

## INFORMATION OF INTEREST

Journal of Aquatic Animal Health Vol. 21, No. 2, June 2009 is now available [online](#)

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## OPTIMIZING COPPER SULFATE TREATMENTS FOR FUNGUS CONTROL ON CHANNEL CATFISH EGGS

David L. Straus, Andrew J. Mitchell, Ray R. Carter, James A. Steeby-2009

Journal of Aquatic Animal Health 21(2): 91-97

## Abstract:

This range-finding study determined the optimum concentration of copper sulfate (CuSO<sub>4</sub>) for fungus control on eggs of channel catfish *Ictalurus punctatus*. The study consisted of five CuSO<sub>4</sub> concentrations (2.5, 5, 10, 20, and 40 mg/L) and an untreated control in a flow-through system. A single spawn was used for each replication (N = 4). Eggs were treated daily until the embryos reached the eyed stage. When hatching was complete for all viable eggs, fry were counted to determine the percent survival in each treatment. Fungal growth was severe in the untreated controls; survival of hatched fry in the control group was approximately 2%. The optimum CuSO<sub>4</sub> treatment, as determined by percent survival of hatched fry, was 10 mg/L daily (69% survival); survival for this treatment group was significantly different from that for the controls. Very little fungus was present in treatments receiving 10-mg/L CuSO<sub>4</sub> or higher except in one replication that had approximately 40% unfertilized eggs. The average survival rates in the 0-, 2.5-, 5-, 10-, 20-, and 40-mg/L CuSO<sub>4</sub> treatments were 2, 34, 50, 69, 59, and 51%, respectively.

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## EVALUATION OF SODIUM CARBONATE PEROXYHYDRATE AS A POTENTIAL CATFISH EGG DISINFECTANT

Brian C. Small-2009

Journal of Aquatic Animal Health 21(2): 117-123

## Abstract:

Two experiments were conducted to evaluate the efficacy of sodium carbonate peroxyhydrate (SCP) in improving the hatching success of channel catfish *Ictalurus punctatus* when used as a prophylactic chemotherapeutant during egg incubation. In the first experiment, the efficacy of SCP was evaluated in 379-L aluminum incubation troughs similar to those used in commercial hatcheries. Egg masses treated daily with 254 mg of SCP/L of water had significantly higher mean hatching success than untreated controls, and a pathogen-inhibiting effect was also evident (i.e., no gross infection was observed on the treated egg masses). In the second experiment, the hatching success of egg masses treated daily with 254 mg/L was compared with that of egg masses treated daily with hydrogen peroxide (70 mg/L). The effects of both treatments on the pH, dissolved oxygen, and hydrogen peroxide concentrations in the trough were also examined. Both SCP and hydrogen peroxide significantly improved hatching success. Unlike in the treatment with hydrogen peroxide, water pH increased during the treatment with SCP;

however, no negative effects on hatching success were observed. The results of this research suggest that SCP acts similarly to hydrogen peroxide in improving channel catfish hatching success and warrants further research to determine whether it could be a practical and effective alternative for managing catfish egg infections in commercial hatcheries.

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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 (English Edition) 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<sup>-1</sup>) 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<sup>-1</sup> 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<sup>-1</sup> 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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NATURAL VARIATION IN LARVAL SIZE AND DEVELOPMENTAL RATE OF THE NORTHERN QUAHOG *MERCENARIA MERCENARIA* AND ASSOCIATED EFFECTS ON LARVAL AND JUVENILE FITNESS

R. Przeslawski, A. R. Webb-2009

Journal of Shellfish Research 28(3):505-510

Abstract:

Larval size and developmental rate can vary tremendously within and among cohorts because of genetics, environment, and maternal investment. This natural variation in larvae may have effects that span multiple life stages. Here we investigate the effects of larval size and developmental stage on the subsequent life stages of the commercially and ecologically important clam *Mercenaria mercenaria*. Fifteen days after fertilization, we divided larvae into two groups based on their developmental stage (umbonal or pediveliger) and recorded survival, size, and developmental stage of individuals over the next 4 months. Results revealed that after four months larvae that had only reached the umbonal stage by Day 15 were significantly smaller than those that had reached the pediveliger stage. These smaller and less developed larvae were less successful than the larger and more developed larvae across late larval and juvenile stages. In particular, smaller and less developed larvae were less likely to metamorphose, required more time to metamorphosis, and had lower survival and growth rates. These results suggest that natural variation in larval size and developmental rate can affect recruitment in a variety of ways: (1) Increased time to metamorphosis may increase the cost to larvae via predation or exposure to environmental stress, (2) Decreased proportion of larvae able to metamorphose may directly reduce the number of settlers, and (3) Decreased growth and survival rates for juveniles may reduce the number of new recruits. We also discuss the persistence of natural variation in larval size and developmental rate in light of the observed negative effects associated with smaller and less-developed larvae.

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#### EARLY WEANING OF SOUTHERN FLOUNDER, *PARALICHTHYS LETHOSTIGMA*, LARVAE AND ONTOGENY OF SELECTED DIGESTIVE ENZYMES

Cynthia K. Faulk, G. Joan Holt-2009

*Aquaculture* 296(3-4) : 213-218

##### Abstract:

There is considerable interest in rearing Southern flounder, *Paralichthys lethostigma*, for commercial production and for stock enhancement. Both goals depend upon excellent larval nutrition for the production of robust juveniles. The current use of live prey for larviculture is an expensive and time consuming process that can be alleviated by weaning larvae onto dry feed. A study was conducted to assess the potential for early weaning of southern flounder larvae onto a microdiet (MD). In addition, the activity of selected digestive enzymes was measured during ontogeny to evaluate the digestive capabilities of the larvae over time. Pancreatic enzyme activities (U larva<sup>-1</sup>) were very low or undetectable at hatching and a marked increase in activity was not observed until the larvae reached 4 mm (~ 11 dph) in standard length for chymotrypsin (24–44,000) and 6 mm (~ 25 dph) for amylase (< 1–24), trypsin (1–18) and bile salt-dependent lipase (0–443). Acid protease activity (~ 1.0) was detected once the larvae were 8.5–9.0 mm (37–39 dph) in length although a sizeable increase in activity (> 10.0) was not observed until after complete metamorphosis (> 11.0 mm; 40–45 dph). Feeding regimes employed for the weaning study consisted of a live feed control (C) and a combination of live feed and MD in which the addition of the MD was initiated on 11 dph and live feed terminated on 17 (T17), 23 (T23) or 29 (T29) dph. At the end of the study (35 dph), mean standard length and the percent of settled fish were significantly greater for fish in the control treatment (8.3 mm; 21.1%) than for fish fed any combination of live prey and MD (6.4 mm; 2.0%). Average survival was 27.7% and no significant differences were noted among treatments. However, the number of fish exhibiting spinal deformities, lordosis, was significantly lower in the control and T29 treatments (1.7%) than the T17 and T23 treatments (25%). The results of this study indicate that southern flounder larvae readily wean onto dry feed prior to the onset of metamorphosis. However, decreased growth and a high incidence of lordosis emphasize the need for the development of a more appropriate MD for this species when digestive enzyme activities are relatively low and gastric digestion is absent.

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#### PERACETIC ACID DEGRADATION AND EFFECTS ON NITRIFICATION IN RECIRCULATING AQUACULTURE SYSTEMS

Lars-Flemming Pedersen, Per B. Pedersen, Jeppe L. Nielsen, Per H. Nielsen-2009

*Aquaculture* 296(3-4) : 246-254

##### Abstract:

Peracetic acid (PAA) is a powerful disinfectant with a wide spectrum of antimicrobial activity. PAA and hydrogen peroxide (HP) degrade easily to oxygen and water and have potential to replace formalin in aquaculture applications to control fish pathogens, for example the ectoparasite, *Ichthyophthirius multifiliis*.

We studied water phase PAA and HP decay in three aquaculture situations, i) batch experiments with two types of system waters, ii) PAA decay at different fish densities, and iii) degradation of PAA in submerged biofilters of recirculating aquaculture systems (RAS). Furthermore, effect of PAA on the nitrification activity and the composition of the nitrifying population were investigated.

PAA and HP decay showed first order kinetics. High dosage PAA/HP in water with low COD inhibited HP removal, which was not observed in water having a higher COD content. PAA decay was

significantly related to fish stocking density, with half life constants for PAA of 4.6 and 1.7 h at 12 and 63 kg m<sup>-3</sup>, respectively.

PAA application to RAS biofilter showed rapid exponentially decay with half life constants of less than 1 h, three to five times faster than the water phase decay rates.

Biofilter surface specific PAA removal rates ranged from 4.6 to 13.9 mg PAA m<sup>-2</sup> h<sup>-1</sup> and was positively correlated to the nominal dosage. Low PAA additions (1.0 mg L<sup>-1</sup>) caused only minor impaired nitrification, in contrast to PAA application of 2.0 and 3.0 mg L<sup>-1</sup>, where nitrite levels were significantly increased over a prolonged period, albeit without fish mortality. The dominant ammonium oxidizer was *Nitrosomonas oligotropha* and the dominant nitrite oxidizer was *Nitrospira*. Based on the present findings and other recent results from field and in vitro studies, application perspectives of PAA are discussed.

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#### RESPONSE AND CONDITION OF LARVAE OF THE SCALLOPS *NODIPECTEN SUBNODOSUS* AND *ARGOPECTEN VENTRICOSUS* REARED AT THE HATCHERY WITH DIFFERENT SEAWATER SOURCES

Fernando Abasolo-Pacheco, José M. Mazón-Suástegui, Pedro E. Saucedo-2009

Aquaculture 296(3-4) : 255-262

Abstract:

Larvae of the scallops *Nodipecten subnodosus* and *Argopecten ventricosus* were reared at a hatchery under five seawater sources: (1) filtered seawater, as the control group; (2) pasteurized seawater; (3) seawater taken from a well; (4) synthetic seawater, and (5) seawater containing a commercial probiotic (Epicin). The quality of each seawater source was measured in terms of counts of *Vibrio* pathogenic bacteria, levels of nitrites, nitrates, and ammonium, and content of suspended and organic matter. Overall response of larvae under each treatment was measured in terms of growth, survival, biochemical composition, and recruitment rate of spat. Differences in all these parameters, as a function of the seawater source, were analyzed with one-way ANOVA. Larvae survived more, grew faster and larger, had higher protein levels, and recruited more in filtered seawater (in *N. subnodosus*) and pasteurized seawater (in *A. ventricosus*), but significant differences between treatments were slight. In *A. ventricosus* cultures, filtered seawater favored significantly higher *Vibrio* counts than pasteurized seawater, but this result did not affect the response and condition of larvae. The well seawater treatment ranked third in terms of low bacterial counts, high nitrate levels, larvae showing relatively high survival and growth rates, and spat reaching the settlement stage (only in *A. ventricosus*). The synthetic seawater and Epicin treatments did not yield satisfactory results for any of the indicators measured. In fact, in the Epicin treatment, larvae survived only the first days of hatchery cultivation and settlement occurred only in *A. ventricosus*. The results from these first set of trials are useful for planning additional experiments aimed to improve the protocol of seawater use at our research hatchery.

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#### EFFECTIVE FLUOROCHROME MARKING OF JUVENILE SEA CUCUMBERS FOR SEA RANCHING AND RESTOCKING

Steven W. Purcell, Bernard F. Blockmans-2009

Aquaculture 296(3-4) : 263-270

Abstract:

Dermal spicules (or 'ossicles') of cultured sea cucumbers can be fluorescently marked with tetracycline and calcein for sea ranching and restocking but optimal immersion conditions are unknown. Lethal and non-lethal effects, and the efficacy of marking spicules in juvenile sandfish (*Holothuria scabra*), were examined under different immersion conditions. Fluorescence brightness and the proportion of marked

spicules generally increased with concentration and duration of immersion. Frequency of burial (an indicator of stress) in sandfish increased with both fluorochromes at concentrations above 50 mg L<sup>-1</sup>. Growth in the two weeks post-marking was unaffected at immersion concentrations of 50 and 100 mg L<sup>-1</sup> compared to controls, but appeared inhibited by immersion in solutions of 200 and 400 mg L<sup>-1</sup> of tetracycline or calcein. Sequential marking by tetracycline (yellow) and calcein (green), in either order, showed that calcein was deposited in a higher proportion of spicules. Three other fluorochromes with disparate colors, alizarin complexone, calcein blue and xylene orange, also marked sandfish spicules and expanded the variety of dichromic combinations. Both tetracycline and calcein fluoresced more brightly when juveniles were marked at 26 or 30 °C than at 21 °C, and this low temperature appears also to reduce the proportion of spicules marked by tetracycline. Our findings show that seawater temperature should be regulated for ex situ immersion marking. The behavioral and biological sensitivities of sandfish demand care in administering the fluorochromes. Fluorochrome immersion at 100 mg L<sup>-1</sup> for 24 h at ≥26 °C provides a practical compromise between minimizing the fitness of released juveniles and ensuring the efficacy of the markers for studies on the growth and survival of sea cucumbers stocked in the wild.

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#### THE FATTY ACID COMPOSITION OF NEREIS DIVERSICOLOR CULTURED IN AN INTEGRATED RECIRCULATED SYSTEM: POSSIBLE IMPLICATIONS FOR AQUACULTURE

Adrian A. Bischoff, Patrick Fink, Uwe Waller-2009

Aquaculture 296(3-4) : 271-276

Abstract:

The potential of solid waste originating from a recirculated fish culture system, i.e. faecal material, uneaten food pellets and bacterial biofilms was examined as food source for the marine polychaete *Nereis diversicolor*. These polychaetes could be a valuable food for fish if they provide essential fatty acids to the fish. Therefore, we analysed the fatty acid profiles from feed and faecal materials, the sediment as well as the cultured organisms — fish and several batches of *N. diversicolor* — from an integrated recirculating aquaculture system.

The major fatty acids (saturated, monounsaturated and polyunsaturated) for all analysed fish feed, fish and faeces samples were C16:0, C18:1 and C22:6 (n – 3), accounting for 48% to 57% of the fatty acids in the samples. The major fatty acids within the sediment were C16:0, C18:1 and C18:3 (n – 3), accounting for 61% of the total fatty acids. The samples of *N. diversicolor* revealed C16:0, C18:1 and C20:5 (n – 3) as the major fatty acids. Combined, they accounted for 56% of the total fatty acids detected within the worm samples.

The results indicate that a recycling or even an upgrade of excreted feed nutrients such as fatty acids, which were otherwise discharged, can be achieved through integrated aquaculture combining fish and worm culture.

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#### CO-FEEDING OF LIVE FEED AND INERT DIET FROM FIRST-FEEDING AFFECTS ARTEMIA LIPID DIGESTIBILITY AND RETENTION IN SENEGALESE SOLE (*SOLEA SENEGALENSIS*) LARVAE

Mônica Giacometti Mai, Sofia Engrola, Sofia Morais, Maria Célia Portella, José Roberto Verani, Maria Teresa Dinis, Luís E.C. Conceição-2009

Aquaculture 296(3-4) : 284-291

Abstract:

The present study intended to evaluate the effects of early introduction of inert diet in lipid digestibility and metabolism of sole, while larval feed intake, growth and survival were also monitored. *Solea senegalensis* larvae were reared on a standard live feed regime (ST) and co-feeding regime with inert

diet (Art R). Trials using sole larvae fed with Artemia enriched with two different lipid emulsions, containing glycerol tri [1-14C] oleate (TAG) and L-3-phosphatidylcholine-1,2-di-[1-14C] oleoyl (PL), were performed at 9 and 17 days after hatching (DAH) to study lipid utilization. Co-feeding did not affect sole survival rates (ST  $59.1 \pm 15.9\%$ ; Art R  $69.56 \pm 9.3\%$ ), but was reflected in significantly smaller final weight at 16 DAH (ST  $0.71 \pm 0.20$ ; Art R  $0.48 \pm 0.14$  mg). Higher feed intake was observed in sole larvae fed on Artemia enriched with labeled PL at 9 DAH but not at 17 DAH. At 17 DAH, the smaller larvae (Art R treatment) ingested proportionally more Artemia in weight percentage, independently of enrichment. At 9 DAH lipid digestibility was equal among treatments and higher than 90%, while at 17 DAH it was higher in ST treatment (around 73%) compared to the Art R group (around 66%). Lipid retention efficiency at 9 DAH was higher in the Art R treatment, reaching values of 50%, while these values almost duplicated at 17 DAH, ranging up to 80% in both treatments without significant differences. These results show that co-feeding of live feed and inert diet from first-feeding in Senegalese sole has a toll in terms of growth and lipid digestibility but does not seem to compromise lipid metabolic utilization.

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#### AN EVALUATION OF THE NUTRITIONAL VALUE OF ALTERNATIVE LIPID SOURCES TO JUVENILE SOUTHERN ROCK LOBSTER, JASUS EDWARDSII

Louise R. Ward, Chris G. Carter-2009

Aquaculture 296(3-4): 292-298

Abstract:

Experimental lobster feeds are currently based on fish meal and fish oil formulations, and although survival and growth similar to that of lobsters fed fresh blue mussels has been achieved, varying the protein level in previous experimental feeds has not increased growth beyond that of lobsters fed natural food. This experiment assessed the growth performance of lobsters fed pelleted feeds containing constant amounts of protein, lipid and energy where the lipid was provided by a range of oil-rich ingredients (fish oil, FO; fish oil with added soybean lecithin, FOL; canola oil, CO; tuna oil, TO; mussel meal, MM; and squid meal, SQM). Feed performance was assessed by lobster growth rate, survival, final biochemical composition, nutrient retention and nutrient efficiency. Twenty tanks containing 15 post-larval lobsters each ( $1.5 \pm 0.04$  g) were randomly allocated one of six test feeds in triplicate, and the two remaining tanks were fed freshly opened blue mussels (FRM) as a reference feed. Lobsters were fed daily to excess for 10 weeks. Final individual weights of whole body and digestive gland were measured, and tissue chemical composition analysed. There were no significant differences in survival ( $88.4 \pm 3.3\%$ ), or specific growth rate ( $1.3 \pm 0.1\% \cdot \text{day}^{-1}$ ) among the formulated feed fed lobsters, which were significantly lower than the survival ( $100 \pm 0.0\%$ ) and SGR ( $2.2 \pm 0.1\% \cdot \text{day}^{-1}$ ) of FRM fed lobsters. The SQM fed lobsters had a significantly lower lipid efficiency ratio and lipid productivity value than lobsters fed TO, FOL and MM feeds. The digestive gland lipid content ( $\text{g} \cdot 100 \text{ g wet tissue}^{-1}$ ) of lobsters fed the feeds TO ( $3.7 \pm 0.4$ ), FO ( $3.5 \pm 0.3$ ) and SQM ( $2.2 \pm 0.2$ ) were significantly lower than lobsters fed feeds MM ( $9.9 \pm 1.1$ ), FOL ( $9.0 \pm 2.3$ ) and FRM fed lobsters contained most digestive gland lipid ( $12.3 \pm 1.5$ ).

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#### BALANCING THE EFFECTS OF REARING AT LOW TEMPERATURE DURING EARLY DEVELOPMENT ON SEX RATIOS, GROWTH AND MATURATION IN THE EUROPEAN SEA BASS (DICENTRARCHUS LABRAX): LIMITATIONS AND OPPORTUNITIES FOR THE PRODUCTION OF HIGHLY FEMALE-BIASED STOCKS

Laia Navarro-Martín, Mercedes Blázquez, Jordi Viñas, Sílvia Joly, Francesc Piferrer-2009

Aquaculture 296(3-4) : 347-358

Abstract:

In many cultured fish species, including the European sea bass, undesirable highly male-biased sex ratios are nevertheless frequent. High temperatures (21 °C), typically used during the larval and early juvenile stages are thought to cause sex-reversal of genotypic females. Rearing at lower temperatures (< 17 °C) has been investigated as a possible solution. However, besides reducing growth, they have sometimes resulted in equivocal sex ratios across different studies. The goal of this study was to find a thermal regime that could maximize the number of females without compromising growth. Four batches were reared at 15 °C during 10 (control group), 30, 60, 90 or 120 (treated groups) days post fertilization (dpf) and then at 21 °C. The controls had excess males (average from 4 batches: 69% males, 31% females). Increasing the duration of rearing at 15 °C doubled the average number of females up to 59% (range 22–90%) and reduced the number of 1-year-old precocious males from 29% in the control to 10–20% in the treated groups. Low temperature retarded growth in groups exposed for 60 or more days; however, fish exposed for 30 days exhibited compensatory growth by 150 dpf. Females reached a marketable size (set at 400 g) during the second year, four months earlier than males. Despite initial slower growth, an increase of 10% in biomass was estimated after rearing at 15 °C for 60 days (850–900 °C-days) by the time of marketing when compared to current industry practices. Finally, we analyzed these and all available data of the effects of temperature on European sea bass sex ratios, and show that exposures to low temperature starting at fertilization do not induce but merely allow female development. In contrast, high temperatures masculinize, on average, over half of the females. The results show that insensitive fish are not males that cannot be feminized by low temperature but instead females that cannot be masculinized by high temperature. Although there is no thermal regime that per se will result in 100% females in the European sea bass, we propose rearing at 17 °C starting soon after fertilization and until 850–900 °C-days, which at that temperature occurs at 53 dpf, as the best balance between the advantages resulting from allowing female development, and the disadvantages of initial slower growth. Combined with genetic selection for high female number and low sensitivity to high temperature, this method offers an opportunity for the routine culture of highly female-biased sex ratios to benefit European sea bass aquaculture.

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## GROWTH AND SURVIVAL OF EEL LEPTOCEPHALI (ANGUILLA JAPONICA) IN LOW-SALINITY WATER

Akihiro Okamura, Yoshiaki Yamada, Naomi Mikawa, Noriyuki Horie, Tomoko Utoh, Toyoji Kaneko, Satoru Tanaka, Katsumi Tsukamoto-2009

Aquaculture 296(3-4) : 367-372

Abstract:

We investigated the effects of low-salinity water on the growth, survival, and activity of artificially reared Japanese eel larvae (*Anguilla japonica*), proceeding from the assumption that such water quality saves energy due to lower cost for osmoregulation. We reared 5-day-old larvae in 0%, 10%, 30%, 50%, 70%, and 100% seawater (SW) with or without diet for 13 days. All larvae reared in 0% and 10% SW died within 6 days, while larvae in 70% and 100% SW survived until 9 days. Larvae in 30% and 50% SW further survived until 13 days without diet. Significant growth in body depth was observed in 30% and 50% SW after 7 days rearing with diet ( $0.65 \pm 0.02$  and  $0.62 \pm 0.02$  mm, respectively) as compared with the initial size ( $0.49 \pm 0.03$  mm), while no significant growth was observed under the other salinity conditions examined. Larvae swam actively in the light (about 2000 lx) in 50%, 70%, and 100% SW, while they were apparently inactive in 0%, 10%, and 30% SW. The long-term rearing trial showed a 2.2-fold higher 2-month cumulative survival rate in 50% SW (18.2%) than in 100% SW (8.2%). The body depth of larvae in 50% SW ( $1.58 \pm 0.47$  mm) was also significantly larger than in 100% SW ( $1.32 \pm 0.35$  mm). These findings indicate that the intermediate salinity can result in better growth and survival performance in Japanese eel larvae.

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CHARACTERIZATION OF PSEUDOMONAS AERUGINOSA ASSOCIATED WITH DISEASED POSTLARVAL ABALONE IN SHENZHEN, CHINA

Junpeng Cai, Shuanyu Lin, Bing Wu-2009

Aquaculture International 17(5): 449-458

Abstract:

Outbreaks of mass mortality of postlarval abalone, *Haliotis diversicolor supertexta*, have occurred in south China since 2002 and have forced many abalone farms to close. About 30 representative bacterial strains were isolated from a sample of five diseased postlarval abalone, taken 25 days post-fertilization during an outbreak of postlarval disease in Shenzhen, China, in October 2006. Bacterial challenge tests showed that the predominant strain, designated as strain 22, was highly virulent to postlarvae with an LD50 value of  $7.8 \times 10^4$  colony forming units (CFU) ml<sup>-1</sup>, while four of the other isolates were weakly virulent with LD50 values ranging from  $1 \times 10^6$  to  $1 \times 10^7$  CFU ml<sup>-1</sup>, and the remaining 25 isolates were classified as avirulent with LD50 values greater than  $1 \times 10^8$  CFU ml<sup>-1</sup>. By means of API 20NE and 16S rDNA and ITS sequencing analyses, strain 22 was identified as *Pseudomonas aeruginosa*. Antibiotic susceptibility tests showed that strain 22 exhibited around 75% of susceptibility to 16 various antibiotics tested. The results of this study show *P. aeruginosa* as one of the bacteria involved in the mortality of abalone postlarvae in Shenzhen, China.

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WHEN LOW GENETIC VARIABILITY IN FERAL AND HATCHERY-REARED TILAPIA BECOMES A VICIOUS CIRCLE: OREOCHROMIS NILOTICUS FROM OAXACA, MEXICO

J. Soto-Hernández, A. Velasco-Murillo, J. M. Grijalva-Chon-2009

Aquaculture International 17(5): 469-478

Abstract:

Several tilapia species in Mexican reservoirs have been used as food source for more than 40 years but little effort has been devoted to the conservation and genetic selection of those species. Our objective was to evaluate genetic variability in feral (n = 59) and hatchery-produced (n = 58) *Oreochromis niloticus* stocked in the Benito Juárez dam. Allozyme analyses for 13 enzymatic systems and general proteins yielded 27 genetic loci. Seventeen loci were polymorphic in at least one sample. The observed heterozygosity was lower than expected and the imbalance in the Hardy–Weinberg equilibrium in almost all loci was caused by heterozygote deficiency. The inbreeding coefficient was 84%. Although the evidence suggests moderate genetic differentiation, it is probable that a great proportion of the source of hatchery organisms is from feral organisms. According to our results, management of the hatchery strain is inadequate, therefore other breeding strategies are needed to increase the genetic variability of *O. niloticus* from Oaxaca.

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STRINGED BED SUSPENDED BIOREACTORS (SBSBR) FOR IN SITU NITRIFICATION IN PENAEID AND NON-PENAEID HATCHERY SYSTEMS

V. J. Rejish Kumar, Cini Achuthan, N. J. Manju, Rosamma Philip, I. S. Bright Singh-2009

Aquaculture International 17(5): 479-489

Abstract:

For establishing nitrification in prawn (non-penaeid, salinity 10–15 ppt) and shrimp (penaeid, salinity 30–35 ppt) larval production systems, a stringed bed suspended bioreactor (SBSBR) was designed, fabricated, and validated. It was fabricated with 5 mm polystyrene and low density polyethylene beads as the substrata for ammonia and nitrite oxidizing bacterial consortia, respectively, with an overall surface area of 684 cm<sup>2</sup>. The reactors were activated in a prototype activator and were transported in polythene bags to the site of testing. Performance of the reactors activated with the nitrifying bacterial consortia AMONPCU-1 (ammonia oxidizers for non-penaeid culture) and NIONPCU-1 (nitrite oxidizers for non-penaeid culture) was evaluated in a *Macrobrachium rosenbergii* larval rearing system and those activated with AMOPCU-1 (ammonia oxidizers for penaeid culture) and NIOPCU-1 (nitrite



oxidizers for penaeid culture) in a *Penaeus monodon* seed production system. Rapid setting up of nitrification could be observed in both the static systems which resulted in a higher relative per cent survival of larvae.

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#### GAMETOGENESIS, REPRODUCTIVE INVESTMENT, AND SPAWNING BEHAVIOR OF THE PACIFIC GIANT OYSTER CRASSOSTREA GIGAS: EVIDENCE OF AN ENVIRONMENT-DEPENDENT STRATEGY

M. Enríquez-Díaz, S. Pouvreau, J. Chávez-Villalba, M. Le Pennec-2009

Aquaculture International 17(5): 491-506

Abstract:

The progress of gametogenesis was studied in oysters *Crassostrea gigas* having the same origin (Tremblade), but cultured during 1 year in two distinctive French marine areas, the Baie des Veys and Marennes-Oléron. We assessed seasonal changes in the reproduction cycle on the basis of stereological techniques to estimate reproductive investment and measurement of gonad evolution area by quantitative histology. From a qualitative point of view, both oyster groups presented typical reproductive stages, but showed differential timing, in particular during the sequence of spawning and duration of the re-absorption stage. Oysters in Baie des Veys had a single partial spawning in August and a re-absorption stage that extended until winter. Oysters in Merennes-Oléron had a partial spawning in July and massive release of gametes during August. Spawning in both the areas were related to maximum temperature (19°C). The quantitative analysis showed, on an annual basis, a higher reproductive investment by oysters from Baie des Veys, 86% against 53% in the other group. Larger gonads, higher gamete production, and more intensive spawning were the characteristics of oysters in Baie des Veys. Recently, the reproduction pattern and investment has been related with summer mortalities; therefore, a quantitative understanding of reproductive processes becomes necessary for *C. gigas*. Environmental conditions at each site may explain differences in the progress and intensity of gametogenesis. While temperature regulated the time and speed of gametogenesis, results suggest that the intensity was influenced by the quantity of available food but may need further research. However, nutrient recycling from unreleased gametes in the gonads of oysters from Baie des Vey is a factor to be considered in the results of this study.

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#### USE OF NEUROACTIVE CATECHOLAMINES TO CHEMICALLY INDUCE METAMORPHOSIS OF HATCHERY-REARED FLAT OYSTER, OSTREA ANGASI, LARVAE

Stephan O'Connor, Natalie Moltschaniwskyj, Wayne O'Connor-2009

Aquaculture Research 40(14): 1567 – 1577

Abstract:

Low numbers and unreliable wild catch of the native flat oyster, *Ostrea angasi*, spat has resulted in the NSW flat oyster industry being reliant on hatchery-produced spat. The need to produce culchless spat in the hatchery stimulated investigation of several catecholamines to induce metamorphosis in *O. angasi* larvae. Larvae were treated with one of four neuroactive catecholamines (epinephrine, epinephrine bitartrate, l-Dopa and GABA) at one of four concentrations (10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> or 10<sup>-6</sup> M) for one of three treatment durations (0.5, 1–2 h) to determine morphogenic action for culchless spat production. Epinephrine bitartrate at 10<sup>-3</sup> and 10<sup>-4</sup> M and epinephrine at 10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup> M, for a treatment duration of 1–2 h, produced significantly greater numbers of spat and culchless spat, compared with any other treatment combination. The other catecholamines tested did not induce a significant increase in the total number of spat or culchless spat, over untreated controls. Separate trials found that long-term treatment (24 h) with epinephrine bitartrate and epinephrine at morphogenic concentrations inhibited metamorphosis. Consecutive daily use of epinephrine bitartrate increased the numbers of spat and

culchless spat produced, but did not affect larval or short-term post-larval survival. Treatment with 10–3 m epinephrine bitartrate or 10–4 m epinephrine for 1 h is recommended for routine commercial production of culchless flat oyster spat.

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#### COMPARING SKELETAL DEVELOPMENT OF WILD AND HATCHERY-REARED SENEGALESE SOLE (*SOLEA SENEGALENSIS*, KAUP 1858): EVALUATION IN LARVAL AND POSTLARVAL STAGES

Paulo Jorge Gavaia, Susana Domingues, Sofia Engrola, Pilar Drake, Carmen Sarasquete, M. Teresa Dinis, M. Leonor Cancela-2009

Aquaculture Research 40(14): 1585 – 1593

Abstract:

The Senegalese sole is a marine pleuronectiform that naturally occurs in Southern Europe and Mediterranean region where it is being produced in aquaculture, in particular in Portugal and Spain. The aim of this study was to assess the quality of hatchery-reared larvae in comparison with those reared in the wild, and determine to which extension wild growing larvae are also affected by skeletal deformities. The main structures affected included those forming the axial skeleton, the caudal fin complex and both anal and dorsal fins, with the most prevalent anomalies affecting caudal vertebrae and arches. Hatchery-reared fish presented a higher incidence of deformities (79%) compared with the 19% observed in wild specimens. In wild postlarvae collected in Autumn no deformities were observed. This work clearly shows that wild Senegalese sole present less skeletal deformities than those hatchery-reared during larval stages, indicating a selective mortality of wild deformed fish and/or an effect of aquaculture-related rearing conditions in the development of skeletal deformities in sole.

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#### FUNCTIONAL RESPONSE OF *AMECA SPLENDENS* (FAMILY GOODEIDAE) FED CLADOCERANS DURING THE EARLY LARVAL STAGE

Fabiola Peña-Aguado, S/ Nandini, S. S. S. Sarma-2009

Aquaculture Research 40(14): 1594 – 1604

Abstract:

We studied the functional response of the goodeid *Ameca splendens*, an endangered species from rivers Ameca and Teuchitlán (Jalisco, Mexico), from birth until 8 weeks old. The cladocerans, *Alona glabra* adults, *Simocephalus vetulus* neonates, *Ceriodaphnia dubia* adults, *Daphnia pulex* juveniles and *Moina macrocopa* adults were used as prey. The prey densities, depending on the species, ranged between 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 16.0 and 32 ind. mL<sup>-1</sup>, with four replicates at each density. We found that all functional responses were Type II. During the feeding period of 30 min, the fish larvae consumed about 600 individuals of the smallest prey *A. glabra* (450 µm). The consumption of larger prey (<1.0 mm) such as *S. vetulus*, *C. dubia*, *D. pulex* and *M. macrocopa* ranged between 150 and 200 prey larva<sup>-1</sup> during the feeding trials. Our study shows that *A. splendens* reached maximal prey consumption at around 4 weeks of age, after which there was no increase in prey consumption during the feeding period with age until the end of the study period of 8 weeks. Our study indicates that prey digestion rather than handling time determines the functional response in this fish species and that *M. macrocopa* is most suited as live food for *A. splendens*.

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#### BENEFICIAL PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM A *RANA CATESBEIANA* HATCHERY

Sergio E. Pasteris, Germán Roig Babot, María C. Otero, Marta I. Bühler, María E. Nader-Macías-2009

Aquaculture Research 40(14): 1605 – 1615

Abstract:

This work addresses the selection of potentially probiotic lactic acid bacteria (LAB) to be used in raniculture. Thus, strains belonging to the genera *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *Lactococcus lactis* and *Enterococcus faecium* isolated from a *Rana catesbeiana* hatchery were evaluated for their inhibitory properties against RLS-associated pathogens (*Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*) and food-borne bacteria. Cell-free supernatants of LAB strains inhibited the growth of at least one of the pathogens by organic acids, but *L. lactis* CRL 1584 also produced a bacteriocin-like metabolite. The ability of LAB strains to produce H<sub>2</sub>O<sub>2</sub> in MRS+TMB medium was also studied. Seventy-eight to ninety six per cent of the strains showed some level of H<sub>2</sub>O<sub>2</sub> production. Moreover, different organic solvents were used to determine the hydrophobicity and Lewis acid/base characteristic of LAB strain surfaces. Most of the strains presented hydrophilic properties, but no acidic or basic surface characters. However, some strains isolated from the skin showed a high degree of hydrophobicity and basic components in the cell surface due to their adhesion to chloroform. These properties were not observed in LAB from balanced feed and freshwater. Taking into account general guidelines and the beneficial properties studied, five strains were selected as potential candidates to be included in a probiotic for raniculture.

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#### VERTEBRAL DEFORMITY IN CULTURED ATLANTIC COD LARVAE: ONTOGENY AND EFFECTS ON MORTALITY

Velmurugu Puvanendran, Colleen Calder-Crewe, Joseph A. Brown-2009

Aquaculture Research 40(14): 1653 – 1660

Abstract:

The effects of different egg incubation densities on the incidences of vertebral deformities in Atlantic cod larvae were investigated. Cod eggs were incubated at four different densities, 3, 6, 12 and 48 mL eggs L<sup>-1</sup>, of water. When all the eggs hatched, larvae were reared in 30 L glass aquaria. Larval samples were taken at 0, 14, 42 and 56 days post hatch (dph) for deformity analysis. Larval samples were stained using bone and cartilage staining methods to determine vertebral deformity. Incubation densities did not have any significant effects on vertebral deformities in Atlantic cod larvae. However, the incidence of larval vertebral deformity was high at hatch and decreased as the larvae grew older until 42 dph, indicating selective mortality of deformed larvae during this period. Larvae at 56 dph, however, showed an increase in the incidence of vertebral deformity, indicating a possible nutritional or prey-type effect. To our knowledge, no studies have documented the occurrence of variable patterns in vertebral deformities in cod at various developmental stages. Overall, our results suggest that broodstock husbandry, genetics and/or nutrition could play a major role in causing vertebral deformities in Atlantic cod at hatch; however, nutrition and prey type may play a major role during metamorphosis.

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#### INVESTIGATION OF THE TEMPORAL EFFECTS OF SPAWNING SEASON AND MATERNAL AND PATERNAL DIFFERENCES ON EGG QUALITY IN ATLANTIC COD GADUS MORHUA L. BROODSTOCK

Dounia Hamoutene, Lynn Lush, Dwight Drover, Andrew Walsh-2009

Aquaculture Research 40(14): 1668 – 1669

Abstract:

A better understanding of the parameters affecting egg quality and larval survival is of importance for continued development of cod broodstock and efficient husbandry practices. Decision tree analysis (DTA) was applied to analyse 3 years of egg quality data in an effort to extract the most important variables (i.e. predictors) in explaining differences in egg quality. The effect of three predictors (spawning time, maternal and paternal differences) has been studied on early cleavage pattern

parameters, egg diameters, fertilization and hatching rates and has shown that females are the dominant variable and that time has a limited and inconsistent impact on the data. When using maternal, paternal differences and batch number (instead of spawning time) as predictors, the results confirm that no particular relationship is found between batch order (i.e. order in time) and egg quality. Moreover, batches with a higher egg quality show a consistency in the parameters assessed (i.e. batches with higher rates of normality in any parameter tend to be normal for other parameters). This is confirmed by the significant correlations found between cleavage parameters. Our results highlight that spawning time is of less importance than female parent contribution in ensuring high rates of fertilization and larval hatch, and maximizing general egg quality.

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