobrachium rosenbergii* broodstock from four different sources: (1) Vietnam wild; (2) Vietnam pond-cultured; (3) Hawaii pond-cultured and (4) China pond-cultured *M. rosenbergii* females were individually followed for 180 days in three 1200-l fresh water recirculation systems and fed a commercial diet. Ovarian development, moulting and spawning events were checked daily. In addition a number of egg and larval quality parameters were determined. The breeding frequency, fecundity, egg laying success rate, egg dimensions and egg hatchability were not significantly different between animals from the four different sources. However, there were significant differences in terms of offspring quality between the different broodstock sources. Individual dry weight, larval development rate, time to reach the postlarval stage, postlarval survival and tolerance to ammonia toxicity were all better in the offspring originating from China pond-reared and Vietnam pond-reared broodstock sources compared to those originating from Vietnam wild and Hawaii pond-reared sources. Moreover, offspring quality from Chinese and Vietnamese pond-reared broodstock proved more stable in terms of ammonia tolerance over three consecutive reproduction cycles. In general, the pond-reared broodstock from China and from Vietnam resulted in better offspring quality than the Hawaii pond-reared and Vietnam wild broodstock. These results indicate that broodstock sourcing deserves proper attention in hatchery operations of *M. rosenbergii*. It furthermore proves that domesticated (pond-reared) animals are not necessarily inferior as breeders compared to wild-sourced animals. The results may also point out the potential to selectively breed stocks with improved characteristics adapted to the local culture environment.

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EFFECT OF ALGAL DIET AND TEMPERATURE ON SURVIVAL, GROWTH AND BIOCHEMICAL COMPOSITION OF SPAT OF THE LION'S PAW SCALLOP *NODIPECTEN SUBNODOSUS*

Ana Nallely Cerón-Ortiz, Beatriz Cordero, Bertha Olivia Arredondo-Vega, Domenico Voltolina-2009

Aquaculture 298(1-2): 64-69

Abstract:

To find the best conditions for production, spat of the scallop *Nodipeecten subnodosus* were grown for 10 weeks at 23, 26 or 29 °C, and fed with 2:1 mixtures (cell:cell) of *Chaetoceros calcitrans* with *Pavlova lutheri* or *Isochrysis* sp. (diets M1 and M2) or with a 1:1 mixture of *P. lutheri* and *Isochrysis* sp. (diet M3). Mortality was 100% after 4 weeks at 29 °C. Survival was significantly higher with diet M1 than with the other diets, and was 51 and 46% at 23 and 26 °C, respectively. With diets M2 and M3, the respective percentages of survival were 39.39 and 33.84% at 26 °C and 23.03 and 19.85% at 23 °C. The best growth in shell height was with diet M2, but there were no differences in final organic weight between diets M1 and M2; diet M3 gave the lowest spat growth. The highest protein content was with diet M1 at 23 °C. This diet gave the lowest carbohydrate contents of the spat at both temperatures, and the lowest spat lipid content at 23 °C. The fatty acid composition of spat was related to that of the respective diets, and there was no evident relation between dietary fatty acids and spat growth. Our results indicate that *N. subnodosus* spat should be kept at 26 °C and fed a mixed diet containing *C. calcitrans* and either *P. lutheri* or *Isochrysis* sp.

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FATTY ACIDS OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) SEMEN: COMPOSITION AND EFFECTS ON SPERM FUNCTIONALITY

Franz Lahnsteiner, Nabil Mansour, Mary A. McNiven, Gavin F. Richardson-2009

Aquaculture 298(1-2): 118-124

Abstract:

The total fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) spermatozoa and seminal plasma was investigated in fresh and in stored semen using gas liquid chromatography. Triglycerides, phospholipids, and cholesterol were measured and the effect of fatty acids, triglycerides, and cholesterol on sperm functionality was evaluated.

In seminal plasma and spermatozoa saturated fatty acids occurred in higher quantities than unsaturated fatty acids. In spermatozoa, the concentrations of monounsaturated fatty acids were higher than the concentrations of polyunsaturated fatty acids. In seminal plasma the concentrations were approximately equal. Myristic acid, palmitic acid, and stearic acid were main saturated fatty acids and oleic acid, vaccenic acid, and linolenic acid main unsaturated fatty acids of spermatozoa and seminal plasma. In spermatozoa linoleic acid, docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, and docosahexaenoic acid were found in high concentrations, too, in seminal plasma palmitoleic acid and eicosenoic acid. During semen storage the fatty acid composition changed in spermatozoa and in seminal plasma indicating that fatty acids were metabolized.

Palmitic acid, the one of the main saturated fatty acids of spermatozoa and seminal plasma, and the unsaturated fatty acids, arachidonic acid and linoleic acid, which occur in spermatozoa and seminal plasma of rainbow trout, too, had a positive effect on sperm viability during immotile, unfrozen storage of spermatozoa as they increased the motility rate and average path velocity, which could be activated. Also the sperm fertility was improved by addition of fatty acids. A similar effect was found by arachidic acid, a saturated fatty acid, which was not detected in spermatozoa and seminal plasma. Therefore, semen short-term storage techniques can be improved by supplementation of storage solutions with fatty acids. Fatty acids had no effect on sperm motility duration. They had also no effect on sperm cryoresistance as the motility pattern and fertility of spermatozoa frozen–thawed with or without fatty acids were similar.

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RELATIONSHIP BETWEEN HATCHING RATE AND THE OUTER EGG MEMBRANES OF THE IN VITRO ARTIFICIALLY FERTILIZED EGGS OF THE JAPANESE MITTEN CRAB *ERIOCHEIR JAPONICA*

Tai Hung Lee-2009

Aquaculture 298(1-2): 168-171

Abstract:

This study shows that by removing the outer membranes of the in vitro artificially fertilized eggs of the Japanese mitten crab *Eriocheir japonica*, the normal zoea hatching rate of the eggs can be raised from 10% up to over 90%. This finding suggests that the normal zoea hatching rate is strongly influenced by the outer egg membranes of the in vitro artificially fertilized eggs. In addition, observations by transmission electron microscopy indicate that the outer egg membranes of the in vitro artificially fertilized eggs are 1.5 to 3 times thicker than those of the naturally spawned eggs. Discussion focuses on the role these thick outer egg membranes play in the normal zoea hatching rate of in vitro artificially fertilized eggs.

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RESPONSE OF THE EARLY DEVELOPMENTAL STAGES OF HATCHERY REARED SALMONIDS TO MAJOR IONS IN A SIMULATED MINE EFFLUENT

Michael S. Stekoll, William W. Smoker, Barbi J. Failor-Rounds, Ivan A. Wang., Valerie J. Joyce-2009

Aquaculture 298(1-2): 172-181

Abstract:

In response to a potential revision by the state of Alaska to increase discharge limits for total dissolved solids (TDS) in the mining industry, we studied various life stages of hatchery reared salmonids exposed to the major ions in a simulated mine effluent at concentrations of salts up to 2500 mg l⁻¹. Effects depended on the developmental stage and the exposure period. For short term (24- to 96-h) exposures the fertilization stage was most sensitive. Fertilization success was reduced with TDS as low as 250 mg l⁻¹. A two-minute exposure to elevated TDS during fertilization was long enough to have deleterious effects on both fertilization success and later development. Embryos exposed to the simulated mine effluent continuously from just after fertilization through to swim-up had high post-hatch mortalities. Thus there may be at least two separate mechanisms of toxicity: an acute response at the moment of fertilization, resulting in low fertilization success, and a response to long-term exposure through swim-up, manifested by mortalities at the alevin stage. Sensitivity to dissolved salts with respect to fertilization success varied among different species of salmonids. Chinook, pink, and coho salmon were most sensitive, and Arctic charr were least sensitive to elevated salts. Specific ion tests indicated that Ca²⁺ was a major contributor to decreased fertilization. We suggest that the current Alaska water quality standards of 1000 mg l⁻¹ for TDS should not be increased and may be too high for some species. In addition, salmonid fertilization trials may be an appropriate, relatively quick, assay for setting site-specific TDS contamination discharge limits.

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