

UPTAKE AND ELIMINATION OF FLORFENICOL IN ATLANTIC COD (*GADUS MORHUA*) LARVAE DELIVERED ORALLY THROUGH BIOENCAPSULATION IN THE BRINE SHRIMP *ARTEMIA FRANCISCANA*

Irja Sunde Roiha, Erling Otterlei, Amund Litlabø, Ole Bent Samuelsen-2010

Aquaculture 310(1-2): 27-31

Abstract:

As an approach to combat bacterial infections, incorporation of florfenicol in Atlantic cod (*Gadus morhua*) larvae, administered orally through bioencapsulation in *Artemia franciscana* nauplii, was investigated. *Artemia* nauplii were enriched with 1000 mg/l florfenicol for 30 min and administered as a single dose, or daily for three consecutive days. The concentration of florfenicol in the medicated nauplii, and in the cod larvae, was analysed by high performance liquid chromatography (HPLC). Following a single oral administration, the elimination half-life ($t_{1/2\beta}$) was calculated to 14 h, C_{\max} to 4.7 µg/g dry weight (14.3 ng/larva), T_{\max} to 0.5 h, and MRT to 20 h, respectively. An hour after administration of batch 1 in the triple dose study, a mean florfenicol concentration of 4.6 µg/g dry weight (14.0 ng/larva) was found in the cod larvae. One hour following administration of batch 2 and 3, corresponding values were 5.9 µg/g dry weight (17.9 ng/larva) and 4.2 µg/g dry weight (12.8 ng/larva), respectively. These data show that medication of cod larvae using *Artemia* nauplii as carriers of florfenicol is a promising approach for treatment.

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A TAQMAN REAL-TIME PCR ASSAY FOR SURVEY OF WHITE SPOT SYNDROME VIRUS (WSSV) INFECTIONS IN *LITOPENAEUS VANNAMEI* POSTLARVAE AND SHRIMP OF FARMS IN DIFFERENT GROW-OUT SEASONS

Xian-Hong Meng, In Kwon Jang, Hyung-Chul Seo, Yeong-Rok Cho-2010

Aquaculture 310(1-2): 32-37

Abstract:

A TaqMan real-time PCR was conducted to quantify the white spot syndrome virus (WSSV) infections of three *Litopenaeus vannamei* hatcheries and 30 commercial grow-out ponds from different farms, during summer and autumn in South Korea. A total of 98.89% postlarvae showed WSSV-positive with very low WSSV infection load, 0.0–34.3 (mean of 3.2) copies/ng DNA, while all farm samples were WSSV-positive with a mean load of 9.8×10^6 and 2.1×10^5 copies/ng DNA in summer and autumn, respectively. In summer, nine (52.9%) farms showed WSSV infections with fewer than 10^3 copies/ng DNA and a mean shrimp production of 1.05 mt/ha; meanwhile, eight (47.1%) farms showed high infections (i.e., $>10^3$ copies/ng DNA) with a mean shrimp production of 0.85 mt/ha. In autumn, a total of eight (61.5%) farms showed infections with fewer than 10^3 copies/ng DNA, with mean shrimp production of 4.53 mt/ha; five (38.5%) farms showed high infection (i.e., $>10^3$ copies/ng DNA), with a mean shrimp production of 0.42 mt/ha. The mean production of autumn farms with infection loads of less than 10^3 copies/ng DNA, versus that of autumn farms with more than 10^3 copies/ng DNA, was significantly different ($P < 0.05$).

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HIGH-THROUGHPUT DNA EXTRACTION FOR PCR-BASED GENOTYPING OF SINGLE *PENAEUS MONODON* EMBRYOS AND NAUPLII

Min Rao, Stuart J. Arnold, Jeff A. Cowley-2010

Aquaculture 310(1-2): 61-65

Abstract:

A simple technique is described for isolating DNA from individual embryos or nauplii of the Black Tiger shrimp, *Penaeus monodon*. The protocol used a commercial prepGEM™ insect DNA extraction

kit in combination with water bath ultrasonic disruption. DNA quality and yields were sufficient for multiplex PCR discrimination of up to six DNA microsatellites in a single test. The utility of the method was demonstrated in defining the parentage of 1279 embryos (7 h old) spawned from 21 females and 684 newly-hatched N1 to N2 nauplii spawned from 12 females each inseminated artificially with the spermatophores of two different males. Overall, parentage assignments based on the six microsatellite markers were deduced to be $89.5 \pm 6.7\%$ and $93.0 \pm 4.4\%$ effective using DNA isolated from individual embryos and nauplii, respectively. The technique is simple, cost-effective and should provide a robust means of isolating DNA from individual embryos and nauplii of aquatic invertebrates suitable for PCR-based genotyping.

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ANNUAL VARIATION IN THE BIOCHEMICAL COMPOSITION OF NEWLY HATCHED LARVAE OF *MAJA BRACHYDACTYLA* IN CAPTIVITY

Mireia Andrés, Alicia Estévez, Carles G. Simeó, Guiomar Rotllant-2010

Aquaculture 310(1-2) : 99-105

Abstract:

Quality of newly hatched larvae (NHL) of *Maja brachydactyla* in captivity has been characterized throughout the year to evaluate their availability for mass production. Spawning took place every month and NHL were collected and analyzed to estimate individual dry weight (DW) and proximate biochemical composition (protein, carbohydrate and lipids). Lipid class, fatty acid composition, amino acid profile, mineral and vitamins A, E and C contents were analyzed seasonally. NHL obtained throughout the year are a potential source for aquaculture purposes, since the increment in the relative protein and lipid (especially phospholipids and n-3 PUFA) content might compensate the decrease in DW of larvae hatched from broodstock kept during one year in captivity. However, the decrease in vitamins A and E as well as in certain essential amino acids (Lys, Val, and His) and trace elements (Cu and Fe) of NHL at the end of the year might be indicative of a nutritional deficiency in broodstock diets. (IRTA Sant Carles de la Ràpita, Ctra Poble Nou, km. 5.5, 43540, Sant Carles de la Ràpita, Spain ; email of Mireia Andrés : mireia.andres@gmail.com)

LIPID CONTENT AND FATTY ACID COMPOSITION IN WILD-CAUGHT SILVER POMFRET (*PAMPUS ARGENTEUS*) BROODSTOCKS: EFFECTS ON GONAD DEVELOPMENT

Xuxiong Huang, Yanqiang Yin, Zhaohong Shi, Weiwei Li, Hongqi Zhou, Weiqun Lv-2010

Aquaculture 310(1-2): 192-199

Abstract:

The fatty acid reserves of broodstocks profoundly affect the fecundity of spawners as well as the quality of the newly hatched larvae. Investigation of the lipid and fatty acid changes in wild-caught broodstocks during the reproductive season should elucidate the most effective strategy with which to enrich broodstocks in artificial breeding programs. This paper investigated the lipid and fatty acid compositions of wild broodstocks of silver pomfret, *Pampus argenteus*, caught at the Zhoushan fishery ground during the reproductive season. The results indicated that there were significant differences in lipid content among gonad, muscle and liver; mean lipid levels in ovary, testis, liver and muscle were 41.85%, 18.54%, 27.80% and 32.31%, respectively. The lipid content in gonads at developmental stages III–V was relatively more stable than that in muscle or liver, although the lipid level increased in ovary and decreased in muscle as the female broodstocks developed from phase III to phase V. There were significant differences in fatty acid levels among broodstock ovary, testis, muscle and liver. The dominant fatty acids in ovary were as follows: 18:1n-7 (16.91%) > DHA (docosahexaenoic acid) (15.46%) > 16:4n-3 (13.14%) > 16:0 (12.65%) > EPA (eicosapentaenoic acid) (6.46%) > 16:1 (5.34%). In female broodstock liver and muscle, the dominant fatty acids were 16:0 (36.42%) > 18:1n-7 (23.26%) > 18:0 (7.50%) > 16:1 (6.98%) > 14:0 (5.10%) > DHA (4.98%) and 18:1n-9 (26.35%) > 16:0 (24.07%) > DHA (8.38%) > 16:1 (6.62%) > 18:0 (5.48%) > 14:0 (4.44%), respectively. The dominant fatty acids in testis were as follows: DHA (29.84%) > 16:0 (23.93%) > 18:1n-9 (8.78%) > 18:0 (7.98%)

> EPA (7.96%) > ARA (arachidonic acid) (3.24%). In the liver and muscle of male broodstocks, the dominant fatty acids were 16:0 (30.14%) > 18:1n9 (25.54%) > 18:0 (8.24%) > 16:1 (7.24%) > 14:0 (5.20%) > DHA (4.75%) and 18:1n9 (29.51%) > 16:0 (23.90%) > DHA (7.80%) > 18:0 (6.34%) > 16:1 (6.22%) > 14:0 (3.88%), respectively. The n-3 PUFAs and n-3/n-6 ratios in ovary and testis of the broodstocks were significantly higher than those in the liver and muscle of the broodstocks. The changes in fatty acid composition in ovary and testis as the broodstocks developed from phase III to phase V were smaller in magnitude than those in muscle or liver. It is therefore suggested that the lipid preferentially transferred to ovary from muscle during the reproductive season, and the PUFAs, such as DHA, EPA, 16:4n-3 and ARA, were selectively transferred to, and conserved in, the ovaries of silver pomfret broodstocks.

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CAN DIETARY AROMATIC AMINO ACID SUPPLEMENTATION BE BENEFICIAL DURING FISH METAMORPHOSIS?

Wilson Pinto, Vera Rodrigues, Maria Teresa Dinis, Cláudia Aragão-2010

Aquaculture 310(1-2): 200-205

Abstract:

Aromatic amino acids (AA, phenylalanine and tyrosine) are the precursors of thyroid hormones, which regulate metamorphosis in all vertebrates. In fish, this is a critical developmental stage where physiological requirements for aromatic AA may increase. Therefore, it is possible that dietary aromatic AA supplementation may be beneficial to accomplish a successful transition from larvae to the juvenile stage. This work aimed to assess the effect of dietary aromatic AA supplementation on tyrosine metabolism in species with an accentuated (Senegalese sole, *Solea senegalensis*) and a less marked (gilthead seabream, *Sparus aurata*) metamorphosis. For this purpose, either species were fed *Artemia* and subsequently received or not an aromatic AA supplement through tube-feeding, together with a L-[U-14C] tyrosine marker, throughout metamorphosis. Tyrosine was elected as a tracer since the metabolic fate of this aromatic AA is directly linked to the production of thyroid hormones and metamorphosis. Results showed that dietary phenylalanine supplementation did not increase tyrosine retention in Senegalese sole larvae during metamorphosis, suggesting that this species may not be able to biosynthesise tyrosine from phenylalanine at a sufficient rate to supply its physiological requirements until after metamorphosis. Furthermore, dietary tyrosine supplementation effectively increased tyrosine availability in Senegalese sole body fluids at metamorphosis, most likely for coping with metamorphosis-related processes, such as production of thyroid hormones. Therefore, dietary tyrosine supplementation may be beneficial for Senegalese sole during metamorphosis. On the other hand, results indicated that gilthead seabream larvae do not seem to require an additional supply of aromatic AA during metamorphosis, probably because the physiological requirements for tyrosine do not increase during this developmental stage. The different results observed for Senegalese sole and gilthead seabream are probably related to the complexity of metamorphosis that each species undergoes and to the needs for production of thyroid hormones, which seems to affect aromatic AA requirements during this critical stage of development. These findings may be important for physiologists, fish nutritionists and for the flatfish aquaculture industry.

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ADVANCED OVARIAN DEVELOPMENT OF MURRAY COD *MACCULLOCHELLA PEELII* *PEELII* VIA PHASE-SHIFTED PHOTOPERIOD AND TWO TEMPERATURE REGIMES

Dane M. Newman, Paul L. Jones, Brett A. Ingram-2010

Aquaculture 310(1-2): 206-212

Abstract:

The timing and characteristics of reproductive development in adult female Murray cod exposed to a simulated seasonal photothermal cycle (14:45 to 09:45 daylight h; 12–26 °C) (CONTROL) were

compared to the development of females exposed to a three month phase-shifted (advanced) seasonal photothermal cycle (PHOTOTHERMAL) and to females exposed to a three month phase-shifted (advanced) photoperiod cycle in combination with constant temperature (19.5 °C) (PHOTOPERIOD). Females in PHOTOTHERMAL and PHOTOPERIOD treatments reached maturity up to three (June) and four (May) months in advance of CONTROL fish (October), respectively. Biannual maturation was also observed in four PHOTOPERIOD females (13%). Mean ovary diameter and relative fecundity of mature females were similar between treatments ($p > 0.05$), and viable eggs were produced in all groups (100% ovulated; 14.02%–39.12% mean survival to hatching). Ovary diameters and plasma levels of E_2 and T in phase-shifted females remained at basal levels and/or were significantly reduced ($p < 0.05$) relative to CONTROL fish throughout the early to mid phases of the maturation period. However, rapid increases in plasma T ($0.54\text{--}4.39\text{ ng ml}^{-1}$) and ovary diameter (20.0–42.4 mm) in the 60 to 90 days preceding the onset of maturity in phase-shifted females revealed a capacity of Murray cod to accelerate development processes to compensate for earlier delays in photo-responsiveness. Low levels of E_2 that persisted throughout the maturation period of PHOTOTHERMAL and PHOTOPERIOD females did not appear to greatly affect ovarian growth. The successful maturation of photoperiodically-manipulated females under constant temperature demonstrates an alternative approach for influencing maturation patterns in Murray cod that may improve the versatility and cost-effectiveness of broodstock conditioning procedures.

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REPLACING ROTIFERS WITH A MICROPARTICLE DIET FROM FIRST FEEDING IN YELLOWFIN SEABREAM, ACANTHOPAGRUS LATUS (HOULTUYN), LARVAE

Behzad Sarvi, Abbas Matinfar, Homayoun Mahmoudzadeh, Gholam Reza Eskandary-2010
Aquaculture Research 41: 1614-1621

Abstract:

The possibility to replace or reduce rotifer use with a microparticle diet (MPD) from first feeding in yellow-fin seabream larvae was investigated. The experiment consisted of five treatments, a rotifer (100%) control treatment (100R), three experimental treatments, which received a fixed ration of MPD supplemented with 75%, 50% and 25% of the amount rotifer fed in the control treatment (treatments 75R-MPD, 50R-MPD and 25R-MPD respectively), and finally a treatment with the fixed MPD ration only (treatment 0R-MPD). The results indicated that feeding regimes 100R, 75R-MPD and 50R-MPD did not differ significantly in the total length, final survival and stress test resistance. The highest dry weight was achieved in treatments 100R and 75R-MPD at the end of the experiment compared with treatments 50R-MPD, 25R-MPD and 0R-MPD. The 0R-MPD treatment showed the lowest growth and survival. Yellow-fin seabream larvae were able to ingest inert food directly from the moment of first feeding.

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A QUANTITATIVE METHOD FOR DETECTING AMMONIA-OXIDIZING BACTERIA IN COASTAL AQUACULTURE SYSTEMS

Kishore K. Krishnani, Velusamy Kathiravan-2010
Aquaculture Research 41: 1648-1657

Abstract:

There is a need to quantify autotrophic nitrifiers in coastal aquaculture systems for evolving a bioremediation strategy. Autotrophic nitrifiers are extremely slow-growing organisms, which cannot be detected by traditional methods as they are notoriously difficult to culture. Molecular techniques based on functional genes could be deployed for the detection of nitrifiers. Ammonia monooxygenase (amoA), that catalyses the oxidation of ammonia to hydroxylamine in the rate-determining step of nitrification is largely unique to ammonia-oxidizing bacteria (AOB). In the present study, a quantitative real-time polymerase chain reaction assay targeting amoA was developed to estimate AOB population size in coastal soil, ammonia-removing bioaugmentors and the solid matrix. To achieve this objective,

different set of primers and a dual labelled probe have been designed for SYBR Green and TaqMan real-time assays. The abundance of AOB ranged from 10⁴ to 10⁶ order of magnitude in the samples. In the present study, biofilm formation of the consortium of nitrifying bacteria onto bagasse has also been quantified. The results demonstrate that the developed method is a rapid and sensitive tool for the quantitative detection of nitrifying bacteria in aquatic and related environment. This helps in making the bioremediation approach for ammonia removal by immobilization of nitrifying bacteria onto the natural substrate.

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REPLACEMENT OF LIVE FEED BY FORMULATED FEED: EFFECT ON THE GROWTH AND SPAWNING PERFORMANCE OF SIAMESE FIGHTING FISH (BETTA SPLENDENS, REGAN, 1910)

Sagar C. Mandal, Narottam P. Sahu, Mahinder P. Singh Kohli, Pronob Das, Sanjay K. Gupta, Sukham Munilkumar-2010

Aquaculture Research 41(11): 1707–1716

Abstract:

Partial replacement of live feed (LF) with formulated feed (FF) was performed in *Betta splendens* to evaluate their growth, survival and reproductive performance. Three hundred *B. splendens* fry of uniform size (mean weight 0.19 g±0.01) were equally distributed in five treatment groups with each of three replicates in glass aquaria of 35 L capacity. Fish were offered diets of different ratio of LF and FF like T1 (control) – 100% LF; T2 – 75% LF, 25% FF; T3 – 50% LF, 50% FF; T4 – 25% LF, 75% FF and T5 – 100% FF. Highest (P<0.05) body weight gain (% BWG) (67.1±1.03) and specific growth rate (2.34±0.02) were observed in the T2 group. Best feed conversion ratio was also found in T2 (2.40±0.11), which was similar to T1 and T3. The highest survival rate (%) was recorded in T1, T2 and T3 groups (97±1.7), which was similar to T4 and the lowest in T5 (49±2.0). The highest gonadal weight (0.12 g±0.01) and gonadosomatic index (15.17±0.50) was observed in the T3 group during the first sampling after 63 days. No significant changes in spawning performance were observed in the second sampling after 21 days after first sampling. However, T4 group registered the highest fecundity and fertilized eggs at the end of 105 days of experiment. From the present study, it concludes that LF can be successfully replaced to the extent of 25% by FF without any detrimental effect on the growth, survival and spawning performance of *B. splendens*.

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ROLES OF MICROALGAE ON TOTAL EGG PRODUCTION OVER FEMALE LIFESPAN AND EGG INCUBATION TIME, NAUPLIAR AND COPEPODITE SURVIVAL, SEX RATIO AND FEMALE LIFE EXPECTANCY OF THE COPEPOD BESTIOLINA SIMILIS

Thomas Camus, Chaoshu Zeng-2010

Aquaculture Research 41(11): 1717–1726

Abstract:

We examined the effects of 10 microalgal diets on a range of productivity-related parameters of *Bestiolina similis*. The diets tested included four monoalgal diets: Tahitian strain *Isochrysis* sp. (T-Iso), *Pavlova salina* (Pav), *Tetraselmis chuii* (Tet) and the diatom *Chaetoceros muelleri* (Chaet), five binary diets: Tet+Pav, T-Iso+Tet, T-Iso+Pav, Chaet+Tet and Chaet+Pav and a tri-algal diet: T-Iso+Tet+Pav. Except for sex ratio, microalgal diets significantly affected all parameters examined (P<0.05). The shortest egg incubation time was found for the tri-algal diet treatment (1.91±0.04 days) while the longest (2.41±0.09 days) was from the monoalgal diet Tet. Survival at the naupliar stage ranged from the highest of 86.3±1.0% for the tri-algal diet to a complete mortality for the Tet diet. Naupliar survival was consistently higher than that of copepodite stages for all diets examined and ranged from 86.3±1.0% for the tri-algal diet to a complete mortality for the Tet diet. Copepodite survival was the highest for the T-Iso treatment (69.0±4.3%) while Chaet+Tet produced the lowest survival (8.8±1.2%).

Sex ratio of *B. similis* was strongly skewed towards females. Adult female life expectancy was highly dependent on diet. The shortest lifespan (3.1 ± 0.3 days) of the Tet treatment was only about half of that of Pav (6.3 ± 0.6 days) and the tri-algal diet (5.9 ± 0.4 days). Daily egg production during female lifespan was generally lower on the first day, but peaked over the subsequent days before decreasing towards the end of their lifespan. The highest total egg output over female lifespan was produced by the tri-algal diet (156.0 ± 11.5 eggs female⁻¹), while the lowest output came from the diet Tet (20.7 ± 2.8 eggs female⁻¹).

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EFFICACY OF FORMALIN AND POVIDONE–IODINE DISINFECTION TECHNIQUES ON THE EGGS OF THREE MARINE FINFISH SPECIES

Kevin R Stuart, Martha Keller, Mark Drawbridge-2010

Aquaculture Research 41(11): e838–e843

Abstract:

Surface disinfection trials were performed on eggs from three marine finfish species: California yellowtail (CYT; *Seriola lalandi*), white seabass (WSB; *Atractoscion nobilis*) and California halibut (HA; *Paralichthys californicus*). All three species were spawned from captive populations maintained at the Hubbs-SeaWorld Research Institute (HSWRI). Five disinfection treatments were used for each species; Treatment 1 included 100 mg L⁻¹ of formalin (F100) for 60 min (current HSWRI treatment), Treatment 2 included 1000 mg L⁻¹ of formalin for 15 min (F1000), Treatment 3 included povidone – iodine of 50 mg L⁻¹ for 15 min (PI50), Treatment 4 included povidone -iodine of 100 mg L⁻¹ for 10 min (PI100) and Treatment 5 involved a control with no chemical treatment (CONT). For each treatment, the per cent egg hatching rate, per cent survival to first feeding and notochord length at the time of hatching to the nearest 0.1 mm were recorded. Bacteria were also cultured from eggs after treatment to determine the effectiveness of each treatment in reducing the bacterial counts (CFU mL⁻¹). Treatments F100, F1000 and CONT yielded the highest hatch rates for each species (70–80%), whereas treatments PI50 and PI100 yielded the lowest hatch rates (0–2%). There were no significant differences in survival to first feeding or notochord length, which suggests that the disinfection treatments did not have a negative effect on the yolk sac larvae. The PI50 and PI100 treatments had the lowest bacterial colony counts, showing almost zero bacterial growth. The highest bacterial growth occurred in the F100, F1000 and CONT treatments. Based on the results from this study, the F100 treatment provided the best balance of disinfection and larval health for CYT, WSB and HA.

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SPAWNING PERFORMANCE AND SEED PRODUCTION FROM HYBRIDIZATION BETWEEN OREOCHROMIS SPILURUS AND THE GIFT STRAIN OF THE NILE TILAPIA OREOCHROMIS NILOTICUS

Mohammad T. Ridha-2010

Aquaculture Research 41(11): e723–e729

Abstract:

This study compared the spawning performance of four crosses between the salt-tolerant tilapia *Oreochromis spilurus* and the fast-growing genetically improved farmed tilapia (GIFT) strain of the Nile tilapia *Oreochromis niloticus*: (1) *O. spilurus*♀ × *O. spilurus*♂ (SS); (2) Nile ♀ × Nile ♂ (NN); (3) Nile ♀ × *O. spilurus*♂ (NS) and (4) *O. spilurus*♀ × Nile ♂ (SN). In each cross, males and females having mean weights of 157 and 115 g, respectively were stocked in 0.43 m³ tanks at a male to female ratio of 1:3. Seeds (unhatched eggs, yolk-sac fry and free swimming fry) were harvested biweekly for 127 days. Viable spawns resulted from all crosses. The NN cross produced the highest total seeds tank⁻¹ (2890), seeds (kg female)⁻¹ day⁻¹ (48.3), seeds m⁻² day⁻¹ (23.3) and seeds female⁻¹ day⁻¹ (7.9), followed by the SS and SN crosses. The NS cross had the lowest fecundity. Lower spawning parameters in the NS and SN crosses may be attributed to the lower social compatibility between the males and females

compared with that of the pure parents. The survival and growth of the offspring from the four crosses will be compared in seawater (37–40 g L⁻¹) to assess whether hybrid vigor was produced. (Mariculture and Fisheries Department, Food Resources and Marine Sciences Division, Kuwait Institute for Scientific Research PO Box 1638, 22017 Salmiyah, Kuwait; email of M. T. Ridha: mridha@mfd.kisr.edu.kw)

NATURAL ZOOPLANKTON AS LARVAL FEED IN INTENSIVE REARING SYSTEMS FOR JUVENILE PRODUCTION OF ATLANTIC COD (*GADUS MORHUA* L.)

Kjersti Eline Tønnessen Busch, Inger-Britt Falk-Petersen, Stefano Peruzzi, Nora Arctander Rist, Kristin Hamre-2010

Aquaculture Research 41(12): 1727–1740

Abstract:

The growth potential of cod larvae is not fully achieved when rotifers (*Brachionus* spp.) are used as live feed. In this experiment, we studied the effect of natural zooplankton (mainly copepods) on the growth of cod (*Gadus morhua* L.) larvae reared in intensive systems. Using a growth model developed for cod larvae, the growth rates observed could be evaluated and compared with growth rates reported previously. The cod larvae showed optimal growth rates until age 19 days post hatch (DPH) when they reached 9.77 ± 0.25 mm standard length (SL). Early weaning (20–25 DPH) resulted in significantly longer larvae at age 30 DPH compared with late weaning (25–32 DPH); however, in this period, the zooplankton concentrations were low. The experimental larvae showed considerably higher growth rates compared with rotifer (*Brachionus* spp.)-reared cod larvae in previous experiments. The nutritional composition of cod larvae was analysed and compared with published results on rotifer-reared larvae. The levels of iodine, manganese, selenium and n-3 PUFA were considerably higher in larvae fed copepods compared with larvae fed rotifers. The differences in nutritional status may well explain the differences in growth observed between copepod and rotifer-reared larvae.

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EFFECTS OF TEMPERATURE ON FERTILIZED EGGS AND LARVAE OF TAWNY PUFFER *TAKIFUGU FLAVIDUS*

Yong-Hai Shi, Gen-Yu Zhang, Ya-Zhu Zhu, Jian-Zhong Liu, Wei-Ling Zang-2010

Aquaculture Research 41(12): 1741–1747

Abstract:

Tawny puffer *Takifugu flavidus* is a species found in China considered to have potential for aquaculture. Experiments were conducted to determine the optimal temperature for its incubation and larval culture. Fertilized eggs collected from cultured broodstocks that were induced to ovulate with a [d-Ala⁶-Pro⁹-Net]-luteinizing hormone-releasing hormone analogue were inseminated. The effect of temperature (19, 20, 23, 26 and 29 °C) on the hatch rate, incubation period, viability of 24 h post-hatch larvae and total mortality rate was assessed. The effect of temperature (20, 23, 26 and 29 °C) on the growth and survival of larvae from 3 to 19 days after hatching (dah) was also assessed. The results showed that the optimal temperature for successful development of fertilized eggs ranged from 23 to 26 °C, and the highest hatch rate, the optimal viability of 24 h post-hatch larvae and the lowest total mortality rate were all predicted using quadratic equations. The relationship between temperature and the incubation period of tawny puffer eggs was determined using the effective degree-day model. The temperature at developmental zero (t₀) was 11.34 °C, and the sum of effective degree-days (k) was 52.356. The survival rate of tawny puffer larvae at 20 °C was significantly lower than among 23, 26 and 29 °C, whereas the survival rate was not significantly different from that at 23, 26 and 29 °C. The larval growth rate increased rapidly as the temperature increased, showing a linear relationship in the range of temperatures investigated. The optimal temperature for larval culture ranged from 23 to 29 °C.

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FERTILIZATION SUCCESS AND BLASTOMERE MORPHOLOGY AS PREDICTORS OF EGG AND JUVENILE QUALITY FOR DOMESTICATED ATLANTIC COD, *GADUS MORHUA*, BROODSTOCK

Øyvind Johannes Hansen, Velmurugu Puvanendran-2010

Aquaculture Research 41(12): 1791–1798

Abstract:

We investigated if simplified routines based on blastomere morphology and fertilization success can be used as predictors of egg and embryo viability in Atlantic cod, and abnormal blastomere morphology is related to juvenile deformities. Egg morphology data from 312 families that were produced by hand stripping of gametes were used. Fertilized eggs from all families were separately incubated in 25 L incubators. Blastomere morphology and fertilization success were assessed at 16–32 cell stage. Embryo mortality was measured until hatch. Fertilization success showed a significant positive correlation with the proportion of normal blastomere and 74% of the variation in the incidence of blastomere abnormality was explained by fertilization success. Juvenile deformities were positively correlated with blastomere abnormality and 8% of the variation in juvenile deformities was explained by incidences of blastomere abnormality. To our knowledge, this is the first direct evidence showing that incidences of blastomere abnormalities may be related to cod juvenile deformities. Our results suggest that fertilization success and the occurrence of blastomere abnormalities could be easily adopted by commercial cod hatcheries for the early determination of egg quality.

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FEEDING, EGG PRODUCTION AND LABORATORY CULTURE OF SCHMACKERIA POPLESIA SHEN (COPEPODA: CALANOIDA)

Guang-xing Liu, Dong-hui Xu-2010

Aquaculture Research, 41: 1817-1826

Abstract:

Copepods are candidates with great potential as live prey for rearing fish larvae and juveniles in aquaculture; however, the techniques for a large-scale culture of copepods are yet to be developed. In this study, we examined the effects of water temperature, salinity, prey concentration and algal species on the grazing and egg production rates of a calanoid copepod *Schmackeria poplesia* (Copepoda: Calanoida). The results showed that the grazing rate of *S. poplesia* was the highest when the copepods were cultured in seawater with temperature of 25 °C, salinity of 20 g·L⁻¹, prey concentration at 105 cells mL⁻¹ and supplied with *Platymonas helgolandica* as the prey. The egg production rates, however, was the highest when copepods were fed with a mixed prey of *Isochrysis galbana* and *Phaeodactylum tricorutum* (cell ratio 1:1, prey concentration 105 cells mL⁻¹) at 25 °C, 20 g L⁻¹ of salinity. A 100 L cultural system was established to culture *S. poplesia* under the condition optimized for egg production. The total number of copepods increased 40–43-fold with the production rates of 87–290 copepods L⁻¹ day⁻¹ in 14 days. This research was the first attempt for a large-scale culture of *S. poplesia* and the technique established can be further applied in aquaculture.

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ONTOGENIC CHANGES IN THE DIGESTIVE ENZYME PATTERNS AND CHARACTERIZATION OF PROTEASES IN INDIAN MAJOR CARP *CIRRHINUS MRIGALA*

R. Chakrabarti, R.M. Rathore-2010

Aquaculture Nutrition 16(6): 569–581

Abstract:

Digestive enzymes of *Cirrhinus mrigala* (Ham.) were studied during ontogenic development. Specific amylase activity was detected in first feeding fish. The enzyme activity decreased up to day-18 and then it increased with the age of fish to reach the highest level on day-34. Protease activity was 28.61 ± 8.90 mU mg protein⁻¹ min⁻¹ on day -4 and increased with the age throughout the study period. Trypsin activity was 31.86 ± 1.12 mU mg protein⁻¹ min⁻¹ on day -4. The activity decreased up to day-10 and

from day-12 onwards increased up to day-26. Chymotrypsin activity was 14.56 ± 2.74 mU mg protein⁻¹ min⁻¹ on day-4 and constantly increased up to day-26. A significant increase in lipase activity was observed between days-24 and 34. SDS-PAGE and substrate SDS-PAGE showed the diversity of protein (17.4–127.8 kDa) and protease activity bands (16.6–88.8 kDa) during ontogenesis. Soybean trypsin inhibitor, phenyl methyl sulphonyl fluoride, N--p-tosyl-l-lysine chloromethylketone and N-tosyl-l-phenylalanine chloromethylketone inhibited the protease activity up to 79.72–97.21, 65.55–94.83, 45.41–75.31 and 40.78–64.72%, respectively. Inhibition study in substrate SDS-PAGE revealed the abundance of serine proteases and the presence of isoforms of trypsin and chymotrypsin. Ethylenediamine-tetraacetate showed 5.56–22.78% inhibition of metal ion-specific enzyme activity. (Aqua Research Lab, Department of Zoology, University of Delhi, Delhi 110007, India; email of Rina Chakrabarti: aquaresearchlab@yahoo.co.in)

THE EFFECT OF FOOD QUALITY ON GLYCOGEN CONTENT, THE FATTY ACID PROFILE AND WINTER MORTALITY IN CULTIVATED OYSTER SPAT (*OSTREA EDULIS*)

M.B. Løfstedt-2010

Aquaculture Nutrition 16(6): 625–636

Abstract:

Winter mortality in hatchery reared oyster spat (*Ostrea edulis*) that received three different diets during the summer period was investigated. Oysters fed a natural type diet had a winter mortality of $18.3 \pm 6.3\%$ while oysters fed cultivated algae (a mixture of *Tetraselmis suecica*, *Isochrysis galbana* and *Chaetoceros muelleri*) had a mortality of $73.0 \pm 9.7\%$. A group of oysters fed a mix between the two diets had a mortality of $54.7 \pm 10.6\%$. Tissue samples were taken at the start of the experiment, after the summer period and after the winter period in order to determine growth and the content of glycogen and fatty acids. The glycogen content decreased for all groups during the winter but the decrease was highest in oysters fed the natural diet. This group also contained the largest variety of fatty acids, but there was no difference in the content of the essential fatty acids EPA, DPA and DHA between the groups. It is concluded that transplantation of spat to the sea in spring and early summer may reduce winter mortality since the feeding period on a more varied natural algal diet is prolonged compared to transplantation of spat later in the season.

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PROTEOME PROFILE COMPARISON OF TWO DIFFERENTLY FED GROUPS OF ATLANTIC COD (*GADUS MORHUA*) LARVAE

H. Sveinsdóttir, A. Gudmundsdóttir-2010

Aquaculture Nutrition 16(6) : 662–670

Abstract:

Proteome analysis was used to study the effects of feeding early Atlantic cod (*Gadus morhua*) larvae with a saithe (*Pollachius virens*) protein hydrolysate (SPH). Protein hydrolysates have previously been shown to beneficially affect fish larval development. Feeding was initiated on day 2 post hatch (ph) or as soon as the larvae opened their mouth and the protein expression was monitored 4 days later or in 6-dph cod larvae. The results demonstrated changes in the abundance of 13 protein spots in the cod larvae fed SPH. Of these, seven protein spots were up-regulated and six protein spots showed down-regulation. Five of the up-regulated proteins in cod larvae are known to be involved in energy metabolism. A few early larval specific proteins were down-regulated in the SPH-fed cod larvae possibly because of an enhanced development in this group relative to the control group. Two trypsin isoforms were detected within the cod larval proteome. The detection of the trypsin spots was made possible by co-electrophoresis of known cod trypsins with the cod larval protein extract. Surprisingly, no difference in trypsin content was observed between the SPH-fed and the control larval groups.

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LONG-TERM PLAN FOR DOMESTICATION OF THE WHITE GROUPER (EPINEPHELUS AENEUS) IN ISRAEL

Sergei Gorshkov-2010

The Israeli Journal of Aquaculture - Bamidgeh 62(4): 215-224

Abstract:

The objective of this article is to highlight culture problems, bottlenecks to broodstock management, and possible genetic consequences to be taken into consideration when planning the domestication and captive breeding program for white grouper (*Epinephelus aeneus*) in Israel. Taking into account the biological features and genetic background of the white grouper, we suggest developing industrial cultivation in several stages. In stage 1, the establishment of broodstock and first steps towards domestication, emphasis should be placed on avoiding inbreeding in captive populations. Establishment of foundation population(s) is the most important step in broodstock management and determines the amount of genetic variation and culture potential of future domesticated generations. In stage 2, existing populations and strains will be tested under experimental and commercial growout conditions. In stage 3, traditional selective breeding methods including mass selection and crossbreeding will be implemented. In stage 4, development of marker-assisted selective breeding molecular techniques will enable assigning parentage to individual fish in mixed-progeny groups and design of an efficient genetic improvement program. Finally, in stage 5, organized and supervised domestication and selective breeding programs will provide Israeli farmers more control of broodstocks and sustainable development of white grouper aquaculture.

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EFFECTS OF PHOTOPERIOD ON GROWTH, FEED CONVERSION EFFICIENCY, AND SURVIVAL OF FRY AND FINGERLINGS OF MAHSEER, TOR PUTITORA (HAMILTON)

Simple Sawhney, Roopma Gandotra-2010

The Israeli Journal of Aquaculture - Bamidgeh 62(4): 266-271

Abstract:

The effect of photoperiod on the growth, feed conversion efficiency, and survival of mahseer (*Tor putitora*) fry and fingerlings was investigated in two simultaneous experiments. In the first experiment, triplicate groups of 30 fry (0.27 ± 0.01 g) were stocked in 100-l plastic tubs. A flow-through system with aerators was used to maintain the optimum dissolved O₂ level. The fish were exposed to one of four photoperiods (18 h light:6 h dark, 12 h light:12 h dark, 6 h light:18 h dark, and the natural photoperiod of 10 h light:14 h dark). The fry were fed a 45% protein diet at the satiation rate of 5% of body weight for 90 days in laboratory conditions. The best weight gain, specific growth rate, feed conversion efficiency, and survival were achieved in 18L:6D treatment. Fry performance was significantly retarded in the 6L:18D treatment. In the second experiment, triplicate groups of eight fingerlings (6.08 ± 0.03 g) were stocked in 100-l tubs and exposed to the same photoperiods as in the first experiment. The fingerlings were fed a 38% protein diet at the rate of 5% body weight for 90 days. The growth performance of the fingerlings was not significantly affected by photoperiod. Results show that *Tor putitora* fry, but not fingerlings, reared in lab conditions are significantly affected by photoperiod regime.

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FACTORS INFLUENCING THE RELATIVE FITNESS OF HATCHERY AND WILD SPRING CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) IN THE WENATCHEE RIVER, WASHINGTON, USA

Kevin S. Williamson, Andrew R. Murdoch, Todd N. Pearsons, Eric J. Ward, Michael J. Ford-2010

Can. J. Fish. Aquat. Sci. 67(11): 1840-1851

Abstract:

Understanding the relative fitness of naturally spawning hatchery fish compared with wild fish has become an important issue in the management and conservation of salmonids. We used a DNA-based parentage analysis to measure the relative reproductive success of hatchery- and natural-origin spring Chinook salmon (*Oncorhynchus tshawytscha*) in the natural environment. Size and age had a large influence on male fitness, with larger and older males producing more offspring than smaller or younger individuals. Size had a significant effect on female fitness, but the effect was smaller than on male fitness. For both sexes, run time had a smaller but still significant effect on fitness, with earlier returning fish favored. Spawning location within the river had a significant effect on fitness for both sexes. Hatchery-origin fish produced about half the juvenile progeny per parent when spawning naturally than did natural-origin fish. Hatchery fish tended to be younger and return to lower areas of the watershed than wild fish, which explained some of their lower fitness.

EXPRESSION OF DMRT FAMILY GENES DURING GONADAL DIFFERENTIATION IN TWO SPECIES OF ARTEMIA (BRANCHIOPODA, ANOSTRACA) FROM URMIA LAKE (IRAN)

Farazmand Ali, Inanloo Kolsoum, Agh Naser-2010

Crustaceana, 83(10): 1153-1165

Abstract:

The DMRT (doublesex- and mab-3-related transcription factor) family of genes, originally implicated in the sex differentiation of *Drosophila* and *Caenorhabditis elegans*, is now known to be involved in early differentiation of sex among species in a variety of phyla. The conserved motif shared by these two genes in all organisms is referred to as the DM domain. In the present study the expression patterns of DMRT-related genes in the differentiating gonads of two species of aquatic crustaceans, *Artemia urmiana* and *Artemia parthenogenetica*, which differ in the paths adopted for reproduction, were compared. Studies using the RT-PCR procedure on the gonads of nauplius larvae of these species at different stages of early development indicated that DMRT genes are expressed only in female gonads, regardless of the mode of reproduction prevalent in the specific species of *Artemia*. In situ hybridization in whole mounts of gonads at different stages of development confirmed our RT-PCR results, suggesting that, unlike the situation in mammals, DMRT genes are involved in the early ovarian, but not in testicular, differentiation.

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TIMING AND SELECTIVITY OF MORTALITY IN REARED ATLANTIC COD REVEALED BY OTOLITH ANALYSIS

Arild Folkvord, Roland M. Koedijk, Vibeke Lokøy, Albert K. Imsland-2010

Environmental Biology of Fishes 89(3-4): 513-519

From the issue entitled "Proceedings of the 4th International Otolith Symposium, 24-28 August 2009, Monterey, California"

Abstract:

In juvenile fish production, large samples of known-aged material can be sampled at pre-determined time intervals from the same population. This enables an accurate determination of size-selective mortality by means of repeated samplings of fish and comparison of otolith size-at-age based on samples from different dates. An example is provided from an experiment with larval and juvenile Atlantic cod, *Gadus morhua*, where groups fed smaller sized enriched rotifers did not reveal any size-selective mortality during weaning to formulated feed, while those groups that were fed larger live natural zooplankton lost a significant fraction of the smaller-sized individuals during the same time period. This was contrary to the overall mortality which was higher among the rotifer fed groups during weaning. Part of this difference may be attributed to size differences between the groups, where larger zooplankton fed larvae were more prone to engage in cannibalistic behaviour.

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EFFECT OF DIETARY MARINE LIPIDS ON FEMALE WHITE BASS OVA COMPOSITIONS AND PROGENY SURVIVAL

H. A. Lewis, J. T. Trushenski, R. L. Lane, C. C. Kohler-2010

Fish Physiology and Biochemistry 36(4): 979-992

Abstract:

We evaluated white bass ovum fatty acid composition as well as embryonic and larval survival after varying n-3 and n-6 long-chain polyunsaturated fatty acid (LC-PUFA) concentrations in maternal diets. Diets containing graded levels (0, 33, 66, or 100%) of squid to menhaden oils were fed daily to apparent satiation to female white bass for 8 weeks prior to spawning. Embryonic survival was negatively related to maternal squid oil intake ($P = 0.015$, $R^2 = 0.970$). Squid oil-fed broodstock produced ova with decreased 20:5n-3 and increased C18 polyunsaturated fatty acid concentrations, largely reflecting the fatty acid profile of squid oil. Within ovum phospholipid, accumulation of 18:2n-6 may have altered biological function resulting in the lower embryonic survival among ova produced from the squid oil-fed broodstock. Our data suggest the importance of feeding white bass broodstock diets high in total n-3 LC-PUFA (at least 4.0% dry matter), and 20:5n-3-rich lipid sources such as menhaden oil can be effectively utilized by female white bass to produce quality ova.

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ORGANOGENESIS OF EXOCRINE PANCREAS IN SHARPSNOUT SEA BREEM (DIPLODUS PUNTAZZO) LARVAE: CHARACTERIZATION OF TRYPSIN EXPRESSION

H. Okan Kamaci, Cüneyt Suzer, Deniz Çoban, Şahin Saka and Kürşat Firat-2010

Fish Physiology and Biochemistry 36(4): 993-1000

Abstract:

The ontogeny and differentiation stages of digestive systems related with trypsin expression in larvae of sharpsnout sea bream, *Diplodus puntazzo*, were investigated from hatching to 40 DAH (days after hatching), and total lengths and weights of larvae were determined. Histologic and enzymatic techniques were used to explain the functional development of the pancreas including trypsin activity. The pancreas was identified as a compact structure located in the region slightly posterior to the liver. At 3 DAH, first anus and then mouth opened. Incipient pancreas secretion polyhedral cells could be first observed as zymogen granules. During larval metamorphosis, the pancreas became diffuse, spreading throughout the mesentery in proximity to the stomach, the anterior intestine and the pyloric caeca. The specific activity of trypsin (42.54 ± 6.8 mU/mg protein⁻¹) was found as early as after hatching at larvae size of 2.87 ± 0.34 mm at 0 DAH. Activity further increased until 10 DAH, especially after exogenous feeding. The highest trypsin activity was detected at 25 DAH as 119.26 ± 11.6 mU/mg protein^l. It is concluded that exocrine pancreas organogenesis is the main critical step in the development of digestive system that results in zymogen granules accumulation and increased trypsin activity.

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A COMPARATIVE STUDY ON THE COMPOSITION AND IMPORTANCE OF FREE AMINO ACIDS IN SEMEN OF GILTHEAD SEA BREEM, SPARUS AURATA, AND PERCH, PERCA FLUVIATILIS

Franz Lahnsteiner-2010

Fish Physiology and Biochemistry 36(4): 1297-1305

Abstract:

A comparative study was conducted on the free amino acid composition of gilthead sea bream, *Sparus aurata*, and perch, *Perca fluviatilis*. Also the effect of 21 free amino acids on sperm motility was investigated. Spermatozoa were incubated in species-specific motility-inhibiting saline solution containing the different amino acids for 48 h. Thereafter, the motility was activated and investigated using computer-assisted cell motility analysis. Twelve free amino acids, respectively, were detected in *S. aurata* and *P. fluviatilis* semen. Arginine, cysteine, glutamic acid, leucine, and methionine occurred in semen of both species. In *S. aurata*, arginine, glycine, hydroxyproline, lysine, and phenylalanine in

concentrations of 1.25 and 2.50 mmol/l, methionine in a concentration of 2.5 mmol/l, and serine in a concentration of 1.25 mmol/l had a positive effect on the motility of spermatozoa. In *P. fluviatilis*, alanine, asparagine, cysteine, glycine, isoleucine, lysine, methionine, serine, threonine, and valine in concentrations of 2.50 mmol/l positively affected motility. From these data, it can be concluded that the amino acid composition and the effect on motility are species specific. Possible consequences for spermatozoa functionality are discussed.

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