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Summary of the 3rd Pangasius Aquaculture Dialogue (PAD) meeting, Can Tho-Vietnam, December 3-4, 2008

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TAQMAN REAL-TIME PCR ASSAY FOR QUANTIFYING WHITE SPOT SYNDROME VIRUS (WSSV) INFECTIONS IN WILD BROODSTOCK AND HATCHERY-REARED POSTLARVAE OF FLESHY SHRIMP, FENNEROPENAEUS CHINENSIS

In-Kwon Jang, Xian-Hong Meng, Hyung-Chu Seo, Yeong-Rok Cho, Bong-Rae Kim, Gopalakannan Ayyaru, Jong-Sheek Kim-2009

Aquaculture 287(1-2): 40-45

Abstract:

In the present study, a highly sensitive and specific TaqMan real-time PCR was developed to quantify white spot syndrome virus (WSSV) infections in wild broodstock and hatchery-reared postlarvae of fleshy shrimp, Fenneropenaeus chinensis. A total of 159 individuals of wild F. chinensis brooders from 3 locations were captured and 210 postlarvae (PL1–8) were obtained from seven commercial hatcheries in 2007 in South Korea. The WSSV infections in 3 broodstocks showed a wide range, from 0 to  $2.28 \times 10^6$  (with a mean of  $1.50 \times 10^4$ ) copies ng<sup>-1</sup> of DNA. Out of 159 brooders assayed, 39 (24.5%) were negative and 120 (75.5%) were positive; 153 (96.2%) showed less than 100 copies

(mean 10.2 copies), 111 (69.8%) showed less than 10 copies and only 6 individuals (3.8%) showed high infections with a range of  $2.36 \times 10^2$  to  $2.28 \times 10^6$  copies  $\text{ng}^{-1}$  of DNA. In 210 postlarvae, a range of 2.6 to 713.6 (with a mean of 220) copies  $\text{g}^{-1}$  of DNA was observed. The mean WSSV copy number in the postlarvae was  $7.9 \times 10^5$ , which was equivalent to  $8.5 \times 10^5$  copies  $\text{mg}^{-1}$  of postlarvae weight. A total of 87.1% of postlarvae were WSSV positive and except for two hatcheries (H4 and H7), the postlarvae of all the other five hatcheries were positive.

Even postlarvae from the same hatchery, especially of hatcheries H4 and H5, showed a wide range of WSSV infection resulting in higher infections than other hatcheries. There were 34.3% of the postlarvae assayed in the present study that showed very low infection, with less than 10 copies  $\text{ng}^{-1}$  of DNA. Based on our results, it is recommended to pre-screen broodstock or larvae for selective breeding, stocking in production systems or stock enhancement.

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#### EFFECT OF DHA CONTENT IN ROTIFERS ON THE OCCURRENCE OF SKELETAL DEFORMITIES IN RED PORGY *PAGRUS PAGRUS* (LINNAEUS, 1758)

F.J. Roo, C.M. Hernández-Cruz, J.A. Socorro, H. Fernández-Palacios, D. Montero, M.S. Izquierdo-2009

Aquaculture 287(1-2): 84-93

Abstract:

Despite being proposed as a potential candidate for diversification of marine aquaculture, limited larval survival and the occurrence of elevated levels of skeletal deformities restrict the commercial production of red porgy. The present study was conducted to determine the effect of rotifer enrichment, particularly on DHA, on growth, survival and occurrence of skeleton deformities in this species. The study included two trials, in one the viability of commercial emulsions for rotifer enrichment was evaluated whereas in another the effect of the elevation of DHA in rotifer emulsions on the larvae was studied. No significant differences were found in growth between larvae fed different rotifers in both trials suggesting that 1.9% dw DHA is enough to fulfil the DHA requirements of red porgy larvae for maximum growth. However, a marked positive effect of rotifer DHA content supplementation on larval survival was found. A low larval survival was found when rotifers had a high DPA content, in agreement with the results obtained for other Sparids. This fatty acid was accumulated in red porgy larval tissues in high levels (0.79% dw DPA) when rotifers rich in DPA were the main source of food. Although the external appearance of the juveniles seemed to be normal, X-ray observations showed elevated levels of bone abnormalities associated, in both trials, to low DHA content in the live prey. Among different anomalies, the presence of fused vertebrae was the most frequent deformity for both rearing trials. Although DHA content in the rotifers used for feeding was the only difference a 50% reduction in the number of deformed fish for each type of deformity studied was obtained when the larvae fed higher DHA levels, denoting the important role of this FA in the prevention of deformities at the rotifer feeding stage. Further studies are needed to elucidate the importance of essential fatty acids on the development of bone deformities in fish, since the functions of HUFA are different, and their absolute levels and ratios among them can lead to very different effects in fish metabolism, including bone formation.

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#### VIABILITY OF SUBITANEOUS EGGS OF THE COPEPOD, *ACARTIA TONSA* (DANA), FOLLOWING EXPOSURE TO VARIOUS CRYOPROTECTANTS AND HYPERSALINE WATER

Cortney L. Ohs, Andrew L. Rhyne, Erik Stenn-2009

Aquaculture 287(1-2): 114-119

Abstract:

Subitaneous eggs were obtained from monocultures of the calanoid copepod *Acartia tonsa* (Dana), Gulf of Mexico strain. Eggs were exposed to methanol, ethylene glycol, propylene glycol, glycerine, and DMSO at 0.0, 0.1, 0.5, 1.0, 2.0, and 5.0 M and hypersaline water at 50, 75, 100, 150, and 200 g/L. Treatments were evaluated after 10 and 20 min of exposure and at 4 and 26 °C. Viability (percent hatched) was determined after 24 h of incubation in 35 g/L saltwater at 26 °C.

Methanol, ethylene glycol, and glycerine had high viability up to 2M, and all experienced large decreases at 5M when the exposure temperature was 26 °C compared to 4 °C. Eggs exposed to propylene glycol had lower mean viability with greater variability at the lower concentrations although viability was greater than 81.4% at 2 M. Significant decreases in viability were observed at 5 M, and the decreases were much greater at an exposure temperature of 26 °C versus 4 °C. DMSO exposed at 26 °C produced high viability up to 1 M before significant decreases occurred, while an exposure temperature of 4 °C produced high viability up to 2 M. Viability of eggs exposed to hypersaline water of 50, 75, and 100 g/L were not significantly different from controls for all treatment combinations except the 26 °C temperature exposed for 20 min, which was significantly lower at 100 g/L. Concentrations of 150 and 200 g/L produced very few to no viable eggs. These results indicate further research is justified to investigate if viability of *A. tonsa* eggs can be protected by these cryoprotectants and hypersaline water after exposure to cryopreservation conditions.

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#### THE EFFECTS OF STOCKING DENSITY ON EGG PRODUCTION AND HATCHING SUCCESS, CANNIBALISM RATE, SEX RATIO AND POPULATION GROWTH OF THE TROPICAL CALANOID COPEPOD ACARTIA SINJIENSIS

Thomas Camus, Chaoshu Zeng-2009

Aquaculture 287(1-2): 145-151

Abstract:

The difficulty in achieving high density copepod cultures is a major bottleneck limiting their utilization in aquaculture hatcheries. The present study evaluated the effects of initial adult stocking density on various productivity-related parameters of the tropical calanoid copepod *Acartia sinjiensis*, a specie with good potential for mass culture as a live feed for marine larvae.

Five stocking densities of 125, 250, 500, 1000 and 2000 adults/L were set-up for all experiments with 5 replicates per treatment. *A. sinjiensis* used for all trials were originated from plankton tows conducted in October 2006 and have since been cultured under laboratory condition. Daily egg production of *A. sinjiensis* was monitored over 12 consecutive days, the results showed that while at the lowest stocking density of 125 adults/L, survival of *A. sinjiensis* over the 12 days period was significantly higher than 250, 500 and 1000 adults/L treatment ( $p < 0.05$ ), average egg output per female was not significantly affected by either stocking density or by the age of the females ( $p > 0.05$ ). Hatching success within 48 h was significantly higher ( $p < 0.001$ ) for eggs produced by females cultured at lower densities. However, when incubation period was increased to 96 h, hatching rates were not significantly different among treatments ( $p > 0.05$ ), indicating a possible delayed effect on hatching of eggs produced at high stocking densities. Cannibalism of adults and late copepodites (C-5) toward nauplii increased steadily with predator density; with naupliar mortality at 2000 ind./L significantly higher than that at 125 and 250 ind./L treatments ( $p < 0.005$ ).

Under optimal culture conditions, population growth of *A. sinjiensis* at different initial adult stocking density was also assessed over a 12 day culture period. The results showed that the sex ratio of the adults on the final day of the experiment was not significantly affected by the initial stocking density ( $p > 0.05$ ). It was also revealed that *A. sinjiensis* can sustain a high culture density of 2000 ind./L, and likely higher, as increases in *A. sinjiensis* populations over the 12 day culture period were found at all densities tested. Nevertheless, the rate of population growth of all post-egg-stages of *A. sinjiensis* was significantly affected by the initial stocking density ( $p < 0.005$ ), over 12 days of culture, the final number of all post-egg-stages increased by 470% and 408% respectively for the lowest initial

stocking densities of 125 and 250 adults/L while at the highest densities of 1000 and 2000 adults/L, the increases were substantially lower at 244% and 171% respectively.

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#### SUCCESSFUL CRYOPRESERVATION OF SPERM FROM SEX-REVERSED DUSKY GROUPEL, EPINEPHELUS MARGINATUS

E. Cabrita, S. Engrola, L.E.C. Conceição, P. Pousão-Ferreira, M.T. Dinis-2009

Aquaculture 287(1-2): 152-157

##### Abstract:

The dusky grouper, *Epinephelus marginatus*, is a protogynous monandric hermaphrodite species, since individuals mature firstly as females and later as males. This makes capture and maintenance of males in captivity difficult due to their large size and older age. Thus, the use of techniques that allow controlling sex, obtaining smaller and younger males, as well as cryopreservation techniques to improve sperm availability, may contribute to improve dusky grouper reproduction in captivity. In the present study, immature fish and young females were sex reversed using 2.5 mg/kg BW 17 $\alpha$ -methyltestosterone ( $\alpha$ -MT) in silastic implants, two months prior gamete extraction. In all individuals a single dose of Lucrin Depot® (20  $\mu$ g/kg BW, GnRH $\alpha$ ) was enough to induce and/or increase sperm production. Volume of sperm, cell concentration, osmolarity, sperm production and motility were registered. Sperm was cryopreserved in 0.5 ml straws using 1% NaCl + 10% DMSO + 10 mg/ml BSA as extender. Post-thawed sperm quality was analysed in terms of motility, viability and fertilization. The sperm volume collected ranged from 5 to 400  $\mu$ l, cell density from 1.2 to 16.3  $\times$  10<sup>9</sup> spz/ml and sperm production from 0.04 to 3.9  $\times$  10<sup>9</sup> spermatozoa. The percentage of motile cells at 15 s post-activation varied from 25% to 93%. Cell viability decreased in post-thawed samples (22.5%) as well as the percentage of motile cells (36.8%). However, sperm velocity (VCL and VSL) and movement pattern (Lin) were not significantly affected by cryopreservation and spermatozoa were able to fertilize the oocytes without a decrease in the fertilization rate. Fertilization rates ranged in thawed samples from 35.9% to 65.1% and only one sample was significantly different from the control (69.5%). Embryo development was impaired in some fertilization trails, registering a significant decrease in the percentage of embryos at G stage when compared with the control. This suggests that the quality of samples may be the principal requirement for improving fertilization rather than the optimization of the cryopreservation protocol. The present study demonstrated that the protocol used for sperm cryopreservation can be successfully used in sex-reversed males for the establishment of a sperm bank. This technique would contribute to the reproduction of this species benefiting production, sea-ranching and species conservation.

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#### GROWTH AND DEVELOPMENT OF VIMBA BREAM (VIMBA VIMBA) LARVAE IN RELATION TO FEEDING DURATION WITH LIVE AND/OR DRY STARTER FEED

J. Hamáčková, M. Prokeš, P. Kozák, M. Peňáz, L.A. Stanny, T. Policar, V. Baruš-2009

Aquaculture 287(1-2): 158-162

##### Abstract:

The effects of the introduction of dry feed upon growth rate and development were studied in vimba bream during larval rearing under controlled conditions. Larvae were fed sequential combinations of brine shrimp (BS) and ASTA starter feed for a total of 20 days as follows: BS alone (group A), 4 d BS followed by 16 d ASTA (B), 8 d BS followed by 12 d ASTA (C), 12 d BS followed by 8 d ASTA (D) and ASTA alone for 20 d (E). No demonstrable difference in fry survival was observed between the groups. The highest average total length (TL = 16.11  $\pm$  1.42 mm), weight (w = 31.37  $\pm$  8.80 mg) and the most advanced ontogenetic development (L5–L6, average 5.65) were attained by larvae fed on brine shrimp nauplii during the whole period. The lowest average growth (TL = 12.42  $\pm$  0.76 mm; w

= 11.01 ± 2.65 mg) and slowest development (L4–L5, average 4.91) was seen in larvae fed for 20 days with starter feed. With an increasing duration of feeding with brine shrimp nauplii, the length and weight of the larvae gradually increased, as did the coefficient of condition (FWC = 0.5648–0.7268). There were very notable differences between the individual samples A–E ( $P \leq 0.001$ ).

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#### CO-FEEDING OF INERT DIET FROM MOUTH OPENING DOES NOT IMPAIR PROTEIN UTILIZATION BY SENEGALESE SOLE (*SOLEA SENEGALENSIS*) LARVAE

Sofia Engrola, Mônica Mai, Maria Teresa Dinis, Luís E.C. Conceição-2009

Aquaculture 287(1-2): 185-190

Abstract:

In most marine species inert diets alone have a poor ability to sustain fish larvae growth and development. Furthermore, results of co-feeding inert diets and live prey are variable, which may be related to the effect of inert diets on digestive maturation and subsequently protein utilization. The aim of the present work was to investigate how different feeding regimes, live feed alone or co-fed with an inert diet, influence protein utilization in Senegalese sole larvae. Feed intake, protein absorption, protein retention and protein catabolism were estimated in sole from 8 to 35 days after hatching (DAH), using <sup>14</sup>C-labelled Artemia protein and posterior incubation in metabolic chambers. Postlarvae that were co-fed with an inert diet from mouth opening ate more than postlarvae fed Artemia alone at most sampling ages. Sole Artemia protein digestibility ranged from 56.97 (16 DAH) to 81.32% (22 DAH). Sole larvae that were fed a second meal had a slightly, though significant, higher digestibility than sole fed a single meal. Digestibility was lower in co-fed sole during metamorphosis climax, and similar between treatments at other developmental stages. Retention efficiency remained almost constant during early development, and was not affected by feeding regime. In short, co-feeding of an inert diet from mouth opening does not impair protein utilization by Senegalese sole larvae.

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#### GONAD DEVELOPMENT DURING SEXUAL DIFFERENTIATION IN HATCHERY-PRODUCED ORANGE-SPOTTED GROUPER (*EPINEPHELUS COIOIDES*) AND HUMPBAC GROUPER (*CROMILEPTES ALTIVELIS*) (PISCES: SERRANIDAE, EPINEPHELINAE)

Min Liu, Yvonne Sadovy de Mitcheson-2009

Aquaculture 287(1-2): 191-202

Abstract:

Juvenile sexual differentiation of two commercially important epinephelines, orange-spotted grouper (*Epinephelus coioides*) and humpback grouper (*Cromileptes altivelis*) (Serranidae, Epinephelinae), produced in hatcheries, was studied until first sexual maturation using gonadal histology. Diandric, protogynous hermaphroditism is confirmed for *E. coioides* with evidence of primary male differentiation directly from the juvenile phase as well as of secondary male development through the sex-change of functional females. All juveniles developed first an ovarian-phase then entered a bisexual-phase gonad. Sexual differentiation occurred in bisexual gonads at around 105 weeks after hatching (wah), with the growth of oocytes beyond the primary-growth stage for females, and the appearance of sperm sinuses and proliferation of spermatogenic cysts in primary males. The minimum size and age of first sexual maturation for females were 355 mm standard length (SL) and 113 wah. No mature males were found by 141 wah, suggesting that first maturation in males takes longer than in females, at least under mariculture conditions. For *C. altivelis*, all juveniles developed an ovarian-phase gonad. Minimum body size and age of first sexual maturation for females were 155 mm SL and 83 wah. In contrast to *E. coioides*, there was no sign of spermatogenic cysts in juvenile gonads and any indication of primary male differentiation in *C. altivelis*. This study reveals the variation in early gonad development in the epinephelines and highlights the importance of studying juvenile sexual differentiation for fully understanding sexual pattern in this fish subfamily.

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#### PROCESS REQUIREMENTS FOR ACHIEVING FULL-FLOW DISINFECTION OF RECIRCULATING WATER USING OZONATION AND UV IRRADIATION

Steven T. Summerfelt, Mark J. Sharrer, Scott M. Tsukuda, Michael Gearheart-2009

Aquacultural Engineering 40(1): 17-27

##### Abstract:

A continuous water disinfection process can be used to prevent the introduction and accumulation of obligate and opportunistic fish pathogens in recirculating aquaculture systems (RAS), especially during a disease outbreak when the causative agent would otherwise proliferate within the system. To proactively prevent the accumulation of fish pathogens, ozonation and ultraviolet (UV) irradiation processes have been used separately or in combination to treat water in RAS before it returns to the fish culture tanks. The objective of the present study was to determine the process requirements necessary to disinfect the full RAS flow, using ozonation followed by UV irradiation, just before the flow was returned to the fish culture tank(s). We found that a proportional-integral (PI) feed-back control loop was able to automatically adjust the concentration of ozone (O<sub>3</sub>) generated in the oxygen feed gas (and thus added in the low head oxygenator) in order to maintain the dissolved O<sub>3</sub> residual or ORP at a pre-selected set-point. We determined that it was easier and effective to continuously monitor and automatically control O<sub>3</sub> dose using an oxidative reduction potential (ORP) probe (in comparison to a dissolved ozone probe) that was located at the outlet of the O<sub>3</sub> contact chamber and immediately before water entered the UV irradiation unit. PI control at an ORP set-point of 450 and 525 mv and a dissolved O<sub>3</sub> set-point of 20 ppb provided almost complete full-flow inactivation of heterotrophic bacteria plate counts (i.e., producing <1 cfu/mL) and improved water quality (especially color and %UVT) in a full-scale recirculating system. Achieving this level of treatment required adding a mean dose of approximately  $29 \pm 3$  g O<sub>3</sub> per kg feed. However, because water is treated and reused repeatedly in a water reuse system, the mean daily O<sub>3</sub> demand required to maintain an ORP of 375–525 mV (or at 20 ppb dissolved O<sub>3</sub>) was 0.34–0.39 mg/L, which is nearly 10 times lower than what is typically required to disinfect surface water in a single pass treatment. These findings can be used to improve biosecurity and product quality planning by providing a means for continuous water disinfection in controlled intensive RAS.

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#### WHOLE-BODY CONCENTRATIONS OF CORTISOL AND SEX STEROIDS IN WHITE STURGEON (*ACIPENSER TRANSMONTANUS*, RICHARDSON 1836) DURING EARLY DEVELOPMENT AND STRESS RESPONSE

Claudia Simontacchi, E. Negrato, M. Pazzaglia, D. Bertotto, C. Poltronieri, G. Radaelli-2008

Aquaculture International 17(1): 7-14

##### Abstract:

In general little is known about hormones and the ontogeny of the stress response in the early developmental stages of chondrosteian fishes and in particular of white sturgeon (*Acipenser transmontanus*, Richardson 1836). In this study, we measured for the first time cortisol and sex steroids (testosterone and estradiol) in eggs, larvae, post-larvae, and fry of white sturgeon by radioimmunoassay (RIA), to elucidate some endocrine aspects of its development. The cortisol, testosterone, and 17 $\beta$ -estradiol of maternal origin found in unfertilized eggs of white sturgeon probably regulate both growth and development of the embryo. Cortisol decreased after fertilization, whereas testosterone and 17 $\beta$ -estradiol did not significantly change. During the late stages of embryo development and immediately after hatching, endogenous production of cortisol and sexual steroids, respectively, occurred. Sex steroids may be physiological inducers of gonad sex differentiation in sturgeon. All steroids showed an increase 10 days post-hatch (dph), near the transition from an endogenous to an exogenous energy source. Cortisol maintained the same basal levels even after

metamorphosis, whereas testosterone and 17 $\beta$ -estradiol declined significantly in post-larvae at 35 and 45 days post-hatch. In addition, to evaluate the ontogeny of a functional hypothalamic-pituitary-interrenal (HPI) axis, larvae and fry were submitted to acute stress. The HPI axis did not seem to be functional on the first day post-hatch, but became so from the third day post-hatch onward.

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#### EFFECT OF DIETARY SOYBEAN LECITHIN ON REPRODUCTIVE PERFORMANCE OF CHINESE MITTEN CRAB *ERIOCHEIR SINENSIS* (H. MILNE-EDWARDS) BROODSTOCK

L. Y. Sui, X. G. Wu, M. Wille, Y. X. Cheng, P. Sorgeloos-2008

Aquaculture International 17(1): 45-56

##### Abstract:

The effect of increasing levels of dietary phospholipids (PL) on the ovarian development and reproductive performance of Chinese mitten crab *Eriocheir sinensis* were investigated using four semipurified formulated diets supplemented with 0%, 1.2%, 2.4%, and 3.6% PL. Four groups of 40 females, with an average individual body weight of 95–120 g, were fed the experimental diets for a period of 7 months. Male crabs were introduced into the female rearing system in March, and mating, spawning, and egg hatching occurred in the following month. After 10 weeks of feeding, females fed the diet with 2.4% PL had a significantly higher gonadosomatic index (GSI) than females fed the diet with 0% PL, whereas females fed the diet with 1.2% PL had a significantly higher hepatic moisture content and lower hepatic lipid content than the other groups ( $P < 0.05$ ). After mating, the spawning rate was higher among females fed diets with 3.6% and 2.4% PL (95% and 92%, respectively) compared with females fed diets with 0% and 1.2% PL (both 81%). The results showed that egg production (total number of eggs/female) and fecundity increased with increasing dietary PL level, with females fed diets with 0% and 1.2% PL supplementation having significantly lower values than females fed the diet with 3.6% PL ( $P < 0.05$ ). In conclusion, our results suggest that dietary PL supplementation has a positive effect on ovarian development and reproductive performance of *E. sinensis* broodstock. Further study should aim to investigate the optimal PL level in the broodstock diet of *E. sinensis* in respect to offspring quality.

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#### USE OF MICROBACTERIUM SP. AND EXIGUOBACTERIUM MEXICANUM TO IMPROVE THE SURVIVAL AND DEVELOPMENT OF ARTEMIA UNDER XENIC CONDITIONS

Araceli Hipólito-Morales, A. M. Maeda-Martínez, S. F. Martínez-Díaz-2008

Aquaculture International 17(1): 85-90

##### Abstract:

The effect of *Microbacterium* sp. strain 8L and *Exiguobacterium mexicanum* strain 8N was evaluated in the diet of *Artemia* under xenic conditions. Viable cultures of bacteria were provided to xenic cultures of *Artemia* in combination with *Sacharomyces cerevisiae*, cornflour or *Spirulina*, and the effect on the survival and growth was recorded. The use of these bacterial strains improves significantly the survival of *Artemia* independently of the used food ( $P < 0.05$ ), and variable results were observed in the growth.

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#### NUTRITIONAL VALUES OF APOCYCLOPS DENGIZICUS (COPEPODA: CYCLOPOIDA) FED CHAETOCEROUS CALCITRANS AND TETRASELMIS TETRATHELE

Omidvar Farhadian, Fatimah Md Yusoff, Suhaila Mohamed-2009

Aquaculture Research 40(1): 74-82

##### Abstract:

The cyclopoid copepod *Apocyclops dengizicus* was isolated from a marine shrimp pond, *Penaeus monodon*, Kuala Selangor, Malaysia, and reared in the laboratory for 3 months to establish a pure population stock. Amino acids and fatty acids of *A. dengizicus* were determined when fed *Chaetoceros calcitrans* (C), *Tetraselmis tetrathele* (T) and their combination (CT) (1:1 by number). The protein contents in *A. dengizicus* that received C, T and CT were 46.8%, 60.5% and 55.3% of dry weight respectively. Correspondingly, the lipid was 19.0%, 17.8% and 19.1% of dry weight for C, T and CT respectively. The *A. dengizicus* cultured on C, T and CT had total essential amino acids without tryptophan measurement of 57.1, 60.3 and 67.8 and total non-essential amino acids of 42.9%, 40.0% and 32.2% of total amino acids. The fatty acid content of *A. dengizicus* showed that it was able to synthesize docosahexenoic acid (22:6n-3, DHA), eicosapentaenoic acid (20:5n-3, EPA) and arachidonic acid (20:4n-6, ARA) from examined microalgal diets. The DHA:EPA:ARA ratios of *A. dengizicus* fed on C, T and CT were 6.8:3.0:1, 14.0:5.8:1 and 11.6:2.6:1 respectively. *Apocyclops dengizicus* could be suitable live food for larval fish and shrimp rearing because it meets their nutritive requirements.

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#### THE POND'S SHAPE MATTERS: DIFFERENTIAL GROWTH, PHYSIOLOGICAL CONDITION AND SURVIVAL OF EPIBENTHIC FARFANTEPENAEUS AZTECUS POSTLARVAE

Ella Vázquez-Domínguez, Andrea Bolongaro-Crevenna, Adolfo Sánchez, Carlos Rosas-2009  
*Aquaculture Research* 40(1): 91-102

##### Abstract:

As a function of the water quality provided by square, circular and oval experimental ponds, the growth, survival and oxygen requirements in epibenthic postlarvae of *Farfantepenaeus aztecus* were analysed in relation to their routine metabolism and apparent heat increment. Temperature, oxygen concentration, pH and salinity were measured daily in two experimental ponds of each shape. The postlarvae oxygen consumption during two 24-h cycles, their growth, physiological condition and survival and the productivity in the ponds were estimated. Low values of pH, oxygen concentration and phytobenthos productivity, and reduced postlarvae relative growth and survival were observed in the square ponds. We suggest that the latter results from a deficient water circulation related to the effect of the pond's shape on dissolved oxygen levels and, consequently, on growth and survival. The postlarvae routine metabolism, including feeding, varied between 1.91 and 2.25 mg O<sub>2</sub> h<sup>-1</sup> g<sup>-1</sup> wet weight, whereas the minimum oxygen concentration needed in the ponds is approximately 4.25 mg O<sub>2</sub> L<sup>-1</sup>. These conditions were achieved in the oval ponds concurrent with higher survival and growth values, in which individuals distributed randomly, for which we suggest that oval-shaped ponds could be the most adequate for the culture of this and other penaeid species.

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#### DIET COMPOSITION OF STURGEON FRY (*ACIPENSER PERSICUS*) REARED IN FERTILIZED EARTHEN PONDS

A. Keramat Amirkolaie-2009

*Aquaculture Research* 40(1): 112-117

##### Abstract:

Artificial rearing of fry has been introduced as an alternative to supply fry and juvenile sturgeon in order to restock the depleted sturgeon population in the Caspian Sea. The aim of this study is to assess the feed selection characteristics of sturgeon (*Acipenser persicus*) fry during the period in which they stay in earthen ponds before being released into the river. Sturgeon fry weighing around 150 mg were introduced into five almost similar-sized earthen ponds. The ponds were enriched with fertilizer to supply natural food for the fries. A weekly sample of six specimens was collected from each pond during the 5 weeks of the experiment. All collected fish were weighed and then dissected for inspection of the stomach and the intestine content. The overall stomach content measured from 150



fish showed that around 74% of the stomach content was composed of chironomids and the rest was daphnia and cyclops. At the beginning of the study (first 2 weeks), sturgeon fry feed more on daphnia and cyclops; this trend, however, reversed in the last 2 weeks of the study. In conclusion, sturgeon fry feed on three prey groups—chironomids, daphnia and cyclops—during their lifespan in earthen pond. Feeding on chironomids larvae increased with sturgeon fry size.

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#### SEASONAL VARIATION IN THE CONDITION INDEX OF PACIFIC OYSTER POSTLARVAE (CRASSOSTREA GIGAS) IN A LAND-BASED NURSERY IN SONORA, MEXICO

Ramón H Barraza-Guardado, Jorge Chávez-Villalba, Héctor Atilano-Silva, Francisco Hoyos-Chairez-2009

Aquaculture Research 40(1): 118-128

Abstract:

This study examined the seasonal variation in the condition index (CI) of *Crassostrea gigas* postlarvae (<5 mm) that were cultivated at a commercial hatchery. Oysters were sampled weekly at the nursery using seawater from a lagoon for the grow-out that precedes commercialization. Temperature, salinity, seston, chlorophyll a, oxygen and pH were recorded at each sampling and water samples were taken to identify phytoplankton groups and their abundance. High levels of primary productivity, chlorophyll a and seston were detected during summer, but the highest CI occurred in winter. During winter, elevated phytoplankton biomass was composed by diatoms and phytoflagellates, which served as the main food source and promoted weight gain in this season. Variations in salinity, oxygen and pH were not related to differences in the CI. However, it appears that the wide temperature variation affected functions, such as feeding activity, apparently enhancing ingestion during winter (mean  $16.5 \pm 1.4$  °C) and reducing ingestion during summer (mean  $31 \pm 1.5$  °C). Winter production resulted in postlarvae with a homogeneous size range and a high CI, indicating that winter is more favourable to start cultivation. The CI represents a practical means to determine the physiological state of postlarvae before transfer to cultivation sites.

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#### ZOOPLANKTON AND THE TOTAL PHOSPHORUS - CHLOROPHYLL A RELATIONSHIP: HIERARCHICAL BAYESIAN ANALYSIS OF MEASUREMENT ERROR

Kamarainen, Amy M., Rowland, Freya E., Biggs, Reinetta, Carpenter, Stephen R.-2008

Canadian Journal of Fisheries and Aquatic Sciences 65(12): 2644-2655

Abstract:

Zooplankton grazing is important in resolving residual variation around the total phosphorus - chlorophyll a relationship. In empirical studies, zooplankton body size is often a better predictor of residual variation than zooplankton biomass. We investigate whether higher measurement error associated with zooplankton biomass may explain its lower predictive ability. We collected five replicate zooplankton biomass samples in 19 lakes, allowing us to quantify measurement error in volumetric zooplankton biomass with greater precision than in previous studies. A hierarchical Bayesian model was used to assess the predictive ability of volumetric zooplankton biomass and mean individual zooplankton length, corrected for measurement error. We found consistent effects of total zooplankton biomass, but not zooplankton length, on chlorophyll a. This finding does not appear to be related to the higher precision with which total zooplankton biomass was measured in our study, but rather to ecological factors. Interlake variation outweighed the effects of measurement error in estimating the strength of relationships between zooplankton variables and chlorophyll a. Our findings therefore suggest that studies to estimate zooplankton effects on phytoplankton should allocate resources to study a larger range of lakes over different time periods than to process replicate samples to reduce measurement error.

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UTILIZATION OF A RAPID DNA-BASED ASSAY FOR MOLECULAR VERIFICATION OF CHANNEL CATFISH, BLUE CATFISH, F1 HYBRIDS, AND BACKCROSS OFFSPRING AT SEVERAL LIFE STAGES

Geoffrey C. Waldbieser, Brian G. Bosworth-2008

North American Journal of Aquaculture 70(4): 388–395

Abstract:

The F1 hybrid offspring of female channel catfish *Ictalurus punctatus* × male blue catfish *I. furcatus* contain many desirable traits for commercial production, such as enhanced growth and increased survivability. Hybrids can be produced through pond spawning (although at a low efficiency), but hybrid catfish cannot always be readily distinguished from channel catfish, especially at early life stages. The present research was designed to produce a rapid DNA-based test for the identification of hybrid catfish. Channel catfish and blue catfish genomic regions were amplified by polymerase chain reaction (PCR) using common primers for the follistatin (*Fst*) and hepcidin antimicrobial protein (*Hamp*) genes, and fragment length polymorphisms between the two species caused by ancestral insertions and deletions were resolved by agarose electrophoresis. The *Fst* amplicons were 348 and 399 base pairs (bp), while the *Hamp* amplicons were 222 and 262 bp from channel catfish and blue catfish, respectively. The mitochondrial cytochrome c oxidase 1 (*Mtco1*) gene was also differentially amplified from each species by using species-specific primers to enable determination of the maternal parent species. The DNA preparation technique provided sufficient genomic DNA to test several life stages. The PCR products were successfully amplified from genomic DNA isolated from embryos at 1, 2, or 5 d after fertilization; from fry 2 d after hatching; from the blood and barbels of juveniles and adults; and from fresh, frozen, and cooked fillet samples. The results from this assay were available as soon as 24 h after receipt of sample. This assay will be useful for management of hybrid populations and postharvest detection of hybrid catfish products.

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HARVESTING AND PROCESSING ZOOPLANKTON FOR USE AS SUPPLEMENTAL CHANNEL CATFISH FRY FEED

Charles C. Mischke, David J. Wise-2008

North American Journal of Aquaculture 70(4): 396–398 |

Abstract:

We present the methods that we used to capture and dry large zooplankton from ponds to feed to channel catfish *Ictalurus punctatus* fry. Using a submersible pump and canister filter, we were able to capture about 1.0 kg (wet weight; 200 g in terms of dry weight) of zooplankton from well-fertilized ponds over 24-h trapping periods. This was a practical method for obtaining the large zooplankton that catfish fry prefer. Fish culturists may be able to use this method to harvest zooplankton throughout the year and store dried zooplankton for the hatchery season. The drying process may also be useful for processing and storing small zooplankton for the culture of other species of fish. The cost was about US\$200 for the submersible pump, \$200 for the canister and filter, and \$40 for the food dehydrator.

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THE EFFECT OF HABITAT EXPOSURE AND ONTOGENY ON THE SURVIVAL SKILLS OF HATCHERY RED DRUM

Jessica L. Beck, Jay R. Rooker-2008

North American Journal of Aquaculture 70(4): 399–409

Abstract:

We examined the influence of prerelease exposure to natural vegetation on the survival skills of a marine finfish, the red drum *Sciaenops ocellatus*. Red drum larvae at 18 d posthatch (10–12 mm standard length [SL]) were collected from a Texas Parks and Wildlife Department hatchery and reared

with and without smooth cordgrass *Spartina alterniflora*, for either 10 or 20 d. High-speed video was then used to quantify a suite of prey capture and antipredator performance variables at days 28 (23 mm SL) and 38 (32 mm SL). Repeated-measures analysis of variance indicated that the timing and distance of key variables (e.g., maximum gape, time to maximum gape, gape cycle duration) increased significantly in older fish, while reaction distance and time to maximum velocity decreased with age. The time to reach maximum velocity was less for individuals reared in vegetation, yet these fish also had a shorter reaction distance to predatory stimuli and took longer to capture prey. Additionally, interindividual variability (as measured by the coefficient of variation) ranged from 0.0% to 101.3% and from 3.4% to 110.1% for the prey capture and antipredator variables, respectively, indicating that performance varied substantially among individuals. Our results indicate that while prerelease exposure to smooth cordgrass habitat does not afford any obvious survival benefits to red drum, ontogeny appears to have a direct effect on the development of key survival skills in this species.

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#### PETRI DISH INCUBATION OF EYED EGGS FROM RAINBOW TROUT AND SPLAKE

Michael E. Barnes, Dan J. Durben-2008

North American Journal of Aquaculture 70(4): 410–414

##### Abstract:

Four experiments were conducted from 2004 to 2006 to evaluate different protocols for incubating eyed eggs of salmonids in petri dishes. In the first two experiments, which used eyed eggs from Erwin strain rainbow trout *Oncorhynchus mykiss* or splake (lake trout *Salvelinus namaycush* × brook trout *S. fontinalis*), dishes were loaded at either 10 or 20 eggs/dish and incubated at 10°C. Water changes occurred every 3 or 7 d until complete hatch; for each species, one group of dishes received no water change. Survival to hatch was not affected by the number of eggs per dish or the frequency of water changes in either species. However, in experiments 1 and 2, hatch was significantly earlier in the dishes containing 20 eggs than in dishes containing 10 eggs. In the last two experiments, 15 eyed eggs from Shasta or McConaughy strain rainbow trout were incubated at either 10°C or 12°C; the water was changed every 3 d or not at all. Shasta strain eggs exhibited no significant difference in survival or time to hatch between any of the treatments. For McConaughy strain eyed eggs, survival to hatch was significantly greater at 10°C than at 12°C, but water change treatment did not affect survival. No significant differences in time (d) to complete hatch were observed between temperature treatments or between water change regimes. To successfully mimic vertical-flow incubation of eyed salmonid eggs in petri dishes, we recommend a density of no more than 15 eggs/dish; no water change is needed at a temperature of 10°C. Daily removal of dead eggs and hatched fry is also required.

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#### INDUCED SPAWNING, ARTIFICIAL FERTILIZATION, AND EGG INCUBATION TECHNIQUES FOR GREEN STURGEON

Joel P. Van Eenennaam, Javier Linares-Casenave, Jean-Benoit Muguet, Serge I. Doroshov-2008

North American Journal of Aquaculture 70(4): 434–445

##### Abstract:

Establishment of hatchery breeding techniques for the threatened green sturgeon *Acipenser medirostris* is important for research and conservation hatcheries. Injections of either gonadotropin-releasing hormone analog (GnRHa) or GnRHa plus domperidone were used to induce ovulation in 13 female Klamath River green sturgeon and to induce spermiation in 19 males. Ovulated eggs were either rinsed in water or not rinsed before fertilization, and the eggs were fertilized with different milt dilutions and for different lengths of time. After fertilization, eggs either were allowed to adhere to the bottom of glass dishes or were silted for 1 h and then incubated in McDonald or upwelling jars. All broodfish ovulated or spermiated in all hormonal treatments, and the best treatment was GnRHa injected alone in a single dose of 10 µg/kg for males or in a 1-µg/kg priming dose and a 19-µg/kg

resolving dose for females. Females were held at 12–13°C, and ovulation was observed  $14 \pm 3$  h (mean  $\pm$  SD) after the second injection. Domperidone was not required for successful ovulation and appeared to reduce the adhesion of ovulated eggs. From 49,000 to 115,000 eggs were collected from each female, and from 30 to 300 mL of milt were collected from each male. Sperm cell concentrations in milt ranged from  $2.9 \times 10^8$  to  $5.4 \times 10^9$  sperm/mL, and the sperm exhibited 90–100% motility for up to 5 min. In all experiments, egg rinsing improved fertilization success by 5–12%. Embryo survival to neurulation in the McDonald jars was lower (5–32%) than that in the upwelling incubators (60–82%). Green sturgeon eggs were sensitive to the high-impact rolling action at the bottom of the McDonald jars, probably due to the large egg diameter (mean  $\pm$  SD =  $4.33 \pm 0.14$  mm) and thinner chorion ( $42 \pm 4$   $\mu$ m) relative to eggs of white sturgeon *A. transmontanus* (diameter =  $3.79 \pm 0.03$  mm; chorion thickness =  $115 \pm 6$   $\mu$ m).

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## EVALUATION OF REPLACEMENT FEEDS FOR THE FRY FEED KYOWA FOR LARVAL WALLEYES

J. Alan Johnson, Robert C. Summerfelt, Richard D. Clayton-2008

North American Journal of Aquaculture 70(4): 446–451

### Abstract:

Because Fry Feed Kyowa (FFK) was the standard and the only commercially manufactured starter feed suitable for intensive culture of larval walleyes *Sander vitreus*, it became imperative to evaluate other commercial feeds after FFK importation to the USA was banned to prevent the spread of bovine spongiform encephalopathy. A project was undertaken at the Rathbun Fish Culture Research Facility (Rathbun), Moravia, Iowa, and Iowa State University (ISU), Ames, using a single-factor experimental design with Lansy and Epac feeds (INVE Aquaculture, Inc., Ogden, Utah) and Gemma feed (Skretting, Vancouver, British Columbia). Walleyes were cultured at both sites from 3 to 28 d posthatch to evaluate the feeds. Performance indicators included presence of feed in gut, gas bladder inflation, survival, total length (mm), weight (mg), growth rate, and incidence of deformities. At both sites, survival was 2.6–3.6 times greater for fish given Gemma than for fish given the INVE feeds; the difference was significant at Rathbun but not at ISU. Length, weight, and growth rate at both sites were also significantly greater for Gemma-fed walleyes than for INVE-fed fish. Weight advantage produced by Gemma was 192% at ISU and 534% at Rathbun. Performance measures of fish given Gemma were similar to those of fish fed FFK in previous years.

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