

COMPARING DENITRIFICATION RATES AND CARBON SOURCES IN COMMERCIAL SCALE UPFLOW DENITRIFICATION BIOLOGICAL FILTERS IN AQUACULTURE

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Aquacultural Engineering 38(2): 79-92

Abstract:

Aerobic biological filtration systems employing nitrifying bacteria to remediate excess ammonia and nitrite concentrations are common components of recirculating aquaculture systems (RAS). However, significant water exchange may still be necessary to reduce nitrate concentrations to acceptable levels unless denitrification systems are included in the RAS design. This study evaluated the design of a full scale denitrification reactor in a commercial culture RAS application. Four carbon sources were evaluated including methanol, acetic acid, molasses and Cerelose™, a hydrolyzed starch, to determine their applicability under commercial culture conditions and to determine if any of these carbon sources encouraged the production of two common “off-flavor” compounds, 2-methylisoborneol (MIB) or geosmin. The denitrification design consisted of a 1.89 m³ covered conical bottom polyethylene tank containing 1.0 m³ media through which water up-flowed at a rate of 10 lpm. A commercial aquaculture system housing 6 metric tonnes of Siberian sturgeon was used to generate nitrate through nitrification in a moving bed biological filter. All four carbon sources were able to effectively reduce nitrate to near zero concentrations from influent concentrations ranging from 11 to 57 mg/l NO₃-N, and the maximum daily denitrification rate was 670–680 g nitrogen removed/m³ media/day, regardless of the carbon source. Although nitrite production was not a problem once the reactors achieved a constant effluent nitrate, ammonia production was a significant problem for units fed molasses and to a less extent Cerelose™. Maximum measured ammonia concentrations in the reactor effluents for methanol, vinegar, Cerelose™ and molasses were 1.62 ± 0.10, 2.83 ± 0.17, 4.55 ± 0.45 and 5.25 ± 1.26 mg/l NH₃-N, respectively. Turbidity production was significantly increased in reactors fed molasses and to a less extent Cerelose™. Concentrations of geosmin and MIB were not significantly increased in any of the denitrification reactors, regardless of carbon source. Because of its very low cost compared to the other sources tested, molasses may be an attractive carbon source for denitrification if issues of ammonia production, turbidity and foaming can be resolved.

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DISTRIBUTION OF LUMINESCENT VIBRIO HARVEYI AND THEIR BACTERIOPHAGES IN A COMMERCIAL SHRIMP HATCHERY IN SOUTH INDIA

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Aquaculture 275(1-4): 13-19

Abstract:

Luminescent *Vibrio harveyi* is a natural microflora of marine and coastal water bodies and is associated with mortality of larval shrimp in penaeid shrimp hatcheries. It is also known that the bacteriophages occur virtually in all places where their hosts exist. In this study, distribution of luminescent *V. harveyi* and the bacteriophages affecting these hosts was examined in a commercial *Penaeus monodon* hatchery during three shrimp larval production cycles, including a cycle affected by luminescent bacterial (LB) disease outbreak.

Out of a total of 1195 samples drawn from seawater source, sand-filtered water, nauplius, zoea, mysis and post larval rearing tanks, maturation and spawning tanks, *Artemia* hatching

tank and algal culture tanks processed using conventional microbiological techniques, 21.4% of the samples harboured luminescent bacteria. During the larval production cycle affected by LB disease (LBD), luminescent *V. harveyi* could be recovered from 52% of the hatchery samples, whereas during luminescent bacterial disease-free larval production cycle (LBDF), these bacteria could be recovered from only about 9% of the samples. The predominant source of luminescent bacteria was the brood shrimp and their rearing tanks in maturation and spawning facilities. 73% of the maturation and 80% of the spawning tank water samples harbored LB during LBD, whereas, only 20% and 32% of the maturation and spawning tanks respectively harbored LB during LBDF. LB could be isolated from 17% of the water samples in tanks from nauplius stage onwards with increasing counts that subsequently lead to LB disease.

Bacteriophages affecting the luminescent *V. harveyi* could be isolated from as many as 36% (21% and 43% of the samples analysed during LBDF and LBD respectively) of a total of 181 water samples drawn from various sources in the hatchery, using 27 luminescent *V. harveyi* hosts by agar overlay technique. The maturation tank water samples were found to be the predominant source of bacteriophages, followed by spawning tank water samples as observed with the LB. Sixty five bacteriophages, 13 during LBDF and 52 during LBD were isolated, which were grouped in to seven types based on their plaque morphology.

The study has indicated that the brooders, maturation and spawning facilities in the shrimp hatchery are the main source of luminescent *V. harveyi* and their bacteriophages and that occurrence of LB even in low counts during early larval stages can possibly lead to development of LB disease despite presence of bacteriophages in the larval rearing tanks.

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CULTURE-INDEPENDENT CHARACTERIZATION OF THE BACTERIAL POPULATIONS ASSOCIATED WITH COD (*GADUS MORHUA* L.) AND LIVE FEED AT AN EXPERIMENTAL HATCHERY FACILITY USING DENATURING GRADIENT GEL ELECTROPHORESIS

Douglas McIntosh, Baijing Ji, Benjamin S. Forward, Velmurugu Puvanendran, Danny Boyce, Rachael Ritchie-2008

Aquaculture 275(1-4): 42-50

Abstract:

Atlantic Cod (*Gadus morhua* L.) represents an attractive species for cold water aquaculture. However, like many so called 'alternative species' early larval culture remains a challenge to commercial production. Indeed in comparison to other cultured finfish species, there exists limited information concerning the bacterial species associated with cod larviculture. Herein, we have employed PCR amplification of 16S rDNA coupled to Denaturing Gradient Gel Electrophoresis (DGGE) and sequencing of isolated amplicons, to profile the bacterial species associated with cod (both eggs and larvae) and the live feed (rotifer and *Artemia*) provided to the larvae during the first 50 days of development. A stable core microflora, comprising a total of six bacterial species with a putative *Arcobacter* sp. as the dominant component, was recorded in the majority of the rotifer samples. In contrast, *Artemia* showed a highly variable microflora composed of *Vibrio* spp, *Alteromonas* spp and *Pseudoalteromonas* spp. An association between *Colwellia* spp and cod eggs was demonstrated, although these species rapidly disappeared from the larval microflora which subsequently showed a reproducible (in 6 tanks over 2 years), and well-defined bacterial succession. *Vibrio logei*, *V. fischeri* and *Listonella anguillarum* predominated during the first 3 weeks of feeding but were gradually replaced by *Flexibacter aurantiacus*, a species of *Sulfitobacter*, and a putative *Mycoplasm*a sp. At 50 days post feeding the putative *Mycoplasm*a sp. emerged as the dominant component of the microflora in all tanks. The culture-independent approach employed in this study

generated a dynamic description of bacterial colonization of cod larvae and live feed and revealed the existence of previously undescribed bacterial associations with eggs and rotifers which would almost certainly have gone undetected using conventional culture based methods.

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IMPACTS OF LIGHT REGIME ON EGG HARVESTS AND 48-H EGG HATCHING SUCCESS OF ACARTIA TONSA (COPEPODA: CALANOIDA) WITHIN INTENSIVE CULTURE

Myron A. Peck, Bianca Ewest, Linda Holste, Philipp Kanstinger, Meike Martin-2008

Aquaculture 275(1-4): 102-107

Abstract:

We examined the effect of light regime on daily egg harvest (EH, eggs tank⁻¹ d⁻¹), and 48-h egg hatching success (HS, %) by *Acartia tonsa* (Copepoda: Calanoida) in intensive 130-l cultures. Since this copepod produces more eggs during darkness than in the light, we tested whether EH could be increased by utilizing unnatural light regimes. Egg harvests were between 0.85 to 1.20 million eggs culture⁻¹ wk⁻¹ and mean EH was not significantly different among tanks maintained at 3 h:3 h, 4 h:4 h, 6 h:6 h and 12 h:12 h light:dark. HS was not significantly different for eggs produced in the different light regimes and incubated at 12 h:12 h. In a second experiment, cohorts were reared (from nauplii) in constant darkness (D) and constant light (L) and eggs produced in each cohort were incubated in darkness (D-D, L-D) or light (D-L, L-L). Mean(\pm SE) HS was significantly different among the treatments, increased with increasing light exposure, and equal to 3.7(1.1), 32.2(15.1), 38.3(0.8) and 52.2(16.5)% for D-D, L-D, D-L and L-L treatments, respectively. These and published data were combined to generate an equation predicting 48-h HS for eggs produced and incubated at photoperiods between 0.5 and 24 h. Our experiments indicated that light can be an important factor affecting the success of intensive cultures of *A. tonsa* and that copepod culture protocols should include information on light regimes used during rearing and incubation of eggs.

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THE EFFECTS OF TEMPERATURE AND SALINITY ON POPULATION GROWTH AND EGG HATCHING SUCCESS OF THE TROPICAL CALANOID COPEPOD, ACARTIA SINJIENSIS

Michael Milione, Chaoshu Zeng-2008

Aquaculture 275(1-4): 116-123

Abstract:

The tropical calanoid copepod, *Acartia sinjiensis*, has good potential for mass culture as a live feed for tropical reef fish larvae. This study was carried out to investigate the effects of temperature and salinity on population growth and egg hatching rates of *A. sinjiensis*. At a salinity of 30 ± 1 psu, temperature effects on population growth of *A. sinjiensis* was determined after 8 day culture at eight temperatures of 10, 15, 20, 25, 28, 30, 34 and 38 °C. Adopting similar experimental protocols, effects of salinity on population growth of *A. sinjiensis* were investigated under nine salinities of 10, 15, 20, 25, 30, 35, 40, 45 and 50 psu, with culture temperature set at 30 ± 1 °C. In addition to population growth experiments, egg hatching rates (%) after 48 h of incubation at various temperature and salinity conditions were also examined. For all experiments, five replicates were set up for each treatment. *A. sinjiensis* were fed an identical binary algal diet of *Tetraselmis chuii* and the Tahitian strain of *Isochrysis* sp. (T-ISO), photoperiod was maintained at 12 L:12D.

The results showed that temperature had significant effects on both population growth and egg hatching rates. Population growth at 30 °C (with an initial number of 10 adults/replicate, final mean population reached 677.2 ± 136.6) was significantly higher ($p < 0.001$) than the other temperature treatments except that of 25 °C and 28 °C treatments. The egg hatching rate did not differ significantly ($p < 0.05$) when incubated at temperature 25, 30 and 34 °C although the highest hatching rate was recorded at 34 °C ($96.3 \pm 2.2\%$). Salinity also had significant effects on both population growth and egg hatching rates. The highest population growth (final mean population: 669.0 ± 111.3) and egg hatching rate ($98.0 \pm 1.4\%$) both occurred at salinity of 30 psu. Population growth at 30 psu was significantly higher ($p < 0.001$) than all other treatments except that of 25 and 35 psu, while there was no significant difference ($p < 0.05$) between egg hatch rates at all salinity levels tested.

The results of this study suggest that for maximum population growth and egg hatching success, *A. sinjiensis* should be cultured at 30 °C with a salinity of 30 psu.

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A LABORATORY-SCALE RECIRCULATING AQUACULTURE SYSTEM FOR JUVENILES OF FRESHWATER PEARL MUSSEL HYRIOPSIS (LIMNOSCAPHA) MYERSIANA (LEA, 1856)

Satit Kovitvadhi, Uthaiwan Kovitvadhi, Pichan Sawangwong, Jorge Machado-2008
Aquaculture 275(1-4): 169-177

Abstract:

Growth and survival rates of juvenile freshwater pearl mussels *Hyriopsis* (*Limnoscapha*) *myersiana* (Lea, 1856) were compared at 0–120 days when reared in two closed recirculating aquacultural systems. System I was composed of a glass aquarium with a filter cabinet (combination of pebbles, ground freshwater mussel shells and nylon fiber), a UV tube, a resting cabinet, and a plastic culture unit. The system II was composed of 5 cabinets: a particulate filter cabinet, a macrophyte (*Limnophila heterophylla*) filter cabinet, a biological filter cabinet, a water resting cabinet and plastic culture units. Water flowed through the juvenile culture units at 20 ml/min in both systems. In each system juveniles were stocked at day 0 with sand at $< 120 \mu\text{m}$ and were fed twice a day on a 1:1 mixture of *Chlorella* sp. and *Kirchneriella incurvata*. Over the 120 days, average growth rate per day and rate of survival were higher in system II. Free carbon dioxide, total ammonia nitrogen, nitrate, phosphate and silica of second system were significantly lower in system II. The relationship between shell length (L) and age of the freshwater pearl mussels cultured in system II was $L = 0.6164 - 0.0809 \text{ Day} + 0.0032 \text{ Day}^2 - 1 \times 10^{-5} \text{ Day}^3$, $R^2 = 0.983$.

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FERTILITY OF CRYOPRESERVED SPERMATOOZOA OF THE JAPANESE PEARL OYSTER, PINCTADA FUCATA MARTENSII

Teruyoshi Narita, Takayuki Kawamoto, Kiyoshi Isowa, Hideo Aoki, Masahiro Hayashi, Akira Komaru, Hiromi Ohta-2008
Aquaculture 275(1-4): 178-181

Abstract:

The aims of this study were to compare the fertility of fresh and cryopreserved spermatozoa of the Japanese pearl oyster, *Pinctada fucata martensii* and to develop artificial fertilization methods using the cryopreserved spermatozoa. The optimal egg density for the fertilization test was found to be 1.0×10^5 eggs ml⁻¹ seawater. When 1.0×10^5 eggs ml⁻¹ seawater

were mixed with various numbers of fresh or cryopreserved spermatozoa (from 0.041 to 63×10^7 spermatozoa), high fertilization rates (about 60%) were obtained following addition of more than 3.5×10^7 spermatozoa when using either fresh or cryopreserved spermatozoa. With reduced numbers of spermatozoa of less than 1.1×10^7 (cryopreserved) or 0.12×10^7 (fresh) spermatozoa, the fertilization rates gradually decreased to 40% or less. More than 10 times the number of cryopreserved spermatozoa was necessary to obtain similar fertilization rates to those arising from the use of fresh spermatozoa.

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PRE-HYDROLYSIS IMPROVES ABSORPTION OF NEUTRAL LIPIDS IN ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*, L.) LARVAE

T.A. Mollan, S.K. Tonheim, K. Hamre-2008

Aquaculture 275(1-4): 217-224

Abstract:

To investigate if digestion is limiting for absorption of dietary neutral lipids in Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae, absorption of triacylglycerol (TAG), diacylglycerol, (DAG), and monoacylglycerol, (MAG) as well as phosphatidylcholine (PC) was studied by tube-feeding larvae with radio-labelled lipids and tracing the radioactivity. PC was included to evaluate any difference in absorption of neutral versus polar lipids. The labelled lipids were deposited by tube-feeding before larvae were incubated individually for 18 h with collection of expired CO₂. At sampling, the gut was separated from the body carcass and radioactivity traced in four different compartments, namely body carcass, gut, incubation water and CO₂ expired from larvae. The relative (%) isotope distribution of each compartment was calculated and the dose size effect analysed by regression analysis.

The results show that there is an increasing amount of evacuated unabsorbed neutral lipids as the complexity of the lipid increases (TAG > DAG > MAG). The larval faecal evacuation ranged from $66 \pm 20\%$ of TAG to $9 \pm 6\%$ of MAG. DAG was intermediate with $52 \pm 21\%$ evacuated. Of the labelled PC, $37 \pm 16\%$ was absorbed, but this can not be directly compared to the neutral lipids due to the different digestive enzymes that specifically hydrolyse neutral and polar lipids. Increasing the administered amount of lipids only slightly increased the total amount of labelled TAG and DAG that were actually absorbed, while there was a linear correlation between fed and absorbed MAG. The absorption of PC was also linearly related to the administered amount. The difference in net absorption of labelled TAG, DAG and MAG diets indicates that digestion is a limiting factor for absorption of neutral lipids in Atlantic halibut larvae.

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DIETARY SUPPLEMENTATION OF SALMON ROE PHOSPHOLIPID ENHANCES THE GROWTH AND SURVIVAL OF PACIFIC BLUEFIN TUNA *THUNNUS ORIENTALIS* LARVAE AND JUVENILES

Manabu Seoka, Michio Kurata, Rakuto Tamagawa, Amal Kumar Biswas, Biswajit Kumar Biswas, Annita Seok Kian Yong, Yang-Su Kim, Seung-Chul Ji, Kenji Takii, Hidemi Kumai-2008

Aquaculture 275(1-4): 225-234

Abstract:

Previous studies have shown that feeding enriched *Artemia* induces growth failure in Pacific bluefin tuna (PBT) *Thunnus orientalis* larvae. This growth failure cannot be improved even if the docosahexaenoic acid (DHA) content in enriched *Artemia* is elevated to the same level as that in yolk-sac larvae, an ideal live feed for PBT larviculture. This might be caused by the

differences in the DHA in the live feeds; i.e., yolk-sac larvae of marine fish have a high level of DHA in the larval phospholipids (PLs) but enriched *Artemia* store DHA in their neutral lipids (NLs). To test this hypothesis two experiments were conducted to evaluate the effect of dietary PL rich in DHA on growth and survival of PBT larvae (Experiment 1) and juveniles (Experiment 2). Three isoproteic and isolipidic artificial test diets (Diets 1, 2 and 3) and two reference live feeds (Diets 4 and 5) were prepared. Diets 1 and 3 were supplemented with NL and PL fractions of commercial salmon *Oncorhynchus gorboscha* roe lipid (SRL) as the lipid source, respectively, whereas Diet 2 was supplemented with a mixture of both lipid fractions. Diets 4 and 5 were enriched *Artemia* and yolk-sac larvae of Japanese parrot fish *Oplegnathus fasciatus*, respectively. Diets 1, 3, 4 and 5 were given to PBT larvae for 10 days, whereas Diets 1, 2 and 3 were fed to PBT juveniles for 10 days. In Experiment 1, the growth and survival of PBT larvae fed Diet 3 (high PL fraction) were significantly improved when compared with larvae fed Diets 1 and 4, although the best growth and survival were obtained in larvae fed Diet 5. Similar results were obtained in Experiment 2; i.e., growth and survival were significantly improved in PBT juveniles fed Diets 2 and 3. In both experiments, fish fed Diets 2, 3 and/or 5 had higher total lipid contents, TAG levels, n-3 HUFA and/or DHA levels in NL fraction of the body when compared with fish fed Diets 1 or 4, while such a difference was not observed in the PL fraction. In juveniles over 90% of deaths were caused by collisions with the tank walls and the significant difference in mortality between treatments implies diet also affects behavior. SRL PL is concluded to enhance the growth and survival of PBT larvae and juveniles along with the accumulation of storage lipid and DHA in the body. (Fisheries Laboratory, Kinki University, Urugami 468-3, Nachikatsuura, Wakayama 649-5145, Japan; email of Manabu Seoka: seoka@nara.kindai.ac.jp)

EFFECT OF DIETARY PHOSPHOLIPID LEVELS ON PERFORMANCE, ENZYME ACTIVITIES AND FATTY ACID COMPOSITION OF PIKEPERCH (*SANDER LUCIOPERCA*) LARVAE

Neila Hamza, Mohamed Mhetli, Ines Ben Khemis, Chantal Cahu, Patrick Kestemont-2008
Aquaculture 275(1-4) : 274-282

Abstract:

This study was carried out to evaluate the effects of dietary phospholipid on the development and rearing performance of pikeperch (*Sander lucioperca*) larvae. From day 10 post-hatching, fish larvae were weaned onto three isoproteic and isolipidic formulated diets with different phospholipid (PL) levels: 1.4 (PL1), 4.7 (PL5) and 9.5% (PL9) of dry matter, as soybean lecithin. Neutral lipid (NL) with inverted gradient was incorporated in diets. Survival, growth and deformities were monitored until day 34 post-hatching, as well as intestinal enzyme activities, leucine alanine peptidase (leu-ala), aminopeptidase N (AN) and alkaline phosphatase (AP), which were used as indicators of digestive tract maturation. This study showed that PL supplementation significantly improved growth but not survival. The increase in dietary PL from 1.4 to 9.5% led to a 50% increase in larval final weight suggesting that high PL levels are needed during larval stages of pikeperch. The incidence of deformities was not affected by dietary phospholipid level. The specific activity of brush border membrane enzymes (AN and AP) increased with dietary phospholipid levels, indicating an earlier or more efficient maturation of digestive structures. A gut maturation index based on the ratio of segmental activity of the brush border membrane enzyme AP related to segmental activity of a cytosolic enzyme, leu-ala, was significantly higher in PL5 and PL9 groups compared to PL1 group indicating that 1% phospholipid incorporation in diet was not sufficient to induce good enterocyte maturation. Diet fatty acid composition was affected by phospholipid incorporation, dietary n – 3 HUFA concentration decreasing with the incorporation of PL. Fatty acid composition in larvae reflected that of corresponding diet. The best results in growth and development obtained in the PL9 group seemed related to the PL entity, independently of its fatty acid composition. The results of this study indicate that pikeperch larvae have a relatively high PL requirement (at least 9.5% of the diet, dry weight).

SALINITY STRESS ON EMBRYOS AND EARLY LARVAL STAGES OF THE POMFRET *PAMPUS PUNCTATISSIMUS*

Zhaohong Shi, Xuxiong Huang, Rongbin Fu, Haiping Wang, Haizhong Luo, Bo Chen, Minghai Liu, Dong Zhang-2008

Aquaculture 275(1-4): 306-310

Abstract:

The pomfret, *Pampus punctatissimus*, is an important fisheries resource in China and a valuable and highly esteemed fish species on the Chinese market. It is currently being evaluated as a candidate for mariculture to meet increasing demand. To better understand the tolerance of this species to varying environmental salinity at early developmental stages, the effects of salinity on egg buoyancy, embryo development, egg hatching, and morphology of early larva were determined. Egg neutral buoyancy was seen at 25–29‰ salinity. Below 20‰ salinity, all eggs sank, and above 30‰ salinity, all floated on the surface. Time to first hatching was 24–28 h at 26–28 °C in all salinities, but eggs/embryos exposed to salinities ≤ 10‰ died. Hatching rates decrease substantially at salinities ≤ 25‰ and ≥ 40‰, and the highest hatching rate (68.2%) occurred at 29–32‰. The percentage of abnormalities of newly hatched larvae was lowest at 29–30‰ salinity, lower (≤ 25‰) and higher (≥ 40‰) salinities resulted in significantly higher percentage of abnormalities. The results on buoyancy, hatching rate, and early larval deformity, indicate that fertilized eggs of *P. punctatissimus* only develop well in a narrow range of salinities. We conclude that the optimal salinity for successful development of fertilized eggs lies between 29 and 32‰ where neutral egg buoyancy, highest egg hatching rate, and lowest incidence of abnormality rate was observed.

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TECHNICAL NOTE

CRYOPRESERVATION OF BRAZILIAN FLOUNDER (*PARALICHTHYS ORBIGNYANUS*) SPERM

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Aquaculture 275(1-4): 361-365

Abstract:

The Brazilian flounder, *Paralichthys orbignyanus*, is being considered for aquaculture due to its high demand and market price. Reproduction and larviculture studies have demonstrated the feasibility of massive fingerling production, and techniques that prolong life and increase gamete viability can assist in the culture development of this species. The aim of this study was to evaluate the efficiency of two different cryosolutions for cryopreservation of Brazilian flounder semen in order to improve broodstock management and consequently augment the potential for its culture. Two different cryosolutions were tested: a) glycerol–saline: glycerol solution (12% or 1.65 M) along a saline-based diluent (423 mM NaCl, 9 mM KCl, 9.25 mM CaCl₂·2H₂O, 22.92 mM MgCl₂·6H₂O, 25.5 mM MgSO₄·7H₂O and 2.15 mM NaHCO₃; pH 8.2; osmolality 900 mOsmol/kg); and b) DMSO–sucrose: DMSO solution (10% or 1.40 M) along a sucrose-based diluent (110 mM Sucrose, 100 mM KHCO₃ and 10 mM Tris-Cl; pH 8.2; osmolality 335 mOsmol/kg). Cryopreservation was made without equilibration time. First, 250 µl-straws were placed 6 cm above the surface of liquid nitrogen for 10 min, then they were maintained for 5 min on the surface of liquid nitrogen (1 cm) before being plunged into liquid nitrogen. The quality of cryopreserved sperm was assessed through the percentage of sperm motility and viability, fertilization capacity, hatching and larval viability. Motility

was estimated with an arbitrary scale, ranging from 0 to 5. Spermatozoa viability was determined using a LIVE/DEAD® sperm viability kit. Motility of fresh sperm (3.5 ± 0.2) was similar to frozen/thawed sperm with DMSO-sucrose (2.5 ± 0.3) ($P > 0.05$). On the other hand, the motility of frozen/thawed sperm with glycerol-saline (1.3 ± 0.4) was lower than the other two treatments ($P < 0.05$). No difference was found in the percentage of live spermatozoa post-thawed between DMSO-sucrose and glycerol-saline solutions ($P < 0.05$). However, fresh sperm had a higher percentage of live spermatozoa than post-thawed sperm with glycerol-saline ($P < 0.05$). Sperm motility was positively correlated with the percentage of live spermatozoa (Adjusted $R^2 = 0.61$, $n = 13$). No difference was found for fertilization and hatching rates and larvae viability among the three treatments ($P > 0.05$). This is the first report on successful cryopreservation of Brazilian flounder sperm. This procedure should improve broodstock management techniques for this species and consequently augment the potential for its culture.

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SHORT COMMUNICATION

A FISH NODAVIRUS ASSOCIATED WITH MASS MORTALITY IN HATCHERY-REARED ASIAN SEA BASS, *LATES CALCARIFER*

V. Parameswaran, S. Rajesh Kumar, V.P. Ishaq Ahmed, A.S. Sahul Hameed-2008

Aquaculture 275(1-4): 366-369

Abstract:

Virus responsible for viral nervous necrosis (VNN) was isolated from infected Asian sea bass (*Lates calcarifer*) larvae during the massive outbreak in sea bass hatcheries located in Chennai and Nagapattinam of Tamilnadu, India. The infected larvae showed anorexia, pale-grey pigmentation of body, loss of equilibrium and corkscrew-like or whirling swimming behavior before to death. The causative organism was isolated using SISK cell line. The typical cytopathic effect of nodavirus characterized by vacuolation was observed in SISK cell line 2 days after inoculating with the filtrate of diseased sea bass larvae. The recovered virus from cell line exhibited $8 \text{ Log TCID}_{50} \text{ mL}^{-1}$ when titrated in the presence of SISK cell line. Electron microscopic observation revealed the vacuolation and aggregation of numerous virus particles in the cytoplasm of nodavirus-infected SISK cells. Histological investigations reveal vacuolations in brain and retina of naturally and experimentally infected sea bass juveniles. Fish larvae challenged by immersion exhibited abnormal swimming pattern and dark coloration 2 days post infection (p.i.) and 100% mortality was observed at 4 days p.i. Intramuscularly challenged juveniles developed dark coloration and abnormal behavior 10 days p.i. and all animals died at 15 days p.i. All naturally and experimentally infected sea bass larvae or juveniles showed positive for piscine nodavirus by RT-PCR.

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A PRELIMINARY STUDY ON THE PROBLEMS IN THE PREPARATION OF ARTEMIA PARTHENOGENETICA CHROMOSOMES FOR SCANNING ELECTRON MICROSCOPY

Tai-Hung Lee, Satoru Shirayama-2008

Journal of Crustacean Biology 28(1): 167-170

Abstract:

The chromosomes of Crustacea, especially decapods, are usually numerous, small in size, and punctiform. These characteristics make it difficult for researchers to further investigate the chromosomes by light microscopy. As an alternative, the scanning electron microscopy

(SEM) is recommended because it has higher resolution and is able to provide valuable three-dimensional data and X-ray microanalysis. The aim of this study is to obtain some fundamental knowledge for the preparation of the chromosomes of crustaceans for SEM observations. Since crustacean chromosomes are so small, impurities such as mucus and cytoplasm easily obscure the chromosome surface making it impossible for researchers to observe their fine structure. We used *Artemia parthenogenetica* as a model and confirmed impurities originating from the nauplii paste and the cytoplasm of the cells, and demonstrate that the nauplii paste can be removed by rinsing and the cytoplasm can be digested by trypsin. (Graduate School of Fisheries Science, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan; email of Tai-Hung Lee: thlee@fish.hokudai.ac.jp)

POST-TRANSPORT RECOVERY OF CULTURED SCALLOP (*PECTEN MAXIMUS*) SPAT, JUVENILES AND ADULTS

Gyda Christophersen, Guillermo Román, Jerry Gallagher, Thorolf Magnesen-2008

Aquaculture International 16(2): 171-185

Abstract:

High mortality associated with transport operations in scallop culture has been a major problem faced by European farmers. Simulated transport with *Pecten maximus* L. spat <2 mm, spat 15–30 mm, juveniles 30–50 mm and adults >100 mm were carried out in Spain, Ireland and Norway. Different time and temperature combinations were studied in order to maximise post-transport survival and establish best practices. Out-of-water transport could result in 100% survival if conditions were right, but the response to emersion stress depended on size, season and location. Post-transport recovery decreased with emersion time and was strongly influenced by temperature. Air exposure was tolerated for a longer time by adult scallops than spat and juveniles, but the results differed among trials in the different countries. The maximum emersion time that gave post-transport survival $\geq 80\%$ was 12 h for the smallest spat, 18 h for larger spat and 24 h for juvenile and adult scallops. Adults were less affected by transport temperatures that deviated from ambient seawater temperature than spat and juveniles. In general post-transport recovery was high when sea temperature was $< 10^\circ\text{C}$, but during warm-water seasons special care should be taken to avoid stressful and lethal transport conditions. A transport temperature $< 12^\circ\text{C}$ was recommended, though not more than 10°C below ambient culture temperature. A maximum transport time of 9 h was suggested for spat and juveniles to attain post-transport survival close to 100%, but 12–24 h was feasible during the cold-water season or at favourable transport temperatures.

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PROBIOTICS FOR SHRIMP LARVICULTURE: REVIEW OF FIELD DATA FROM ASIA AND LATIN AMERICA

Olivier Decamp, David J. W. Moriarty, Patrick Lavens-2008

Aquaculture Research 39 (4): 334–338

Abstract:

Disease problems have emerged as major constraints in aquaculture production. The prophylactic application of antibiotics is expensive and detrimental, i.e. selection of bacteria that are drug-resistant or more virulent and the prevalence of drug residues in reared animals. Probiotics, which compete with bacterial pathogens for nutrients and/or inhibit the growth of pathogens, could be a valid alternative to the prophylactic application of chemicals. A mixture of specific *Bacillus* strains was designed following a research programme on the ability of numerous *Bacillus* strains to inhibit a range of pathogenic *Vibrio* strains, to grow under conditions prevailing in shrimp hatcheries and to degrade waste products. These strains were then included in bioassays and challenge tests in order to confirm the lack of toxin production and pathogenicity to humans, target organisms and the environment. Here, we report on the

performance of a commercially available mixture of Bacillus strains (SANOLIFE® MIC), using data from Asian and Latin-American hatcheries, with *Penaeus monodon* (Fabricius 1798) and *Litopenaeus vannamei* (Boone 1931). These results show that probiotics may be a suitable alternative to the prophylactic use of antibiotics. Obviously, minimizing the risk of vibriosis demands a multi-disciplinary approach, including good hygiene and sanitation measures to reduce the input of potential pathogens, as well as a suitable farm management. (INVE Technologies NV, Hoogveld 93, 9200 Dendermonde, Belgium; email of O. Decamp: o.decamp@inve.be)

DIGESTIVE ENZYME ACTIVITY AT DIFFERENT DEVELOPMENTAL STAGES OF BLACKSPOT SEABREAM, *PAGELLUS BOGARAVEO* (BRUNNICH 1768)

Laura Ribeiro, Ana Couto, Mercedes Olmedo, Blanca Álvarez-Blázquez, Fátima Linares, Luísa M P Valente-2008

Aquaculture Research 39(4): 339–346

Abstract:

Blackspot seabream, *Pagellus bogaraveo* (Brunnich), has been identified as a potential species to diversify European aquaculture production. Although rearing aspects have been widely investigated, little information exists on the nutritional requirements for this species. The aim of this study was to build up information on the activity of digestive enzymes at certain developmental stages of blackspot seabream in order to understand the nutritional needs of larvae and post larvae. Fish larvae were reared from hatching to 55 days after hatching (dah), and the feeding plan consisted in rotifers (5–35 dah), *Artemia* naupli (30–35 dah) metanaupli (35–45) and Gemma microdiet (45–55 dah). At 7, 11, 21, 45 and 55 days after hatching (dah), pooled samples of fish larvae were collected for analysis of trypsin, amylase, lipase, alkaline phosphatase and leucine–alanine peptidase activity. Up to 21 dah, the whole larvae body was used for enzymatic analysis, whereas in older larvae only the dissected abdominal cavity was used. Blackspot seabream body dry weight growth was exponential, increasing from 60 µg at 5 dah to 30±9.7 mg at 55 dah. Amylase specific activity decreased significantly during development, exhibiting at 11 dah (0.6 U mg⁻¹ protein) an average value 2.7 times lower than at 7 dah, and remaining stable between 45 and 55 dah (0.7 U mg protein⁻¹). Trypsin specific activity remained constant until 21 dah (between 38 and 44 mU mg protein⁻¹), which could be related to the larvae feeding regime. At later stages of development, lipase-specific activity exhibited a significant increase ($P < 0.05$), being three times higher at 55 dah (8 U mg protein⁻¹) than at 45 dah. The total activity of the studied digestive enzymes increased significantly during larval development (until 21 dah), whereas afterwards only lipase and leucine–alanine peptidase increased significantly between 45 and 55 dah. The pattern of digestive enzymes activity was related to organogenesis and the type of food used at different developmental stages.

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MARKING LIVE FEEDS WITH INERT METAL OXIDES FOR FISH LARVAE FEEDING AND NUTRITION STUDIES

Matthew A. Cook, Ronald B. Johnson, Pete Nicklason, Harold Barnett, Michael B. Rust-2008

Aquaculture Research 39 (4): 347–353

Abstract:

Yttrium oxide (Y₂O₃), ytterbium oxide (Yb₂O₃), lanthanum oxide (La₂O₃) and dysprosium oxide (Dy₂O₃) were evaluated as potential live feed markers for feeding and nutrition studies with fish larvae, by determining the uptake and depletion of markers over time in two trials, and quantifying ingestion of Y₂O₃-marked rotifers (*Branchionus plicatilis*) by Atlantic cod (*Gadus morhua*) in a third trial. In the first two trials, *Artemia* nauplii and rotifers quickly took up markers within 10 min to concentrations useful for nutrition studies (>2% dry

weight). There was no significant difference ($P>0.05$) among temperatures in depletion of markers (10, 15, 20 °C) with *Artemia* or rotifers. Depletion from rotifers was not significantly different ($P>0.05$) between 5 and 20 min nor between 5 and 30 min for *Artemia* when marked at a concentration of 50 mg of marker per litre of seawater. In the second trial, rotifers and *Artemia* were marked with a higher concentration (250 mg L⁻¹) and allowed to deplete for a longer time (90 min). In the third trial, visual estimates of *Artemia* consumed by Atlantic cod larvae were similar to consumption estimates determined by analysis of Y2O3-marked *Artemia* using inductively coupled plasma optical emission spectroscopy ($r^2=0.77$). (Aquaculture Research Institute, University of Idaho, 3059F National Fish Hatchery Rd., Hagerman, ID 83332, USA; email of M. B. Rust: Mike.Rust@noaa.gov)

EFFECTS OF TWO CULTURING TECHNIQUES ON THE GROWTH, SURVIVAL AND LARVAL QUALITY OF DENTEX DENTEX LINNAEUS, 1758

Gemma Giménez, Alicia Estévez-2008

Aquaculture Research 39 (4): 354–361

Abstract:

Common dentex larvae were reared using two culturing techniques, mesocosms and intensive rearing, to determine the principal culture parameters involved in the differences observed in growth, skeletal deformations and survival between the two rearing techniques. In growth, only dry weight of larvae of 40 days post-hatching (dph) from mesocosms was significantly higher than larvae from intensive rearing. Significant differences were observed in survival at 40 dph (6.58% in mesocosms and 1.58% in intensive rearing) and in the incidence of skeletal deformations, both for percentage of deformed larvae and for some deformation types such as those related to vertebral column and to the caudal complex. Initial larval density and initial prey density and quality are the factors suspected to affect growth and survival performance, while skeletal deformities might also be affected by tank hydrodynamics.

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DOES THE PRESENCE OF MICROALGAE INFLUENCE FISH LARVAE PREY CAPTURE ?

Rui J. Rocha, Laura Ribeiro, Ricardo Costa, Maria Teresa Dinis-2008

Aquaculture Research 39 (4): 362–369

Abstract:

The green water technique has been widely shown to improve fish larvae growth, survival and feed ingestion. Therefore, fish larvae (*Sparus aurata* L. and *Solea senegalensis* Kaup) feeding behaviour was studied through gut content analysis, when using different species of microalgae, as the 'green water' technique. Six treatments were used: Stain – food green stain; Tetra – microalgae *Tetraselmis chuii*; Iso – microalgae *Isochrysis galbana*; Tetra Sup – *T. chuii* supernatant (obtained from centrifugation); Phyto – a microalgae paste, *Nannochloropsis oculata*, (Phytobloom®); and C water – clear water, as control. At 9, 16 and 23 days after hatching (DAH) for *S. aurata*, and 4, 9 and 14 DAH for *S. senegalensis*, 40 unfed fish larvae were transferred to 3 L experimental tanks, filled with the different 'green water' technique. Fish larvae were sampled 2 h after being fed with live prey, anaesthetized and fixed in buffered formaldehyde for posterior gut content determination. Feeding was evaluated by the feeding rate, percentage of larvae with prey items in the digestive tract and feeding intensity, number of prey in each larva digestive tract. Fish larvae feeding ability was influenced by the interaction between light conditions and substances provided by the presence of microalgae during fish larvae development. *Sparus aurata* was more dependent on microalgae addition than *S. senegalensis* larvae, which may be related to the type of prey, larval behaviour, ontogeny and physiology. The presence of microalgae influenced the selection of larger prey (*Artemia* over rotifers) by *S. aurata* aged 23 DAH.

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STABLE CARBON ($\Delta^{13}\text{C}$) AND NITROGEN ($\Delta^{15}\text{N}$) ISOTOPES AS NATURAL INDICATORS OF LIVE AND DRY FOOD IN PIARACTUS MESOPOTAMICUS (HOLMBERG, 1887) LARVAL TISSUE

Rosângela Kiyoko Jomori, Carlos Ducatti, Dalton José Carneiro, Maria Célia Portella-2008
Aquaculture Research 39 (4): 370–381

Abstract:

This study proposed the use of the stable isotope technique to track the type of food utilized by pacu *Piaractus mesopotamicus* larvae during their development, and to identify the moment when the larvae start using nutrients from the dry diet by retaining its carbon and nitrogen atoms in their body tissues. Five-day-old pacu larvae at the onset of exogenous feeding were fed *Artemia nauplii* or formulated diet exclusively; nauplii+formulated diet during the entire period; or were weaned from nauplii to a dry diet after 3, 6 or 12 days after the first feeding. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *Artemia nauplii* were -15.1‰ and 4.7‰ , respectively, and -25.0‰ and 7.4‰ for the dry diet. The initial isotopic composition of the larval tissue was -20.2‰ and 9.5‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively. Later, at the end of a 42-day feeding period, larvae fed *Artemia nauplii* alone reached values of -12.7‰ and 7.0‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively. Larvae that received the formulated diet alone showed values of -22.7‰ for $\delta^{13}\text{C}$ and 9.6‰ for $\delta^{15}\text{N}$. The stable isotope technique was precise, and the time at which the larvae utilized *Artemia nauplii*, and later dry diet as a food source could be clearly defined.

(Aquaculture Center, Sao Paulo State University, Via Prof. Paulo D. Castellane, Jaboticabal, SP 14.884-900, Brazil; email of M. C. Portella: portella@caunesp.unesp.br)

METABOLIC AND DIGESTIVE ENZYME ACTIVITY PROFILES OF NEWLY HATCHED SPOTTED WOLFFISH (ANARHICHAS MINOR OLAFSEN): EFFECT OF TEMPERATURE

Arianne Savoie, Nathalie R Le François, Chantal Cahu, Pierre U Blier-2008
Aquaculture Research 39 (4): 382–389

Abstract:

Three groups of newly hatched spotted wolffish (*Anarhichas minor*) were held at three different temperatures in order to determine relationships between metabolic, digestive and growth response in rapidly developing larvae. Growth rates were successfully modulated by temperature (5, 8 and 12 °C). Activity levels of trypsin and lactate dehydrogenase (LDH) were positively linked to specific growth rates at all temperatures. Trypsin showed a positive compensation (higher activity at lower temperature) whereas glycolytic enzymes (pyruvate kinase and Lactate dehydrogenase) and aspartate aminotransferase (AST) showed a negative compensation (lower activity at lower temperature). Citrate synthase was not affected by growth rate, indicating that the level of aerobic capacity was adequate in sustaining the high energetic needs associated with rapid growth early in the life of the spotted wolffish. In light of our results, it is suggested that protein digestion, as demonstrated by the activity profile of trypsin in relation to growth rate and temperature, is likely a key growth-limiting agent during the early-life stages of wolffishes. Our results are discussed in comparison with *A. lupus*, a closely related species displaying different temperature preferences and growth capacities.

(Université du Québec à Rimouski/MAPAQ, Centre Aquacole Marin, 6, rue du Parc, Grande-Rivière, Québec, Canada G0C 1V0; email of N. R. Le François: Nathalie_Le-Francois@uqar.ca)

SURVIVAL OF BLUE KING CRAB *PARALITHODES PLATYPUS* BRANDT, 1850, LARVAE IN CULTIVATION: EFFECTS OF DIET, TEMPERATURE AND REARING DENSITY

Bradley G. Stevens, Sara Persselin, Julie Matweyou-2008

Aquaculture Research 39 (4): 390–397

Abstract:

Blue king crab (*Paralithodes platypus*) larvae were cultivated to test the effects of diet, temperature and rearing density. Dietary treatments included no feeding (unfed), *Artemia nauplii* enriched with diatoms *Thalassiosira nordenskiöldii* (THAL), unenriched *Artemia* fed in addition to *Thalassiosira* (A+THAL) and a control diet of *Artemia* enriched with frozen *Isochrysis* paste (ISO 6). Trials were conducted at 6 °C, and a rearing density of 10 zoea L⁻¹, with six replicates per treatment. The ISO 6 diet was also tested at 3 °C (ISO 3) and 9 °C (ISO 9), and at densities of 20 (ISO 20) and 40 (ISO 40) zoea L⁻¹. Survival of zoea larvae fed the A+THAL diet (91.7%) was significantly higher than all others, whereas unfed zoea larvae died within 2 weeks. Temperature and rearing density had no significant effects on survival. Time required to reach stage C1 was significantly greater at 3 °C (109 days) than at 6 °C (70 days), but did not decrease further at 9 °C. After reaching the postlarval (glaucothoe) stage, half of the replicates in the ISO 20 and ISO 40 treatments were fed continuously, but survival did not differ significantly from unfed glaucothoe. We conclude that blue king crab larvae are not lecithotrophic and can be cultivated with high survival using the proper diet. These techniques can be used to produce large numbers of juvenile crab for laboratory research, or could be modified for use in stock-enhancement programmes.

(National Marine Fisheries Service, Kodiak, AK, USA)

PERFORMANCE OF *FARFANTEPENAEUS PAULENSIS* (PÉREZ-FARFANTE, 1967) BROODSTOCK IN TANKS WITH SAND AND HARD SUBSTRATE

Cintia L. Nakayama, Silvio Peixoto, Adalto Bianchini, Ricardo B. Robaldo, Ronaldo O. Cavalli-2008

Aquaculture Research 39 (4): 398–405

Abstract:

This study evaluated the reproductive performance, physical condition and tissue biochemical composition of wild-caught *Farfantepenaeus paulensis* kept for 50 days in maturation tanks provided with sand or hard substrate. The use of sand in *F. paulensis* maturation tanks had limited impact on the performance and biochemical composition of broodstock. Females in the hard-bottom tank produced more spawns and more eggs in total, but those kept in the presence of the sand substrate produced a larger number of nauplii because an improvement in mating success was observed. An increased survival of females was also related to the presence of the sand substrate, which agrees with improvements in water quality and the physical condition of females. On the other hand, sourcing mature females was more difficult and time consuming in sand-bottom tanks. The decision on whether or not to use sand substrates in *F. paulensis* maturation tanks must take into account not only productivity and animal welfare but also considers ease of operation and costs. These findings may have implications for the design of maturation systems for closed thelycum species.

(Fundação Universidade Federal do Rio Grande – FURG, Programa de Pós-Graduação em Aqüicultura, CP, Rio Grande, Brazil; email of R. O. Cavalli: cavalli@mikrus.com.br)

USE OF ENRICHED ROTIFERS AND ARTEMIA DURING LARVICULTURE OF ATLANTIC COD (*GADUS MORHUA* LINNAEUS, 1758): EFFECTS ON EARLY GROWTH, SURVIVAL AND LIPID COMPOSITION

Alexandre Sachida Garcia, Christopher C. Parrish, Joseph A. Brown-2008

Aquaculture Research 39 (4): 406–419

Abstract:

A feeding experiment was conducted to evaluate the effect of rotifers (*Brachionus plicatilis*) and *Artemia* sp. enriched differently on early growth, survival and lipid class composition of Atlantic cod larvae (*Gadus morhua*). Rotifers enrichments tested were: (1) AlgaMac 2000®, (2) AquaGrow® Advantage and (3) a combination of Pavlova sp. paste and AlgaMac 2000®. The same treatments were tested with *Artemia* as well as a combination of DC DHA Selco® and AlgaMac 2000® as a fourth treatment. After rotifer feeding, the larvae from treatment 3 [1.50 ± 0.11 mg dry weight (dw)] were significantly heavier than larvae from treatment 2 (1.03 ± 0.04 mg dw). After feeding *Artemia*, the larvae from treatment 1 were significantly heavier (12.06 ± 2.54 mg dw) than those from treatments 3 (6.5 ± 0.73 mg dw) and 4 (5.31 ± 1.01 mg dw). Treatment 3 resulted in the best survival through the 59 days of larviculture. After rotifer feeding, high larval concentrations of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid (AA) and ω 6 docosapentaenoic acid (ω 6DPA) could be linked to better larval growth and survival while after feeding *Artemia*, high larval DHA/EPA ratios (3) and high DPA/AA ratios (>1) could be linked to better survival. (Ocean Sciences Centre, Memorial University of Newfoundland, St John's, NF, Canada A1C 5S7; email of A. S. Garcia: w24asg@mun.ca)

INFLUENCE OF TANK VOLUME ON VITELLOGENESIS AND SPAWNING PERFORMANCES IN SEA BASS *DICENTRARCHUS LABRAX* L.

Vincent Buchet, Elodie Coquard, Armelle S ev ere, Herv e Barone-2008
Aquaculture Research 39 (4): 420–426

Abstract:

Sea bass, *Dicentrarchus labrax* (mean weight: 748 ± 13 g), were maintained before and during vitellogenesis in 1, 3, 8, 16 and 32 m³ tanks, and then they were transferred to 2 m³ tanks, for the spawning season. During the first 2 months of the experiment, the growth rates were significantly lower in smaller tanks (1 m³). In August, the oocyte diameters were significantly lower in smaller tanks (1, 3 and 8 m³) than in larger (16–32 m³) tanks. At the end of the experiment, the fish mean weight in the 1 m³ tanks was significantly lower than in the 3 m³ tanks, but oocyte diameters and plasma oestradiol concentrations were not significantly different between the volumes. This shows a longer acclimation requirement in smaller volume rearing. Although all the females had not spawned, one spawn at least was collected in each volume. The variation in conditioning volume has not blocked the spawning process. The qualitative and quantitative characteristics of spawns were not significantly different between volumes. The conditioning volume of 3 m³ seems to be a minimal volume required to obtain good reproduction of sea bass.

(UMR 1067, Nutrition Aquaculture and Genomics of Fish, Ifremer, BP 70, 29280 Plouzan e, France; email of V. Buchet: Vincent.Buchet@ifremer.fr)

EFFECT OF THE DUSK PHOTOPERIOD CHANGE FROM LIGHT TO DARK ON THE INCUBATION PERIOD OF EGGS OF THE SPOTTED ROSE SNAPPER, *LUTJANUS GUTTATUS* (STEINDACHNER)

Neil J. Duncan, Leonardo Ibarra-Castro, Ricardo Alvarez-Villase nor-2008
Aquaculture Research 39 (4): 427–433

Abstract:

Spotted rose snapper, *Lutjanus guttatus* (Steindachner), eggs were incubated under different photoperiods to examine the effect of photoperiod on incubation. The eggs from two fish were incubated under five artificial photoperiods: constant dark (D), constant light (L) from 06:00 hours and 6, 10 and 14 h of light from 06:00 hours. The eggs from seven other fish were incubated under a natural photoperiod. Different spawning times (21:00 – 01:00 hours) and different photoperiods combined to give the start of the dusk photoperiod change after 11–23 h of incubation. Constant light or applying the dusk photoperiod change after ≥ 20 h of incubation appeared to extend the hatching period. The mean hatching period for groups of

eggs incubated in darkness or that received the dusk photoperiod change after ≤ 19 h of incubation ($n=8$ different groups) was $2\text{ h }15\pm 10$ min, which was significantly lower ($P<0.05$) than the mean hatching period of $4\text{ h}\pm 37$ min for groups that did not receive the dusk photoperiod change or that received the dusk photoperiod change after ≥ 20 h of incubation ($n=9$ groups). However, despite these differences, the majority of the eggs hatched during a 2–3 h period from 17 to 20 h of incubation, and a sigmoid regression ($r^2=0.9$) explained the relationship between percentage hatch and hours of incubation for all photoperiod groups. (CIAD, A.C. Unidad Mazatlán, A.P. 711, Mazatlán, C.P. 82000, Sinaloa, México; email of N. J. Duncan: neil.duncan@irta.es)

CHANGES IN ATLANTIC COD (GADUS MORHUA L.) SPERM QUALITY DURING THE SPAWNING SEASON

Catherine Rouxel, Marc Suquet, Jacky Cosson, Armelle Severe, Loic Quemener, Christian Fauvel-2008

Aquaculture Research 39 (4): 434–440

Abstract:

The biology of cod reproduction is well described in the scientific literature. However, sperm biology and spermatozoa management are poorly studied in this species. Because of its recent farming expansion, a better knowledge of cod gametes is becoming especially useful. This work aimed at establishing tools to study sperm biology in cod, and also investigated the existence of changes in cod sperm quality during the spawning period. We showed that sperm concentration could be assessed using spectrophotometry at 260 nm. Sperm motility significantly decreased after a 168-h storage at 4 °C. A 1:9 dilution of sperm in a non-activating medium (1/3 seawater and 2/3 freshwater, osmotic pressure: 360 mOsm kg⁻¹) improved sperm storage. Sperm concentration, sperm velocity and storage capacity at 4 °C peaked during the medium period of the spawning season and then decreased to values close to those observed at the beginning of the reproductive period. The measured values of osmotic pressure, pH, protein, Na⁺, Cl⁻ and Ca²⁺ concentrations of the seminal fluid were modified along the spawning period. Cell damage was noted at the end of the spawning period: local blebs were observed on the flagellum but also loops at its distal part. On the other hand, spermatocrit did not vary with the sampling date. In conclusion, cod sperm quality is modified during the spawning period, the highest-quality samples being collected during the medium part of this season.

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DECAPSULATED ARTEMIA AS AN INITIAL FEED ITEM FOR COLORADO RIVER CUTTHROAT TROUT

Ronney Arndt, Eric Wagner-2007

The Ichthyogram 18(2): 7-8

Colorado River cutthroat trout *Oncorhynchus clarkii pleuriticus* are routinely cultured at the Fisheries Experiment Station (FES), Logan, to meet the needs of both conservation and sportfishing efforts. For production, eggs are taken and fertilized using wild brood fish and then shipped to the FES, where eye-up and hatch are generally above 85 %. However, mortalities for the first month or two after first feeding may exceed 25 %. Newly hatched *Artemia nauplii* can be a good source of nutrition for larval fish, and have been a mainstay of marine-fish larval culture for several decades (Sorgeloos et al. 2001). Previous research at the FES has demonstrated that a feeding cycle using *Artemia nauplii* can improve the survival of Colorado River cutthroat trout (Arndt and Wagner 2007). The culture and maintenance of live *Artemia* cultures can be a time consuming endeavour, however. By feeding dried decapsulated *Artemia* cysts to chub, *Leuciscus cephalus* (L.), Harzevili et al. (2003) obtained superior survival compared to other feeding regimes including live *Artemia nauplii*. By

decapsulating cysts and subsequently drying them, a good source of larval nutrition could be obtained at a labor savings compared to live nauplii culture. The purpose of this research was to determine whether or not dried, decapsulated Artemia cysts would be a good source of nutrition to first-feeding cutthroat trout.

The source of the fish for this test was from a wild brood lake, Sheep Creek Lake, located on the north slope of the Uinta Mountains, which ultimately drains to the Colorado River. The eggs were fertilized on site and then brought to the Fisheries Experiment Station, where they further developed until hatching. When approximately 50 % of the recently hatched fish had absorbed their yolk sac and were swimming in the water column they were randomly distributed (200/tank) into twelve 26 L tanks that had a flow of 0.5-0.7 L/min. All four treatments were represented in triplicate. This tank system was housed inside a hatchery building provided with 10 h artificial light per day and with additional, incidental light from a nearby window. Mortalities were removed daily in between feeding events. Beginning at 0700 through 1600, the fish were offered feed hourly. The amount of feed was not quantified as wasted feed is ascertained at this early stage of development. The feed treatments consisted of a commercial swim up diet (Silver Cup, Nelson and Sons, Inc.), live Artemia nauplii, dried decapsulated Artemia cysts, and a second commercial swim up diet (Skretting, Vancouver, Canada). The Silver Cup diet served as a negative control of sorts because it had been used previously with poor results, and the Skretting diet was being tested as a higher end larval diet whose use had been suggested by biologists from the U.S. Fish and Wildlife Service.

The brine shrimp (Great Salt Lake Artemia franciscana) were cultured as outlined by Treece (2000), and the decapsulated, dried cysts were prepared as outlined by Harzevili et al. (2003). The fish were fed the above diets for a total of 18 days, after which the trial was ended and fish were inventoried from all tanks.

Over the course of the 14-day test, survival was not impacted one way or another by treatment application. All treatments exhibited an average survival of 90 % (± 1.4). However, growth was influenced by treatment type. Fish fed the Skretting diet had the highest individual fish weight, 0.32 g/fish, compared to 0.24 for the Silver Cup diet, 0.18 for fish fed dried cysts, and 0.14 for fish fed Artemia nauplii. Growth values for each treatment were significantly different from the other treatments ($P < 0.001$). The amount of time and labor involved was not quantified, but it was evident that larger batches of the dried cysts could be produced more quickly than producing live Artemia nauplii.

For the culture of fresh water species, Artemia can play an important role in early life survival. When European chub *Leuciscus cephalus* were fed decapsulated Artemia cysts, Artemia nauplii, rotifers and Daphnia, and an artificial diet, they grew best on the Artemia nauplii diet (Harzevili et al. 2003), but survival was better within the group fed the dried decapsulated cysts. Because Artemia are a live prey item, it is possible that larval cutthroat trout may prefer them to feed particles. Live nauplii survive for 1-2 hours when placed into fresh water. During that time they actively swim and being positively phototactic, may be more inclined to orient themselves in mid water column or even close to the surface. Commercial feed items may only remain suspended momentarily in the water column after which they fall to the tank bottom where they may remain uneaten by the fish. The theory that larval fish prefer live or active feed items has been supported by the work of Fernandez-Diaz et al. (1994) who determined gilthead sea bream larvae *Sparus aurata* ingested more living prey items compared with inert particles. The concluding results from this small test indicated that either commercial diet exhibited similar survival and superior growth to either Artemia treatment. The dried, decapsulated Artemia were superior to live nauplii with respect to growth, so their use may be desirable in conditions where it is known that the use of brine shrimp is necessary.

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