

EXPERIMENTAL STUDIES TO EVALUATE LARVAL SURVIVAL OF THE FIRE SHRIMP, *LYSMATA DEBELIUS*, TO THE JUVENILE STAGE

Matthew R. Palmtag, G. Joan Holt-2007

Journal of the World Aquaculture Society 38(1): 102–113.

Abstract:

Many coral reefs are threatened because of anthropogenic impacts such as destructive fishing methods for marine ornamentals. The marine ornamental industry is currently almost completely dependent upon wild collections. The development of commercially feasible methods to culture ornamental species could help to reduce the need for wild-collected specimens and reduce pressure on coral reefs. Advances in larval rearing and nutrition would help overcome the bottlenecks that impede the commercial production of many marine ornamental species. This article focuses on research for improving the production of the fire shrimp, *Lysmata debelius*. Experiments were performed with larval fire shrimp to evaluate (1) the importance of essential fatty acid enrichment of live prey (rotifers and *Artemia*) and (2) feeding penaeid shrimp muscle tissue to improve survival to metamorphosis. We also provide the fatty acid composition of Day-1 posthatch fire shrimp larvae. The enrichment of live prey with docosahexaenoic acid did not improve larval growth, survival, or time to metamorphosis, but larvae fed live prey together with pureed penaeid shrimp muscle had survival rates of $9.8 \pm 1.2\%$. The data and protocols developed in this study provide a foundation and baseline for future fire shrimp larval studies.

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GONADAL DEVELOPMENT AND DIFFERENTIATION IN CULTURED JUVENILE WINTER FLOUNDER, *PSEUDOPLEURONECTES AMERICANUS*

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Journal of the World Aquaculture Society 38 (1): 114–121

Abstract:

Winter flounder, *Pseudopleuronectes americanus*, is currently being evaluated as a stock enhancement candidate in New Hampshire, USA; however, little is known about the gonadal development or the sex ratio of cultured juveniles. To determine the size at gonadal differentiation, 327 cultured fish ranging from <20 to 110 mm total length (TL), in 10-mm-TL size classes, were examined histologically. Gonads had differentiated into testes and ovaries in fish ≥ 41 mm TL (98%), whereas the majority of fish (81%) smaller than 40 mm TL possessed undifferentiated gonads. A total of 313 cultured fish >40 mm TL were analyzed for sex ratio. In 2003, 67 females and 164 males were identified, yielding a sex ratio that was significantly skewed toward male ($\chi^2 = 40.7$, $df = 1$, $P < 0.001$). This trend held true when cultured fish were sorted by age and length, with the exception of those fish 61–70 mm TL. This aberration probably was because of a small sample size in this length category. However, in both the 2004 and the 2005 cultured populations, flounder sex did not deviate from a 1:1 ratio (2004 $\chi^2 = 0.12$, $df = 1$, $P = 0.724$ and 2005 $\chi^2 = 0.02$, $df = 1$, $P = 0.881$). The 2003 data suggest that environmental or genetic factors may affect winter flounder sex determination; rearing manipulation studies in the hatchery are needed to confirm this hypothesis.

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EFFECTS OF DIETARY B-1,3 GLUCAN AND FEED STIMULANTS IN JUVENILE OLIVE FLOUNDER, *PARALICHTHYS OLIVACEUS*

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Journal of the World Aquaculture Society 38 (1): 138–145

Abstract:

The present study was conducted to investigate the effects of dietary supplementation of β -1,3 glucan and a laboratory developed feed stimulant, BAISM, as feed additives for juvenile olive flounder, *Paralichthys olivaceus*. Eight experimental diets were formulated to be isonitrogenous and isocaloric and to contain 50.0% crude protein and 16.4 kJ of available energy/g with or without dietary β -1,3 glucan and BAISM supplementation. β -1,3 glucan (G) and BAISM (B) were provided at 0% in the control diet (G0B0) and at 0.05% G + 0.45% B (G0.05B0.45), 0.05% G + 0.95% B (G0.05B0.95), 0.1% G + 0.90% B (G0.1B0.9), 0.10% G + 1.90% B (G0.1B1.9), 0.15% G + 1.35% B (G0.15B1.35), 0.15% G + 2.85% B (G0.15B2.85), and 0.30% G + 2.70% B (G0.3B2.7) in experimental diets. After the feeding trial, fish fed G0.1B0.9, G0.1B1.9, and G0.15B1.35 diets had higher percent weight gain (WG), feed efficiency ratio (FER), specific growth rate (SGR), protein efficiency ratio (PER), and condition factor (CF) than those fed G0B0, G0.05B0.45, G0.05B0.95, G0.15B2.85, and G0.3B2.7 diets ($P < 0.05$); however, there was no significant differences among fish fed G0.1B0.9, G0.1B1.9, and G0.15B1.35 diets. Fish fed G0.1B0.9 and G0.1B1.9 diets had higher chemiluminescent responses (CL) than those fed the other diets ($P < 0.05$). Lysozyme activity of fish fed G0.1B0.9 diet was significantly higher than that of fish fed the other diets ($P < 0.05$). These results indicated that the optimum dietary supplementation level of β -1,3 glucan and BAISM could be approximately 0.10% β -1,3 glucan + 0.90% BAISM (G0.1B0.9) of diet based on WG, FER, SGR, PER, CF, CL, and lysozyme activity in juvenile olive flounder, *P. olivaceus*.

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EFFECT OF MANGANESE AND IRON ON GROWTH AND FEEDING OF JUVENILE GIANT RIVER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE-MAN)

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Journal of the World Aquaculture Society 38 (1): 161–168

Abstract:

The effect of either manganese or iron on survival, growth, and feeding of giant river prawn, *Macrobrachium rosenbergii* (De-Man), juveniles was studied in two separate experiments. Survival rates of *M. rosenbergii* juveniles (4.58 ± 0.48 g) following 60-d exposure to 0.01 (control), 0.3, 0.6, and 1.2 mg/L of total manganese (Mn) were 100, 93.3 ± 3.4 , 83.3 ± 4.3 , and $83.3 \pm 4.3\%$, respectively, while the same were 100, 83.3 ± 3.6 , 73.3 ± 3.3 , and $63.3 \pm 4.7\%$, respectively, at the total iron levels of 0.02 (control), 0.32, 0.65, and 1.2 mg/L. Average daily growth of the prawn was significantly ($P < 0.05$) lower at 0.3 mg/L and higher levels of total manganese compared to control (0.01 mg/L). Average growth of the prawn exposed to 0.65 and 1.2 mg/L total iron was significantly lower ($P < 0.05$) than in control (0.02 mg/L iron) and 0.32 mg/L treatments after 60 d of exposure. Feed utilization was significantly ($P < 0.05$) reduced in *M. rosenbergii* juveniles exposed to 0.3 mg/L and higher levels of manganese. Feed utilization was significantly ($P < 0.05$) reduced in the prawns at all the iron treatments compared to control (0.02 mg/L iron). The accumulation of Fe and Mn was minimum in the muscle and maximum in the hepatopancreas of the prawns.

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CHARACTERIZATION OF DIGESTIVE ENZYMES DURING LARVAL DEVELOPMENT OF RED DRUM (*SCIAENOPS OCELLATUS*)

J.P. Lazo, R. Mendoza, G.J. Holt, C. Aguilera, C.R. Arnold-2007

Aquaculture 265(1-4) : 194-205

Abstract:

The digestive capacity of red drum larvae throughout development was evaluated by characterizing and quantifying digestive enzyme activities using biochemical and substrate-SDS-PAGE techniques with specific inhibitors. Results showed that red drum larvae possess a nearly complete set of alkaline proteases since first feeding (3 days post-hatch). Alkaline proteases played a major role in digestion during the first days of feeding, while acid proteases became more important toward the end of the

larval period, concomitant with the appearance of a functional stomach. Enzymes for the digestion of proteins (trypsin-like), lipids (lipase) and carbohydrates (amylase) were already present in the larvae before exogenous feeding commenced, and their activity subsequently increased with age and length. At some stages of development, digestive enzymes of red drum larvae seem to have a temporal distribution mediated by underlying genetic mechanisms, rather than controlled by feeding activity. Intracellular digestion, measured as the activity of leucine–alanine peptidase, was high early in the larval stage and decreased as development progressed. In contrast, aminopeptidase activity, which is present in the intestinal brush border membrane and is indicative of intestinal maturity, was lowest at first feeding and subsequently increased with age. Using specific inhibitors the relative contribution of the alkaline proteases to the overall alkaline proteolytic activity was assessed. Interestingly, chymotrypsin relative contribution to alkaline proteolytic activity was higher than that for trypsin by 22 DAH. Substrate-SDS-PAGE analysis indicated that although the proteolytic enzymes measured were at least in part comprised of serine-type proteases, other types such as metallo-proteases may play a significant role in the digestive process in red drum larvae and should be further characterized. (CICESE — Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, B.C., México; email of J.P. Lazo : jplazo@cicese.mx)

THE EFFECT OF GREEN WATER AND LIGHT INTENSITY ON SURVIVAL, GROWTH AND LIPID COMPOSITION IN ATLANTIC COD (GADUS MORHUA) DURING INTENSIVE LARVAL REARING

Terje van der Meer, Anders Mangor-Jensen, Jana Pickova-2007

Aquaculture 265(1-4): 206-217

Abstract:

The effects of green water and light intensity on establishment of exogenous feeding, survival, growth, and lipid and fatty acid composition were investigated for larvae of Atlantic cod in two replicate experiments. In each experiment, six tanks received “low” irradiance (12–20 $\mu\text{W cm}^{-2}$) and another six tanks “high” irradiance (240–283 $\mu\text{W cm}^{-2}$). In three of the tanks within each light treatment, the alga *Isochrysis galbana* was added to make green water, and the other three tanks had clear water. Cod larvae were stocked into the tanks at day 3 post-hatch, and fed the rotifer *B. plicatilis*. Larval survival and gut filling at initiation of exogenous feeding were significantly improved by use of green water, feeding incidence was not significantly affected by any of the treatments, although low light conditions gave a marginal increase in feeding incidence. Growth was not affected by any of the treatments, but a tendency to density-dependent growth was observed within green vs. clear water, yielding some improvement in growth with use of algae. However, a significant signal from the algal treatment was detected in the fatty acid composition of the phospholipids and in the relative amount of triacylglycerols in the larval lipids of cod. Thus, the data demonstrate a nutritional vector of algae in larval cod rearing, and the possibly benefit of this on survival. Lack of effects of light intensity contradicts previous studies on larval cod, but point out possible genetic adaptations in this species to variable environmental conditions throughout its distribution area.

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INDUCED SPAWNING OF KUTUM, RUTILUS FRISII KUTUM (KAMENSKII, 1901) USING (D-ALA6, PRO9-NET) GNRHA COMBINED WITH DOMPERIDONE

Fatemeh Paykan Heyrati, Hossein Mostafavi, Hossein Tolooe, Salar Dorafshan-2007

Aquaculture 265(1-4):288-293

Abstract:

Kutum, *Rutilus frisii kutum* (Kamenskii, 1901), Cyprinidae is an endemic fish of the Caspian Sea. The Iranian Fisheries Organization (Shilat) produces up to 200 million fry (1–2 g b.w.) to restock the Caspian Sea population annually. These fish are produced by artificial breeding using carp pituitary extract (CPE). The objective of this study was to assay the effectiveness of a gonadotropin releasing

hormone analogue (D-Ala6, Pro9-NEt GnRH) alone or in combination with the dopamine antagonist domperidone (DOM) on spawning success, latency period, ovulation index (OI), weight of stripped egg mass/weight of stripped egg mass + remnant ovaries, and fertilization success in kutum. Ninety fish were divided into nine groups and injected intraperitoneally as follows: 2 mg kg⁻¹ b.w. of CPE as positive control, 20 µg GnRH_a kg⁻¹ b.w. in single injection, 5 µg + 2.5 mg, 10 µg + 5 mg and 20 µg + 10 mg kg⁻¹ b.w. of GnRH_a + DOM in single or double injection (10–90%) 24 h apart. Propylene glycol injected fish were used as negative controls. The results showed that the highest doses of GnRH_a and DOM in single injection lead to higher spawning success and latency periods in comparison with positive control ($P < 0.05$), while no significant differences in the OI and fertilization success were found ($P > 0.05$). Only 2/10 fish were ovulated in the group which received GnRH_a 20 µg kg⁻¹ b.w. alone suggesting dopaminergic tone on gonadotropin (GtH) secretion in this fish at the preovulation stage. Therefore, it can be concluded that like many other cyprinids, dopamine inhibitory tone is active in kutum and it is necessary to combine GnRH_a with a dopamine antagonist for spawning induction.

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ENERGETICS AND METABOLISM OF YELLOWTAIL KINGFISH (*SERIOLA LALANDI* VALENCIENNES 1833) DURING EMBRYOGENESIS

Damian Moran, Brendan Gara, Rufus M.G. Wells-2007

Aquaculture 265(1-4): 359-369

Abstract:

A study was undertaken to measure changes in oxygen consumption and metabolite concentration (ammonia, free amino acids, glucose, glycogen, ninhydrin positive substances and protein) in embryos of yellowtail kingfish (*Seriola lalandi*) incubated at different temperatures (17, 19, 21 and 23 °C). The oxygen uptake at hatch and total oxygen consumed during embryogenesis was negatively correlated with temperature, and resulted in Q₁₀ values less than 1. This was attributed to the fact that embryos and first hatch larvae have been reported to be smaller at warmer incubation temperatures and therefore consumed less oxygen. Free amino acids were present in high concentrations immediately after spawning (188 ± 15.3 nmol ind⁻¹) and were dominated by the neutral amino acids alanine, glycine, isoleucine, leucine, serine, and valine. The free amino acid pool was rapidly depleted until hatch (20–40 nmol ind⁻¹), after which the pool was dominated by glycine, serine, valine and arginine/taurine (unresolved). The loss of positive buoyancy around the time of hatch was likely to be correlated with the decrease in free amino acid content during embryogenesis. The sequence of metabolic fuel use during embryogenesis is likely to be similar to that observed in other marine fish species with pelagic eggs, namely that carbohydrates, lipid and protein are of relatively minor importance compared to the catabolism of free amino acids for energy. Eggs incubated at 17, 19 and 21 °C had similar profiles of metabolite concentration during embryogenesis, however, eggs incubated at 23 °C were found to have a considerably different pattern of substrate utilisation, possibly indicative of abnormal physiological development at a temperature above that which is routinely encountered in the wild.

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SHORT COMMUNICATION: GROWTH, BODY COMPOSITION, RESPIRATION AND AMBIENT AMMONIA NITROGEN TOLERANCE OF THE JUVENILE WHITE SHRIMP, *LITOPENAEUS VANNAMEI*, AT DIFFERENT SALINITIES

Erchao Li, Liqiao Chen, Ceng Zeng, Xuemin Chen, Na Yu, Qiuming Lai, Jian G. Qin-2007

Aquaculture 265(1-4): 385-390

Abstract:

Trials were conducted in laboratory to investigate the growth performance, body composition, respiration and ammonia-N tolerance of the white shrimp, *Litopenaeus vannamei*, at 3.0, 17.0 and 32.0‰, respectively. In the growth trial, 40 juvenile shrimps were stocked into each tank with four replicates at each salinity, and were fed with a commercial diet for 50 d. Shrimp weight gain at 17.0‰ was the highest, and significantly higher than that of shrimps at 3.0‰. Shrimp survival rate at 3.0‰ was significantly lower than that of other two groups. However, hepatosomatic index and condition factor were not significantly affected by the ambient salinity. Shrimp body protein and ash content were not affected significantly by salinity, while body moisture increased at high salinity, and crude lipid in shrimps was lowest at 32.0‰. After being exposed to the above three salinities for 30 d prior to the test, shrimp oxygen consumption and respiratory quotient of the shrimps at 3.0‰ were significantly higher than those of shrimps at medium and high salinities, while salinity did not significantly affect CO₂ production. When juvenile *L. vannamei* were exposed to seven ammonia-N concentrations (0, 4.00, 6.67, 9.33, 12.00, 14.67, and 17.33 mg l⁻¹) at the three above salinities to which shrimps had been separately acclimated for 10 d at pH 8.30 and 29 ± 0.5 °C, shrimps at 3‰ were the most susceptible to ambient ammonia-N, and the 96 h LC₅₀ with 95% confidence limit to ambient ammonia-N was 9.33 (8.39–10.37) mg l⁻¹. This study suggests that *L. vannamei* could adapt to a wide range of salinity, but the animals would be more susceptible to ammonia toxicity and spend more energy to compensate the cost for osmoregulation at low salinity.

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REARING OF OCTOPUS VULGARIS PARALARVAE: PRESENT STATUS, BOTTLENECKS AND TRENDS

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Aquaculture 266(1-4): 1-15

Abstract:

Due to the high mortality rates and poor growth generally observed in *Octopus vulgaris* paralarval rearing experiments, it was decided to organize a working group in order to formulate recommendations to tackle this problem. Over a dozen scientists representing the most active current research groups related to this subject attended the meeting in Vigo, Spain, in November 2005. The aim of this working group was to determine the bottlenecks that prevent success in paralarval rearing, define the most appropriate rearing conditions, and identify required future research.

This paper describes rearing techniques for the *O. vulgaris* paralarvae used by the different research participant teams, with regard to tank systems, feeding environment, and diets (*Artemia*, crustacean zoeae, sandeel flakes, copepods, etc.). Additionally, it includes other related themes such as the culture of *Artemia* and copepods, organisms that are commonly used in paralarval rearing.

When embarking on *O. vulgaris* rearing it is advised to use prey rich in DHA (docosaenoic acid, 22:6n-3) and EPA (eicosapentaenoic acid, 20:5n-3), and with high DHA/EPA ratio. Such prey could be enriched *Artemia*, accompanied or not by crustacean zoeae or any microdiet. It is also recommended that, in future studies, values of growth and survival rates are recorded at the beginning of the benthic phase, in order to compare them to successful previous studies. Dry weight and DHA/EPA ratio of paralarvae may also be good criteria to define paralarval viability and evaluate success of the rearing system.

It is further concluded that the nutritional aspect is the most important factor influencing larval mortality. Certain lipids (phospholipids, cholesterol, and polyunsaturated fatty acids), amino acids (lysine, leucine and arginine), and essential elements (e.g. copper) play a relevant role in the larval nutrition. It is believed that the PUFA content, especially DHA and EPA, constitutes one of the basic nutritional requirements.

Regarding new research lines, no standardized system for paralarval rearing exists, and it is essential to make progress on this issue. Research on nutritional requirements is considered an area of highest priority, especially the development of a specific enrichment for *Artemia*, the search for alternative live preys, and the development of suitable formulated diets.

OBSERVATIONS ON THE SPAWNING BEHAVIOR OF ARTIFICIALLY MATURED JAPANESE EELS *ANGUILLA JAPONICA* IN CAPTIVITY

S.Z. Dou, Y. Yamada, A. Okamura, S. Tanaka, A. Shinoda, K. Tsukamoto-2007

Aquaculture 266(1-4): 117-129

Abstract:

Spawning behavior of artificially matured Japanese eels *Anguilla japonica* in captivity was investigated using a DVD Video image system. Following a routine hormone treatment technique for this fish, female eels were artificially matured by weekly intramuscular injections of salmon pituitary extracts (SPE) at a dosage of 40 mg kg⁻¹ BW for a total of 7–11 doses to induce ovarian maturation, while male eels received weekly intramuscular injections of human chorionic gonadotropin (HCG) at a dosage of 1000 IU kg⁻¹ BW for a total of 6–11 doses at 18 °C to induce testicular maturation in a separate aquarium. In this experiment, three pairs of such hormone-treated matured eels were acclimatized in seawater in 1.5 m³ experimental aquaria with or without shelters at 20 °C for 24 h. Twenty four hours after the acclimatization terminated, the females received SPE injections to boost maturation and ovulation. Twenty four hours following these injections, the females received injections of HCG (1000 IU per fish, HCG injection) and 17 α -hydroxyprogesterone (2 mg per fish) to induce ovulation, while males were given HCG injections (1000 IU per fish, HCG injection) to induce spermiation. Video taping started after the 24 h acclimatization terminated and last for a total of 96 h. Before the HCG injections, both sexes were inactive, staying on the bottom or in shelters if available. Following these HCG injections, they became active and frequently left the bottom swimming in the water column. During the 24 h following HCG injections, activity accounted for 67% and 45% of the total activity in no shelter treatment for females and males, respectively, in comparison with 77% and 78% in shelter treatment. Activity was significantly more pronounced during this phase than during other phases for each sex in either shelter treatment. Egg release and sperm ejection occurred in the water column around the time eels' activity reached peaks. Eels either returned into the shelters or stayed motionlessly on the bottom of the aquaria after egg release and sperm ejection. Eight out of nine (89%) females in no shelter treatment spontaneously released eggs with a total of 11 batches 14–18 h following HCG injections, in contrast with four out of nine (44%) females releasing eggs for 4 batches 16–20 h in shelter treatment. Males arrived at activity peaks 11–13 h following HCG injections in no shelter treatment, 2–4 h ahead of the females (14–16 h), in comparison with 8–11 h in shelter treatment with 5–6 h ahead of the females (14–17 h). Courtship behavior indicative of spawning such as pairing, chasing and touching bodies was not observed in the eels in this study. However, on many occasions, eels of both sexes (male–female or female–female) were found to “cruise together” in water column for a short time period or frequently come together prior to releasing eggs and ejecting sperm, suggesting the possibility of group mating in artificially matured Japanese eels.

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SPAWNING INDUCTION AND EARLY DEVELOPMENT OF THE CARIBBEAN SCALLOPS *ARGOPECTEN NUCLEUS* AND *NODIPECTEN NODOSUS*

L.A. Velasco, J. Barrosa, E. Acosta-2007

Aquaculture 266(1-4): 153-165

Abstract:

Argopecten nucleus and *Nodipecten nodosus* are two commercially valuable bivalve species from the Caribbean region, and are interesting candidates for mass culture in the sea based on laboratory production of juvenile “spat” organisms. An experiment of artificial spawning was performed with the two species as the initial part of a study on the feasibility of producing their spat. Stimulation of spawning was carried out, determining the percentages of individuals spawning, response time,

numbers of gametes produced, and fertilization of the oocytes. Five external stimuli and eight combinations of those were tested: slow changes in temperature (ST), fast changes in temperature (FT), desiccation (D), high concentrations of microalgae (M), exposure to water currents (C), ST + D, ST + M, ST + C, FT + D, FT + M, FT + C, D + M and D + C. Four internal stimuli were applied, including intragonadal injection of serotonin (Se), dopamine (Da), Da + prostaglandin E2 (Da + PE2) and Se + PE2. The results showed that *A. nucleus* spawned in response to all 17 stimuli tested, while *N. nodosus* responded to only eight of these (47%), suggesting that spawning in the first species was more sensitive to both external and internal stimuli. Larger percentages of individuals of *A. nucleus* spawned, with specially high values (100%) obtained with the ST + M, ST + D and Da stimuli; in *N. nodosus*, the Da stimulus was the most effective. *N. nodosus* gave shorter response times to stimuli than *A. nucleus*, with the shortest times in both scallop species (16 to 32 min) obtained using the internal stimuli. The production of gametes was similar between the two species, with about 5×10^9 spermatozoa, and 1.9×10^6 oocytes released per individual. *A. nucleus* produced higher numbers of spermatozoa using the ST + M stimulus, and higher numbers of oocytes using the FT + M, ST + D and Se stimuli; these values did not differ among treatments in *N. nodosus*. Abnormal spawning was observed in some of the internal stimuli tested, including release of immature gametes, simultaneous release of both male and female gametes, or sole production of male gametes. Fertilization was about 63% for both species, and was not influenced by the type of stimulation used to obtain the gametes. In summary, the most efficient stimuli for inducing the normal spawning of viable gametes in *A. nucleus* were ST + M and ST + D, and Se + PE2 in *N. nodosus*. Early embryonic and larval development in *A. nucleus* were significantly more rapid than in *N. nodosus*, while the sizes of early embryos and larvae were similar in both species, and comparable to previous descriptions of scallops' early development. (Instituto de Investigaciones Tropicales (INTROPIC), Universidad del Magdalena, Carrera 2 No 18-27, Taganga, Santa Marta, Colombia; email of L. Velasco: luza.velasco@unimagdalena.edu.co)

COMBINED EFFECTS OF DIETARY HUFA LEVEL AND TEMPERATURE ON SEA BASS (*DICENTRARCHUS LABRAX*) LARVAE DEVELOPMENT

M. Vagner, J.H. Robin, J.L. Zambonino Infante, J. Person-Le Ruyet-2007

Aquaculture 266(1-4) :179-190

Abstract:

The purpose of this study was to investigate the combined effect of the incorporation of vegetable products in diet and temperature on enzymatic pathways for high unsaturated fatty acids (HUFA) desaturation in sea bass larvae. Four replicated groups were fed a low (LH; 0.8% EPA + DHA) or a high (HH; 2.2% EPA + DHA) n-3 HUFA microparticulated diet from mouth opening, six days post-hatching and were reared at 16 or 22 °C. The four experimental conditions (LH16, HH16, LH22 and HH22) were tested for 45 days. At the end of the experiment, body weight, total length and biomass were affected by temperature ($P < 0.001$), while biomass as well as fresh body weight was also influenced by diet ($P < 0.05$ and $P < 0.001$ respectively). This always led to the same ranking of experimental conditions: HH22 > LH22 > HH16 > LH16. The larval skeletal development was more advanced in 22 °C-groups than in 16 °C-ones ($P < 0.001$), while it was not affected by diet. Amylase and trypsin pancreatic secretions did not vary between d-25 and d-45, indicating that pancreatic maturation was achieved at d-25. Low temperature combined with low dietary HUFA delayed intestinal maturation ($P < 0.001$), while low temperature combined with high HUFA diet allowed larvae compensating for the initial intestinal maturation retardation. Lipase gene expression was down-regulated in HH16 group at d-25 ($P < 0.05$) and in the two 16 °C-groups at d-45 ($P < 0.001$), while lipase enzymatic activity was similar in all groups. This suggested the presence of a post-transcriptional regulation of this gene. PPAR α and PPAR β were not affected neither by temperature, nor by diet, suggesting that lipid metabolism was not significantly affected by a lowering in dietary n-3 HUFA when isolipidic diets were used. A higher DHA content was found in larvae than in their diets ($\times 2$ for LH; $\times 1.5$ for HH) but the DHA content in PL of d-45 LH larvae was lower than the initial one, which revealed a HUFA deficiency in this group. Delta 6-desaturase ($\Delta 6D$) gene expression was significantly up-regulated by HUFA deprived diet ($P < 0.05$) whatever the temperature was. This was supported by the increase in 18:3n-6 in LH larvae ($P < 0.001$), which indicated a desaturation from 18:2n-6 by the $\Delta 6D$. This study clearly showed that larvae were able to

adapt to an n-3 deprived diet by a stimulation of enzymatic pathways for HUFA desaturation, and that this adaptation was not affected by temperature.

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OXIDATIVE STABILITY AND CHANGES IN THE PARTICLE SIZE OF LIPOSOMES USED IN THE ARTEMIA ENRICHMENT

Óscar Monroig, Juan Carlos Navarro, Francisco Amat, Pedro González, Francisco Hontoria-2007
Aquaculture 266(1-4): 200-210

Abstract:

The oxidative stability and the particle size of several types of liposomes were assessed in order to characterize their behaviour when submitted to the aggressive conditions of the Artemia enrichments. Results show that all liposomes tested in this study were much more oxidatively stable than a commercial product based on fish oil emulsion. Whereas the initial thiobarbituric acid reactive substances (TBARS) concentration in the emulsion was only slightly higher than in liposomes, the concentration of TBARS in the emulsion increased up to values three orders of magnitude above those registered by liposomes after 21 h of incubation. Among the different liposome formulations, results indicate that vesicles composed of phospholipids containing long-chain highly unsaturated fatty acids (krill phospholipid extract) were generally less stable than those composed of shorter length-chain and more saturated acyl chains.

In regards to the particle size changes during enrichment, all liposomes maintained their original size during the experimental period when incubated without nauplii. In the presence of nauplii, liposomes did not exhibit notable changes in their size, except for unilamellar vesicles prepared by the extrusion methodology and formulated with soybean phosphatidylcholine. The implications of the results on the capability of liposomes to be used in Artemia nauplii enrichments are discussed.

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GROWTH AND REPRODUCTIVE PERFORMANCE IN CULTURED NEARSHORE ROCKFISH (SEBASTES SPP.)

Aquaculture 266(1-4): 236-245
Wai Ning Tsang, Peter M. Chaillé, Peter M. Collins-2007

Aquaculture 266(1-4): 236-245

Abstract:

The present study evaluates the extent to which the viviparous reproductive strategy that characterizes the genus *Sebastes* (rockfish) can be expressed in culture. In addition, the growth patterns of rockfish were examined during the early phases of growth pertinent to the culture of the species. Using grass rockfish (*Sebastes rastrelliger*) as our model, it was demonstrated that all phases of ovarian seasonal cyclicity could proceed under controlled environmental conditions. Rockfish successfully spawned in our facility and subsequently passed through successive phases of oocyte development during the normal reproductive season before follicular atresia was observed in the absence of fertilization. Fish introduced into culture in an advanced stage of vitellogenesis became pregnant in the absence of males 14–55 days later. This observation is consistent with the view that delayed fertilization occurs in rockfish and establishes that wild caught specimens for spawning purposes are not confined to pregnant females. By monitoring growth parameters in two groups of brown rockfish (*S. auriculatus*) introduced into culture during their first and second year of development a comprehensive picture of early growth was established. Overall changes in both weight and length followed a sigmoid curve comprising an early phase of exponential growth transitioning to a phase of exponential decay. The constituent phases of this curve, as expressed in the two age groups, and the combined growth data over a period of 1083 culture-days were precisely described by the Gompertz equation. This equation also described the growth of young-of-the-year copper rockfish (*S. caurinus*) with similar accuracy. Validation of the applicability of our Gompertz equation was achieved by reference to growth parameters of brown rockfish of known age raised from birth at UCSB. Our mathematical model

precisely depicted growth with age from birth to the period of sexual maturity. These data on growth in relation to age may find broad application in fisheries management plans allowing for evaluation of population changes over time and be useful in determining and improving the culture potential of nearshore rockfish species.

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ONTOGENETIC CHANGES IN TOLERANCE TO ACUTE AMMONIA EXPOSURE AND ASSOCIATED GILL HISTOLOGICAL ALTERATIONS DURING EARLY JUVENILE DEVELOPMENT OF THE BLUE SWIMMER CRAB, *PORTUNUS PELAGICUS*

Nicholas Romano, Chaoshu Zeng-2007

Aquaculture 266(1-4): 246-254

Abstract:

The current study was performed to determine the LC50 values through early juvenile ontogeny of the blue swimmer crab *Portunus pelagicus*, which forms an important fisheries industry and is currently a targeted species throughout the Indo-Pacific region. Additionally, through examination of the gills we sought to investigate cause of death as well as explaining ontogenetic changes in tolerance to acute ammonia-N exposure. The juvenile stages tested were crab one (C1) stage (mean weight 0.002 g), crab three (C3) stage (mean weight 0.028 g), crab five (C5) stage (mean weight 0.187 g) and crab seven (C7) stage (mean weight 0.732 g). The C1 stage was defined as the first molt from the megalopa larvae stage to the first crab stage. The developmental duration from the C1 to C7 stage took approximately 40 days. When the desired stage was reached the crabs were randomly selected and exposed to 5, 10, 20, 40, 60, 80, 100 mg/l ammonia-N and a control (no ammonia-N added) for 96-h according to the "static renewal method". For each juvenile experiment, a total of 30 crabs, each acting as a replicate, were tested at each ammonia-N treatment and control at a salinity of 30‰, temperature of 28 °C and pH of 8.1.

The results demonstrate that ammonia-N tolerance significantly ($p < 0.01$) increases with the juvenile ontogenetic development of *P. pelagicus*. The 96-h LC50 (mg/l) values of total ammonia-N were 23.10 (1.65 NH₃-N mg/l), 25.23 (1.80 NH₃-N mg/l), 37.43 (2.67 NH₃-N mg/l) and 50.65 (3.62 NH₃-N mg/l) for the C1, C3, C5 and C7 crabs respectively, showing an over two-fold increase in ammonia-N tolerance from the C1 to C7 crab stage. Furthermore, in comparison to other crustacean species, *P. pelagicus* early juveniles exhibit a considerably higher tolerance to ammonia-N.

The histological results show that following an ammonia-N exposure concentration of 5 mg/l, localised infiltration of haemocytes and epithelial changes of the lamellae on the anterior gills of the C1 crabs were observed. However, at the same ammonia-N concentration, no obvious gill histopathological changes were observed for the C3, C5 or C7 crabs. In general, the severity of morphological and physiological changes to the gill lamellae, including extensive infiltration of haemocytes, necrosis, epithelial changes, disrupted pillar cells and lamellae collapse, increased with increasing ammonia-N concentrations for all crab stages. However, at the same ammonia-N concentration, the severity of the changes to the gill lamellae were less with more developed crabs.

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CHANGES ON CORTISOL LEVEL AND DIGESTIVE ENZYME ACTIVITY IN JUVENILES OF JAPANESE FLOUNDER, *PARALICHTHYS OLIVACEUS*, EXPOSED TO DIFFERENT SALINITY REGIMES

Sergio Néstor Bolasina, Masatomo Tagawa, Yoh Yamashita-2007

Aquaculture 266(1-4): 255-261

Abstract:

The aim of this work was to evaluate the effect of salinity changes on digestive enzyme activity and whole-body cortisol concentration in juveniles (46 DAH) of Japanese flounder.

Two different salinity patterns were designed: a control group exposed to a constant flux of seawater ($S = 32$) and a treatment group exposed to changes from full seawater ($S = 32$) to low salinity water ($S = 10$) each 6 h. The duration of the experiment was 2 days. Samples were taken every 6 h for enzyme activity and cortisol determination.

Survival in both treatments was 92% at the end of the experiments. Cortisol levels were significantly higher ($P < 0.05$) in fish exposed for the first time to low salinity. Cortisol concentrations in the test group were also higher during the remainder of the experiment, especially during exposure to low salinity, but these differences were not significant ($P > 0.05$) between control and treatment groups. Trypsin activity decreased significantly ($P < 0.05$) during the first exposure to low salinity water, the comparison with the control group also showed significant differences ($P < 0.05$). In the case of lipase activity, no significant differences ($P > 0.05$) were found between treatment and control group.

This work showed that juveniles of Japanese flounder could survive fluctuating exposure to low salinities ($S = 10$) at least in short periods. The results suggest that juveniles of *P. olivaceus* may retain abilities to survive exposure to low salinities through shared phylogenetic relationship.

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SPAWNING PERFORMANCE AND EGG QUALITY OF ASIAN CATFISH CLARIAS BATRACHUS (LINN.) AT VARIOUS DOSES OF HUMAN CHORIONIC GONADOTROPIN (HCG) INJECTION AND LATENCY PERIODS DURING SPAWNING INDUCTION

S.K. Sahoo, S.S. Giria, S. Chandra, A.K. Sahu-2007

Aquaculture 266(1-4): 289-292

Abstract:

Attempts were made to spawn *Clarias batrachus* by injecting human chorionic gonadotropin (HCG) at different doses (1000, 2000, 3000, 4000 and 5000 IU/kg body weight) in combination with latency periods (11, 14, 17, 20 and 23 h). The females could not be stripped at 11 h latency when injected with 1000–3000 IU of HCG per kg body weight. A significant ($P < 0.05$) reduction of stripped egg number was observed when females were injected with a dose of 5000 IU HCG and stripped at 20–23 h post-injection. The eggs obtained from the females with injection of 4000–5000 IU per kg body weight and stripped at 11 h post-injection, did not fertilize. The highest fertilization (75–89%) and hatching (66–78%) could be obtained at 3000–5000 IU doses of HCG with 14–17 h post-injection. Although the highest working fecundity was obtained with 4000 IU HCG at 14 and 17 h latency, the response from 3000 IU HCG was only slightly less at these latency periods, and was effective for up to 23 h.

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