

## INFORMATION OF INTEREST

Electronic newsletter – [Fish Health section / Asian Fisheries Society](#) – August 2008

New COST Action FA0801: Critical success factors for fish larval production in European Aquaculture: a multidisciplinary network (LARVANET) – see [website](#)

DIPNET (Disease Interactions and Pathogen exchange NETwork): [website](#) with documents and reports

Interesting [website](#) on practical fish keeping

AuthorAid: supporting developing-country researchers in publishing their work: see [Resource Library](#)

VLIZ Library Acquisitions no

[405 Aug 14, 2008](#)

[406 Aug 22, 2008](#)

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## PROBIOTICS IN AQUACULTURE: CHALLENGES AND OUTLOOK

Yan-Bo Wang, Jian-Rong Li, Junda Lin-2008

Aquaculture 281(1-4): 1-4

Abstract:

With increasing demand for environment friendly aquaculture, the use of probiotics in aquaculture is now widely accepted. However, there is clearly a need in increasing our knowledge of intestinal microbiology and of effective preparation and safety evaluation of probiotics. This review provides a summary of the status and challenges of probiotic application in aquaculture. Our review is from the perspective of digestive tract, thereby taking into account the experiences in introducing the preparation, use and safety evaluation of probiotics in aquaculture. A better understanding of rationale, preparation and safety of probiotics in aquaculture may be of interest for commercial aquaculture.

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## INTENSIVE LARVAL HUSBANDRY AND FINGERLING PRODUCTION OF COBIA RACHYCENTRON CANADUM

Daniel D. Benetti, Bruno Sardenberg, Aaron Welch, Ronald Hoenig, M. Refik Orhun, Ian Zink-2008

Aquaculture 281(1-4): 22-27

Abstract:

Methods and results of two larval rearing trials of cobia (*Rachycentron canadum*) are presented. These trials were designed to test the efficacy of protocols developed over several years of research in cobia larviculture at the UMEH. The protocols incorporate the use of probiotics and prophylaxis, minimize microalgae use, and include commercially available ingredients for live feed enrichment. During the trials, fertilized eggs were stocked at 400/L and incubated in 1000-L cylinder-conical tanks with flow-through seawater at 500% daily exchange rate. Moderate aeration and pure oxygen were used to maintain dissolved oxygen concentrations above saturation (6.5 mg/L at 26 °C). Hatching occurred at 22–24 h post fertilization. Two day-post-hatch (dph) yolk-sac larvae were stocked in four 12,000-L cylinder-conical tanks at 5 and 10 larvae/L. Beginning on 3 dph, larvae were fed microalgae (*Isochrysis galbana* C-strain) at low concentrations (5–10,000 cell/ml) and enriched rotifers (*Brachionus plicatilis*) at 3–5/mL through 9 dph. Beginning on 7 dph, enriched *Artemia* (*Artemia franciscana* GSL Strain) nauplii were fed to larvae at 0.1–1/mL. Cobia larvae were reared at water temperatures ranging from 24.3 to 31.8 °C. Water quality parameters were within normal ranges for seawater: salinity 26–34 ppt, pH 7.92–8.16, and NH<sub>3</sub> < 0.18 mg/L. Vigorous aeration and supplemental oxygen were used at all times during both larval rearing trials to maintain adequate water movement and levels of dissolved oxygen (DO) (7.0–9.0 mg/L). Water was filtered down to 10

µm using standard sand filters filled with broken glass media and bag filters prior to entering the tanks. Daily water exchange rates in the tanks ranged from 100% at 3 dph to 500% from 17 dph onwards. Between 20 and 22 dph, all post-larvae were fully weaned onto dry starting diets. Survival rates of post-larvae measuring 1.5–2.0 cm SL and weighing 0.5 g at 20–22 dph were estimated to be ≥ 50%. Further mortality during the nursery stage to 3–5 cm and 1–3 g fingerlings prior to shipping at 27 dph brought the overall survival rate to an average of 25.7%. Survival rates of fingerlings cultured in tanks initially stocked at lower densities (5 larvae/L) was significantly higher ( $P = 0.0078$ ). From 15 dph, post-larvae and fingerlings were daily graded by size with large individuals singled out and stocked into another tank. These trials generated 125,328 fingerlings in four tanks in just two months, levels of production that could sustain a commercial operation and indicate that cobia aquaculture can be viable in the Americas.

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#### THE REPRODUCTIVE CYCLE OF WHITE CLAM *SPISULA SOLIDA* (L.) (MOLLUSCA: BIVALVIA): IMPLICATIONS FOR AQUACULTURE AND WILD STOCK MANAGEMENT

Sandra Joaquim, Domitília Matias, Belisandra Lopes, William S. Arnold, Miguel B. Gaspar-2008

Aquaculture 281(1-4): 43-48

Abstract:

The dynamics of the white clam's (*Spisula solida*) reproductive cycle along with its nutrient storage and exploitation strategy in the Algarve coast (Portugal) was studied throughout the year 2003. The timing of gametogenic development and spawning of *S. solida* were analysed through histological preparation using qualitative and quantitative criteria. Condition index and biochemical composition were determined in order to provide information on energy storage and utilization. Seawater temperature is a primary environmental factor determining reproductive development and spawning of *S. solida*; reproductive activity occurred during low temperatures. The spawning period began in late winter as a consequent response to the increase in seawater temperature and extended through spring. During this period, the condition index and the gonadal index decreased. In June, most of the population was spent and big resting oocytes appear dispersed in the gonad. In summer, the specimens were found to be in the resting phase and condition index increased to its maximum value as a consequence of reserves storage. Gametogenic activity was initiated coincident with decreasing temperature in September, but a sudden increase of this environmental parameter in October disturbed the gametogenic process and a second spawning occurred. In this period, the synchronism between males and females of the population was lost. The striking consumption of glycogen reserves developed during the previous August and consequent biosynthesis of lipids during gamete formation occurred. In the following two months, reproductive synchronism was restored, and storage of reserves and gametogenesis took place concurrently. In December the entire population was in the ripe stage of gonadal development. Moreover than a consequence of gametogenesis during autumn/winter, lipid behaviour reflected the energy accumulation process and its conversion to somatic development in spring/summer. The reproductive strategy adopted by *S. solida* makes possible broodstock manipulation in terms of conditioning in aquaculture. The information obtained in this study is important for assessing sustainable management of wild stocks as well as for estimating its potential for aquaculture production.

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#### PROMISE AND PITFALLS OF LOCALLY ABUNDANT SEAWEEDS AS BIOFILTERS FOR INTEGRATED AQUACULTURE

Nicholas A. Paul, Rocky de Nys-2008

Aquaculture 281(1-4): 49-55

Abstract:

Seaweeds from the genus *Caulerpa* offer promise for bioremediation in integrated tropical aquaculture in northern Australia, as they are common on shallow reefs adjacent to where aquaculture is developing and their propagation is readily manipulated through fragmentation. Fragments of five varieties of *Caulerpa* had high growth rates (between 3 and 7% day<sup>-1</sup>) and high nitrogen content (up to 3% dry weight/0.2% fresh weight for *Caulerpa taxifolia*) in tank-based culture. These attributes combined confirm the promise for *Caulerpa* in integrated aquaculture, especially as certain species (*Caulerpa lentillifera* and *Caulerpa racemosa*) are valuable products. However, this potential was not realised when *Caulerpa* spp. were cultured in an in situ aquaculture context. Only a limited proportion of fragments (13%, predominantly *C. taxifolia*) persisted during a 6 week in situ experiment in a flow-through settlement (treatment) pond from an 800 tonne year<sup>-1</sup> fish production facility. Mean growth of persisting pond fragments (less than 0.3% day<sup>-1</sup>) was much less than concurrent tank cultures (3–7% day<sup>-1</sup>). The factor most strongly influencing pond culture was the negative (smothering) impact of blooming filamentous algae (*Cladophora* and *Chaetomorpha* spp.). Poor pond growth of *Caulerpa* was further substantiated in an additional test, determining that persistence and growth (or lack thereof) was independent of initial seeding size of fragments. These results suggest that *Caulerpa* culture will not be easily integrated into settlement ponds in tropical aquaculture. However, because some species of *Caulerpa* grew well in tank-based systems (*C. racemosa* grew at > 7% day<sup>-1</sup>) and others are capable of luxury uptake (*Caulerpa serrulata* and *C. taxifolia* almost doubled internal nitrogen in nutrient-rich water), *Caulerpa* species have application in bioremediation of intensive tank-based aquaculture and perhaps treated pond aquaculture effluent.

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#### RESPONSE OF EARLY STAGE SPINY LOBSTER *JASUS EDWARDSII* PHYLLOSOMA LARVAE TO CHANGES IN TEMPERATURE AND PHOTOPERIOD

Michel Bermudes, Arthur J. Ritar-2008

Aquaculture 281(1-4): 63-69

##### Abstract:

The influence of temperature and photoperiod on the survival, intermoult period, moult increment and feeding activity were examined in early stage *Jasus edwardsii* phyllosoma larvae. In the first experiment, growth increment of newly-hatched larvae to Stage II was similarly high after rearing at 14.3 and 18.2 °C and lowest at 10.5 and 21.5 °C, whereas survival to Stage II was not significantly different between temperatures. By contrast, almost all Stage II phyllosoma died at 21.5 °C but there was no difference in survival from Stages II to IV at 14.3 and 18.2 °C, while larvae at the coldest temperature (10.5 °C) did not moult beyond Stage II during the experiment. The biological zero temperature of Stage I larvae was estimated at 9.4 °C by the Bělehrádek's expression fitted to the relationship between intermoult period and temperature ( $V = 48.716 (T - 9.425) - 0.579$ ). The growth rate and moult increment remained greater at 18.2 °C than at 14.3 °C throughout subsequent development to Stage IV. Consumption of *Artemia nauplii* by Stage I larvae escalated from 10.5 to 18.2 °C but not at higher temperature. An energetic imbalance at 21.5 °C may explain the reduced growth in Stage I larvae compared to animals reared at 18.2 °C. In the second experiment, daily photoperiods with 0, 6, 12, 18 or 24 h light did not affect larval survival through the first three stages of development but had a marked effect on intermoult period, growth and feeding. The response to increasing photoperiod changed during development, with Stages I and II larvae tending to grow faster and feed more under increasing light conditions whereas Stage III larvae required some light/dark phase to optimise growth (shortest intermoult period and highest moult increment at 6, 12 or 18 h light) and feeding. These findings will assist in refining the culture conditions for early stage *J. edwardsii* larvae.

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#### EFFECTS OF PHOTOPERIOD ON GROWTH, MORTALITY AND DIGESTIVE ENZYMES IN MIIUY CROAKER LARVAE AND JUVENILES

Xiujuan Shan, Zhizhong Xiao, Wei Huang, Shuozen Dou-2008  
Aquaculture 281(1-4): 70-76

Abstract:

The growth, mortality and digestive enzymes (trypsin, amylase and lipase) in miiuy croaker *Miichthys miiuy* larvae and juveniles (253 dph) were investigated at four photoperiods: 24L:0D, 18L:6D, 12L:12D and 0L:24D. Larvae could not feed at 0L:24D and did not survive up to dph. In the 24L:0D, 18L:6D, 12L:12D groups, photoperiod had not significant effects on the growth of the miiuy croaker younger than dph. However, their total length and specific growth rate (SGR) were significantly larger at 18L:6D and 24L:0D than 12L:12D after dph. Photoperiod also affected the mortality of the first feeding larvae (dph), being apparently higher in 27%), but no significant differences in mortality were found among other photoperiods. High mortality of the miiuy croaker in 12L:12D, 18L:6D and 24 L:0D groups mainly occurred from 5 (2027%) to 16%) and tended to decrease gradually from dph onwards. Digestive enzymes activities in the miiuy croaker larvae and juveniles had a similar change trend with age at all photoperiods. They underwent drastic changes with age. The specific activity of lipase was significantly higher at 18L:6D and 24L:0D than 12L:12D, but there were no significant differences in specific activities of either trypsin or amylase among photoperiods. With regard to the total length, SGR, survival and digestive enzyme activities, our findings suggested that the optimal light regime for the culture of miiuy croaker during the early life stage was 18L:6D.

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#### ARTEMIA NAUPLII AND TWO COMMERCIAL REPLACEMENTS AS DIETARY SUPPLEMENT FOR JUVENILE SIGNAL CRAYFISH, PACIFASTACUS LENIUSCULUS (ASTACIDAE), FROM THE ONSET OF EXOGENOUS FEEDING UNDER CONTROLLED CONDITIONS

A. González, J.D. Celada, R. González, V. García, J.M. Carral, M. Sáez-Royuela-2008

Aquaculture 281(1-4): 83-86

Abstract:

Two 100-day experiments were carried out under controlled conditions to evaluate effects of different dietary supplements on survival and growth of juvenile *Pacifastacus leniusculus* from the onset of exogenous feeding (stage 2). In the first experiment, a dry diet for salmonids was supplemented with six levels of freshly hatched *Artemia nauplii*, starting at 50, 100, 300, 500, 1000 nauplii per crayfish per day increased by 15% every 20 days, and nauplii in excess. Survival did not show significant differences, ranging from 75 to 90%. Crayfish receiving nauplii in excess had significantly higher carapace length (16.63 mm) and weight (1238.43 mg) than the other treatment groups. No significant differences were observed among the treatment groups starting at 300, 500 and 1000 nauplii per crayfish per day, averaging 15.18 mm carapace length and 975.60 mg weight. Considering costs and these results, an initial amount of 300–500 nauplii per crayfish per day increased by 15% every 20 days could be a reasonable option during the first 100 days of rearing. In a second experiment, partial substitution (50%) of nauplii with two commercial *Artemia* replacements was tried as supplement to the dry diet alone. Treatments were 500 nauplii per crayfish per day, 250 nauplii + 250 Artemac and 250 nauplii + 250 Proton, increased by 15% every 20 days. During the trial (at days 20, 40, 60 and 80) no significant differences were found, either in survival or in growth. At the end of the experiment, similar survival (approximately 80%) and growth (approximately 14.50 mm and 850 mg) were obtained with both *Artemia* replacements and with *Artemia* only. Results showed that these replacements can be used from the onset of exogenous feeding.

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#### EVALUATION OF FOUR DIETARY PROTEIN SOURCES FOR USE IN MICROBOUND DIETS FED TO MEGALOPAE OF THE BLUE SWIMMER CRAB, PORTUNUS PELAGICUS

Sarah Castine, Paul C. Southgate, Chaoshu Zeng-2008

Aquaculture 281(1-4): 95-99

Abstract:

Introduction of formulated diet particles for larval culture will simplify hatchery protocols and promote consistent survival, while decreasing total operating costs. The blue swimmer crab, *Portunus pelagicus*, is a commercially important species with substantial aquaculture potentials in the Indo-Pacific region. Feeding trials aimed at investigating protein nutrition of *P. pelagicus* larvae were undertaken with the megalopae instar. Microbound diets (MBD) with four different dietary protein sources (fish meal, squid meal, krill meal and soybean meal) were formulated and fed to newly molted megalopae until they metamorphosed to the first crab stage. Thirty megalopae were reared individually for each dietary treatment, with the addition of two control treatments of megalopae fed live *Artemia nauplii* and an unfed treatment.

Survival was high for all fed treatments (73.3–93.3%), while total mortality was recorded for the unfed control. Megalopae fed the fish meal based MBD demonstrated higher survival than that of megalopae fed live *Artemia*, suggesting that MBD have the potential to completely replace live food for the culture of *P. pelagicus* megalopae without adversely affecting their survival. These results also suggest that *P. pelagicus* megalopae are able to adapt to a range of dietary protein sources. For megalopae fed live *Artemia nauplii*, mean development time was significantly shorter ( $4.0 \pm 0.4$  days) and the mean carapace width and dry weight of newly settled first stage crabs were significantly greater, ( $3.1 \pm 0.0$  mm,  $1.3 \pm 0.1$  mg, respectively) than megalopae in all other fed treatments. Among MBD treatments, no significant difference in mean development time was detected, however, the mean carapace width of crabs that metamorphosed from megalopae fed fish meal based MBD ( $2.8 \pm 0.0$  mm) was larger than those fed squid meal based MBD ( $2.6 \pm 0.0$  mm) and the mean dry weight of individuals fed fish meal based MBD ( $0.87 \pm 0.04$  mg) was also significantly heavier than those fed soybean meal based MBD ( $0.72 \pm 0.04$  mg). The outcome of this research provides valuable information regarding protein requirements of *P. pelagicus* megalopae and will aid in optimising formulated diet particles for potential use in commercial hatcheries.

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EFFECTS OF FEEDING COPEPOD AND ARTEMIA ON EARLY GROWTH AND BEHAVIOUR OF THE SELF-FERTILIZING FISH, *RIVULUS MARMORATUS*, UNDER LABORATORY CONDITIONS

Maria Vivian Camacho Grageda, Tomonari Kotani, Yoshitaka Sakakura, Atsushi Hagiwara-2008  
Aquaculture 281(1-4): 100-105

Abstract:

Growth and survival have often been used as parameters to assess the effects of live feeds on marine finfish, however, behavioural effects, which entail energy cost and may have consequences on fish growth have been given less emphasis. Thus, a 20-day feeding experiment was conducted to determine the effects of copepod *Acartia tsuensis* (104–732  $\mu\text{m}$ ), unenriched, and docosahexaenoic acid, DHA-enriched, first instar *Artemia franciscana nauplii* (656–906  $\mu\text{m}$ ) on growth and behaviour of the mangrove killifish *Rivulus marmoratus*. Growth was significantly higher in *Acartia*-fed larvae compared with larvae fed *Artemia* (unenriched and DHA-enriched) until day 10. On day 20, *Acartia*-fed larvae had significantly lower growth than fish fed DHA-enriched *Artemia*. Feeding success was highest in larvae fed *Acartia* on day 1. Ingestion rate and satiation time did not differ among fish fed different types of feeds until day 20. Swimming activity before feeding was significantly lower in larvae fed *Acartia* compared with larvae fed *Artemia* (unenriched and DHA-enriched) until day 10. Higher growth in *Acartia*-fed fish on day 10 is probably due to the suitable size and high DHA content of *A. tsuensis*, and lower swimming activity of the larvae. However, on day 20, lower growth observed in *Acartia*-fed fish may be attributed to the shift in the food size preference of the fish. The present study was able to demonstrate the effects of copepods on growth and behavioural development of marine finfish using *R. marmoratus* as a model animal.

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THE EFFECTS OF THE GNRH AGONIST, AZAGLY-NAFARELIN (GONAZON™), ON OVULATION AND EGG VIABILITY IN THE EUROPEAN GRAYLING (THYMALLUS THYMALLUS L.)

Tomasz Mikolajczyk, Mirosława Sokolowska-Mikolajczyk, Pawel Szczerbik, Michal Duc, Krzysztof Goryczko, Stefan Dobosz, Jan Glogowski, Piotr Epler, William J. Enright-2008

Aquaculture 281(1-4): 126-130

Abstract:

In 2005–2007, four separate experiments in three different hatcheries were performed on the effects of azagly-nafarelin, a commercially-available (Gonazon™) GnRH agonist (pGlu-His-Trp-Ser-Tyr-[d-Nal(2)]-Leu-Arg-Pro-[Aza-Gly]) on ovulation and egg viability of the European grayling (*Thymallus thymallus* L.). The experiments were performed on cultured as well as captured wild grayling broodstock. Gonazon was injected intraperitoneally at doses of 16, 32 or 48 µg kg<sup>-1</sup> body weight. Control fish were injected with phosphate-buffered saline (PBS). In the first experiment performed on cultured 2-year-old females (first reproduction), there was no significant effect of Gonazon on ovulation rate between the control and the 16 µg kg<sup>-1</sup> Gonazon groups. In three other experiments performed on older females (3 or more years old), Gonazon at all doses (especially 16 µg kg<sup>-1</sup>) significantly accelerated and synchronized ovulation (90–100% of ovulation occurred 10–14 days post injection) in comparison with the control group (only 50–60% ovulated during the same period). No differences in mortality rate were observed between the control and Gonazon groups. In two experiments, the egg viability was monitored during the whole incubation period. There were no significant differences in the percentage of fertilized eggs between Gonazon and control fish; however, there was a 4 to 5% higher egg mortality from Gonazon fish during the whole incubation period. It is concluded that Gonazon (especially at a dose of 16 µg kg<sup>-1</sup>), given at the beginning or in the middle of the spawning period, is highly effective and a safe inducer of ovulation in cultured and wild European grayling.

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ADVANCES IN STUDIES OF THE EFFECTS OF STARVATION ON GROWTH AND DEVELOPMENT OF FISH LARVAE

Xiujuan Shan, Wei Huang, Liang Cao, Yunfei Wu-2008

Journal of Ocean University of China 7(3): 319-326

Abstract:

Starvation has important effects on early development of fish. It determines the survival and growth of fish larvae, and plays an important role in the dynamics of fish population and fisheries recruitment. In this review, we discuss the current studies about the effects of starvation on growth and development of fish larval stage. The goals of this review are to understand some adaptive mechanisms and ecological countermeasures of starved fish larvae and to provide the scientific guideline for exploring early life history processes, evaluating the nutrition condition and growth of larval fish, protecting fish resource and breeding fish larvae.

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EFFECTS OF NUTRITIONAL FACTORS ON THE GROWTH AND HETEROTROPHIC EICOSAPENTAENOIC ACID PRODUCTION OF DIATOM NITZSCHIA LAEVIS

Xiaohong Cao, Songyao Li, Chunling Wang, Meifang Lu-2008

Journal of Ocean University of China 7(3): 333-338

Abstract:

The effects of several nutritional factors on the growth and eicosapentaenoic acid (EPA) production of diatom *Nitzschia laevis* were studied. 4 LDM (quadrupled concentration of the nutrient salt) was the optimal concentration of nutrient salt for the growth and EPA production of *N. laevis*. The growth of

*N. laevis* was inhibited when the glucose concentration was either lower than 10 gL<sup>-1</sup> or higher than 15 gL<sup>-1</sup>. Both sodium nitrate and urea were good nitrogen sources for the growth and EPA production, while ammonium chloride seriously decreased the dry cell weight (DW) and the EPA content. Silicate seriously influenced the growth of *N. laevis*. The maximum DW of 2.34 gL<sup>-1</sup> was obtained in the presence of 150 mgL<sup>-1</sup> Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O. The EPA content remained almost the same when the silicate concentration was lower than 150 mgL<sup>-1</sup>; however, higher silicate concentrations resulted in a steady decrease of EPA content. Low medium salinity (□29) did not seem to influence the DW of *N. laevis*, and high salinity resulted in a decrease of DW. The highest EPA content (4.08%) and yield (110 mgL<sup>-1</sup>) were observed at the salinity of 36 and 29, respectively. (Tianjin Key Laboratory of Food Nutrition and Safety, College of Food Science and Biotechnology, Tianjin University of Science and Technology, Tianjin, 300457, P. R. China; email of Xiaohong Cao: caoxh@tust.edu.cn)

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#### INBREEDING EFFECTS ON HATCHERY AND GROWOUT PERFORMANCE OF PACIFIC WHITE SHRIMP, *PENAEUS (LITOPENAEUS) VANNAMEI*

Dustin R. Moss, Steve M. Arce, Clete A. Otoshi, Shaun M. Moss-2008  
Journal of the World Aquaculture Society 39(4): 467 – 476

##### Abstract:

In animal breeding programs, selection coupled with a narrow genetic base can cause high levels of inbreeding to occur rapidly (in one or two generations). Although the effects of inbreeding have been studied extensively in terrestrial animals and to a lesser extent in aquaculture species, little is known about the effects of inbreeding on penaeid shrimp. The objective of this study was to investigate the effects of inbreeding on hatchery and growout performance of the Pacific white shrimp, *Penaeus vannamei*. The experiment was conducted over 2 yr, and data from two successive generations (G2 and G3) of inbred (sibling–sibling mating) and outbred families were analyzed. There were 11 inbred and 12 outbred families in G2 and 9 inbred and 10 outbred families in G3. Inbreeding coefficients (F) for outbred and inbred families were 0.00 and 0.25, respectively, for G2 and 0.00 and 0.375, respectively, for G3. Growth rates for outbreds and inbreds were similar in both G2 and G3. Hatch rate for inbred families was 33.1% lower than for outbred families in G2 and 47.1% lower in G3. Inbreeding depression (IBD) (relative change in phenotype per 0.1 increase in F) ± 95% CI for hatch rate was  $-12.3 \pm 10.1\%$ . Hatchery survival for inbred families was 31.4% lower than for outbred families in G2 and 38.8% lower in G3. IBD for hatchery survival was  $-11.0 \pm 5.7\%$ . Growout survival was 1.9% lower for inbred families than for outbred families in G2 and 19.6% lower in G3. IBD for growout survival was  $-3.8 \pm 2.9\%$ . There was also a significant linear relationship between IBD estimates for survival traits and mean outbred survival. At high outbred survival, IBD was low (e.g., growout survival in G2), but IBD appeared to become more severe when outbred survival was lower. This suggests that stress (related to environment and/or life stage) may worsen IBD for survival traits. Results also indicate that moderate to high levels of inbreeding (>10%) should be avoided in commercial shrimp hatcheries because the cumulative effect of IBD on hatch rate and hatchery survival will significantly reduce postlarvae production. Thus, IBD can be significant enough to justify the use of inbreeding as a germplasm protection strategy (under certain scenarios) for genetic improvement programs.

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#### EFFECTS OF DIETARY BINDERS ON SURVIVAL AND GROWTH PERFORMANCE OF POSTLARVAL TONGUE SOLE, *CYNOGLOSSUS SEMILAEVIS* (GÜNTHER)

Feng Liu, Qinghui Ai, Kangsen Mai, Beiping Tan, Hongming Ma, Wei Xu, Wenbing Zhang, Zhiguo LiuFu-2008

Journal of the World Aquaculture Society 39 (4): 500 – 509

##### Abstract:

A 30-d feeding experiment was conducted in tanks to investigate the effects of four different binders (2% in microdiet) on the survival, growth performance, and specific activity of digestive enzymes in tongue sole, *Cynoglossus semilaevis* Günther, postlarvae (24 d after hatching, with initial average wet

weight of  $22.4 \pm 6.65$  mg [mean  $\pm$  SD]). Five approximately isonitrogenous and isoenergetic microbound diets were formulated with carrageenan (Car), sodium carboxymethylcellulose (CMS), sodium alginate (SA), and gelatin (Gel) as binders, and a diet with no special binder as a control. A commercial diet (RQ Com., manufactured by Marubeni Nisshin Feed Co., Ltd., Chita, Japan) was used as another tested diet, and *Artemia nauplii* was used as live prey control. The results showed that the survival of the fish fed SA diet (31.3%) was significantly higher than that of fish fed CMS (21.8%), Car (10.8%), and control (21.8%) diets ( $P < 0.05$ ), but significantly lower than that of the fish fed the live prey (54.5%) diet ( $P < 0.05$ ). The results of growth followed the similar pattern as that of survival, although there were no significant differences in specific growth rate (SGR) among fish fed SA, CMS, Gel, the control, and RQ diets (7.2, 6.8, 7.0, 6.9, and 7.3% per day;  $P > 0.05$ ). However, fish fed Car diet had a significantly lower SGR (5.9% per day) than fish fed other diets. Percentages of secreted amylase in fish fed artificial diets were not significantly different, but fish fed artificial diets had significantly higher ( $P < 0.05$ ) secreted amylase than live prey group. Percentage of secreted trypsin in fish fed SA diet (54.9%) was relatively higher compared with other binders, and not significantly different from RQ Com. and live prey treatments. However, trypsin in fish fed Car diets (29.3%) was significantly lower than in other groups. Specific activities of alkaline phosphatase (AP) in the intestine and AP and leucine aminopeptidase N in brush border membranes in fish fed SA diet (264.6, 1882.8, and 187.2 mU/mg protein) were relatively higher compared with other binders. These results indicate that SA is one of suitable binders compared with carrageenan, CMS, and gelatin in microdiet of postlarval tongue sole.

(The Key Laboratory of Mariculture (Ministry Education of China), Ocean University of China, Qingdao 266003 China)

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#### CHARACTERIZATION OF SMALL-SIZE EGG-BEARING THAI SS-STRAIN ROTIFERS BRACHIONUS ROTUNDIFORMIS AND THEIR FIRST OFFSPRING

Agus Somamihardj, Amrit Bart-2008

Journal of the World Aquaculture Society 39 (4): 528 – 534

Abstract:

This study selected small (100–120  $\mu\text{m}$ ) egg-bearing females from a local Thai ss population and characterized their first offspring's life history in an attempt to produce small-size rotifers. Mean lorica length of selected small adult females was  $120 \pm 6$   $\mu\text{m}$  at the time of collection, and this mean length did not increase over their lifetime. While their first offspring life span was only  $5.11 \pm 0.35$  d, the reference population longevity was higher,  $9.65 \pm 0.4$  d. The length of reproductive period of offspring from the small-size egg-bearing female was  $2.58 \pm 0.38$  d, shorter than that of reference population  $6.83 \pm 0.58$  d. Although the rate of egg production was not different between small egg-bearing female and reference population, the total number of eggs produced by the small egg-bearing female's first offspring was lower ( $4.78 \pm 0.60$ ) compared to the reference population ( $11.83 \pm 1.26$ ). The small egg-bearing female's first offspring had a higher egg to lorica length ratio ( $76.1 \pm 1.64\%$ ) than the reference population ( $56.6 \pm 1.3\%$ ), indicating a relatively high investment in reproduction. The small egg-bearing female's first offspring reached only  $125 \pm 6$   $\mu\text{m}$  in 36 h, and further culture of their offspring over 35 d resulted in a mean offspring size,  $163 \pm 11$   $\mu\text{m}$ , similar to the reference population ( $159 \pm 16$   $\mu\text{m}$ ). This shows that population mean size reduction is not likely to result from selecting small egg-bearing females within a single generation. The unique reproductive characteristics of small egg-bearing female place it in its own category.

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#### IMPROVEMENT OF WATER QUALITY BY THE MACROALGA, GRACILARIA LEMANEIFORMIS (RHODOPHYTA), NEAR AQUACULTURE EFFLUENT OUTLETS

Yongjian Xu, Jianguang Fang, Qisheng Tang, Junda Lin, Guanzong Le, Lv Liao-2008

Journal of the World Aquaculture Society 39(4)::549 – 555

Abstract:



Raft culture of the seaweed, *Gracilaria lemaneiformis* (Bory) Dawson, was used as biofilter for purifying effluent and removing nutrients from the nearby animal aquaculture. The results showed that the cultivated seaweed grew well outside effluent discharge outlets for growout ponds and a hatchery plant. The biomass (fresh weight) of *Gracilaria* from the 0.5-ha area outside the growout ponds increased about 60 times, from 80 to 4750 g/m during the 65 d of experiment (with the daily special growth rate [SGR] of 3.87%/d); the yield was 30.4 tons and 93.97 kg N and 12.81 kg P were removed; the seaweed biomass from the 0.2-ha area outside the hatchery outlet increased 37.5 times, from <100 to 3600 g/m (with SGR of 3.4%/d); the yield was 10.4 tons and 31.7 kg N and 4.33 kg P were removed. Several water quality parameters such as dissolved oxygen, secchi disk depth, and pH value in the algal cultivation area were substantially higher than those in the noncultivation zone, whereas chemical oxygen demand, dissolved inorganic nitrogen, and dissolved inorganic phosphorous at both areas were similar.

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#### FAIRY SHRIMP (BRANCHIOPODA: ANOSTRACA) OF CHILE

D.Christopher Rogers, Patricia de los Ríos, Oscar Zuñiga

Journal of Crustacean Biology 28(3): 543-550

Portions of Chile were surveyed for Anostraca. Including the two species of *Artemia* previously reported from Chile, we found 5 new localities and range extensions for 5 species of Branchinecta. Previous studies on Chilean Anostraca have focused on *Artemia*, and although Branchinecta was previously reported in Antarctica close to Chilean Air Force installations, and in a few temporal ponds in Southern Patagonia, the species were never determined. Brief habitat descriptions, as well as conservation status, and identification keys are presented and discussed. Additionally, we describe a new species from the Atacama Desert, *Branchinecta papillata* n.sp.

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#### BACTERIAL GENETIC DIVERSITY ON SETTLEMENT SUBSTRATES DURING MASS MORTALITY OF LARVAE OF NONA-POROUS ABALONE (*HALIOTIS DIVERSICOLOR SUPERTEXTA*)

Ying Ma, Zhiyong Wang, Zengxiang Zhang, Lin Wang-2008

Aquaculture 282(1-4): 1-6

Abstract:

Settlement substrate samples for nona-porous abalone seed attachment from diseased and nondiseased abalone hatcheries during mass mortality occurrence were collected, the 16S rRNA gene clone libraries of the attachments were constructed using primers universal for the domain Bacteria. In total, 83 clones randomly selected were screened by PCR-restriction fragment length polymorphism (PCR-RFLP) analysis and clones with unique RFLP patterns were sequenced. Phylogenetic analysis revealed bacterial components different from previous studies in such environments. Apart from sequences originating from chloroplast of eukaryotic diatom (occupying 29.33%), all the cloned sequences fell into five lineages of the bacterial domains, the  $\alpha$ -Proteobacteria,  $\gamma$ -Proteobacteria, Flavobacteria, Acidobacteria and an uncultured candidate division TM7. The  $\alpha$ -Proteobacteria was predominant (56%) and was mainly comprised of Sphingomonadales, Rhodobacterales, and Rhizobiales affiliated clones. Other groups appeared just as minor components. Most of our sequences were closely related to sequences from attached bacteria, or sequences retrieved from extreme environments or specific environments, indicating that these bacteria might play an important function in bacterial attachment or in extreme environments. The community structure and the clone abundance in different groups of the two clone libraries were different, further research focusing on these different bacteria will help to reveal the potential pathogens of the abalones' mass mortality.

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## LABORATORY PRODUCTION OF EARLY HATCHING ARTEMIA SP. CYSTS BY SELECTION

Elizabetha Briski, Gilbert Van Stappen, Peter Bossier, Patrick Sorgeloos-2008

Aquaculture 282(1-4): 9-25

Abstract:

The goal of this study was to test whether it is possible to produce early hatching cysts of *Artemia franciscana* from San Francisco Bay (SFB) by truncation selection. The starting material was an SFB cyst sample, harvested in nature. After selection of early hatching nauplii had taken place, these selected individuals were cultured to maturity, and the hatching rate of their offspring (F1) was compared with that of the parental generation and with the non-selected control F1. The possible differences in hatching rate were used to estimate heritability.

Two different selection experiments were run, accompanied with a number of additional tests studying the influence of the hatching set-up, the hatching temperature and diapause termination using hydrogen peroxide, on the hatching rate. Also the influence of different culture salinities on hatching rate and on possible success of selection was studied.

The results revealed an influence of all those abiotic factors on the hatching rate, but also a marked interference of the hatching percentage with the hatching rate. Choosing the individuals based on their own phenotypic values (hatching within a certain time span of hatching incubation) and using those to produce the next generation, revealed a positive response. The selection in different salinities showed a different response. Depending on the strength of the selection pressure, the samples were advanced in time, starting with the highest selection pressure, and ending with the control and the parental sample. In spite of the strong interference of environmental factors, our results suggest that selection of early hatching cysts is possible.

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## SEAHORSE BEHAVIOUR AND AQUACULTURE: HOW TO IMPROVE HIPPOCAMPUS GUTTULATUS HUSBANDRY AND REPRODUCTION?

Filipa Faleiro, Luís Narciso, Luís Vicente-2008

Aquaculture 282(1-4): 33-40

Abstract:

In recent years, aquaculture development has been based on the use of new tools and technologies from different scientific areas. Animal behaviour can also constitute a useful tool for aquaculture and contribute to the improvement of rearing protocols. In this study, a behavioural approach was developed for the long-snouted seahorse (*Hippocampus guttulatus*) culture, in order to improve broodstock husbandry and reproduction. The optimisation of laboratory conditions (such as water column depth, holdfast type and availability, animal density, sex ratio and male/female size) was analysed based on seahorse behaviour. Social and reproductive behaviours were studied in terms of space use preferences, activity levels, distribution patterns, mate competition and selection, courtship, male pregnancy and juvenile birth. In captive conditions, seahorses were diurnally active and more isolated during the night. They were mainly located in the water inlet area, near the bottom, and preferred vertical holdfasts and natural plants. *H. guttulatus* reproduction in captivity was achieved. Seahorse behaviour and reproductive output were affected by the different rearing conditions but not animal welfare. Animal density increased seahorse activity and influenced the number of groups, while holdfast availability affected the group size. Seahorses were generally found in male–female pairs that were not stable over time and tend to select similar size or larger mates. Male competition was rather frequent and was affected by the sex ratio. Courtship occurred during all day and no daily greetings were observed throughout male pregnancy. Water column depth and male competition resulted in mating interruption and juvenile number and size decreased in artificial conditions.

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MOLECULAR CLONING AND FUNCTIONAL ANALYSIS OF CATHEPSIN B IN NUTRIENT METABOLISM DURING LARVAL DEVELOPMENT IN MERETRIX MERETRIX

Xiaomei Wang, Baozhong Liu, Guodong Wang, Baojun Tang, Jianhai Xiang-2008

Aquaculture 282(1-4): 41-46

Abstract:

Seed rearing is an important part in large scale clam culture industry. Since the nutritional history affects early development in bivalve, the condition of larval nutrition plays a key role in successful seed rearing. So far, the molecular mechanism of nutrient uptake in bivalve larvae is unclear. As one of the important proteolytic enzymes, cathepsin B of several organisms has been reported to be involved in digestion. We intended to analyze whether cathepsin B is involved in larval nutrient metabolism in the economic bivalve, clam *Meretrix meretrix*. The full length of *M. meretrix* cathepsin B (MmeCB) cDNA was cloned, which is 1647 bp with an open reading frame of 1014 bp. The deduced amino acid sequence encoded a preproenzyme of 337 residues with Cys-114, His-282 and Asn-302 composing cathepsin B activity center. The temporal and spatial expressions of MmeCB mRNA were examined from trochophore to post larva stages by whole mount in situ hybridization. In trochophore stage, no detectable signal was found. In the later three stages, MmeCB mRNA was detected in the digestive gland, suggesting a possible role of MmeCB in digestion. Moreover, MmeCB mRNA was also observed in the epidermal cells in D-veligers. Cathepsin B specific inhibitor (CA074 methyl ester) was applied to block the activity of cathepsin B in unfed larvae. The average shell lengths of treated larvae were smaller than that in control groups. The results of mRNA epidermal distribution and inhibitor treatment in D-veligers indicated that MmeCB may be also associated with other pathway of nutrient metabolism in larval epidermis. The overall results in this paper revealed that MmeCB might play a role in larval nutrient metabolism.

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A FLOW-THROUGH REARING SYSTEM FOR ECOPHYSIOLOGICAL STUDIES OF PACIFIC OYSTER *CRASSOSTREA GIGAS* LARVAE

B. Rico-Villa, P. Woerther, C. Mingant, D. Lepiver, S. Pouvreau, M. Hamon, R. Robert-2008

Aquaculture 282(1-4):54-60

Abstract:

While literature is relatively abundant on adult shellfish, ecophysiological studies at the larval stage are scarce because of both technical difficulties and inadequate methodology. A tool dedicated to provide basic information for larval ecophysiology was accordingly developed. Two steps were followed: first a flow-through method of *Crassostrea gigas* larval culture was perfected during a set of experiments in which rearing systems and larval density were assessed. Then a continuous hydrobiological data recorder was adapted with modifications to comply with our experimental aim. SILO (Système d'Instrumentation des Larves en flux Ouvert) allowed the successive acquisition of hydrobiological parameters in ten 150-l larval tanks in which larvae were reared using flow-through techniques under controlled environmental conditions. An automated system enabled alternate measurement of hydrobiological parameters from one larval culture to the next. A chamber system contained several probes for measurement of temperature, salinity, pH and fluorescence. The electronic system allowed real time acquisition, storage and transfer of data. SILO was successful as a larval rearing device, reducing larval disturbance that could result from transfer and handling for measurement. It was also efficient as a tool to provide basic information for larval ecophysiology research. The effect of temperature (17, 22, 25, 27, 32 °C) on growth and ingestion of microalgae was studied to test SILO. Maximum growth occurred at 27 °C ( $16 \pm 2 \mu\text{m d}^{-1}$ ) and 32 °C ( $15 \pm 3 \mu\text{m d}^{-1}$ ) whereas larvae reared at 22 and 25 °C showed lower development ( $8.6 \pm 3.1$  and  $11.7 \pm 2.5 \mu\text{m d}^{-1}$ , respectively). Moreover, metamorphosis exhibited no significant difference at 27 or 32 °C with  $87.5 \pm 7.1$  and  $85.9 \pm 9.6\%$  respectively, while at 22 or 25 °C lower metamorphosis was recorded with  $55.2 \pm 8.3$  and  $57.6 \pm 9.6\%$ , respectively. The lowest temperature (17 °C) strongly inhibited ingestion

whereas the highest temperature (32 °C) stimulated maximum feeding activity over the whole larval rearing period.

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#### PARTICLE SIZE PREFERENCE, GUT FILLING AND EVACUATION RATES OF THE ROTIFER BRACHIONUS "CAYMAN" USING POLYSTYRENE LATEX BEADS

Andreas Baer, Chris Langdon, Scott Mills, Carsten Schulz, Kristin Hamre-2008

Aquaculture 282(1-4): 75-82

Abstract:

A rotifer strain commonly used in Norwegian cod hatcheries was identified as *Brachionus* "Cayman" using genetic barcoding techniques. This rotifer is used extensively in hatcheries around the globe being a member of the *Brachionus plicatilis* species complex. It is of medium lorica size ( $168 \pm 10 \times 111 \pm 9 \times 81 \pm 8 \mu\text{m}^3$ ) and is most closely related to *B. "Tiscar"*, its closest named relative being *B. ibericus*. Size selective feeding by *Brachionus* "Cayman" was investigated using polystyrene latex beads with diameters of 1.6 to 20  $\mu\text{m}$ . Different sizes of latex beads were offered to the rotifers, in combination with a standard 6  $\mu\text{m}$  bead, and Jacobs selectivity indices (D) were calculated based on the relative numbers of the different sized beads consumed. In addition, experiments were carried out to determine the optimal feeding period for short-term enrichment of rotifers in order to maximize the volume of ingested beads. Gut evacuation rates at different water temperatures (4, 10 and 26 °C) were also determined.

*B. "Cayman"* showed the highest positive selectivity ( $D = + 0.54$ ) for 4.5  $\mu\text{m}$  latex beads compared to other beads in the size range 1.6 to 20  $\mu\text{m}$ . The maximum diameter of latex bead ingested was 10  $\mu\text{m}$ , while 12 and 15  $\mu\text{m}$  beads were captured but not ingested. Rotifers ingested a mean bead volume of 18,000  $\mu\text{m}^3$  (S.D. + 2051) after 35 min of feeding on 4.5  $\mu\text{m}$  diameter beads at a volumetric concentration of  $40 \times 10^7 \mu\text{m}^3 \text{ mL}^{-1}$  at 26 °C. This corresponds to approximately 1.18% of the volume of the whole body of the rotifer. Rotifers emptied their guts at slower rates when culture temperatures were lowered from 26 to 4 °C. These results will facilitate optimization of enrichment protocols for rotifers in order to improve their nutritional value as live feed for marine fish larvae.

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#### IMPROVED MATURATION OF POND-REARED, BLACK TIGER SHRIMP (PENAEUS MONODON) USING FISH OIL AND ASTAXANTHIN FEED SUPPLEMENTS

Chalee Paibulkichakul, Somkiat Piyatiratitivorakul, Patrick Sorgeloos, Piamsak Menasveta-2008

Aquaculture 282(1-4): 83-89

Abstract:

*Penaeus monodon* female (49 g) and male (37 g) shrimp were fed formulated diets supplemented with 3 or 8% fish oil and 100 or 500 mg kg<sup>-1</sup> astaxanthin, in addition to fresh squid during a 120 day trial using a 2 × 2 × 2 factorial design. Four formulated diets were provided with different combinations of high and low concentrations of lipid (fish oil) and astaxanthin. We found that fish oil addition, at the concentrations we used did not significantly ( $P > 0.05$ ) affect shrimp growth, but there was significantly greater growth of male shrimp and higher astaxanthin concentration. Female shrimp growth was not significantly different at either astaxanthin concentration. Likewise, there were no significant interactions on growth between fish oil and astaxanthin or fish oil and shrimp sex, but there was significant interaction between astaxanthin and sex of shrimp. Reproductive performance, as measured by number of eggs in gravid females and number of spermatozoa in male shrimp was significantly enhanced by both higher concentration of fish oil and astaxanthin. There was no significant interaction between fish oil and astaxanthin on number of eggs or spermatozoa. Likewise, there was no significant interaction between fish oil and astaxanthin, or fish oil and astaxanthin concentrations in shrimp muscle, hepatopancreas, ovaries or shell tissues. Astaxanthin concentrations in these respective tissues were similar for both levels of dietary fish oil. There were, however,

significant interactions between astaxanthin and shrimp sex with these tissues. Greater dietary astaxanthin concentration resulted in significantly greater astaxanthin concentration in female shrimp muscle, hepatopancreas and ovarian tissues, but not in their shells. Female shrimp had significantly greater astaxanthin concentration in hepatopancreas tissue compared with males, but not in muscle or shells. Shrimp fed diets containing high levels of fish oil and astaxanthin had significantly greater 22:6n-3, total n-3 PUFA and total n-3 HUFA concentrations in muscle and ovary, whereas 20:4n-6, 20:5n-3, 22:6n-3, total n-6 PUFA, total n-3 PUFA and total n-3 HUFA concentrations were significantly greater in hepatopancreas of shrimp fed diet containing high level of fish oil. We concluded that dietary supplementation of formulated diets with 8% fish oil (12% total lipid) and at least 280 mg kg<sup>-1</sup> astaxanthin will significantly improve *Penaeus monodon* maturation and spawning success.

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#### EVALUATION OF FLUOROGENIC SUBSTRATES IN THE ASSESSMENT OF DIGESTIVE ENZYMES IN A DECAPOD CRUSTACEAN MAJA BRACHYDACTYLA LARVAE

Guiomar Rotllant, Francisco Javier Moyano, Mireia Andrés, Manuel Díaz, Alicia Estévez, Enric Gisbert-2008

Aquaculture 282(1-4): 90-96

Abstract:

The potential use of fluorescent substrates for measuring digestive enzyme activities has been adapted to decapod crustacean larvae and compared to traditional analytical techniques based on spectrophotometry. The ontogeny of three digestive enzymes (trypsin, amylase, and esterase) from the hepatopancreas of *Maja brachydactyla* has been selected to illustrate the advantages and disadvantages of both spectrophotometric (SP) and fluorometric (FL) methods. The three enzyme activities showed the same pattern with either of the techniques used. Standardised discriminant function coefficients indicated that the stage of development in spider crab larvae was most strongly discriminated by the activity of amylase, whichever method of analysis was considered. The FL method allows the detection of up to three enzyme activities in a single larva (< 100 µg dry weight), whereas SP needs more than 20 times this weight. The FL method allows studying the inter-individual variability, while the SP approach only permits one to evaluate the inter-population variability (pool of individuals). Nevertheless, the higher sensitivity of the FL method implies a larger variability in the results compared to SP. There are no differences in feasibility between the two methods when using kinetic analyses. Although data from different experiments assayed by SP methods are easily standardised in units of dry weight or soluble protein concentration, this standardisation is difficult when using FL substrates, since differences between the sensitivity of the equipment used for the analysis might be higher than one order of magnitude. The availability of FL substrates in the market is constantly increasing, although it is still scarce in relation to the high number and types of substrates normally used in SP methods. This may be a limitation to the study of certain enzyme activities. The cost of the analysis is based on the price of the specific substrates, FL substrates being more expensive than SP ones. Both SP and FL methods are useful to measure digestive enzymes in decapod crustacean larvae. Selection of the method used will depend on the objectives and interests of the researcher. For studies based on the characterization of enzyme activities in crustacean populations without any limitation on the number and amount of samples, the SP method is recommended. When several experimental treatments, dietary regimes, and/or moulting stages are considered, the FL analysis might be more appropriate due to the low number of individuals needed for sampling. If the study is conducted to assess the individual variability of the population, then, the FL method needs to be applied.

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PAVLOVA LUTHERI: PRODUCTION, PRESERVATION AND USE AS FOOD FOR CRASSOSTREA GIGAS LARVAE

E. Ponis, G. Parisi, G. Chini Zittelli, F. Lavista, R. Robert, M.R. Tredici-2008

Aquaculture 282(1-4): 97-103

Abstract:

The haptophyte *Pavlova lutheri* was cultivated in a 4-l flat alveolar photobioreactor (FAP) in semi-continuous mode during 35 days, achieving a mean volumetric productivity of 0.4 g (dry wt) l<sup>-1</sup> day<sup>-1</sup>. The microalgal culture was harvested from the photobioreactor at an average concentration of 3.5 g (dry wt) l<sup>-1</sup> and subjected to different storage conditions in which the effects of temperature (1 °C, 4 °C and 25 °C), air supply and preservative (ascorbic or propionic acid) addition on the viability of the stored suspensions were investigated. Low temperatures (1 and 4 °C) and air-bubbling extended the shelf-life of the stored suspension, while no beneficial effects of ascorbic or propionic acid were found. The influence of temperature and air-bubbling on fatty acid content and profile was also evaluated over a 23-day period of storage. Air-bubbled suspensions exhibited, on the average, a 70% decrease of total fatty acid content (TFA) during storage. Non-bubbled suspensions showed a much lower decrease. The decrease mainly involved the saturated 14:0 and 16:0 and the monounsaturated 16:1n-7 and 18:1n-9. The polyunsaturated eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) decreased significantly at the end of the storage period.

A feeding trial on Pacific oyster (*Crassostrea gigas*) larvae was carried out with fresh and preserved *P. lutheri* biomass kept under the best storage conditions previously defined (1 °C; darkness; air-bubbling). After 14 days of rearing no significant differences in growth or survival were detected when fresh or preserved *P. lutheri* was used in a monospecific diet or in association with the diatom *Chaetoceros calcitrans* forma *pumilum*.

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EVALUATION OF THE EFFECTS OF RETINOIDS AND CAROTENOIDS ON EGG QUALITY USING A MICROINJECTION SYSTEM

Satoru Haga, Susumu Uji, Tohru Suzuki-2008

Aquaculture 282(1-4): 111-116

Abstract:

Preparation of high quality eggs is essential in aquaculture. While egg quality is widely regarded as being largely dependent on the nutrients that are stored in the yolk, the identity of the factors responsible for affecting this egg quality is not yet known. Thus, in order to ensure the quality of better quality eggs, it is essential to elucidate how the nutrients in yolk influence embryogenesis. Using a microinjection system, we performed two tests with zebrafish embryos to evaluate the effects of nutrients on embryogenesis. To determine whether retinoic acid, excess retinoids and carotenoids in yolk cause malformations, we injected retinoic acid, retinal, retinol and  $\beta$ -carotene into the yolk of 1–4 cell stage embryos, and compared their effects on embryogenesis. The injection of retinal into yolk causes similar anomalies to those induced by retinoic acid in the brain, jaw, heart, as well as suppressing hatching rate. Retinol had a lower teratogenic effect than retinal and toxicity was not detected for  $\beta$ -carotene. These findings suggest that excess retinal and retinol in yolk can impair egg quality by causing malformations and decreasing hatching rate. We then compared the protective effects of astaxanthin,  $\beta$ -carotene and  $\alpha$ -tocopherol against oxidative stress in embryos. After injecting one of these compounds into the yolk of a 1–4 cell-stage embryo, embryos were exposed to H<sub>2</sub>O<sub>2</sub> for 2 h from 24 h post-fertilization (hpf) and mortality and apoptosis were observed at 48 hpf. Astaxanthin conferred embryos with a marked tolerance against mortality and apoptosis due to H<sub>2</sub>O<sub>2</sub> exposure, suggesting its potential application to the improvement of egg quality by increasing the tolerance of embryos toward oxidative stress. The methodology presented herein describes a quick method for evaluating the effects of nutrients on egg quality.

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UTILIZATION OF FREE AMINO ACIDS, YOLK PROTEIN AND LIPIDS IN DEVELOPING EGGS AND YOLK-SAC LARVAE OF JAPANESE EEL ANGUILLA JAPONICA

Nobuyuki Ohkubo, Sayumi Sawaguchi, Kazuharu Nomura, Hideki Tanaka, Takahiro Matsubara-2008  
Aquaculture 282(1-4): 130-137

Abstract:

To elucidate the utilization of major yolk nutrients in eggs and yolk-sac larvae of Japanese eel *Anguilla japonica*, contents of free amino acids (FAA), 380 kDa lipovitellin (oLv; the major yolk protein in ovulated eggs), and lipids were measured. All larvae hatched by the 2nd day after fertilization at a water temperature of 23 °C, and the hatched larvae absorbed almost all of the yolk mass by the 10th day. The FAA composition in ovulated eggs is unique in that glutamine (Gln) is markedly high (32% of total FAA) in comparison to other pelagic eggs of marine teleosts suggesting the importance of Gln for early development of the Japanese eel. Total FAA content decreased to 45% of the initial level by the 4th day after fertilization. Gln content showed a rapid decrease to 5% of the initial level within 2 days after fertilization. oLv content, measured by enzyme-linked immunosorbent assay using an antiserum against lipovitellin, decreased until the 4th day and was, thereafter, undetected. Total content of triacylglycerol (TG) was stable until the 2nd day, and then decreased until the 8th day. On the other hand, the content of phospholipids (PL) gradually decreased until the end of yolk sac absorption. From these data, we divided the pattern of nutrient utilization in embryos and larvae of Japanese eel into two phases: first, utilization of FAA (especially Gln) and oLv from the 1st day, followed by TG utilization from the 2nd to 4th day by which time more than 30% of the FAA and the oLv stock have been utilized. In addition, PL was utilized as a subsidiary energy source throughout development. Almost all the yolk nutrient were absorbed by the 8th day after fertilization, and it is considered that the larvae need to start feeding before this period.

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