CRYOPROTECTANT MICROINJECTION TOXICITY AND CHILLING SENSITIVITY IN GILTHEAD SEABREAM (SPARUS AURATA) EMBRYOS

J. Beirãoa V. Robles, M.P. Herráez, C. Sarasquete, M.T. Dinis, E. Cabrita-2006

Aquaculture 261(3): 897-903

Abstract:
Cryopreservation of fish embryos requires an optimal distribution of cryoprotectants inside all embryo compartments. Traditional techniques for the incorporation of cryoprotectants (CPAs) have failed to protect all fish compartments, especially the yolk sac which has been considered the principal point of embryo chilling sensitivity. In the present study, microinjection was used to incorporate cryoprotectants into the yolk sac of gilthead seabream (Sparus aurata) embryos at tail bud stage. The effect of microinjection viability, cryoprotectant toxicity and chilling resistance was evaluated through the hatching rate. Larval survival at first feeding was also determined in microinjection viability and cryoprotectant toxicity studies. Permeabilized seabream embryos were microinjected with 2.35 nl dimethyl sulfoxide (Me2SO), methanol (MeOH), ethylene glycol (EG) (5 M, 10 M and pure) or sucrose (10% and 15%). In a second experiment, 29.5 nl and 154.0 nl of the highest concentration of each cryoprotectant were used in the same embryo stage. To test the effect of microinjected cryoprotectants on embryo chilling resistance, 29.5 nl of pure Me2SO or 15% sucrose was microinjected into the yolk sac of tail bud stage embryos and then at a later stage, (tail-bud-free), were exposed to 3 M Me2SO solution at − 10 °C for 30 min. Our results showed that microinjection technique did not affect the viability of tail bud stage embryos as is shown by the high hatching and survival rates. Hatching and larval survival rate at first feeding were not affected with any of the CPAs tested, showing percentages higher than 75% and 90%, respectively, when embryos were microinjected with a smaller quantity of cryoprotectant. Sucrose was the cryoprotectant better tolerated at higher concentration and volume. Cryoprotectant concentration inside the yolk higher than 1.18 M for Me2SO, 1.5 M for EG and 2 M for methanol decreased the hatching rate. Microinjection allowed the delivery of high concentrations of CPAs into the yolk sac without deleterious effects on the embryo, but did not provide a significant level of protection for the whole embryo against chilling injury.

(FCMA and Center for Marine Sciences–CCMAR, University of Algarve, Campus Gambelas, 8005-139 Faro, Portugal; email of E. Cabrita: cabrita@ualg.pt)

EFFECT OF VARYING DENSITY AND WATER LEVEL ON THE SPAWNING RESPONSE OF AFRICAN CATFISH CLARIAS GARIEPINUS: IMPLICATIONS FOR SEED PRODUCTION

Gamal O. El Naggar, George John, Mahmoud A. Rezka, Waheed Elwan, Mohammed Yehia-2006

Aquaculture 261(3): 904-907

Abstract:
The development of a reliable methodology for spawning of African catfish, Clarias gariepinus without the use of hormone injections would greatly improve the prospects of aquaculture in Africa. Earlier work has shown that it is possible to produce C. gariepinus fingerlings by subjecting the broodfish to a physical stress of reduced water depth and/or increased temperature. The hypothesis that C. gariepinus could be induced to spawn through a combined physical stress of lowered water level and increased stocking density was tested in concrete tanks. Three water levels (25, 50 and 75 cm) and three stocking densities (2, 4 and 6 pairs of broodfish at a 1:1 sex ratio in each hapa) were tested. Water depth in the tanks and brood fish density in the hapas affected spawning success. The percentage of spawning females was significantly higher when broodfish were stocked at 2 and 4 pairs in each hapa at water levels of 25 cm or 50 cm. There was no significant difference in spawning response between the 25 and 50 cm depths while a significant difference was seen between the 75 cm and both 25 and 50 cm depths. The results indicate optimum levels and densities for enhancing spawning success in C. gariepinus.

(WorldFish Center, Regional Research Center for Africa and West Asia, Abbassa, Abou-Hammad, Sharkia, Egypt; email of Gamal O. El Naggar: g.naggar@cgiar.org)

MORPHOLOGICAL CHARACTERIZATION OF LARVAL STAGES AND FIRST JUVENILE OF THE FRESHWATER PRAWN CRYPHIOPS CAEMENTARIUS (MOLINA, 1782) (DECAPODA: PALAEMONIDAE) UNDER LABORATORY CONDITIONS

María C. Morales, Miguel Rivera, Jaime Meruane, Cesar Galleguillos, Hidetsuyo Hosokawa-2006

Aquaculture 261(3): 908-931

Abstract:
Research has been in progress for several years on various aspects of the biology and ecology of the freshwater prawn Cryphiops caementarius, an inhabitant of rivers in northern Chile. The commercial value of this prawn fomented the accomplishment of studies on its reproduction and development with the aim of producing juveniles under controlled conditions, to be followed by growout to commercial size in managed culture systems. The present study describes larval culture of this species from eggs of gravid females obtained in the field, from the first developmental stage (Zoea I) through the first juvenile stage. The larvae were cultured at 25 °C in UV sterilized water at variable salinities based on the requirements of the developmental stages. Larvae were fed with Nannochloris, Isochrysis and Artemia nauplii as required. This report describes in detail the 18 larval stages of this prawn, as well as its first juvenile form.

(Departamento de Acuicultura, Facultad de Ciencias del Mar, Universidad Católica del Norte. Larrondo 1281, Casilla 117, Coquimbo, Chile; email of M. Morales: mcmorale@ucn.cl)

PRODUCTIVITY AND PHOTOSYNTHETIC EFFICIENCY OF OUTDOOR CULTURES OF TETRASELMIS SUECICA IN ANNULAR COLUMNS

Graziella Chini Zittelli, Liliana Rodolfi, Natascia Biondi, Mario R. Tredici-2006

Aquaculture 261(3): 932-943

Abstract:
In this study, 120-l annular columns were used to cultivate Tetraselmis suecica outdoors. The mass transfer at different aeration rates and the influence of the harvest rate on productivity and biochemical composition were investigated. The potential of the system was evaluated by estimating productivity at full-scale. Two different arrangements to simulate a full-scale plant and determine the "overall areal productivity" (OAP) were experimented with. In August 2003, one experimental column (full-scale column) was placed between seven dummy columns. All the reactors were positioned at a distance of 0.8 m wall to wall and centred at the vertices of equilateral triangles. A second experimental column (isolated column) was placed in a separate area under full sunlight. In August 2004, the columns were placed side by side in an east–west oriented row at a distance of 0.24 m wall to wall.
In the first experiment, the mean volumetric productivity of the full-scale column was not significantly lower than that achieved by the isolated column (0.46 against 0.49 g l− 1 day− 1) in spite of the shading by the dummy units. The average OAP and efficiency of conversion of visible solar radiation (PE) were 36.3 g m− 2 day− 1 and 9.4%, respectively. In the second experiment, the full-scale column attained a mean volumetric productivity of 0.42 g l− 1 day− 1. The OAP and the PE were 38.2 g m− 2 day− 1 and 9.3%, respectively.

(Istituto per lo Studio degli Ecosistemi, CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Firenze, Italy; email of M. Tredici: mario.tredici@unifi.it)

CHILLED STORAGE OF WHITE SHRIMP (LITOPENAEUS VANNAMEI) SPERMATOPHORES

Aquaculture

Subuntith Nimrat, Sopon Siriboonlamom, Shicui Zhang, Yuyan Xu, Verapong Vuthiphandchai-2006

Volume 261(3): 944-951

Abstract:

Chilled storage of spermatophores from white shrimp (Litopenaeus vannamei) is needed to generate a consistent and reliable supply of spermatozoa for domestication purposes. The objective of this study was to develop a protocol for the chilled storage of white shrimp spermatophores and to evaluate bacterial propagation during such storage. In the first experiment, spermatophores were immersed in four extenders, mineral oil, Ringer's solution, phosphate buffer and 0.85% NaCl, and stored at low temperature (2–4 °C) for 35 days. Characteristics of preserved spermatophores changed the least and viable sperm was highest when spermatophores were stored in mineral oil. Spermatophores preserved with mineral oil appeared morphologically normal. Bacillus circulans, Staphylococcus hominis and S. lugdunensis, S. sciuri, S. xylosus and Micrococcus spp. were identified as the predominant bacteria during chilled storage, and total bacterial counts gradually increased during the experiment. A second experiment investigated the effect of antibiotic on chilled storage. Spermatophores were preserved in only mineral oil or mineral oil with 0.1% penicillin–streptomycin. These were evaluated for changes in external morphology of spermatophores, sperm viability and total bacteria count every week during a 35-day experimental period. Percentages of viable sperm (69.5 ± 3.9%) were significantly higher ($P < 0.05$) among spermatophores preserved in mineral oil with 0.1% antibiotic compared with those preserved only in mineral oil (57.7 ± 3.4%) over 35 days. The number of total bacteria in the treatment with mineral oil ranged between 28.3 ± 4.8 and 2416.7 ± 299.4 CFU/g, but in mineral oil containing antibiotic bacteria were undetectable. This study suggests that chilled storage of spermatophores is a feasible approach for the management and spawning of white shrimp broodstock.
(Department of Microbiology, Environmental Science Program, Faculty of Science, Burapha University, Chonburi 20131, Thailand; email of Subuntith Nimrat:subunti@buu.ac.th)

CHARACTERIZATION OF SENEGALESE SOLE, SOLEA SENEGALENSIS, MALE BROODSTOCK IN TERMS OF SPERM PRODUCTION AND QUALITY
E. Cabrita, F. Soares, M.T. Dinis-2006
Aquaculture 261(3): 967-975
Abstract:
Sperm quality and production have never been characterized in Solea senegalensis males. Reproduction in captivity in this species has been obtained mostly with wild-captured animals, because it is common that the F1 generation fails to reproduce. However, there is no information on sperm quality from both types of broodstocks. The aim of the present study was to characterize sperm production and to describe the profiles of spermiation in individual wild-captured males. Also, sperm quality and production were determined in two types of broodstocks established in our facilities; wild-captured and F1 individuals. The males were analyzed for their fluency and identified as fluent or non-fluent. The sperm volume, cell concentration, sperm production and motility were recorded from mid February until mid November in both broodstocks. Results showed that S. senegalensis males can produce motile sperm during all this period, with specific peaks of high spermiation and a high percentage of fluent males. This fact was observed in both male broodstocks. There was a large variability in terms of sperm profiles in males maintained under the same conditions. Sperm volume collected in this species was very small and ranged from 5 to 20 μl in F1 broodstock and 10 to 80 μl in wild-captured broodstock. Cell density ranged from 0.7 to 1.2 × 109 spermatozoa/ml in F1 males to values of 1–2 × 109 spermatozoa/ml for the wild-captured males. Sperm production (total spermatozoa per stripping) was also very low and ranged from 20 × 106 spermatozoa for F1 broodstock to 40–60 × 106 spermatozoa for wild-captured broodstock. Our results demonstrated that sperm production in this species is very low and variable according to the type of males. These results suggest that a previous selection of males according to their fluency, sperm production and provenience (wild-captured or F1) should be taken into account in the establishment of a S. senegalensis broodstock.
(Center for Marine Sciences-CCMAR, University of Algarve, 8000 Faro, Portugal; emai of E. Cabrita: ecabrita@ualg.pt)

THE INFLUENCE OF WATER TEMPERATURE ON SPAWNING PATTERNS AND EGG QUALITY IN THE ATLANTIC HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS L.)

N.P. Brown, R.J. Shields, N.R. Bromage-2006
Aquaculture 261(3): 993-1002

Abstract:
A three-year study was conducted to investigate the effects of water temperature on Atlantic halibut broodstock reproductive performance. Two groups of fish held under ambient photoperiod were established onto contrasting temperature regimes. The 'chilled' group were held at below 9 °C from late October and at approximately 6 °C from December until the end of the spawning period whilst the 'ambient' group received no temperature control. The temperature profiles for the 'ambient' group changed over the 3 years but were generally characterised by warmer conditions prior to spawning and an earlier temperature rise in the spring. Total egg production was higher in the 'chilled' group each year. Absolute fecundity was significantly reduced in the 'ambient' group compared to the 'chilled' group every year of the study (0.6 million eggs/female for 'chilled' group vs. 0.3 to 0.4 million eggs/female for 'ambient' group) and egg viability, in terms of fertilisation and hatch rate, was significantly impaired in the 'ambient' group in years 2 and 3 (mean fertilisation rate in the 'ambient' group was between 27.0% and 54.8%; vs. 63.4% to 77.4% in the 'chilled' group, mean hatch rate in the 'ambient' group was between 3.1% and 25.6% vs. 60.7% to 71.7% in the 'chilled' group). Eggs spawned at high temperatures, later in the season were generally of low viability. In the 'ambient' group the spawning season became progressively delayed during the study and average duration of spawning season over 3 years was shorter (between 23.5 to 26.3 days for the 'ambient' group vs. between 30.5 and 41.2 days for 'chilled' group). It is hypothesised that high temperature during the vitellogenesis period caused a delay in spawning and a reduction in quantity and quality of eggs and that this effect was exacerbated by high temperature during spawning.

(Center for Cooperative Aquaculture Research, 33 Salmon Farm Road, Franklin, Maine, 04634, USA; email of N. Brown: npbrown@maine.edu)

OZONATION OF SEAWATER IMPROVES THE SURVIVAL OF LARVAL SOUTHERN ROCK LOBSTER, JASUS EDWARDSII, IN CULTURE FROM EGG TO JUVENILE

Arthur J. Ritar, Greg G. Smith, Craig W. Thomas-2006
Aquaculture 261(3): 1014-1025

Abstract:
Phyllosoma larvae of the southern rock lobster, Jasus edwardsii, were cultured from egg to juvenile. For larvae reared from hatch to Stage III, survival was highest and bacterial contamination was lowest in seawater ozonated at low and moderate levels (400 and 500 mV oxidation–reduction potential, ORP). By contrast, at high ozonation (600 mV), all larvae suffered deformities at the moult to Stage II and terminally starved, while in unozonated water (about 300 mV), all larvae died at Stage III probably as a consequence of Vibrio bacteria proliferation. In a second experiment between Stages VI to VIII, larval survival was highest in ozonated water that had been filtered through activated charcoal and coral sand, compared to ozonated water with no filtration or filtered only through activated charcoal. Ozonated water with the combined filtration was used subsequently but there were ongoing deformities, so the level was progressively reduced from 400 mV at Stage VIII to 330 mV at Stage X, at which time ozonation was discontinued. Larvae were then cultured in unozonated water to metamorphosis of eight pueruli at 377 to 437 days after hatch, of which two survived to juvenile. Ozonation was thus effective up to Stage IX in improving culture water to minimise bacterial disease without problems of larval deformities.

(Marine Research Laboratories, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Nubeena Crescent, Taroona, Tasmania 7053, Australia; email of A. Ritar: Arthur.Ritar@utas.edu.au)

EVALUATION OF B-(1 → 3, 1 → 6)-GLUCANS AND HIGH-M ALGINATE USED AS IMMUNOSTIMULATORY DIETARY SUPPLEMENT DURING FIRST FEEDING AND WEANING OF ATLANTIC COD (GADUS MORHUA L.)

Jorunn Skjermo, Trond R. Størseth, Karina Hansen, Aleksander Handå, Gunvor Øie-2006

Aquaculture 261(3): 1088-1101

Abstract:

Stimulation of the non-specific defence enhances the disease resistance and growth, and has good potentials as a measure for increased microbial control in juvenile production of marine fish and shellfish. So far, the most commonly used immunostimulants are β-(1 → 3, 1 → 6)-glucans, and in this study the stimulatory potential of a β-(1 → 3, 1 → 6)-glucan of marine origin, the storage polysaccharide from the marine diatom Chaetoceros mülleri, was examined. The glucan (chrysolaminaran) was extracted from cultures of C. mülleri, and used as a dietary supplement in two first feeding experiments with larvae of Atlantic cod Gadus morhua L. In one experiment the microalgal glucan was compared to the commercial yeast-glucan product MacroGard®, and in the other to an alginate with a high content of mannuronic acid (High-M alginate) isolated from Durvillaea antarctica. The stimulants were given via rotifers, and weaning to formulated feed was initiated at day 17 or 18 after hatching. The survival ± SEM at day 27 after hatching was 24.5 ± 2.0%, 14.8 ± 4.5% and 13.1 ± 1.4% for the groups fed C. mülleri-glucan, yeast glucan and for the control, respectively, in the first experiment. The group fed C. mülleri-glucan group had higher survival compared to the control ($P < 0.05$) group, whereas the yeast glucan had no positive effect on the survival ($p > 0.05$). The dry weights of the groups at day 27 were low, with 203.2 ± 52.2, 165.2 ± 43.4 and 198.5 ± 58.1 μg per larva for the C. mülleri-glucan, yeast glucan and control groups, respectively. In the second experiment the survival in the period of feeding formulated feed (days 18–30) were 44.6 ± 4.3%, 44.7 ± 1.3%, and 33.8 ± 4.1% survival for the C. mülleri-glucan, High-M alginate and control group, respectively. The cod larvae fed C. mülleri-glucan reached an average weight of 531.6 ± 17.2 μg at day 30, which was significantly higher ($p < 0.05$) than the control group that had an average of 473.6 ± 3.5 μg. The larvae fed High-M alginate had an average weight of 470.3 ± 31.6 μg per larva at day 30, and not significantly different from the control ($p > 0.05$). The early weaning to formulated diet had detrimental effect on the growth of the larvae. In both experiments the C. mülleri-glucan group was the only group showing a positive growth rate in the period of weaning to dry feed. The microbial conditions in larval gut and water were monitored with respect to total colony forming units on Marine agar, and Vibrio- and Pseudomonas-like species on selective agars (TCBS and marine Pseudomonas Agar with CFC-supplement). The larvae were rapidly colonised after hatching, but no or weak effects of the stimulants were observed on the colonisation rates or the composition. The total CFU varied from 101 to 102 CFU per μg larva after initiation of the first feeding. The percentages of Pseudomonas-like bacteria increased throughout the period, whereas the levels of Vibrio-like bacteria were low and stable. The chrysolaminaran from the diatom C. mülleri was shown to be a promising candidate for use as an immunostimulatory feed additive, and which should be further explored.

(Department of Marine Resources Technology, SINTEF Fisheries and Aquaculture, N-7465 Trondheim, Norway; email of Jorunn Skjermo: Jorunn.Skjermo@sintef.no)

PREPARATION OF MONOCLONAL ANTIBODY AGAINST MACROBRACHIUM ROSENBERGII NODAVIRUS AND APPLICATION OF TAS-ELISA FOR VIRUS DIAGNOSIS IN POST-LARVAE HATCHERIES IN EAST CHINA DURING 2000–2004

Dong Qiana, Wen Liu, Wu Jianxiang, Lian Yua-2006

Aquaculture 261 (4): 1144-1150

Abstract:

“Whitish muscle disease” of Macrobrachium rosenbergii, also called “whitish disease” or “white tail disease”, is a new serious epizootic disease that has occurred in recent years in giant freshwater prawn culture regions, mainly in southern China. This disease occurred in post-larvae 3–5 days to 3 weeks after desalting. Clinical signs include the development of white spot in muscles or milky muscles throughout the body, causing serious loss in few days, with a mortality rate of 40–90%. A 26–27 nm icosahedral non-enveloped virus, identified as M. rosenbergii Nodavirus (MrNV), was confirmed as the aetiological agent. Twelve hybridomas strongly secreting monoclonal antibodies (Mabs) against

MrNV were shown to be specific for MrNV and reacted with MrNV 42 kDa coat protein by Western blot. A triple antibody enzyme-linked immunosorbent assay (TAS-ELISA) was developed and shown to be a useful diagnostic tool for MrNV infection.
(Institute of Preventive Medicine, Zhejiang University, Hangzhou, 310029, China; email of Luan Yu: qdomonas@163.com

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BACTERIAL FLORA ASSOCIATED WITH THE GIANT FRESHWATER PRAWN MACROBRACHIUM ROSENBERGII, IN THE HATCHERY SYSTEM
Bob Kennedy, M.N. Venugopal, Indrani Karunasagar, Iddya Karunasagar-2006
Aquaculture 261(4): 1156-1167
Abstract:
The objective of this study was to understand the microbial flora associated with the hatchery system of giant fresh water prawn, Macrobrachium rosenbergii during an entire rearing cycle. Bacteriological and physico-chemical analysis was done for different samples of water, larvae, and Artemia. The total bacterial load in well water, seawater and inlet water varied from 101 to 105 cfu ml− 1 with higher counts seen in larval rearing tank (LRT) water. The Vibrio count ranged between 101 to 103 cfu ml− 1. Larval samples harboured a bacterial load of 106 to 107 cfu/10 larvae. The bacterial load in Artemia hatching water ranged from 4.90 × 104 to 5.63 × 106 cfu ml− 1 while Artemia had a load ranging from 1.08 × 107 to 2.09 × 109 cfu g− 1. Vibrio count in the LRT water ranged from 101–103 cfu ml− 1 while the count in larvae ranged from 102 to 104 cfu/10 larvae. The bacterial genera were predominantly Gram-negative and comprised of Aeromonas spp., Pseudomonas spp., Vibrio spp. and Bacillus spp. and non-spore formers (NSF) were the dominant Gram-positive bacteria. This study documents the bacterial flora associated with Macrobrachium hatchery system during a regular normal run. Knowledge of the qualitative and quantitative aspects of bacterial flora in the hatchery would help to understand disturbances, if any, brought about during disease outbreaks.
(Department of Fishery Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore - 575 002, India; email of Iddya Karunasagar: karuna8sagar@yahoo.com)

STUDIES ON THE ROTIFER (BRACHIONUS URCEUS LINNAEUS, 1758) AS A VECTOR IN WHITE SPOT SYNDROME VIRUS (WSSV) TRANSMISSION
Jia-Song Zhang, Shuang-Lin Dong, Xiang-Li Tian, Yun-Wei Dong, Xiang-Yi Liu, Dong-Chun Yan-2006
Aquaculture 261(4): 1181-1185
Abstract:
The rotifer Brachionus urceus (Linnaeus, 1758) was experimentally infected with the white spot syndrome virus (WSSV) by the virus–phytoplankton adhesion route in order to assess the possibility of rotifer acting as a vector of WSSV to infect the shrimp Fenneropenaeus chinensis (Osbeck, 1765) larvae at zoea stage III. The nested-PCR test revealed WSSV-positive results in the rotifers exposed to WSSV by the virus–phytoplankton adhesion route. Among 10 replicates in the infection treatment, 40% of F. chinensis larvae became WSSV-positive when fed with WSSV-positive rotifers, whereas all were WSSV-negative for F. chinensis when fed with WSSV-free rotifers. Though the mortality of shrimp larvae in the infection treatment (39.47 ± 15.44%) was higher than that in the control treatment (34.67 ± 15.11%), there was no significant difference in the mortality between them ($P > 0.05$). These results indicated that the rotifer could serve as a vector in WSSV transmission when ingested.
(The Key Laboratory of Mariculture, Ministry of Education Fisheries College, Ocean University of China, Qingdao 266003, PR China; email of Dong-Chun Yan: dongsl@mail.ouc.edu.cn)

PRODUCTIVITY IMPROVEMENT OF LYSMATA SETICAUDATA (RISSO, 1816) LARVAL REARING PROTOCOL THROUGH MODELLING

Joana Figueiredo, Luís Narciso-2006
Aquaculture 261(4): 1249-1258
Abstract:
The Monaco shrimp Lysmata seticaudata (Risso, 1816) is a marine ornamental species whose ecology and biology, as well as its larval culture has previously been addressed. The objective of the study was to predict and improve productivity of this species rearing protocol through modelling. The models developed intend to help aquaculturists to maximize survival to postlarva, decrease larval duration and increase synchronism of metamorphosis and newly metamorphosed postlarvae size by manipulating temperature, diet, first feeding period and stocking density.
The models developed allow us to conclude that the L. seticaudata rearing protocol productivity can be improved by raising larvae at a density of 40 larvae L− 1 and fed newly hatched Artemia nauplii since hatching to zoea V, and with Algamac 2000™ enriched Artemia metanauplii from zoea V to metamorphosis to postlarvae.
By providing more productive protocols to aquaculturists, destructive practices and wild collection may be reduced.
(Laboratório Marítimo da Guia / IMAR, Dep. de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Estrada do Guincho, Forte N. S. da Guia, 2750-374 Cascais, Portugal; email of Joana Figueiredo: joana_figueiredo@portugalmail.pt)

EFFECT OF NITRITE ON LARVAL DEVELOPMENT OF GIANT RIVER PRAWN MACROBRACHIUM ROSENBERGII
Margarete Mallasena, Wagner Cotroni Valenti-2006
Aquaculture 261(4): 1292-1298
Abstract:
The effects of ambient nitrite concentrations on larval development of giant river prawn Macrobrachium rosenbergii were evaluated. The trials were conducted in two phases: phase 1, larvae from stages I through VIII and phase 2, larvae from stage VIII until post-larvae. In both phases larvae were kept in water with nitrite (NO2–N) concentrations of 0, 2, 4, 8 and 16 mg/L. Oxygen consumption was analyzed for larvae in stage II at nitrite concentrations of 0, 4, and 8 mg/L. Survival, weight gain, larval stage index and metamorphosis rate decreased linearly with increasing ambient nitrite concentration. However, there was no significant difference between larvae subjected to 0 and 2 mg/L NO2–N. In phase 1, there was total mortality at 16 mg/L NO2–N, while in phase 2 larval development stopped at stage X in this treatment. The oxygen consumption in stage II increased significantly at NO2–N concentration from 0 to 4 mg/L, but there was no difference between 4 and 8 mg/L NO2–N. In conclusion, increasing ambient nitrite up to 16 mg/L NO2–N delays larval development, reduces larval growth rate and causes mortality, whereas no significant effect occurs for levels below 2 mg/L NO2–N. However, the establishment of a general safe level of nitrite to M. rosenbergii hatchery may be difficult due to the great variability in larvae individual sensitivity.
(Centro Avançado do Pescado Continental, Instituto de Pesca, Caixa Postal 1052, 15025-970, São José do Rio Preto, SP, Brazil; email of Margarete Mallasena: maga@pesca.sp.gov.br)

MICROINJECTION OF THE ANTIFREEZE PROTEIN TYPE III (AFPIII) IN TURBOT (SCOPHTHALMUS MAXIMUS) EMBRYOS: TOXICITY AND PROTEIN DISTRIBUTION V. Robles E. Cabrita, L. Anel, M.P. Herráez-2006
Aquaculture 261(4): 1299-1306
Abstract:
Fish embryo cryopreservation has not been achieved. Different methods and alternative cryoprotective agents (CPAs) should be explored in order to succeed in this purpose. Antifreeze proteins (AFPs) are naturally expressed in sub-arctic fish species, and they inhibit the growth of ice crystals as well as recrystallization during thawing. Therefore, their introduction into embryos can be highly beneficial for vitrification purposes. In this study, AFP type III was introduced into turbot embryos, by microinjection into the yolk sac and the perivitelline space at F stage (tail bud).Toxicity

and distribution of protein in microinjected embryos were established before testing the protein effect on embryo cryopreservation. AFP-FITC distribution within the embryo was analyzed by confocal microscopy at 5 min and 24 h after microinjection in F stage embryos. To test the sensitivity of microinjected embryos to CPAs, embryos were subjected to a protocol for the incorporation of a vitrifying solution that was specially designed for turbot embryos. Hatching rates after CPA incorporation were determined. Results indicate that embryos at late developmental stages are more resilient to microinjection, with embryo survival rates between 60 and 82%. Confocal microscopic images demonstrated that the protein was homogeneously distributed within the microinjected embryo compartment, but did not enter any other compartment. On the other hand, microinjected embryos successfully surmounted their incubation in the CPAs. This study explores new alternatives for cryopreservation suggesting the use of natural cryoprotectants (AFPs) in the protection of intra-embryo compartments, which are usually unprotected with the conventional cryopreservation protocols for fish embryos.
(CCMAR, Center for Marine Sciences, University of Algarve, Campus de Gambelas, Faro, 8005-139, Portugal; email of Robles E. Cabrita: dbcvrr@unileon.es)

THE EFFECTS OF SUPPLEMENTAL DIETARY CHOLESTEROL ON GROWTH, DEVELOPMENT AND SURVIVAL OF MUD CRAB, SCYLLA SERRATA, MEGALOPA FED SEMI-PURIFIED DIETS

May-Helen Holme, Chaoshu Zeng, Paul C. Southgate-2006

Aquaculture 261(4): 1328-1334

Abstract:
The effects of varying levels of dietary cholesterol on growth, development time and survival of mud crab, Scylla serrata megalopa were investigated using semi-purified microbound diets (MBD). Five iso-energetic diets containing different level of cholesterol ranging from 0.14% to 1% of dry weight of the diet were tested. Fifteen megalopa were reared individually for each dietary treatment, and development time and survival were recorded on a daily basis. More than 25% of megalopa from all treatments were able to metamorphose into the first crab stage, suggesting that the endogenous level of cholesterol in the basal diet (0.14%) was sufficient to support development of the megalopa stage of this species. Widest mean carapace width (3.53 ± 0.08 mm) and highest mean dry weight (2.11 ± 0.22 mg) were recorded for juveniles that molted from megalopa fed live Artemia, whereas no megalopa in the unfed control treatment metamorphosed into crabs. The average development time from megalopa to the juvenile crab stage varied between the treatments, where megalopa fed live Artemia or MBD containing 0.2%, 0.4% or 0.8% total cholesterol showed the most synchronized molting (between 8.0 and 9.9 days). Longest development time was recorded for the megalopa fed diets containing 0.14% or 1% total cholesterol (both 11 days). Highest survival (74.3%) was recorded for the megalopa fed a diet containing 0.8% cholesterol. The results of this study are valuable in research to develop formulated diets for mud crab larvae as a replacement for live food in hatchery culture.
(AIMS@JCU, Australian Institute of Marine Science, School of Marine Biology and Aquaculture, James Cook University, Townsville, Queensland 4811, Australia; email of May-Helen Holme: mayhelen.holme@jcu.edu.au

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SUCCESSFUL CULTURE OF LARVAE OF LITOPENAEUS VANNAMEI FED A MICROBOUND FORMULATED DIET EXCLUSIVELY FROM EITHER STAGE PZ2 OR M1 TO PL1

Louis R. D′Abramo, Elifonso Isiordia Perez, Ravi Sangha, Ana Puello-Cruz-2006

Aquaculture 261(4): 1356-1362

Abstract:
In three separate experiments, harpaticoid copepods Tisbe monozota (alive and dead) and a microparticulate microbound diet were evaluated as alternatives to live Artemia nauplii as food, beginning at either stage PZ2 or M1, in the larval culture of Litopenaeus vannamei. Larvae were

cultured in 2 L round bottom flasks at a density of 150 L− 1 (Experiment 1) and 100 L− 1 (Sections 3.2 and 3.3) at 28 °C, 35‰ salinity and 12:12 LD photoperiod, and fed 4×/day− 1. Larvae were initially fed a mixture of phytoplankton to stages PZ2 or M1 and then fed either live Artemia, live or dead copepods, or a microparticulate microbound diet. The experiments were terminated and all larvae were harvested when more than 80% of larvae had molted to postlarvae 1 (PL1) within any flask representing any of the treatments. The comparative value of the different diets and feeding regimes was determined by mean survival, mean dry weight and total length of individual larva, and percentage of surviving larvae that were PL1. Trypsin activity of samples of larvae from each treatment was also determined. The microparticulate microbound diet effectively served as a complete substitute for Artemia nauplii when fed beginning at stage M1. When fed at the beginning of the PZ2 stage, survival was comparable to that of larvae fed Artemia, but mean dry weight, mean total length, and percent of surviving larvae that were PL1 generally were significantly less. Responses to the feeding of copepods, whether fed dead or live, as a substitute were generally significantly less than those of larvae fed either the Artemia nauplii or the microparticulate diet. Values of trypsin activity (10− 5 IU/μg− 1 dry weight) corresponded to the relative proportions of the different larval stages within a treatment, with higher activity being characteristic of early stages. Previously demonstrated successful results with another species of crustacean suggest that the microparticulate microbound diet has characteristics that should be effective in the culture of the carnivorous stages of other crustacean and fish larvae that are currently fed live Artemia nauplii.
(Department of Wildlife and Fisheries, Mississippi State University, Box 9690, Mississippi State, MS, 39762 USA; email of Ana Puello-Cruz: puello@victoria.ciad.mx)

EFFECTS OF NA+/K+ AND MG2+/CA2+ RATIOS IN SALINE GROUNDWATERS ON NA+–K+-ATPASE ACTIVITY, SURVIVAL AND GROWTH OF MARSUPENAEUS JAPONICUS POSTLARVAE
Lu-Qing Pan, Zhi-Hua Luan, Cai-Xia Jin-2006
Aquaculture 261(4): 1396-1402
Abstract:
The effects of the Na+/K+ and Mg2+/Ca2+ ratios in saline groundwaters on Na+–K+-ATPase activity, survival and growth of Marsupenaeus japonicus postlarvae were investigated. The results indicate that the Na+–K+-ATPase activity, survival rate and weight gain of postlarvae were significantly affected by the Na+/K+ and Mg2+/Ca2+ ratios ($P < 0.05$). The Na+–K+-ATPase activity of postlarvae, in every treatment, changed corresponding to Na+/K+ and Mg2+/Ca2+ ratios, and came to a stable level after 24 h. There was a negative relation between Na+–K+-ATPase activity and Na+/K+ ratio, while there was a positive relation between Na+–K+-ATPase activity and Mg2+/Ca2+ ratio. Compared with seawater (the Na+/K+ and Mg2+/Ca2+ ratios are 27.8 and 4.64 respectively), the Na+–K+-ATPase activity of the Na+/K+ ratio 30 treatment showed no significant difference, while the Mg2+/Ca2+ ratio 4.5 treatment showed distinct difference. The survival rates and weight gain of postlarvae increased markedly when the suitable amount of K+ and Ca2+ was added to test water, and arrived at their maximum in the Na+/K+ ratio 20–30 or Mg2+/Ca2+ ratio 4.5 treatment, having no significant difference compared with normal seawater. Therefore, considering the Na+/K+, Mg2+/Ca2+ ratios and the absolute concentration of Mg2+, Ca2+ in the experimental saline groundwaters applied to Marsupenaeus japonicus farming, it should be modulated to around 30, 4.5 and 1312 mg/l, 291 mg/l, respectively.
(The Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China; email of Lu-Qing Pan: panlq@ouc.edu.cn)

DEVELOPMENTAL REGULATION OF GASTRIC PEPSIN AND PANCREATIC SERINE PROTEASE IN LARVAE OF THE EURYHALINE TELEOST, OREOCHROMIS MOSSAMBICUS
Ming-Ji Lo, Ching-Feng Weng-2006
Aquaculture 261(4): 1403-1412

Abstract:
Most recent research on teleost digestive enzymes has focused on the immunohistochemistry of flounder and catfish, fewer studies have been done of gene expression. The present study is made an attempt to clarify the expression of digestive enzymes in tilapia (Oreochromis mossambicus) during larval development. This work was done by cloning the digestive enzymes, semi-quantifying the expression of genes by RT-PCR-Southern blot, detecting the expression of proteins (western blot) and investigating enzymatic activity. Under microscopic observation, the mouths of tilapia larvae were seen to open on the third day after hatching and on the fifth day after hatching, the actinotrichia were reabsorbed and the larvae began feeding. The partial nucleotide sequences of pepsinogen, trypsinogen and chymotrypsinogen were obtained. After RT-PCR and southern blotting analysis, the expressing pattern of pepsinogen mRNA appeared on day 2 after hatching, while mRNAs of trypsinogen and chymotrypsinogen were both detected 1 day after hatching. The proteins of larval pepsin, trypsin and chymotrypsin appeared 1 day after hatching, and specific activities of these enzymes were detectable on day 3 (for pepsin) and day 5 (for trypsin and chymotrypsin) after hatching. The transcription of the genes for these three digestive enzymes gradually increased after hatching and that all protein detected on day 1, suggesting that all proteins may result from maternal sources.
(Institute of Biotechnology, National Dong Hwa University, Hualien, Taiwan 974; email of Ching-Feng Weng: cfweng@mail.ndhu.edu.tw)

SUSPENSION OF ANNUAL GAMETOGENESIS IN NORTH AMERICAN GREEN SEA URCHINS (STRONGYLOCENTROTUS DROEBACHIENSIS) EXPERIENCING INVARIANT PHOTOPERIOD—APPLICATIONS FOR LAND-BASED AQUACULTURE

S. Anne Böttger, Michael G. Devin, Charles W. Walker-2006

Aquaculture 261(4): 1422-1431

Abstract:
Sea urchin fisheries are valuable commercial resources in the United States with processed gonads sold in Japanese and American markets and maximum US sales of $150M US dollars in 1996. Wild populations of sea urchins on all coasts of the US have been dramatically over-fished. Aquaculture of sea urchins in land-based facilities can help restore commercial populations and preserve this ecologically important herbivore. In this study, we used invariant summer photoperiod to prevent gametogenesis in the North American green sea urchin (Strongylocentrotus droebachiensis) maintained in a land-based aquaculture system and provided a commercially available formulated feed that promotes maximum growth of intra-gonadal somatic nutrient storage cells called nutritive phagocytes. Results were compared with individuals fed the same formulated feed under ambient photoperiod in cages in the ocean. Monthly samples of gonads from both treatments were evaluated for gonad index, volume fractions of cellular constituents of the germinal epithelium, oocyte diameters and taste. Over the 5 months of this study, gonad indices increased significantly ($p < 0.001$) in both treatments from 4.8% ± 0.9 (all values ± SE) initially to 20.5% ± 2.1 under invariant and 23.2% ± 1.4 under ambient photoperiod with no significant difference between treatments ($p = 0.55$). Volume fractions of nutritive phagocytes increased to 80.3% ± 5.9 (initial 37.9% ± 7.1) in males and 71.0% ± 6.7 (initial 10.3% ± 4.0) in females ($p < 0.001$) only under invariant photoperiod. Nutritive phagocyte lengths increased under both photoperiod treatments, but the volume fraction containing nutrients was higher under invariant photoperiod. Volume fractions of gonial/gametogenic cells increased significantly ($p < 0.001$) only under ambient photoperiod from 20.4% ± 5.5 to 37.8% ± 1.8 in males and 0% to 22.6% ± 3.6 in females. The volume fraction of residual oocytes from last year's oogenesis increased under invariant photoperiod while that of both residual and new oocytes increased under ambient photoperiod. Residual oocyte diameters increased from 56.2 μm ± 2.2 initially to 93.5 μm ± 3.7 under invariant and those of residual and new oocytes to 126.0 μm ± 7.3 under ambient photoperiod. Invariant photoperiod yields gonads in both sexes of S. droebachiensis that do not initiate fall gametogenesis but attain large size as their nutritive phagocytes grow substantially in size. A Canadian study of wild-collected S. droebachiensis indicated that gonads taste best when they contain pre-dominantly nutritive phagocytes and not copious gametes, however gonad

taste in our study was unsatisfactory suggesting that the only commercially available sea urchin diet requires modification to support commercial development of land-based aquaculture.
(Department of Zoology, Center for Marine Biology and Marine Biomedical Research Group, University of New Hampshire, Durham, New Hampshire 03824, United States; email of Charles W. Walker: cwwalker@christa.unh.edu)

EARLY DEVELOPMENT OF THE SHORTFIN SILVERSIDE CHIROSTOMA HUMBOLDTIANUM (VALENCIENNES, 1835) (ATHERINIFORMES: ATHERINOPSIDAE)
Ma. Cecilia Hernández-Rubio, Gerardo Figueroa-Lucero, Irene de los A. Barriga-Sosa, José Luis Arredondo-Figueroa, Thalía Castro-Barrera-2006
Aquaculture 261(4): 1440-1446
Abstract:
The shortfin silverside Chirostoma humboldtianum has been considered for culture in Mexico, but success has been limited by a poor knowledge of its early development. First synthesis of the early development of the shortfin silverside is presented to determine conditions suitable for rearing. Brooder maturation was induced through photothermal cycles. C. humboldtianum ova were fertilized in vitro. The eggs were incubated in reconstituted water (160–180 mg/L CaCO3) at 18 °C and 5 gm of NaCl per litre. During the hatching day, 300 shortfin silversides were stocked and followed up until metamorphosis in order to establish the timing of exogenous feeding, changes in food type, growth and development during critical periods for survival, according to the theory of saltatory ontogeny. Free embryos hatched 12 days after fertilization at 18 °C. First critical point for survival is the beginning of exogenous feeding. Free embryos started mixed feeding on day four of post-hatching (dph), point of no-return was presented towards the end of mixed feeding on 6 dph, larval period began at six (dph) when the anus is opened, and metamorphosis to juvenile was presented at 65 dph with a SL of 19.34 ± 2.28 mm, when scales and fins were well developed. Differences in growth between periods were detected: free embryos growth slower than larvae but mouth size depicted a larger growth rate in the former. Cephalic length and mouth size were negatively related to standard length in embryos and larvae. Mouth size was positively related to cephalic length in free embryos but negative in larvae. Results suggest that during the free embryo phase, growth priorities are directed to the development of apparatuses and systems; whereas, during the larval period, energy is directed to growth in length, mouth size and development of fins, which allows them to increase their swimming velocity, grants them a greater capacity to obtain exogenous food and, in consequence, increases fitness for survival.
(Laboratorio de Hidrobiología Experimental, Departamento de Zoología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Prolongación de Carpio y Plan de Ayala, Santo Tomás, México, D. F., C. P. 11340, México; email of Ma. Cecilia Hernández-Rubio: mhernaru@encb.ipn.mx)

SEROTONIN STIMULATES OVARIAN MATURATION AND SPAWNING IN THE BLACK TIGER SHRIMP PENAEUS MONODON
Kanokpan Wongprasert, Somluk Asuvapongpatana, Pisit Poltana, Montip Tiensuwan, Boonsirm Withyachumnarnkul-2006
Aquaculture 261(4): 1447-1454
Abstract:
Serotonin (5-hydroxytryptamine, 5HT) has been reported to induce ovarian maturation and spawning in the crayfish Procambarus clarkii and white Pacific shrimp Litopenaeus vannamei. The aim of this study was to explore the role of exogenous 5HT on the reproductive performance of the black tiger shrimp Penaeus monodon. 5HT solution was injected into domesticated P. monodon broodstock at 50 μg/g body weight and ovarian maturation and spawning were recorded. The presence of 5HT in the ovary and oviduct of P. monodon was also studied by immunohistochemistry and its levels in the ovary by enzyme link immunoabsorbance assay (ELISA). The 5HT-injected P. monodon developed ovarian maturation and spawning rate at the level comparable to that of unilateral eyestalk-ablated

shrimp. Hatching rate and the amount of nauplii produced per spawner were also significantly higher in the 5HT-injected shrimp, compared to the eyestalk-ablated shrimp. 5HT-positive reactions were found in the follicular cells of pre-vitellogenic oocytes, in the cytoplasm of early vitellogenic oocytes and on the cell membrane and cytoplasm of late vitellogenic oocytes. 5HT in the ovary was present at 3.53 ± 0.26 ng/mg protein level in previtellogenic stage and increased to 17.03 ± 0.57 ng/mg protein level in the mature stage of the ovary. The results suggest a significant role of 5HT, possibly directly on the ovary and oviduct, on the reproductive function of female P. monodon.
(Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Rd, Rajdhevi, Bangkok 10400, Thailand; email of K.Wongprasert: sckbp@mahidol.ac.th)

PREDATION BY SCAVENGING AMPHIPODS TO INJURED HATCHERY-RAISED JUVENILE JAPANESE FLOUNDER PARALICHTHYS OLIVACEUS UNDER LABORATORY CONDITIONS

Keiichiro Ide, Kazutaka Takahashi, Koichi Sasaki, Michio Omori-2006
Fisheries Science 72(6): 1209-1214
Abstract:
The attacking potential of the scavenging amphipod Scopelocheirus onagawae on artificially injured hatchery-raised Japanese flounder Paralichthys olivaceus juveniles was investigated in relation to the degree of injury on the fish. All injured flounder juveniles were attacked by amphipods regardless of the degree of injury, while non-injured juveniles were not attacked. The attack by amphipods on the juveniles generally depended on the amount of glycine, a main feeding stimulant for the amphipod, released from the injury opening. The swimming ability of flounder juveniles was important to avoid the attack of amphipods. Furthermore, an area of injury allowing the amphipods to cling to the fish affects to the vulnerability of juveniles against the predation of amphipods. This study suggests that scavenging amphipods are potentially involved in the rapid reduction of the number of hatchery-raised juveniles.
(Tohoku National Fisheries Research Institute, Shiogama, Miyagi 985-0001, Japan ; email of Keiichiro Ide: keiichir@affrc.go.jp)

MEASUREMENT OF TEMPERATURE AND SALINITY EFFECTS ON OXYGEN CONSUMPTION OF ARTEMIA FRANCISCANA K., MEASURED USING FIBRE-OPTIC OXYGEN MICROSENSORS

Sandra Irwin, Vanessa Wall, John Davenport-2006
Hydrobiologia 575(1): 109-115
Abstract :
Oxygen consumption rates of nauplii of the brine shrimp Artemia franciscana Kellogg 1906 were determined over a range of salinities from 10 to 110 ppm, in temperatures from 0 to 30°C, using a multi-factorial design. The oxygen micro-sensors employed have a fast response time and are capable of accurately measuring oxygen concentrations at temperatures well below 0°C. Oxygen uptake rate ranged from 0.03 to 0.66 μmol O2 mg−1 h−1 and was sensitive to changes in both salinity and temperature. Temperature was the dominant factor affecting oxygen consumption rates, which showed a significant increase with increasing temperature. A slight decrease was measured in oxygen consumption with increasing salinity related to differential solubility of oxygen in waters of different salinities. Thermal sensitivity of oxygen consumption determined from calculations of Q 10, indicated physiological adaptation of Artemia nauplii to the ranges of temperatures tested.
(Department of Zoology, Ecology & Plant Science and Environmental Research Institute, University College Cork, Distillery Fields, North Mall, Cork, Ireland; email of Sandra Irwin: s.irwin@ucc.ie)
