

WHY WON'T THEY GROW? – INHIBITORY SUBSTANCES AND MOLLUSC HATCHERIES

B. Jones-2006

Aquaculture International 14(4): 395-403

Abstract: Molluscs are known to be seriously affected by trace amounts of environmental pollutants such as tributyltin at concentrations in seawater that are below the level of detection by all but the most sensitive chemical analytical techniques. This extreme sensitivity by molluscs has led to use of both adults and larvae as biomonitors for environmental pollution. Mollusc aquaculture has led to an increasing demand for commercial hatcheries to supply seed stock, including selected genetic lines of spat and juveniles. It is becoming apparent that many of the unexplained “crashes”, ill thrift or failures of larvae to metamorphose in such hatcheries are primarily due to their being compromised for a range of reasons including traces of inhibitory or toxic substances in the water supply. Because dead and dying larvae are ideal substrates for bacterial and ciliate growth, such invaders are often assumed to be the primary cause of the problem and this hinders finding a solution. In addition, many of the toxins which may be implicated in crashes are sporadic in occurrence and are both difficult to detect and hard to remove from the water supply. This paper provides evidence for these toxic effects and suggests ways of reducing the problems.

(Department of Fisheries, Government of Western Australia, P.O. Box 20, North Beach, WA, 6920, Australia; email of J.B. Jones: bjones@agric.wa.gov.au)

CHEMICAL CUES PROMOTE SETTLEMENT IN LARVAE OF THE GREEN-LIPPED MUSSEL, Perna canaliculus

Andrea C. Alfaro, Brent R. Copp, David R. Appleton, Shane Kelly, Andrew G. Jeffs-2006

Aquaculture International 14(4): 405-412

Abstract:

The large (70,000 tonnes) mussel aquaculture industry for the endemic New Zealand green-lipped mussel, *Perna canaliculus* Gmelin, currently relies almost entirely (80%) on wild caught seed that is often harvested attached to great quantities of floating algae. Natural vagaries in catches of wild mussel seed frequently result in shortages of mussel seed that, at times, have severely affected commercial aquaculture production. Both field and laboratory experiments were used to establish that chemical cues derived from algae significantly increase settlement of mussel larvae on artificial substrates. This is the first time an algal chemical cue has been implicated in the settlement behavior of *P. canaliculus* larvae. These results have potential commercial implications for improving mussel seed supply through inducing higher or more reliable levels of mussel settlement for aquaculture seed collection.

(Division of Applied Sciences, Auckland University of Technology, Private Bag 92006, Auckland, 1020, New Zealand; email of Andrea C. Alfaro: andrea.alfaro@aut.ac.nz)

REARING ZEBRAFISH (DANIO RERIO) LARVAE WITHOUT LIVE FOOD: EVALUATION OF A COMMERCIAL, A PRACTICAL AND A PURIFIED STARTER DIET ON LARVAL PERFORMANCE

António Paulo Carvalho, Leonor Araújo, Miguel M Santos-2006

Aquaculture Research 37(11): 1107-1111

Abstract:

Owing to the increasing importance of zebrafish as a vertebrate model in many fields of research, efforts must be made to breed and maintain this species in laboratory. Zebrafish larvae are traditionally reared on cultured live paramecia during the first 9 days of exogenous feeding, followed by a combination of paramecia and artemia nauplii until day 21, making larval rearing expensive, labour intensive and unpredictable. Thus, a trial was conducted with zebrafish larvae in order to evaluate the suitability of artificial diets as an alternative to live food during the first 21 days of

exogenous feeding. Five dietary treatments were tested: (1) artemia nauplii; (2) a commercial; (3) a purified; (4) a practical diet, all delivered continuously; (5) the same practical diet delivered manually. The best overall larval performance was achieved in the group fed artemia nauplii (86% survival, 14.3 mm standard length, 46.1 mg wet weight). Compared with existing results obtained with the traditional live food schedule, our results suggest that paramecia might not be the most suitable first food for zebrafish, and that artemia nauplii could be used as the only live food. The present work demonstrates that zebrafish larvae can be reared without live food with a significant growth and a high survival, provided that an appropriate artificial diet is presented in a continuous way. Among the diets tested, the practical diet, if continuously delivered, led to the best performance assuring a mean standard length of 72% of that obtained with artemia and a similar survival rate. Moreover, the purified diet, supporting over 50% survival and an appreciable growth, could be useful in some toxicological studies in which a well-defined diet is needed.

(Departamento de Zoologia e Antropologia, Faculdade de Ciências da Universidade do Porto, Pr. Gomes Teixeira, 4099-002 Porto, Portugal; email of A Paulo Carvalho: apcarval@fc.up.pt)

LARVAL DEVELOPMENT IN EUROPEAN HAKE (MERLUCCIUS MERLUCCIUS L.) REARED IN A SEMI-INTENSIVE CULTURE SYSTEM

Reidun Marie Bjelland, Anne Berit Skiftesvik-2006

Aquaculture Research 37(11): 1117-1129

Abstract :

Eggs of European hake (*Merluccius merluccius* L.) were stripped from fish caught at sea. Larvae were kept under semi-intensive conditions at around 12°C. In addition, eggs were incubated in single wells at 9.2, 12.7 and 14.5°C, where hatching, development and survival were closely examined. During the larval stage, a total of 299 larvae were sampled to follow development and growth. In addition a small number of juveniles were sampled. Larvae hatched approximately 4 days after fertilization, and were 2.9 mm in total length (TL). At 6-day post hatching (dph), the larvae were 4.1 mm (TL), the jaw apparatus was developed, and the larvae had started to feed. Most of the growth during the early larval period is restricted to the head, and there is almost no increase in length for the first 3–4 weeks post hatching. Teeth and pelvic fins appear at 25 dph. Development of unpaired fins at approximately 30 dph marks the start of the larval–juvenile transition. Weaning to formulated feed was accomplished 50 dph, when external morphology was similar to that of adult hake.

(Institute of Marine Research, Austevoll, N-5392 Storebø, Norway; email of R M Bjelland: reidun.bjelland@imr.no)

BACTERIAL LOAD REDUCTION OF LIVE PREY FOR FISH LARVAL FEEDING USING OX-AQUACULTURE©

Gemma Giménez, Francesc Padrós, Ana Roque, Alicia Estévez, Dolores Furones-2006

Aquaculture Research 37 (11): 1130-1139

Abstract :

Reduction of the bacterial load in live prey is paramount to achieving efficient microbiological control during fish larval rearing. Several methods have been tested using physical and chemical disinfection procedures. Nevertheless, chemicals are difficult to deal with because of the frequent problems encountered when disposing of their residual products and because of the sensitivity of the live prey. Hydrogen peroxide is a good disinfectant that has been used for a long time in other fields of research and decomposes in non-toxic products. However, it has not been applied for disinfection in aquaculture until recent years. The effects of a hydrogen peroxide-based product, Ox-Aquaculture©, on rotifers and *Artemia* nauplii survival and on their associated microbial population have been tested in the present study, a disinfection protocol is proposed, and its effects on live prey fatty acids composition after enrichment were checked. More than 80% survival and a 90% reduction of total heterotrophic bacteria and Vibrionaceae were obtained in rotifers exposed for 15 min to 40 mg L⁻¹ of the product. In the case of *Artemia* nauplii, 90% survival and 94.5% reduction of heterotrophic bacteria were obtained after 5 min exposure to 8000 mg L⁻¹ with a further 82.8% reduction of

Vibrionaceae. No differences in the fatty acid composition of the live prey were detected after disinfection with hydrogen peroxide. No oxidation of polyunsaturated fatty acids was observed. A short time exposure and easy removal of the product from the treated live prey make hydrogen peroxide an interesting chemical for industrial application.

(Centre d'Aquicultura-IRTA, Ctra. Poble Nou Km 7.5, 43540 Sant Carles de la Ràpita, Tarragona, Spain; email of A Estévez: alicia.estevez@irta.es)

EMBRYONIC AND LARVAL DEVELOPMENT OF HONEYCOMB GROUPEP EPINEPHELUS MERRA BLOCH

I. Jagadis, Bobby Ignatius, D. Kandasami, Md. Ajmal Khan-2006

Aquaculture Research 37(11): 1140-1145

Abstract :

Spawning and successful rearing of larvae of honeycomb grouper *Epinephelus merra* Bloch 1793 upto juvenile stage was accomplished at the finfish hatchery of Mandapam Regional Centre of Central Marine Fisheries Research Institute during 2004. The fertilized eggs were free, spherical and buoyant with size ranging from 710 to 730 µm. Complete early embryonic development took place within 24–27 h and hatching occurred. The hatchlings measured 1.5 mm. Mouth opening (115 µm) appeared at 72 h when the larvae were 2.2 mm in size. Pectoral fin developed on the fifth day. Complete metamorphosis took place and by the 60th day the larvae transformed into juveniles (45 mm) and attained skin colouration and honeycomb pattern.

(Regional Centre of Central Marine Fisheries Research Institute, Marine Fisheries PO, Mandapam Camp, Tamil Nadu, India; email of I. Jagadis: ijagadis@sify.com)

ROLE OF FREE NEUROMASTS IN LARVAL FEEDING OF WILLOW SHINER GNATHOPOGON ELONGATUS CAERULESCENS TELEOSTEI, CYPRINIDAE

Yukinori Mukai-2006

Fisheries Science 72(4): 705-709

Abstract:

It has been reported that the larvae of willow shiner *Gnathopogon elongatus caeruleus* have many free neuromasts on their body surface. This study examined the ability of the willow shiner larvae to feed on zooplankton by mechanoreception by the free neuromasts. Feeding experiments using untreated larvae and larvae treated with streptomycin, which impairs free neuromast function, were conducted under light and completely dark conditions. The larvae were put into Petri dishes, then nauplii of *Artemia salina* were introduced. The average number of *Artemia* eaten by the larvae was expressed as the average ingestion rate of *Artemia*/10 min. The ingestion rate of *Artemia* for untreated larvae was 12.3 under light conditions and 10.6 *Artemia*/10 min. even in complete darkness. The ingestion rate in the larvae treated with streptomycin was 11 under light conditions and only 0.8 *Artemia*/10 min under dark conditions. The low rate in the treated larvae under dark conditions must be due to impairment by the streptomycin. Therefore, the high ingestion rate for the untreated larvae under dark conditions would be surely dependent on mechanoreception. The results indicate that larval willow shiner is able to feed on zooplankton under limited light conditions.

(Borneo Marine Research Institute, Universiti Malaysia Sabah, Locked Bag 2073, 88999 Kota Kinabalu, Sabah, Malaysia; mukai9166@yahoo.co.jp)

LARVAL MOLTING AND GROWTH OF THE JAPANESE SPINY LOBSTER PANULIRUS JAPONICUS UNDER LABORATORY CONDITIONS

Hirokazu Matsuda, Taisuke Takenouchi-2006

Fisheries Science 72(4): 767-773

Abstract:

Ten newly hatched phyllosoma of *Panulirus japonicus* were cultured individually to monitor body length (BL) and intermolt period, and 2000 were cultured in groups to sample specimens for

measurement of body weight. Phyllosoma were fed with *Artemia* and mussel gonad; the culture seawater temperature was 24–26°C. The individually cultured phyllosoma showed an increment in body length by the first molt of approximately 0.5 mm, and the molt increment increased to approximately 1 mm at 5 mm BL; it was constant to 15 mm BL. Thereafter, the molt increment increased exponentially. The duration of the first instar was 6–7 days. Instar duration increased with development up to approximately 2 weeks at the 20th instar (16 mm BL) and then became constant. Of the 10 larvae reared individually, five metamorphosed to the puerulus stage. The entire phyllosoma life ranged from 245–326 days (mean 289.0 days), and the number of instars ranged from 22–29 (mean 26.2). Body length in the final instar ranged from 28.50–33.10 mm (mean 30.280 mm). For the phyllosoma cultured in groups, relationships between BL and wet/dry body weights (WW/DW, mg) were expressed as exponential equations: $WW = 0.0686BL^{2.2023}$ and $DW = 0.0209BL^{2.1905}$. (Fisheries Research Division, Mie Prefectural Science and Technology Promotion Center, 3564-3 Hamajima, Shima Mie 517-0404, Japan; email of Hirokazu Matsuda: matsuh07@pref.mie.jp)

GROWTH HORMONE-LIKE SUBSTANCE IN THE ROTIFER BRACHIONUS PLICATILIS
Wenresti G. Gallardo, Atsushi Hagiwara, Kenji Hara, Kiyoshi Soyano-2006

Fisheries Science 72(4): 781-786

Abstract:

A growth hormone (GH)-like substance was extracted from the rotifer *Brachionus plicatilis* and subsequently purified by gel filtration and ion exchange chromatography. The GH-like substance had a molecular weight of approximately 28 kDa and had cross-reactivity with salmon GH antibody. In vivo bioassay showed a higher intrinsic rate of increase and net reproduction rate of *B. plicatilis* treated with the GH-like substance.

(Graduate School of Marine Science and Engineering, Nagasaki University, Bunkyo, Nagasaki 852-8131, Faculty of Fisheries, Nagasaki University, Bunkyo, Nagasaki 852-8521, Japan; email of Wenresti G. Gallardo: gallardo@ait.ac.th)

SHORT PAPER

PRODUCTION OF DOCOSAHEXAENOIC ACID BOUNDED PHOSPHOLIPIDS VIA PHOSPHOLIPASE A2 MEDIATED BIOCONVERSION

Shinichi Awano, Koji Miyamoto, Masashi Hosokawa, Mitsumasa Mankura, Koretaro Takahashi-2006

Fisheries Science 72(4): 909-911

(Graduate School of Fisheries Sciences, Hokkaido University, Hakodate 041-8611, Japan; email of Koretaro Takahashi: kore@fish.hokudai.ac.jp)

EFFECTS OF LOCALLY-ISOLATED MICRO-PHYTOPLANKTON DIETS ON GROWTH AND SURVIVAL OF SEA SCALLOP (*PLACOPECTEN MAGELLANICUS*) LARVAE

Rajashree Gouda, Ellen Kenchington, Bruce Hatcher, Benedikte Vercaemer-2006

Aquaculture 259 (1-4): 169-180

Abstract:

The micro-phytoplankton *Navicula pelliculosa*, *Chaetoceros septentrionalis* and *Prymnesium* sp., indigenous to the coastal waters of Nova Scotia, Canada were isolated, cultured and used in laboratory feeding experiments with larvae of the giant sea scallop (*Placopecten magellanicus*) to evaluate their dietary benefits. The effects of these micro-phytoplankton on larval growth and survival were compared with three species of micro-algae commonly used as commercial feed for bivalve larvae: Tahitian *Isochrysis* sp., *Pavlova lutheri* and *Chaetoceros calcitrans*. The experiments were conducted at 14 °C over approximately one month. Larvae were fed at concentrations of 25,000 cells ml⁻¹ from Day 3 to Day 9 and 35,000 cells ml⁻¹ from Day 10 to Day 35. Total growth and survival were determined by sampling the larvae at intervals of 4 to 5 days. Eight dietary formulations were compared for larval survival and growth. Two way, repeated measures ANOVA using both larval shell height and percent larval survival as dependent variables and diet and time as factors resulted in

significant variation ($p < 0.001$) in both larval growth and survival. *Prymnesium* sp. supported significantly better growth as a monospecific diet than local *Chaetoceros* or *Navicula*, and when used in combination with the commercial diets which included other flagellates. Local *Navicula* and *Chaetoceros* were found to be better diets for the later stages of larval growth. Our studies showed that these two local diatoms *Navicula pelliculosa* and *Chaetoceros septentrionalis* could also be used as a component of multispecies diets along with flagellates.

(Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4 J1, Canada; email of Ellen Kenchington: kenchington@mar.dfo-mpo.gc.ca)

LARVAL REARING AND WEANING OF THICK LIPPED GREY MULLET (CHELON LABROSUS) IN MESOCOSM WITH SEMI-EXTENSIVE TECHNOLOGY

I. Ben Khemis, D. Zouiten, R. Besbes, F. Kamoun-2006

Aquaculture 259 (1-4): 190-201

Abstract:

The mullets are consumers of low trophic layers and can develop in a variety of biotopes. Despite research efforts on artificial propagation, mullet aquaculture still relies on wild captured juveniles and mass propagation techniques are still needed for aquaculture development. The objective of this study was to evaluate the feasibility of juvenile mullet mass production using the semi-extensive technology in mesocosms. Larvae of thick lipped grey mullet (*Chelon labrosus*) were reared in pilot scale mesocosms (20 m³), from the first day post-hatching until a size compatible with impoundment (day 71). Length and weight growths and protein content changes of the larvae and juveniles are discussed in relation with development stages. Zootechnical aspects of the trial are detailed and changes in larvae and juveniles behaviour during development and growth are described. Evolution of alkaline phosphatase activity is used as an indicator of digestive tract maturation and studied using a semi-quantitative micro-method.

Analysis of growth pattern revealed an extended initial low growth phase, lasting until flexion of notochord around 14 days post hatch (p. h.). This low growth occurred although the larvae were feeding actively since mouth opening at day 5 (p. h.), and still possessed energetic reserves at least until day 10 (p. h.), as the oil droplet was still visible at that age. It is suggested this low growth phase is related with major physiological changes during early larval development. The switch from low to fast linear growth and from low to elevate protein deposition rates occurred concomitantly with the end of an elevated peak of the alkaline phosphatase activity, assayed in whole larvae homogenate. It seems thus related with development of larval digestive function which permits a better nutritional status and hence a higher growth. The larvae of *C. labrosus* seem to develop the intestinal adult mode of digestion between day 14 and day 20. This suggests they might support early co-feeding and weaning strategies which could be initiated as early as the second week of life. A total of 25,000 metamorphosed and weaned juveniles were produced during this pilot scale trial. The semi-extensive rearing in mesocosm appeared an interesting and simple technology for mass production of *C. labrosus* juveniles, until a size of 34.9 ± 1.3 mm, compatible with impoundment.

(INSTM (Institut National des Sciences et Technologies de la Mer), Laboratoire Aquaculture, BP 59, 5000 Monastir, Tunisia; email of I. Ben Khemis: ines.benkhemis@instm.rnrt.tn)

EFFECTS OF INCUBATION TEMPERATURES ON EMBRYONIC DEVELOPMENT IN THE ASIAN YELLOW POND TURTLE

Xin-Ping Zhua, Chen-Qing Wei, Wei-Hua Zhao, He-Jun Du, Yong-Le Chen, Jian-Fang Gui-2006

Aquaculture 259 (1-4): 243-248

Abstract:

The Asian yellow pond turtle, *Mauremys mutica* (Cantor), is a potential aquaculture target in China owing to the higher values for food and remedy than other species of turtle. In this study, color and morphological changes of fertilized eggs were observed during embryogenesis, and the effects of incubation temperature on embryonic development were analyzed. Both calcium layer and membrane layer are thicker in the middle portion of egg-shell than that in the terminal portion, and become

thinner after embryo hatching than before embryonic development. Significant change in the white spot and subsequent white ring on the egg-shell occurs during embryonic development. Of five different incubation temperatures used to investigate the effects of incubation temperatures on embryonic development, 29.0 ± 0.5 °C was optimal for embryo survival and development. Moreover, the incubation temperature of 33.0 ± 0.5 °C was harmful effect to embryonic development. The data provide important and useful information for husbandry and management of the Asian yellow pond turtle.

(State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, Wuhan 430072, China; email of Jian-Fang Gui: jfgui@ihb.ac.cn)

GROWTH OF THE DIATOM CHAETOCEROS CALCITRANS IN SEDIMENT EXTRACTS FROM ARTEMIA FRANCISCANA PONDS AT DIFFERENT CONCENTRATIONS OF NITROGEN AND PHOSPHORUS

Chau Minh Khoi, Vo Thi Guong, Roel Merckx-2006

Aquaculture 259 (1-4): 354-364

Abstract:

This study was carried out with the aim to optimize the growth of the diatom *Chaetoceros calcitrans* (*C. calcitrans*) to feed *Artemia franciscana*. This is based on management of both quantities and ratios of nutrients dissolved in the water column, especially nitrogen (N) and phosphorus (P). To this end, algae were inoculated in media prepared from the extracts of *Artemia* pond sediments to relate their growth with the availability of N and P present in these extracts. Algal responses to different levels of dissolved inorganic nitrogen (DIN), dissolved reactive phosphorus (DRP) and to widely varying DIN:DRP ratios in the water column were examined by adding inorganic N ($6.6\text{--}76$ mg N L⁻¹) and P ($0.01\text{--}0.2$ mg P L⁻¹) to the media at different combinations. In all media, inoculation of algae at a density of 50×10^3 cells mL⁻¹ resulted in an exhaustion of DRP after five days. Adding inorganic P to the media stimulated algae to assimilate more N and increased algal densities ($R^2 = 0.70$, $P < 0.001$) and biomass ($R^2 = 0.68$, $P < 0.001$). In contrast, adding inorganic N decreased algal production. The densities of algae decreased logarithmically when DIN:DRP ratios increased from 50 to 910. Below DIN:DRP 150, the densities and biomass of algae were strongly dependent on the initial concentrations of DRP. When DIN:DRP ratios exceed 150, algal densities and biomass were constantly low, regardless the availability of DRP. In these media, supplying DRP to reach concentrations above 0.06 mg P L⁻¹, while maintaining DIN:DRP below 150, is suitable for the growth of *C. calcitrans*.

(Department of Soil Science and Land Management, College of Agriculture and Applied Biological Sciences, Cantho University, 3/2 Street, Cantho city, Vietnam; email of Chau Minh Khoi: cmkmoi@ctu.edu.vn)

EFFECT OF STOCKING DENSITY ON GROWTH, DIGESTIVE ENZYME ACTIVITY AND CORTISOL LEVEL IN LARVAE AND JUVENILES OF JAPANESE FLOUNDER, PARALICHTHYS OLIVACEUS

Sergio Bolasina, Masatomo Tagawa, Yoh Yamashita, Masaru Tanaka-2006

Aquaculture 259 (1-4): 432-443

Abstract:

The aim of this work was to evaluate the effect of stocking density on growth, digestive enzyme activity and cortisol level in larvae (2–35 DAH) and juveniles (46–65 DAH) of Japanese flounder. The body weight and body length were recorded, enzyme activities (trypsin and lipase) and cortisol levels were examined from frozen samples anesthetized with 0.1% MS 222.

In the first experiment using larvae, two different densities (1000 and 5000 individuals in triplicate 100 l tanks) were examined.

The weight and total length of the low density group was significantly larger ($P < 0.05$) than that of high density group after 15 DAH. No significant differences ($P > 0.05$) were found in cortisol

concentrations before 30 DAH, while a significantly higher concentration ($P < 0.05$) was found in the high density group on 35 DAH. No differences were found in digestive enzyme activity.

In the second experiment, juveniles of 46 DAH were kept in 100 l triplicate tanks at two different densities (200 and 2000 larvae in 100 l tanks, bottom area 0.22 m²), and reared for 15 days. Bottom settled fish and swimming fish were separately sampled. On 61 DAH, the fish swimming were selectively captured in the high density tanks and 100 individuals were transferred to 30 l tanks and further reared for 4 days. The growth in total length and weight of swimming juvenile flounder was significantly lower ($P < 0.05$) comparing with settled fish. Growth in total length at 61 DAH was significantly higher ($P < 0.05$) in the high density bottom settled fish, compared with the low density one. Swimming fish transferred from high density tanks, showed a significant increase ($P < 0.05$) in body weight 4 days after the transfer. Transferred fish also showed an increase in total length during this period but this difference was not significant ($P > 0.05$).

In juveniles, specific activity of trypsin was significantly higher ($P < 0.05$) in the swimming fish than the bottom settled in the high density group. Trypsin level was significantly lower ($P < 0.05$) after two days of being transferred to lower densities. Swimming fish reared at high density presented the highest cortisol levels. Bottom settled fish in the high density group had higher levels than those in the lower density. On swimming fish transferred from high density to low density, cortisol levels tended to decrease after 4 days.

These results showed that the rearing density has a significant influence on Japanese flounder juveniles, and results in social hierarchy (bottom settled > swimming) at high densities, that further causes higher cortisol levels and lower growth rates in low rank individuals.

(Maizuru Fisheries Research Station, Kyoto University, Nagahama, Maizuru, Kyoto 625-0086, Japan; email of Sergio Bolasina: bolasina@mdp.edu.ar)

BIOFILM DEVELOPMENT WITHIN A LARVAL REARING TANK OF THE TROPICAL ROCK LOBSTER, PANULIRUS ORNATUS

David G. Bourne, Lone Høj, Nicole S. Webster, Jennie Swan, Michael R. Hall-2006

Aquaculture 260 (1-4): 27-38

Abstract:

The role of bacterial biofilms in disease processes is becoming increasingly recognised in both clinical and environmental settings. Biofilm development within a rearing tank of the tropical rock lobster *Panulirus ornatus* was studied to evaluate if the biofilm is a reservoir for potentially pathogenic bacteria that cause mass larval mortalities. Within a 5000 L larval rearing tank, fiberglass microscope slides were systematically distributed during a standard rearing attempt to assess biofilm development. Culture-based counts for two media types, TCBS and Marine Agar (MA), demonstrated increased bacterial densities until days 11 and 13 respectively. For both media types, a drop in the plate counts was followed by a subsequent increase towards the end of the experiment. Scanning electron microscopy (SEM) confirmed that cell densities decreased between days 13 and 17, most likely due to sloughing of the biofilm into the water column. SEM images revealed distinct changes in dominant morphologies reflecting a succession of bacterial populations. A dynamic succession of microbial species during biofilm development was also demonstrated using denaturing gradient gel electrophoresis (DGGE) profiling of bacterial 16S rRNA genes in combination with statistical ordination analysis. Prominent changes in the DGGE profiles coincided with the decrease in bacterial numbers observed by SEM and plating on MA between days 13 and 17. Fluorescence in situ hybridization (FISH) identified α -Proteobacteria as being numerically abundant in the biofilm. This was supported by results from DGGE analysis, which retrieved only sequences affiliated with α - and γ -Proteobacteria. DGGE bands affiliated with *Vibrio* became dominant towards the end of the larval run (days 21 to 24). A *Vibrio harveyi* strain isolated from the biofilm late in the larval rearing trial (day 24) demonstrated increased larval mortality in small scale phyllosoma survival studies. The detection of Vibrionaceae at the end of the larval trial coincided with mass phyllosoma mortality and show that the biofilm is a reservoir for potentially pathogenic bacteria.

(Australian Institute of Marine Science, Townsville, QLD, Australia; email of David G. Bourne: d.bourne@aims.gov.au)

EFFECTS OF ILLUMINATION ON EARLY LIFE DEVELOPMENT AND DIGESTIVE ENZYME ACTIVITIES IN COMMON PANDORA PAGELLUS ERYTHRINUS L. LARVAE

Cüneyt Suzer, Şahin Saka, Kürşat Fırat-2006

Aquaculture 260 (1-4): 86-93

Abstract:

The activities of main digestive enzyme (proteases, amylase and lipase) and animal husbandry (mainly growth and survival) were studied in common pandora *Pagellus erythrinus* larvae until 30 days after hatching (DAH). Three different illumination levels (10, 30 and 100 lx) were compared in triplicate and green water technique was carried out. At the end of the experiment, illumination did not affect neither survival nor growth except 10 lx treatment. Similarly, specific growth rate (SGR) was different in 10 lx treatment ($p < 0.05$), although no differences were found in group 30 and 100 lx treatments ($p > 0.05$). In all groups, trypsin and chymotrypsin specific activities were firstly detected on day 3 related with mouth opening and slightly increased until 20 and 25 DAH respectively, and after this date specific activities of those decreased. Although, there was a significant difference between 10 lx treatment and other experimental groups ($p < 0.05$), no differences were found in other treatments ($p > 0.05$). Pepsin was firstly detected on day 25 related with stomach formation and sharp increase was observed until 30 DAH and then slight decrease was measured from this date and no differences were found between all groups. Amylase was firstly determined on day 2 and increased to day 5. After this date, slight decreases were measured in all groups and continued until end of experiments. The highest specific activity of amylase was determined in 30 lx treatment and no significant differences were found between groups ($p > 0.05$). Lipase was firstly detected on day 4 and increased to day 10. Then, activity of lipase decreased until day 15 and increased again until 25 DAH. Slight decreases were found in all groups until day 30 and continued to end of experiments. No significant differences were detected among groups ($p > 0.05$).

Finally, the significant improvement in survival, larval development and specific enzyme activities of larvae were determined in 30 lx treated group. It is thought that this phenomenon is related to optimal keeping conditions provided by the medium illumination level for *Pagellus erythrinus* larvae.

(Ege University, Faculty of Fisheries, Aquaculture Department, 35100 Bornova, Izmir, Turkey; email of Cüneyt Suzer: cuneyt.suzer@ege.edu.tr)

EFFECTS OF STARVATION AND RECOVERY ON THE SURVIVAL, GROWTH AND RNA/DNA RATIO IN LOLIGINID SQUID PARALARVAE

Érica A.G. Vidal, Paul DiMarco, Phillip Lee-2006

Aquaculture 260 (1-4): 94-105

Abstract:

The ability of *Loligo opalescens* paralarvae to resist and recover from starvation was examined by measuring their survival, growth rate and RNA/DNA ratios during starvation and refeeding. Paralarvae were fed *Artemia* sp. nauplii, zooplankton and mysid shrimp. Fourteen days after hatching they were separated into five feeding treatments: a control treatment (food was always available) and treatments starved for 2, 3, 4 and 5 days, and then refed. Each day, 5–7 paralarvae from each treatment were anesthetized to measure mantle length and wet weight (WW), and then RNA and DNA were extracted using an ethidium bromide fluorometric technique. Paralarvae did not survive 4 and 5 days of starvation, showing that at 15 days of age and at 16 °C the limit to recovery was 3 days of starvation. Paralarvae starved for 2 and 3 days showed compensatory growth that mitigated the effects of starvation, in that at the end of the experiment (10 days), they attained mean final body weights similar to the control treatment. Differences in the RNA/DNA ratios between control and starved paralarvae were detected within 2 days of food deprivation. For paralarvae starved 2 and 3 days, it took 1 day after refeeding to attain RNA/DNA ratios not significantly different from the control treatment. Additionally, RNA/DNA ratios were highest during the day (0800, 1200, 1600 h) and lowest at night (0000, 0400 h), suggesting daytime feeding activity. Growth rates ranged from –14% to 21% WW day⁻¹ and the resulting equation between RNA/DNA ratio and growth rate (GR)

of paralarvae was $GR = 1.74 \text{ RNA/DNA} - 11.79$ ($R^2 = 0.70$). After starvation, there was a reduction in growth variability in all starved treatments, while growth variability remained high in the control treatment. Findings from the present study indicate that nucleic acids are a valid indicator of nutritional condition and growth in squid paralarvae.

(National Resource Center for Cephalopods, Marine Biomedical Institute, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-1163, USA; email of Érica A.G. Vidal: ericavidal2000@yahoo.com.br)

SURVIVAL OF CLARIAS GARIEPINUS FRY IN EARTHEN PONDS: EFFECTS OF COMPOSTS AND LEAKS

S. Yong-Sulem, E.T. Tomedi, S. Mouchili, S. Tekeng, R.E. Brummett-2006

Aquaculture 260 (1-4): 139-144

Abstract:

To inform decisions on improving the yields of African catfish *Clarias gariepinus* fingerlings in earthen ponds, the hypothesis that composts and leaks were partly responsible for usually low and variable fry survivals was tested, through comparison of treatments and simple regression. The occurrence of amphibians was significantly higher ($P < 0.05$) and survival of fry was significantly lower ($P < 0.02$) in ponds with composts than in those without. The survival of fry and fingerlings in earthen ponds was negatively correlated with their night leaking rates and regression analysis yielded the equations $y = -13.31x + 82.56$ for fry and $y = -6.97x + 95.29$ for fingerlings, where y is the survival of fry or fingerlings in a holding pond and x is the leaking rate of the pond. Realizing how negatively the existence of composts and leaks could affect the survival of fry and fingerlings, fertilizing unfenced ponds with composts and sterilizing nursery ponds by drying were proscribed.

(Institute of Agricultural Research for Development (IRAD), P.O. Box 255, Foumban, Cameroon; email of S. Yong-Sulem: yongsulem@yahoo.com)

EFFECTS OF CHLORAMPHENICOL, ERYTHROMYCIN, AND FURAZOLIDONE ON GROWTH OF ISOCHRYSIS GALBANA AND CHAETOCEROS GRACILIS

A.I. Campa-Córdova, A. Luna-González, F. Ascencio, E. Cortés-Jacinto, C.J. Cáceres-Martínez-2006

Aquaculture 260 (1-4): 145-150

Abstract:

This study focused on determining the effects of antibiotics on microalgae used as food for scallop larvae. Six different dose levels of chloramphenicol, erythromycin, and furazolidone were added to cultures of *Isochrysis galbana* and *Chaetoceros gracilis*. An *in vivo* experiment was subsequently conducted to determine the effect of chloramphenicol and erythromycin on larval survival of the Pacific calico scallop *Argopecten ventricosus* in tanks and on the population of its associated bacteria. Results showed that growth of *I. galbana* was not significantly affected by chloramphenicol or erythromycin at the test doses of 0.5, 1.0, 3.0, 6.0, 9.0, and 12.0 mg/l. *C. gracilis* was significantly sensitive to erythromycin and chloramphenicol at doses higher than 0.5 and 3.0 mg/l, respectively. Furazolidone inhibited the growth of both *I. galbana* and *C. gracilis* at all test doses. Results showed that exposure of scallop larvae to a dose of 6 mg/l chloramphenicol or erythromycin did not significantly affect growth of *I. galbana*, significantly enhanced survival of the scallop larvae, and inhibited the growth of *Vibrio* spp. in tanks. This study demonstrated the adverse effect of chloramphenicol, erythromycin and furazolidone on *I. galbana* and *C. gracilis* microalgae but the positive effect on survival of the scallop larvae, decreasing associated bacterial population.

(Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo 195, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23090, Mexico; email of A.I. Campa-Córdova: angcamp04@cibnor.mx)

THE EFFECTS OF SALINITY ON THE SURVIVAL, GROWTH AND HAEMOLYMPH OSMOLALITY OF EARLY JUVENILE BLUE SWIMMER CRABS, PORTUNUS PELAGICUS

Nicholas Romano, Chaoshu Zeng-2006

Aquaculture 260 (1-4): 151-162

Abstract:

The blue swimmer crab, *Portunus pelagicus*, is an emerging aquaculture species in the Indo-Pacific. Two experiments were performed to determine the effects of salinity on survival, growth and haemolymph osmolality of early juvenile *P. pelagicus* crabs. The salinities tested for the first experiment were 10, 15, 25 and 40 ppt, and for the second experiment 5, 20, 30, 35 and 45 ppt. Each salinity experiment was triplicated, with each replicate consisting of 10 stage 4 juveniles. Each experiment lasted 45 days. Mortalities and incidence of “molt death syndrome” were recorded daily, while the intermolt period, carapace length, carapace width and wet weight were measured at each molt. At the end of the experiments the haemolymph osmolality and dry weights were measured.

Results demonstrate that salinity significantly affects both the survival and growth of early *P. pelagicus* juveniles. Mortality was significantly higher ($p < 0.01$) for juveniles cultured at salinities ≤ 15 ppt and at 45 ppt. At a salinity of 5 ppt a complete mortality occurred on day 20. In all salinity treatments, the majority of mortalities were due to “molt death syndrome”. In experiment 1, immediate effects of salinity on growth and development were seen at 10 ppt as the intermolt period was significantly longer ($p < 0.01$) and the mean carapace size increase was significantly less ($p < 0.01$) at the first molt compared to the other treatments. Meanwhile, the specific growth rates (carapace length, width and wet weight) were significantly lower ($p < 0.05$) at high salinities (≥ 40 ppt) due to longer intermolt periods and significantly lower ($p < 0.05$) carapace size or wet weight increases.

The haemolymph osmolality exhibited a positive linear relationship with the culture medium with an isosmotic point of 1106 mOsm/kg, equal to a salinity of approximately 38 ppt. Based on the osmolality graph, high metabolic cost for osmoregulation due to increased hyper- and hypo-osmotic stress appeared to cause lower survival and specific growth rates of the crabs. The results demonstrate that a salinity range of 20–35 ppt is suitable for the culture of early juvenile *P. pelagicus*.

(Tropical Crustacean Aquaculture Research Group, School of Marine Biology and Aquaculture, James Cook University, Townsville, Qld 4811, Australia; email of Nicholas Romano: Nicholas.Romano@jcu.edu.au)

A SOFT TECHNOLOGY TO IMPROVE SURVIVAL AND REPRODUCTIVE PERFORMANCE OF *LITOPENAEUS STYLIROSTRIS* BY COUNTERBALANCING PHYSIOLOGICAL DISTURBANCES ASSOCIATED WITH HANDLING STRESS

Nelly Wabete, Liet Chim, Dominique Pham, Pierrette Lemaire, Jean-Charles Massabuau-2006

Aquaculture 260 (1-4): 181-193

Abstract:

The consequences of handling stress (fishing, transfer, eyestalk ablation) on shrimp broodstock are poorly documented. The weakness of farmed shrimp, *Litopenaeus stylirostris*, during winter is a major problem in New Caledonia, because of seasonal climate (tropical–sub-temperate). The transfer of broodstock in winter from earthen outdoor ponds to indoor maturation tanks in the hatchery ($T = 20$ °C, Salinity = 35‰, fed shrimp) usually leads, after 48 h, to high mortality (up to 70%). Eyestalk ablation to induce ovarian maturation in females leads to further mortality.

Starting from a background analysis of physiological disturbances (initial osmoregulatory imbalance) associated with handling stress (Wabete, N., Chim, L., Lemaire, P., Massabuau, J.-C., 2004. Caractérisation de problèmes de physiologie respiratoire et d'échanges ioniques associés à la manipulation chez la crevette pénéide *Litopenaeus stylirostris* à 20 °C. *Styli* 2003. Trente ans de crevetticulture en Nouvelle-Calédonie. Ed. Ifremer. Actes Colloq. 38, 75-84.), we developed a protocol using a soft technology, based on modifications of water salinity, temperature and feeding regime. The aim was to minimize problems of osmoregulatory imbalance and associated mortalities. The protocol we developed, called the LSD OT protocol (Low Salinity and Diet, Optimal Temperature), was first evaluated on sub-adult shrimp (20–25 g) and then applied to broodstock. Survival after transfer and following eyestalk ablation, as well as reproductive achievement (spawning rate, nauplii number) was considerably improved when shrimps were transferred under “physiological comfort” i.e. warmed isosmotic water (26 °C and 26‰) and unfed for 3 d. This new handling protocol, based on a better control of salinity, temperature and feeding conditions, has been

transferred successfully to private hatcheries and already contributes to an increased profitability of New-Caledonian shrimp industry.

(IFREMER, Département d'Aquaculture en Calédonie, B.P. 2059, 98846 Nouméa, New Caledonia; email of Liet Chim: liet.chim@ifremer.fr)

SUBSTITUTION OF CHAETOCEROS MUELLERI BY SPIRULINA PLATENSIS MEAL IN DIETS FOR LITOPENAEUS SCHMITTI LARVAE

Barbarito J. Jaime-Ceballosa, Alfredo Hernández-Llamas, Tsai Garcia-Galano, Humberto Villarreal-2006

Aquaculture 260 (1-4): 215-220

Abstract:

The nutritional response of *Litopenaeus schmitti* larvae to substitution of *Chaetoceros muelleri* by *Spirulina platensis* meal (SPM) was evaluated. The substitution levels (S) were 0%, 25%, 50%, 75% and 100%, dry weight basis. Final larval length (FL) ranged from 1.98 to 3.16 mm for the different substitution levels. There was a significant relationship between S and FL, described by the following quadratic equation: $FL = 2.853 + 0.01598S - 0.000233S^2$. The substitution level (S) yielding maximum FL was 34.2%. Development index (DI) values ranged from 2.84 to 3.93 and were dependent on substitution level. The corresponding equation was $DI = 3.799 + 0.00945S - 0.000189S^2$ ($P < 0.01$). Maximum DI was obtained at 25.0% substitution. Survival was high (82–87%) and no significant differences were found between treatments. Protein digestibility of either microalgae was high, with 92% for SPM and 94% for *C. muelleri*, with no significant differences between them. The results in this study indicate that an adequate balance of nutrients in relation to the requirements of the species is critical. To simultaneously improve FL and DI, a 30% substitution of *C. muelleri* by SPM is suggested. This is equivalent to feeding 0.15 mg larvae⁻¹ day⁻¹ dry weight basis of a 70% *C. muelleri*/30% SPM diet, representing 0.078 mg protein larvae⁻¹ day⁻¹, 0.026 mg lipids larvae⁻¹ day⁻¹ and 2.732 J larvae⁻¹ day⁻¹.

(Departamento de Maricultivo, Centro de Investigaciones Pesqueras (CIP), 5ta Avenida y 248, Barlovento, Santa Fe, Ciudad de la Habana, Cuba; email of Humberto Villarreal: humberto04@cibnor.mx)

EGG QUALITY CRITERIA IN COMMON DENTEX (DENTEX DENTEX)

G. Giménez, A. Estévez, F. Lahnsteiner, B. Zecevic, J.G. Bell, R.J. Henderson, J.A. Piñera, J.A. Sanchez-Prado-2006

Aquaculture 260 (1-4): 232-243

Abstract:

The spawning quality, in terms of hatching rate, larval mortality at 3 and 5 days post-hatching (dph) and day of total mortality of two broodstock groups of common dentex was evaluated for 1 month in 2005. Several biochemical parameters including total lipid content, lipid class and fatty acid composition, carbohydrate content and metabolic enzyme activities were analysed in all the egg batches collected. Comparison was carried out between low- (mortality at 3 dph higher than 35%) and high-quality (mortality at 3 dph lower than 10%) batches. No differences were observed in lipid content and/or lipid class and fatty acid composition although a slightly higher content of neutral lipids was detected in high-quality batches. However, significant differences were obtained regarding carbohydrate composition and the activity of enzymes such as alkaline phosphatase and pyruvate kinase being higher in low-quality egg batches.

(Centro de Acuicultura-IRTA, Ctra. Poble Nou Km 6, 43540 Sant Carles de la Rápita, Tarragona, Spain; email of A. Estévez: alicia.estevez@irta.es)

ONTOGENETIC DEVELOPMENT OF DIGESTIVE ENZYMES IN YELLOWTAIL KINGFISH SERIOLA LALANDI LARVAE

Ben N. Chen, Jian G. Qin., Martin S. Kumar, Wayne G. Hutchinson, Steven M. Clarke-2006

Aquaculture 260 (1-4): 264-271

Abstract:

The development of digestive enzymes was examined in laboratory-reared yellowtail kingfish larvae from hatching to 36 days after hatching (DAH). The specific activities of amylase, lipase, and alkaline phosphatase showed three distinct phases: a sharp increase in enzyme activity from hatching to the onset of exogenous feeding on 3 DAH, followed by a fluctuation and a general decline toward 18 DAH, and then a period of low activity from 18 to 36 DAH. The total activities of these three enzymes showed a gradual increase from hatching to 18 DAH, followed by a sharp increase toward 36 DAH. In contrast to other enzymes, the specific and total activities of trypsin reached the maximum on 15 DAH and 24 DAH, respectively, and then both activities declined to low levels toward 36 DAH. The dynamics of digestive enzymes corresponded to the anatomical development of the digestive system. The enzyme activities tend to be stable after the formation of gastric glands in the stomach on 15 DAH. The composition of digestive enzymes indicates that yellowtail kingfish is able to digest protein, lipid and carbohydrates at an early stage. However, due to the low level of amylase specific activity after 18 DAH, the carbohydrate component should remain at a low level in formulated diets for fish larvae.

(School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide SA 5001 Australia; email of , Jian G. Qin: jian.qin@flinders.edu.au)

COMPARISON OF EARLY LIFE HISTORY STAGES OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS*: EFFECTS OF MICROALGAL DIETS ON GROWTH AND BIOCHEMICAL COMPOSITION

Lisa M. Milke, V. Monica Bricelj, Christopher C. Parrish-2006

Aquaculture 260 (1-4): 272-289

Abstract:

The culture of bay scallops, *Argopecten irradians*, is limited by a reliable and affordable supply of spat and the ability to ensure that animals attain market size within a single growing season. The main goals of our study were thus: (1) to develop growth-optimizing algal diets for implementation in hatcheries, and (2) to identify and compare bay scallop postlarval and juvenile dietary requirements, especially of lipids and fatty acids, which if met may enhance production. Nutritional needs of postlarval bay scallops (present study) are compared with those of sea scallops, *Placopecten magellanicus*, offered the same diets in a previous companion study. To this end, postlarval (initial shell height, SH = 240 μm) and juvenile (initial SH = 10 mm) bay scallops were offered 6–7 microalgal diet combinations at 20 °C, for 3 weeks. A similar growth ranking among diets was observed between the two developmental stages. A combination diet of Pavlova sp. (CCMP 459) and *Chaetoceros muelleri* was far superior to any other diet tested, yielding growth rates of 58 and 357 $\mu\text{m day}^{-1}$ which were 65% and 25% higher than the next highest performing diet of *Tetraselmis striata*/*C. muelleri* in postlarvae and juveniles, respectively. The *T. striata*/*C. muelleri* diet, which is limited in the n-3 fatty acid docosahexaenoic acid (DHA), yielded very poor growth of sea scallop postlarvae in a prior study, indicating that bay scallops may have less stringent requirements for DHA than sea scallops. The Pav 459/*C. muelleri* diet, which also supported the highest growth of sea scallop postlarvae, is characterized by elevated levels of the n-6 fatty acids, arachidonic (AA) in *C. muelleri* and 4,7,10,13,16-docosapentaenoic (DPA) in Pav 459. The two diets deficient in AA and n-6 DPA, Pavlova lutheri/*Thalassiosira weissflogii* and *P. lutheri*/*Fragilaria famila*, yielded the lowest growth rates in both bay scallop postlarvae and juveniles. Tissue enrichment of these two fatty acids relative to the diet, as well as overall enrichment in \sum n-6 fatty acids was observed across developmental stages and dietary treatments. A similar pattern has previously been observed in sea scallop postlarvae, suggesting a dietary requirement for n-6 fatty acids in pectinids that has often been overlooked in the past.

(Institute for Marine Biosciences, National Research Council, 1411 Oxford St, Halifax, NS, Canada B3H 3Z1; email of Lisa M. Milke: lisa.milke@noaa.gov)

AN IMPROVED ENZYME PREPARATION FOR RAPID MASS PRODUCTION OF PROTOPLASTS AS SEED STOCK FOR AQUACULTURE OF MACROPHYTIC MARINE GREEN ALGAE

C.R.K. Reddy, Shikh Dipakkore, G. Rajakrishna Kumar, Bhavanath Jha, Donald P. Cheney, Yuji Fujita-2006

Aquaculture 260 (1-4): 290-297

Abstract:

Preparation of protoplasts and their subsequent applications for both basic and applied research of marine macroalgae remains largely under developed due to lack of development of reliable methods with consistent yields of viable protoplasts. An improved enzyme preparation with a single commercial enzyme, e.g. 2% Cellulase Onozuka R-10 in 1% NaCl solution, was developed to produce protoplasts rapidly from different green algal genera of *Ulva*, *Enteromorpha* and *Monostroma*. The simple dissolution of enzyme powder in 1% NaCl resulted in exclusion of 2% Macerozyme R-10 from the mixture consisting of 2% Cellulase Onozuka R-10 with 3% NaCl earlier reported as superior for the same algae. Optimal conditions for the isolation of maximum yields of viable protoplasts were found to be with 2% Cellulase Onozuka R-10 incubated at 20 °C for 2 h in 1% NaCl solution with 0.8 M mannitol adjusted to pH 6.0. The protoplast yield with optimized enzyme mixture was as high as 102.8×10^6 cells g⁻¹ f. wt for *M. oxyspermum* while it was in the range of $74.4\text{--}88.6 \times 10^6$ cells g⁻¹ f. wt thallus for seven species of *Ulva*, and $82.5\text{--}95.4 \times 10^6$ cells g⁻¹ f. wt for three species of *Enteromorpha*. The regeneration rate of protoplasts isolated using this method ranged from 89 to 92% with normal morphogenesis. The seeding of nylon threads with isolated protoplasts of *M. oxyspermum* was successful and after 3–4 weeks the entire frame with nylon threads became thick green in color with tiny germlings in laboratory culture. Thus, the method described in the present study allow for rapid mass production of viable protoplasts that could be potentially used as a source for seed material for mariculture and for other applied phycological research.

(Central Salt and Marine Chemicals Research Institute, Bhavnagar 364 002, India; email of C.R.K. Reddy: crk@csmcricri.org)

EXTENDERS AND CRYOPROTECTANTS FOR COOLING AND FREEZING OF PIRACANJUBA (*BRYCON ORBIGNYANUS*) SEMEN, AN ENDANGERED BRAZILIAN TELEOST FISH

A.N. Maria, A.T.M. Viveiros, R.T.F. Freitas, A.V. Oliveira-2006

Aquaculture 260 (1-4): 298-306

Abstract:

The aim of this study was to develop a protocol for semen storage of piracanjuba (*Brycon orbignyanus*) by both cool storage at 4 °C and cryopreservation at – 196 °C. Semen was diluted in some fish semen extenders (Exp. 1) or in extenders combined with the antibiotic gentamycin sulfate (Exp. 2) and stored at 4 °C. Sperm motility was estimated every 24 h. Then, the effects of egg yolk (0 and 5%), cryoprotectants (dimethyl sulphoxide — DMSO, methanol, and methylglycol) and extenders (NaCl 154 mM, BTS™ Minitub and M III™ Minitub) on semen cryopreservation were evaluated (Exp. 3). Semen was added to each of eighteen cryosolutions (2 yolk concentrations × 3 cryoprotectants × 3 extenders), aspirated into 0.5-mL straws, frozen in nitrogen vapor (Taylor-Wharton, CP 300, “dry shipper”) and stored at – 196 °C. Sperm motility was evaluated after thawing at 60 °C-water bath for 8 s. The three cryosolutions that produced the highest post-thaw sperm motility were used again to freeze semen. Post-thaw semen quality was then evaluated under three tests: sperm motility, the percentage of live spermatozoa and hatching rate (Exp. 4). Piracanjuba semen diluted (1:10 total volume) in NaCl 200 mM or in Saad solution (NaCl 200 mM, Tris 30 mM) maintained motility above 35% for as long as 7 days, at 4 °C. Motility of only 7% was observed on undiluted semen after 3 days at 4 °C. There was neither beneficial nor detrimental effect of gentamycin on sperm motility at 250 µg/mL. Egg yolk addition to the cryosolution was beneficial in samples cryopreserved in NaCl 154 mM and in M III™, but detrimental for samples cryopreserved in BTS™. Methylglycol was the most effective cryoprotector compared to DMSO and methanol. Motility and percentage of live spermatozoa were similar among semen cryopreserved in NaCl–yolk,

M IIITM-yolk and BTSTTM, all containing 10% methylglycol, but lower than fresh control. Hatching rates of eggs fertilized with sperm cryopreserved in NaCl-yolk or BTSTTM were higher than for eggs fertilized with sperm cryopreserved in M IIITM-yolk, but lower than control fertilizations. The semen cryopreservation protocols developed here will be used to set up a gene bank for endangered piracanjuba populations.

(Depto Zootecnia, Federal University of Lavras, caixa postal 3037, Lavras, MG, 37200-000, Brazil; email of A.T.M. Viveiros: ana.viveiros@ufla.br)

INVOLVEMENT OF TRYPSIN AND CHYMOTRYPSIN ACTIVITIES IN ATLANTIC COD (GADUS MORHUA) EMBRYOGENESIS

Hólmsfríður Sveinsdóttir, Helgi Thorarensen, Ágústa Gudmundsdóttir-2006

Aquaculture 260(1-4): 307-314

Abstract:

Proteases play a key role in yolk formation and degradation during embryogenesis of marine fish. This study presents the first clear data on the involvement of trypsin and chymotrypsin activities in the embryogenesis of Atlantic cod (*Gadus morhua*). Both enzyme activities were shown to be present in unfertilized eggs followed by a significant decline ($P < 0.01$) in trypsin activity during the first 4 days post fertilization (dpf). Thereafter, the trypsin- and chymotrypsin activities increased to a maximum around day 9 pf. A decline in trypsin and chymotrypsin activities was observed from day 10 pf with minimal activity just prior to first feeding (day 15 pf). Western blot analysis, using polyclonal antibodies raised to Atlantic cod trypsins I and Y, mostly coincided with the trypsin activity profile. The novel trypsin Y was previously shown to have both trypsin- and chymotrypsin-like activities. Thus, some of the chymotrypsin activity observed in the samples may be originated from trypsin Y. The low trypsin and chymotrypsin activities just prior to first feeding (13–15 dpf) may indicate insufficient digestive function as trypsin has been shown to be a suitable short-term indicator reflecting the nutritional quality of marine fish larvae.

(Department of Food Science and Nutrition, Science Institute, University of Iceland, Vatnsmýrarvegur 16, 101 Reykjavík, Iceland; email of Ágústa Gudmundsdóttir: ag@hi.is)

SEROTONIN INDUCES OVARIAN MATURATION IN GIANT FRESHWATER PRAWN BROODSTOCK, MACROBRACHIUM ROSENBERGII DE MAN

Prasert Meeratana, Boonsirm Withyachumnarnkul, Praneet Damrongphol, Kanokphan Wongprasert, Anchalee Suseangtham, Prasert Sobhon-2006

Aquaculture 260 (1-4): 315-325

Abstract:

This study investigated the effects of serotonin (5-hydroxytryptamine or 5HT) on ovarian development in *Macrobrachium rosenbergii* de Man. Adult female prawns at the ovarian stage I (spent) were injected with 5HT at 1, 5, 10, 20 and 50 $\mu\text{g g}^{-1}$ body weight (BW) intramuscularly on days 0, 5 and 10, and sacrificed on day 15. The doses as related to the effect could be categorized into three levels: low (1 and 5 $\mu\text{g g}^{-1}$ BW of 5HT), medium (10 and 20 $\mu\text{g g}^{-1}$ BW of 5HT) and high (50 $\mu\text{g g}^{-1}$ BW of 5HT). The low-dose, especially at 1 $\mu\text{g g}^{-1}$ BW, caused prawns to exhibit a significant increase in ovarian index (ovarian weight/body weight $\times 100$) ($5.79 \pm 0.09\%$) as compared to the control (1.49%). The ovaries of most of these prawns could develop to stage IV (mature) and contained synchronously mature oocytes while most of the control ovaries remained at stage I and II (proliferative), and contained only oogonia to previtellogenic (Oc1, Oc2) and early vitellogenic oocytes (Oc3). The medium- and high-dose treated prawns exhibited ovaries that could reach stages III and IV and contained various types of oocytes of different maturity. Pretreatment with 5HT receptor antagonist, cyproheptadine (CYP), at 10 $\mu\text{g g}^{-1}$ BW before 5HT injection significantly suppressed the effect of 5HT. Intramuscular injection of the 5HT-primed thoracic ganglion culture medium into CYP-pretreated prawns resulted in the increase of ovarian index about 5–6 times more than in the control, and in the groups injected with 5HT-primed media from muscle strip, eyestalk and brain. The ovaries of most prawn could develop up to stage IV and contained synchronously

developed vitellogenic (Oc4) and mature oocytes (Oc5). These findings suggest that 5HT indirectly induces ovarian development and oocytes maturation in *M. rosenbergii*, probably via a putative ovarian stimulating factor released from the thoracic ganglia.

(Department of Medical Science, Faculty of Science, Burapha University, Chonburi, 20131, Thailand; email of Prasert Meeratana: g3936465@yahoo.com)

THYROID GLAND DEVELOPMENT IN SENEGALESE SOLE (*SOLEA SENEGALENSIS* KAUP 1858) DURING EARLY LIFE STAGES: A HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL APPROACH

J.B. Ortiz Delgado, N.M. Ruane, P. Pousão-Ferreira, M.T. Dinis, C. Sarasquete-2006

Aquaculture 260(1-4): 346-356

Abstract:

A key to success in the culture of marine fish species is the mass production of high quality fry, a process largely dependent on successful first feeding and normal development and growth of fish larvae. In this regard it is important to examine the structural and functional development of the endocrine system (pituitary, thyroid, interrenal glands) during early ontogeny of marine fish. Particularly, the thyroid hormones, thyroxine (T4) and triiodothyronine (T3), influence numerous metabolic processes, such as growth, differentiation, metamorphosis, reproduction, respiration, migratory behaviour, central nervous system activity, seasonal adaptation, etc. Therefore the aim of this study was to describe the development of the thyroid gland and the ontogeny appearance of the thyroid hormones in *Solea senegalensis* larvae by means of histological and immunohistochemical techniques. The first thyroid follicle was present at 4 days-post-hatch (dph) coinciding with first feeding. During metamorphosis (12–20 dph) the follicles increased in both number and size, and by 30 dph presented the same characteristics as that seen in adult fish. Tissue immunostaining of both thyroid hormones decreased during the endogenous larvae development to nearly undetectable levels at the completion of yolk-sac absorption. During larvae exogenous phase, T3 and T4 immunostaining was first detected by 6 dph and an increase of specific staining for both hormones was detected between 12 and 20 dph, during metamorphosis phase.

(Institute of Marine Sciences of Andalucía, CSIC, Polígono Río San Pedro Apdo Oficial, 11510 Puerto Real, Cádiz, Spain; email of C. Sarasquete: carmen.sarasquete@icman.csic.es)

ONTOGENY OF THE DIGESTIVE TRACT IN SHI DRUM (*UMBRINA CIRROSA* L.) REARED USING THE MESOCOSM LARVAL REARING SYSTEM

Mario M. Zaiss, Ioannis E. Papadakis, Eric Maingot, Pascal Divanach, Constantinos C. Mylonas-2006

Aquaculture 260 (1-4): 357-368

Abstract:

Histological changes of the digestive tract were studied in shi drum (*Umbrina cirrosa*) from hatching until 41 days post hatching (dph), when the fry had a mean (\pm S.D.) total length (TL) of 32 ± 2 mm and wet weight (WW) of 0.42 ± 0.07 g. Larvae were reared using the mesocosm technique, the most natural among commercially employed rearing methods for marine larvae. Shi drum opened their mouth at 2 dph (2.78 ± 0.09 mm TL), at which time 90% of the larvae already had an inflated swim bladder. The differentiation of the digestive tract into buccopharynx, esophagus, and anterior and posterior intestine was completed by 3 dph (2.82 ± 0.07 mm TL), 1 day after the onset of exogenous feeding. The alimentary canal started coiling and formed its first loop at 2 dph, while the pancreas and liver were differentiated at 3 dph. Yolk sac reserves lasted until 7 dph (4.3 ± 0.1 mm TL), suggesting a brief period of endogenous and exogenous feeding. The first esophageal goblet cells appeared at 7 dph containing acid mucins and at 8 dph taste buds appeared on the buccopharyngeal epithelium. The stomach was morphologically differentiated at 9 dph (5.5 ± 0.1 mm TL) when gastric glands became abundant in the cardiac region, and the first pyloric caeca appeared at 14 dph (10.1 ± 0.9 mm TL). Supranuclear eosinophilic vacuoles were observed in the posterior intestine between 3 and 11 dph (6.3 ± 0.9 mm TL). Their number decreased as the stomach differentiated, suggesting a change in the protein digestion mechanism. The results of the study suggest a rapid development of shi drum and its

digestive system and underline the possibility of weaning larvae to artificial feed even earlier than the 12 dph employed in the present study.

(Hellenic Center for Marine Research, Institute of Aquaculture, P.O. Box 2214, Iraklion, Crete 71003, Greece; email of Constantinos C. Mylonas: mylonas@her.hcmr.gr)

EFFECT OF INCREASED WATER RECIRCULATION RATE ON ALGAL SUPPLY AND POST-LARVAL PERFORMANCE OF SCALLOP (*PECTEN MAXIMUS*) REARED IN A PARTIAL OPEN AND CONTINUOUS FEEDING SYSTEM

Gyda Christophersen, Lise Torkildsen, Terje van der Meeren-2006

Aquacultural Engineering 35 (3): 271-282

Abstract:

In a commercial scallop hatchery spat production depends on a culture system which ensures high survival and good growth. Reuse of water with algae may increase the food exploitation and hence reduce the costs. Post-larvae of great scallop (*Pecten maximus*) were studied in a commercial hatchery using a partial open and continuous feeding tank system. Three different water recirculation rates (67, 83 and 92%) were tried out in two experiments with post-larvae originating from three spawning groups of ages between 43 and 57 days post-spawn, 316–886 μm shell-height and 1.1–9.6 μg ash-free dry weight. The post-larvae were held in sieves in tanks of 2500 l where a downwelling flow was maintained by airlifts. New water with a mix of monocultured algae was continuously added to the tanks at algal concentrations of 10 and 15 cells μl^{-1} in experiment 1 (groups 1 and 2) and 2 (group 3), respectively. The algal supply to each sieve was reduced along with increased recirculation rate, but was kept between 6 and 13 cells μl^{-1} . Generally no significant differences in survival, growth or chemical content were found between the three recirculation rates, while few differences were found between and within groups. Large variation in survival was found between and within groups (1–81%). Highest survival was found in experiment 1, and where post-larvae from two settlements were used, the first settlement survived better than the second. The daily growth ranged from 15 to 62 μm shell-height and from 0.3 to 2.6 μg ash-free dry weight. The scallop post-larvae could well be reared at all three recirculation rates studied as an increase from 67 to 92% did not seem to affect the post-larval performance seriously. The algal supply, however, had to be compensated by an increasing number of cells (>10 cells μl^{-1}) when increasing the recirculation rate.

(Department of Biology, University of Bergen, P.O. Box 7800, N-5020 Bergen, Norway; email of Gyda Christophersen: gyda.christophersen@bio.uib.no)

PRODUCTION OF MICROALGAL CONCENTRATES BY FLOCCULATION AND THEIR ASSESSMENT AS AQUACULTURE FEEDS

Richard M. Knuckey, Malcolm R. Brown, René Robert, Dion M.F. Frampton-2006

Aquacultural Engineering 35(3): 300-313

Abstract:

A novel technique was developed for the flocculation of marine microalgae commonly used in aquaculture. The process entailed an adjustment of pH of culture to between 10 and 10.6 using NaOH, followed by addition of a non-ionic polymer Magnafloc LT-25 to a final concentration of 0.5 mg L⁻¹. The ensuing flocculate was harvested, and neutralised giving a final concentration factor of between 200- and 800-fold. This process was successfully applied to harvest cells of *Chaetoceros calcitrans*, *C. muelleri*, *Thalassiosira pseudonana*, *Attheya septentrionalis*, *Nitzschia closterium*, *Skeletonema* sp., *Tetraselmis suecica* and *Rhodomonas salina*, with efficiencies $\geq 80\%$. The process was rapid, simple and inexpensive, and relatively cost neutral with increasing volume (cf. concentration by centrifugation). Harvested material was readily disaggregated to single cell suspensions by dilution in seawater and mild agitation. Microscopic examination of the cells showed them to be indistinguishable from corresponding non-flocculated cells. Chlorophyll analysis of concentrates prepared from cultures of ≤ 130 L showed minimal degradation after 2 weeks storage.

Concentrates of *T. pseudonana* prepared using pH-induced flocculation gave better growth of juvenile Pacific oysters (*Crassostrea gigas*) than concentrates prepared by ferric flocculation, or centrifuged concentrates using a cream separator or laboratory centrifuge. In follow up experiments, concentrates prepared from 1000 L *Chaetoceros muelleri* cultures were effective as supplementary diets to improve the growth of juvenile *C. gigas* and the scallop *Pecten fumatus* reared under commercial conditions, though not as effective as the corresponding live algae. The experiments demonstrated a proof-of-concept for a commercial application of concentrates prepared by flocculation, especially for use at a remote nursery without on-site mass-algal culture facilities.

(Department of Primary Industries and Fisheries, Northern Fisheries Centre, GPO Box 5396, Cairns, Qld 4870, Australia; email of Richard M. Knuckey: Richard.Knuckey@dpi.qld.gov.au)

DEVELOPMENT OF DIGESTIVE SYSTEM AND SWIM BLADDER OF LARVAL NASE (CHONDROSTOMA NASUS L.)

P. Sypa, T. Ostaszewska, M. Olejniczak-2006

Aquaculture Nutrition 12(5): 331-339

Abstract :

Digestive tract and swim bladder differentiation during nase (*Chondrostoma nasus* L.) larval development was evaluated using histological methods. The mouth, pharynx and oesophagus were impervious at the time of hatching. The straight tubular intestine consisted of undifferentiated cells and was situated over the yolk sac. The intestine, lined with a single layer of columnar epithelial cells, opened on 2 day posthatching (dph). First evidence of protein digestion and absorption by the enterocytes was observed on the sixth day, while lipid digestion and absorption began on the ninth day of larval development. Intestinal mucous cells developed and became active by the fourth day. The posterior chamber of the swim bladder inflated on the third day, while the anterior one filled 12 dph. Complete yolk sac resorption took place on the ninth day.

(Division of Histology and Embryology, Faculty of Veterinary Medicine, Warsaw Agricultural University, Nowoursynowska 159, 02-776 Warsaw, Poland; email of P. Sypa: sypa@alpha.sggw.waw.pl)

EFFECT OF SUPPLEMENTAL L-ASCORBYL-2-POLYPHOSPHATE IN ENRICHED LIVE FOOD ON THE ANTIOXIDANT DEFENSE SYSTEM OF PENAEUS VANNAMEI OF DIFFERENT SIZES EXPOSED TO AMMONIA-N

W.-N. Wang, A.-L. Wang, Y. Wang-2006

Aquaculture Nutrition 12(5): 348-352

Abstract:

The effects of supplemental l-ascorbyl-2-polyphosphate (APP) in enriched live food (*Artemia*) on reactive oxygen intermediate (ROI) and free radical scavenging enzyme (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione transferase) activities in the muscle of *Penaeus vannamei* of two sizes exposed to ambient ammonia-N, were investigated. Significantly, decreased ROI value was found in prawns fed on enriched *Artemia* compared with those fed on starved *Artemia* ($P < 0.05$); the decrease was 24% and 36%, respectively. In both size classes, the antioxidant enzyme activities in prawns fed on enriched *Artemia* were higher than in those fed on starved *Artemia* ($P < 0.05$). The results demonstrated that the supplementation of ascorbic acid in enriched live food (*Artemia*) enhanced the antioxidant capacity of prawn, increasing its defense system that may fight against environmental stress, leading to impaired ammonia toxicity.

(College of Life Science, South China Normal University, Guangzhou 510631, P.R. China; email of W.-N. Wang: weina63@yahoo.com.cn)

EFFECT OF DIETARY PHOSPHOLIPID LEVEL ON THE DEVELOPMENT OF GILTHEAD SEA BREAM (SPARUS AURATA) LARVAE FED A COMPOUND DIET

I. Seiliez, J.S. Bruant, J.L. Zambonino Infante, S. Kaushik, P. Bergot-2006

Aquaculture Nutrition 12(5): 372-378

Abstract:

The aim of the study was to determine the influence of dietary phospholipid (PL) levels on survival and development of first feeding gilthead sea bream (*Sparus aurata*) larvae. Larvae were fed from day 4 to 23 posthatching with an isoproteic and isolipidic formulated diet with graded levels of PL from 90–150 g kg⁻¹ dry matter (DM). A dietary PL content of more than 90 g kg⁻¹ DM seems to be necessary for sustaining growth of first feeding sea bream larvae. The survival rates of larvae fed the formulated diets (31–40% at day 23) were similar to those generally observed in marine aquaculture hatcheries with live prey feeding sequence. However, this high survival rate was not associated with high growth and the larvae showed, at the end of the study, a high proportion of individuals with abnormal liver and calculi in the urinary bladder. It is concluded that although the diets used here cannot be used in total replacement of live preys, they constitute a solid starting point for further nutritional studies with first feeding gilthead sea bream larvae.

(INRA Fish Nutrition Laboratory, UMR NUAGE, 64310 St Pée-sur-Nivelle, France; email of Iban Seiliez: seiliez@st-pee.inra.fr)
