#### OBITUARY KENNETH L. SIMPSON

Dr. Kenneth L. Simpson, Professor Emeritus in the Department of Nutrition and Food Science, University of Rhode Island, USA, died at the age of 78 on Saturday, July 25, 2009, surrounded by family members. Ken received his B.S. (1954), M.S. (1960) and Ph.D. (1963) degrees from the University of California, Davis, having also served with the U.S. Army in Germany after receiving his B.S. degree. Following a post-doctoral year at University College in Aberystwyth, Wales, he joined the University of Rhode Island in 1964 as an Assistant Professor in Agricultural Chemistry. As a very successful researcher, Ken progressed rapidly through the academic ranks, becoming Associate Professor (1969) and Professor (1972) in Food and Resource Chemistry, and spent a sabbatical year (1971-72) as a Visiting Professor at the University of Liverpool. His initial research interest was the study of carotenoids, pigments which color such diverse foods as salmon, carrots, sweet potatoes, butter and egg yolks. Besides imparting colors to food, these compounds also serve as precursors to vitamin A. His work centered on aspects of the biosynthesis, chemical properties, occurrence, analysis, stereochemistry, and the discovery of new compounds. Ken developed rapid and cheap analytical methods for carotenoids that could be applied in laboratories overseas. Ken's research and collaborations took him all over the world, including Iran and Rumania for the carotenoid work. In the 1970's, his research focus expanded to include nutritional problems in aquaculture. In particular, he became a founding member of the International Study on Artemia (ISA). The ISA research established that fatty acids, rather than pollutants, amino acids, carotenoids, or other chemicals, were the primary determinants of the nutritional quality of Artemia for marine aquaculture organisms. Graduate students from many countries came to Ken's lab to learn the intricacies of fatty-acid analysis. Aquaculture provided a whole new set of collaborators for Ken, including valued colleagues at the Southeast Asian Fisheries Development Center in the Philippines and at Kagoshima University in Japan, among others, as well as the ISA team. Ken retired from URI in 1995 with an impressive record of production: over 120 academic publications and at least 40 graduate students. He leaves his wife Jill, children Pam, Valerie and Andrew and their spouses, and ten grandchildren.

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THERMAL MODELING OF GREENHOUSE AQUACULTURE RACEWAY SYSTEMS Shuhai Li, Daniel H. Willits, Craig L. Browdy, Michael B. Timmons, Thomas M. Losordo-2009 Aquacultural Engineering 41(1): 1-13 Abstract:

A mechanistic model was developed to describe the thermal behavior of an indoor raceway system with an inflated double polyethylene cover. The model describes the heat balances of the two covers, the inside air, the water in the raceway and the soil beneath the raceway. On-site measurements were made with an experimental system at the Waddell Mariculture Center in South Carolina. The collected data were used to calibrate the model. Comparison of the predictions with observations showed that the average absolute errors of air temperature and water temperature were 1.4 and 0.5 °C, respectively and was 8% for the relative humidity. The accuracies are regarded as sufficient for the model to be useful for more general application. Model simulations were used to investigate the effects of the greenhouse on the air and water temperatures, to examine the heat fluxes and to calculate the heat consumption and costs at four different climatic locations. The results suggest that under the mild weather conditions in January near Charleston, SC where the daily mean temperature

is 7.6 °C and solar radiation is 121 W m-2, the inside air temperature increases by 5.6 °C and water temperature increases by 9.7 °C on average for the system with the 0.85 m deep raceway covering 70% of the greenhouse floor. An examination of the heat fluxes suggests that thermal radiation is a major mechanism of heat loss for the greenhouse covers and the water surface. Convection from the water surface is also a significant mechanism for latent and sensible heat loss from the raceway. Reducing these heat flows will help conserve and utilize energy. The yearly heating requirements to keep the water temperature at 28 °C for the experimental system were estimated to be 870, 520, 274 and 221 kWh per square meter of raceway for Syracuse, NY, Roanoke, VA, Charleston, SC and Baton Rouge, LA, respectively. The model was deemed to be a useful tool for exploring the performance of greenhouse raceway systems under different scenarios, such as different cover materials, sizes and climates.

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### A DEVICE THAT CONVERTS AQUEOUS AMMONIA INTO NITROGEN GAS

Ramsey Kropp, Dean Tompkins, Timothy Barry, Walter Zeltner, Greg Pepping, Marc Anderson, Terence Barry-2009

Aquacultural Engineering 41(1): 28-34

Abstract:

A photoelectrocatalytic oxidation (PECO)3 device was developed for converting aqueous ammonia into nitrogen gas. The device uses a germicidal UV lamp to activate a titania (TiO2)-coated anode (photoanode) that is connected to a titanium or platinum wire cathode to form an electrolytic cell. When a small bias (2 V DC) was applied between the anode and cathode, ammonia (-3 oxidation state) was oxidized primarily into nitrogen gas (0 oxidation state) rather than nitrite (+3 oxidation state) or nitrate (+5 oxidation state). The ammonia oxidation rate changed as a function of the crystalline structure of the titania coating on the anode, the salinity of the water, the applied voltage, and the disruption of boundary layers near the photoanode surface. There was no ammonia removal in water without at least some NaCl in solution, suggesting that the device works by converting chloride ions into chlorine and hypochlorous acid, which then react with ammonia to form nitrogen gas. Varying the pH between pH = 5 and pH = 10 had no effect on the rate of ammonia removal. A continuous flow-through PECO reactor was tested using aquariums spiked with ammonium chloride or stocked with seawater-adapted tilapia fed a high protein diet, and found to effectively remove ammonia and limit nitrite and nitrate accumulation in the tanks.

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### ARTEMIA FRANCISCANA ENRICHMENT MODEL — HOW TO KEEP THEM SMALL, RICH AND ALIVE?

Joana Figueiredo, Robert van Woesik, Junda Lin, Luís Narciso-2009 Aquaculture 294(3-4): 212-220

Abstract:

Artemia nauplii are among the most commonly used cultured prey in aquaculture, but they may lack certain essential fatty acids pertinent to their target species. While enrichment can improve Artemia nutritional profile, enrichment procedures can cause undesired effects such as mortality and rapid growth, which is problematic for larvae with a small mouth gape. In this study we tested the effect of salinity (3–33), temperature (16–28 °C) and enrichment time (0–24 h) on survival, total length and fatty acid profile of Artemia franciscana nauplii (marine strain) using a factorial design. Results were used to construct an A. franciscana nauplius enrichment model. Temperature was the most important forcing function influencing mortality, growth and fatty acid incorporation; temperature increase caused higher mortality, faster growth and more fatty acid incorporation. Salinity affected primarily growth and arachidonic acid (ARA) incorporation; lower salinities reduced growth and maintained higher ARA levels. The model allowed us to test different combinations of temperature and salinity, predict their outcomes, and consequently, choose the optimal combination of these abiotic factors and

enrichment time to produce a prey with the desired properties (a specific total length and fatty acid profile), while minimizing mortality.

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COUPLING OSTEOLOGICAL DEVELOPMENT OF THE FEEDING APPARATUS WITH FEEDING PERFORMANCE IN COMMON SNOOK, CENTROPOMUS UNDECIMALIS, LARVAE: IDENTIFYING MORPHOLOGICAL CONSTRAINTS TO FEEDING

Matthew L. Wittenrich, Nicole R. Rhody, Ralph G. Turingan, Kevan L. Main-2009

Aquaculture 294(3-4): 221-227

Abstract:

Identifying bottlenecks to feeding in marine finfish larvae is becoming a dominant theme as commercially important fish stocks collapse worldwide. The transition from endogenous yolk reserves to feeding exogenously is perhaps the largest constraint to developing aquaculture technologies in closed systems. Mass mortality during early larval development is generally attributed to a lack of suitable prey during the first feeding stage, however, empirical evidence identifying a causal link between morphology and performance remains scarce. In this study, we examined the link between osteological development of the feeding apparatus and feeding performance, expressed as (1) the median number of prey consumed by larvae and (2) the median size of prey consumed by larvae, during larval development of the common snook, Centropomus undecimalis. Cluster analysis, nMDS, and SIMPER analysis allowed us to identify functional intervals of the feeding apparatus through larval development. Results revealed that first feeding larvae exhibited rudimentary skeletal elements and selected only one or two of the prey types available relative to older larvae, which included more and larger prey types in their diet. Upon complete formation of the hyoid apparatus, around 8 dph, a dietary shift to rotifers was observed suggesting that high rates of mortality observed in closed culture systems may be attributed to the absence of a suitable small, non-elusive food organism during the first feeding stage. First feeding larvae exhibit a poorly developed feeding apparatus that may constrain their ability to consume elusive prey as an initial diet. Based on the association between stage-specific characteristics of the feeding apparatus and corresponding stage-specific metrics of feeding performance established in this study, we propose a stage-specific feeding-management scheme for snook hatchery aquaculture.

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COMPARISON OF DIETARY PHOSPHOLIPIDS AND NEUTRAL LIPIDS ON SKELETAL DEVELOPMENT AND FATTY ACID COMPOSITION IN ATLANTIC COD (GADUS MORHUA) Elin Kjørsvik, Cecilia Olsen, Per-Arvid Wold, Katja Hoehne-Reitan, Chantal L. Cahu, José Rainuzzo, Atle Ivar Olsen, Gunvor Øie, Yngvar Olsen-2009

Aquaculture 294(3-4): 246-255

Abstract:

Dietary essential fatty acids may affect larval skeletal formation. The aim of this study was to compare effects on growth and osteological development of dietary docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) being incorporated in the phospholipid (diets PL1 and PL3) or in the neutral lipid (diet NL1) fraction of the larval diet for Atlantic cod (Gadus morhua). The diets were labelled according to the estimated percentage of total n-3 fatty acids contained in the dietary neutral lipid (NL1-1.3% of dietary dry matter) or in the phospholipid fraction (PL1-1.1% and PL3-2.3%). Larvae were weaned to the isoenergetic and isolipidic microdiets from 17 days post hatching (dph). They were co-fed enriched rotifers until 24 dph and received thereafter only the experimental diets until 45 dph (at 12 °C).

Dry weight on 45 dph was significantly higher in the PL1- and PL3-larvae (mean  $2.74 \pm 0.2$  mg DW) than in the NL1-larvae (mean  $2.17 \pm 0.1$  mg DW). Larvae fed the PL3-diet had a significantly higher

DHA content than larvae from the other treatments. No differences were found in larval content of EPA and arachidonic acid (ARA). Larvae fed both PL-diets showed a significantly faster ossification of the vertebral column compared to larvae fed the NL1-diet, according to both larval size and age. On 45 dph, PL3-larvae also had a significantly higher number of fin rays than the other groups at comparable larval sizes. Deformities (mainly kyphosis and shortened vertebrae) were observed in 11% (all groups) of the larvae on 45 dph, with no significant differences among the groups.

Ossification of the vertebral column in the cod larvae was first observed in the neural arches on the anterior part of the spine (21 dph, > 6.9 mm standard length, SL), followed by the vertebrae (25 dph, > 7.9 mm SL), haemal arches (31 dph,  $\ge$  8.4 mm SL) and parapophysis (35 dph, > 8.7 mm SL). Ossification of all vertebral elements was observed in 45-day-old larvae  $\ge$  11.4 mm SL. Large variations between larvae in number of ossifying structures between 31 and 35 dph demonstrated that this was the period of most rapid skeletal change (size range 8.2–11.4 mm SL), and that the ossification process was more susceptible to fatty acid lipid source rather than to quantity of dietary fatty acids. We suggest that an optimal dietary content of n–3 HUFA in feed for cod larvae is higher than in the PL1-diet.

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OPTIMAL LEVELS OF DIETARY VITAMIN A FOR REDUCED DEFORMITY INCIDENCE DURING DEVELOPMENT OF EUROPEAN SEA BASS LARVAE (DICENTRARCHUS LABRAX) DEPEND ON MALFORMATION TYPE

David Mazurais, Nomiki Glynatsi, Maria J. Darias, Stavroula Christodoulopoulou, Chantal L. Cahu, Jose-Luis Zambonino-Infante, Giorgos Koumoundouros-2009

Aquaculture 294(3-4): 262-270

Abstract:

The purpose of this study was to examine the impact of graded levels of dietary vitamin A on sea bass larval performances and to determine optimal retinol levels at different larval stages to avoid specific skeletal malformations. Retinol was incorporated into larval feeds at 0, 5, 10, 15, 25, 35 and 70 mg kg- 1 dry matter (giving rise to RET0, RET5, RET10, RET15, RET25, RET35, RET70 groups, respectively). Analysis of the several types of deformities affecting the skull, vertebral column or fins of the fish were observed depending on experimental groups. On one hand, the incidence of skull malformations affecting the maxillary and premaxillary bones, dentaries, operculum, branchiostegal rays and glossohyal was lower for the RET0 and RET5 groups. On the other hand, the frequency of vertebral (slight fusions and kyphosis of the anterior five vertebrae, over-mineralization and lordosis of the haemal vertebrae, the transformation of the last pre-haemal vertebra into haemal) and fin (deformations of the dorsal and anal pterygiophores, deviations of the related rays, modifications of the anatomy of the caudal supporting elements, partial to complete lack of the pelvic fins) deformities were lower for the RET5-70 groups. In the RET0 group, lower level of Hoxd-9 expression coincided with partial or complete lack of pelvic fin. Our results suggest that the optimal level of retinol for harmonious ontogenesis fluctuate along sea bass larvae development and that inadequate dietary retinol levels alters morphogenesis through the modulation of Hox gene expression, at least for the

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DIGESTIVE ENZYME RESPONSE TO NATURAL AND FORMULATED DIETS IN CULTURED JUVENILE SPINY LOBSTER, JASUS EDWARDSII

Cedric J. Simon-2009

Aquaculture 294(3-4): 271-281

Abstract:

A better understanding of the nutritional requirements and digestive processes are essential for improving the consumption and growth of spiny lobsters on formulated diets used in commercial culture. Differences in the enzymatic digestive response (i.e., post-prandial changes in digestive enzyme activity, digestive fluid pH and digestive gland structure) of juvenile Jasus edwardsii fed either a natural (mussel flesh) or a formulated diet were investigated. Digestive enzyme activities (total protease, trypsin,  $\alpha$ -amylase and  $\alpha$ -glucosidase) and soluble protein concentration in the foregut after a single feeding event for the two dietary treatments displayed a common peak at 4 h, and a second peak at 18 h post-prandial for the mussel fed lobsters only. In lobsters fed the formulated diet, the lumen size of the digestive gland tubules was 88% larger, the number of B-cells per tubule at 18 h post-feeding was 269% higher, and the pH of the digestive gland was lower (6.20 versus 6.34) than in lobsters fed mussel flesh. These results indicated an intensified intracellular digestion in the digestive gland on the formulated diet that could have played a role in the poor appetite revival (>18 h) exhibited on this diet. Rearing of juvenile lobsters on the formulated diet for 6 months resulted in a marked decrease in the digestive capacity (i.e., total and specific enzyme activity of the foregut and digestive gland) and nutritional condition of lobsters. Overall, these results suggest that difficulty in the digestive processing of formulated feeds may help to explain the bottlenecks encountered in developing more effective formulated diets for juvenile spiny lobster culture. Improvements in the dissolution of dietary ingredients upon entering the foregut, and in the digestibility of dietary carbohydrate sources, may assist in further improving the performance of formulated diets for lobsters.

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# EFFECTS OF FEEDING REGIME AND PROBIONTS ON THE DIVERTING MICROBIAL COMMUNITIES IN ROTIFER BRACHIONUS CULTURE

Zizhong Qi, Kristof Dierckens, Tom Defoirdt, Patrick Sorgeloos, Nico Boon, Zhenmin Bao, Peter Bossier-2009

Aquaculture International 17(4): 303-315

Abstract:

Rotifer growth performance and microbial community changes associated with rotifer cultures were monitored while different feed types (Nannochloropsis oculata paste and the commercial yeast based feed CS-3000), different regimes (daily changes, changes per batch and no changes) and mixtures of three probionts (Phenylobacterium sp.; Gluconobacter sp. and Paracoccus denitrificans) were provided. It was shown that the dominant bacterial species in the cultures receiving either N. oculata or CS-3000 were different. However, in cultures receiving both feeds (either switching between feeds on a daily basis or on a batch basis), a high similarity in microbial community fingerprint was found. The presence of probionts was detected by the end of four batch culture cycles in spite of strong shifts of the bacterial community. By group discriminant analysis, it was found that Phenylobacterium sp. and Paracoccus sp. contributed positively to the CS-3000-fed group, while Gluconobacter sp. contributed positively to the N. oculata-fed group, although they did not appear as very dominant species.

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# MINIMALLY INVASIVE SURGICAL TECHNIQUE FOR EGG COLLECTION FROM THE PERSIAN STURGEON, ACIPENSER PERSICUS

Mohsen Pourasadi, Bahram Falahatkar, Ghobad Azari Takami-2009

Aquaculture International 17(4): 317-321

Abstract:

A minimally invasive surgical technique (MIST) for the removal of ovulated eggs from Persian sturgeon Acipenser persicus was tested on broodstocks caught from the wild to determine whether it affected fecundity or hatching rates compared with the traditional stripping method of killing and removing the eggs (commonly used in hatchery). Morphological parameters of females, germinal

vesicle (GV) position, weight of obtained egg, number of eggs/gram, fertilization rate, and percentage of hatching during incubation were not significantly different between the MIST and traditional stripping methods. Obtained ova were  $4.8 \pm 0.4$  kg female-1 in the MIST and  $4.6 \pm 0.5$  kg female-1 in traditional stripped groups, respectively; the fertilization rate was  $83.4 \pm 11.2\%$  and  $80.0 \pm 7.2\%$  in groups, respectively. The results of this study showed that the minimally invasive surgical technique approach is efficient, practical, and less stressful to Persian sturgeon broodstocks during artificial propagation than other reported egg collection procedures.

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### SENSITIVITY OF STRIPED TRUMPETER, LATRIS LINEATA, EMBRYOS TO MECHANICAL SHOCK AND SIMULATED TRANSPORT

M. Jones, S. C. Battaglene, P. M. Pankhurst-2009 Aquaculture International 17(4): 331-340

Abstract:

The sensitivity of striped trumpeter, Latris lineata, embryos to transport was assessed through mechanical shock at four stages of embryonic development, and simulated transport at different densities and durations. Fertilised egg mortality due to mechanical shock was greatest for embryos shocked 3 and 27 h post-fertilisation (48.6%  $\pm$  4.1 and 36.0%  $\pm$  3.2, respectively; mean  $\pm$  SE), and prior to blastopore closure. Mortality of embryos shocked 51 and 75 h post-fertilisation, and following blastopore closure, did not differ from un-shocked controls (9.5%  $\pm$  0.7). Density and duration of incubation affected the survival of embryos in a simulated transport trial. Embryonic mortality remained below 3% for all density treatments up to 24 h post-fertilisation. Significant mortality of fertilised eggs occurred when incubated at 5,000 embryos l-1 (17.2%  $\pm$  6.9) for 48 h and was correlated with a drop in dissolved oxygen (DO) levels. Final DO concentrations decreased with increasing embryonic density and incubation duration. DO of embryos incubated at 5,000 embryos l-1 was significantly lower than those incubated at 500, 1,000 and 2,500 embryos l-1, at all incubation durations. Mortality was prevalent in treatments that had a final DO level less than 72% saturation at 14°C. Transportation of fertilised striped trumpeter eggs in seawater is recommended from 51 h post-fertilisation at 14°C, after blastopore closure and neural keel development and at densities of no more than 2,500 embryos l-1 for 24 h transportation duration.

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# FEEDING OF SCLERACTINIAN CORAL, GALAXEA FASCICULARIS, ON ARTEMIA SALINA NAUPLII IN CAPTIVITY

Yii-Siang Hii, Chen-Lin Soo, Hock-Chark Liew-2009

Aquaculture International 17(4): 363-376

Abstract:

Satisfying nutrient requirement of corals is still a major constraint for maintaining corals in marine aquariums. Corals are polytrophic in nature. Heterotrophic feeding on zooplankton is one of the corals' strategies to overcome nutrient deficiency. Artemia salina nauplii are commonly used as biocarriers for many fish larvae in aquaculture and can also serve as a biocarrier for coral in aquariums, provided coral acceptability, optimal feeding rate, and digestibility of the nauplii are well understood. Feeding rate and digestibility of coral fed on A. salina nauplii at 100, 2,000, 4,000, 6,000, and 10,000 ind. l-1 under light and dark conditions was assessed in this study. The maximum feeding rates of Galaxea fascicularis under light and dark conditions was 113.6 ind. polyp-1 h-1 and 76.9 ind. polyp-1 h-1, respectively. The daily feeding rates of G. fascicularis varies and depends on nauplii density. Light plays an important role in coral feeding. Nevertheless, the quantity of A. salina nauplii consumed by the coral under light and dark conditions was not significantly different (P > 0.05). A. salina nauplii are well accepted by G. fascicularis. Complete nauplii digestion was observed after 180 min. Digestibility of A. salina nauplii by G. fascicularis was positively correlated with digestion time.

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#### CRYOPRESERVATION OF SPERM FROM SPOTTED WOLFFISH

Snorri Gunnarsson, Sindri Sigurdsson, Helgi Thorarensen, Albert K. Imsland-2009 Aquaculture International 17(4): 385-389

Abstract:

The effects of different concentrations of cryoprotectant (dimethyl sulfoxide; DMSO), cooling rate and straw size on the post-thaw motility of frozen sperm from spotted wolffish, Anarhichas minor, were studied. There was no significant difference in the post-thaw motility of sperm treated with three different concentrations of DMSO (10, 20 and 30%). Similarly, there was no significant difference in the post-thaw motility of spermatozoa when using different freezing rates (i.e. distance of straws from the surface of liquid N2, 4.7, 5.5 and 7.1°C min-1) and the straw size (0.5 and 1.0 ml) did not affect survival. The cryopreservation of sperm can be used to make up for the frequent lack of sperm and/or the unsynchronised timing of sperm production in spotted wolffish males and the ovulation time in females. The results show that sperm from spotted wolffish can be frozen to secure access to viable sperm, but further experiments are needed in order to reveal the effect of different parameters on the post-thawing mortality and define the optimum conditions for cryopreservation.

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EFFECT OF COMBINED PHOTOPERIOD, WATER CALCIUM CONCENTRATION AND PHON SURVIVAL, GROWTH, AND MOULTING OF JUVENILE CRAYFISH (PROCAMBARUS CLARKII) CULTURED UNDER LABORATORY CONDITIONS

Cai-Feng Yue, Ting-Ting Wang, Yu-Feng Wang, Yu Peng-2009

Aquaculture Research 40(11): 1243 – 1250

Abstract:

The red swamp crayfish, Procambarus clarkii (Girard), is one of the most commonly farmed freshwater species in inland China due to its high market value and consumer demand. The aim of this study was to determine the optimum combinations of photoperiod, water calcium concentration and pH for juvenile survival, growth and moulting. In our orthogonal experiment, the three environmental factors were varied at three levels (photoperiod: 16L:8D, 12L:12D and 8L:16D; calcium concentration: 45.5, 65.5 and 85.5 mg L-1; and pH: 6.8, 7.8 and 8.8). Range analysis showed that the maximum survival of juvenile crayfish occurred at photoperiods of 16L:8D or 8L:16D, water calcium concentration of 45.5 mg L-1 and pH of 7.8; maximum weight gain at photoperiod 16L:8D, water calcium concentration 65.5 mg L-1 and pH 7.8; maximum length increase at photoperiod 16L:8D, water calcium concentration 65.5 mg L-1 and pH 7.8; and the highest moult frequency at photoperiod 12L:12D, water calcium concentration 65.5 mg L-1 and pH 7.8. Analysis of variance indicated that photoperiod, water calcium concentration and pH significantly influenced only the weight gain of juvenile crayfish (P<0.05). Taking growth into consideration, we suggest that a photoperiod of 16L:8D, calcium concentration of 65.5 mg L-1 and pH 7.8 might be optimal conditions for rearing juvenile P. clarkii.

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VITAMIN CONTENTS OF ECCS THAT DOODLICE LADVAE SHOWING A HIGH SHOWI

VITAMIN CONTENTS OF EGGS THAT PRODUCE LARVAE SHOWING A HIGH SURVIVAL RATE IN THE JAPANESE EEL ANGUILLA JAPONICA

Hirofumi Furuita, Tatsuya Unuma, Kazuharu Nomura, Hideki Tanaka, Tsuyoshi Sugita, Takeshi Yamamoto-2009

Aquaculture Research 40(11): 1270 – 1278

#### Abstract:

This paper describes the relationship between the egg vitamin concentrations and the egg quality in the Japanese eel Anguilla japonica. No notable relation was found between any vitamin and the fertilization rate. Hatching and survival rates of larvae, however, significantly increased with an elevated level of egg vitamin C (VC). In contrast to VC, the relation between vitamins E (VE) and A (VA) concentrations and survival rate showed a clear peak, with a reduced survival rate at both higher and lower vitamin concentrations. The ratio of VE to lipid or highly unsaturated fatty acid (HUFA) in eggs positively correlated with hatching and survival rates of larvae. High-quality (HQ) eggs were determined as eggs that produced larvae having a survival rate higher than 80% at 8 days post hatch, and low-quality (LQ) eggs were determined as eggs that did not hatch. The level of VC of HQ was significantly higher than LQ. The results of this study suggest that HQ eggs, which produce larvae having a high survival rate, must have high levels of VC and VE/HUFA ratio and contain optimum levels of VA and VE in Japanese eel.

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RELATIONSHIP BETWEEN SEMEN CHARACTERISTICS AND BODY SIZE IN BARBUS BARBUS L. (TELEOSTEI: CYPRINIDAE) AND EFFECTS OF IONS AND OSMOLALITY ON SPERM MOTILITY

S.M. Hadi Alavi, Marek Rodina, Tomáš Policar, Otomar Linhart-2009

Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 153(4): 430-437

#### Abstract:

The objectives of the present study were to determine the relationships among length and weight of males, sperm volume, spermatozoa concentration, total number of spermatozoa, ionic contents and osmolality of seminal plasma in Barbus barbus. The effect of osmolality on sperm motility parameters after activation in NaCl, KCl, or sucrose solutions was also examined. There were significant correlations between spermatozoa concentration – length (R = +0.7) and – weight (R = +0.8) of males. No significant correlations were observed between the total number of spermatozoa, sperm volume, and length and weight of males. Seminal plasma osmolality was higher when the total number of spermatozoa (R = +0.6) and sperm volume (R = +0.6) were higher. Sperm motility and velocity was positively correlated with osmolality (R = +0.5). The correlation between sperm motility and K+ was negative (R = 0.5), but positively correlated with Ca2+ (R = 0.8), Na+ (R = 0.8), and Cl-(R = 0.8). There was a rapid decrease (P < 0.05) in sperm motility parameters after sperm activation. Just after sperm activation, beating waves propagated along the full length of flagella. At latter stages post sperm activation, the waves appeared only in proximal part of the flagellum. The highest spermatozoa velocity and percentage of motility were observed at 215–235 mOsmol kg- 1 in NaCl, KCl or sucrose. The tip of the flagellum became curled into a loop shape which shortened the flagellum after activation of sperm in distilled water. B. barbus sperm is very similar to that of other cyprinids in terms of ionic contents and osmolality of the seminal plasma, mechanism of sperm activation and behavior and motility of sperm during swimming period.

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FOLLICLE STIMULATING HORMONE (FSH) AND LUTEINIZING HORMONE (LH) GENE EXPRESSION DURING LARVAL DEVELOPMENT IN SENEGALESE SOLE (SOLEA SENEGALENSIS)

José M. Guzmán, María J. Bayarri, Jesús Ramos, Yonathan Zohar, Carmen Sarasquete, Evaristo L. Mañanós-2009

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#### Abstract:

The gonadotropins (GTHs), follicle stimulating hormone (FSH) and luteinizing hormone (LH), determine the reproductive competence of adult breeders, but also participate in the establishment of the reproductive axis at early stages of life. The present study aimed at studying, by real-time qPCR, the gene expression levels of GTH subunits (FSH $\beta$ , LH $\beta$  and the common glycoprotein  $\alpha$  -GP $\alpha$ -subunit) during early development in Senegalese sole, from 1 to 100 days post hatching (dph). The FSH $\beta$ , LH $\beta$  and GP $\alpha$  transcripts were first detected at 1, 5 and 3 dph, respectively. Transcript levels of FSH $\beta$ , and GP $\alpha$ , increased continuously to peak levels at mid metamorphosis (15 dph), decreasing thereafter; levels were maintained low until a second increment detected at 90 and 100 dph. Contrarily, transcript levels of LH $\beta$  were very low and only detectable around metamorphosis. All three subunits were highly expressed in 1-year old soles, with FSH $\beta$  and GP $\alpha$  transcript levels 10-fold higher than those of LH $\beta$ . These results suggest, i) activity of the reproductive axis early after hatching (1 dph), which was highest during the metamorphic climax and, ii) a predominant role of FSH, rather than LH, in the early development of the reproductive axis in Senegalese sole.

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## CLONING AND EXPRESSION OF STAR DURING GONADAL CYCLE AND HCG-INDUCED OOCYTE MATURATION OF AIR-BREATHING CATFISH, CLARIAS GARIEPINUS

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Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 154(1): 6-11 Abstract:

Complementary DNAs encoding steroidogenic acute regulatory protein (StAR) have been isolated from different fish species, yet the relevance of StAR during gonadal cycle and more importantly in final oocyte maturation has not been assessed so far. A cDNA encoding StAR was isolated from the ovarian follicles of air-breathing catfish, Clarias gariepinus. Catfish StAR exhibited 55 to 72% identity at nucleotide level with other vertebrate orthologs. RT-PCR analysis of tissue distribution pattern demonstrated the presence of StAR mRNA in various tissues including gonads, kidney, liver, brain and intestine of catfish. Real-time RT-PCR analysis revealed high expression of StAR mRNA in the pre-spawning phase of ovary while it was low in preparatory, spawning and regressed phases. In testis, maximum expression was noticed during the preparatory phase. During human chorionic gonadotropin (hCG)-induced oocyte maturation, both in vitro and in vivo, StAR mRNA levels were augmented by 2 h and then declined gradually to reach basal levels by 12 h as that of saline-treated controls. Taken together, high level of expression during hCG-induced oocyte maturation vis-à-vis in spawning suggests a role for StAR, in addition to the steroidogenic enzyme genes in final oocyte maturation.

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## CLONING, CHARACTERIZATION AND EXPRESSION OF FERRITIN SUBUNIT FROM CLAM MERETRIX MERETRIX IN DIFFERENT LARVAL STAGES

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Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 154(1): 12-16

Abstract:

Shell formation is one of the important events during larval development and metamorphosis in bivalves. However, the molecular mechanisms and environmental cues regulating shell initiation and growth are unclear. Here, we report that ferritin, a principal protein for biological iron storage and metabolism, might play a role in larval shell development of the bivalve mollusk Meretrix meretrix. A full-length ferritin subunit cDNA, named as MmeFer, was cloned and characterized. The MmeFer mRNA expression in different developmental stages, from trochophore to post larvae, was analyzed

by real-time reverse transcription polymerase chain reaction (RT-PCR). MmeFer mRNA expression in larvae of later developmental stages increased at least 8-fold following trochophores. Moreover, the temporal and spatial expressions of MmeFer mRNA were examined by whole mount in situ hybridization. In the trochophore stage, MmeFer was detectable where it was supposed to be for shell initiation. In the later developmental stages, MmeFer was found near digestive glands and mantle that secret larval shell. MmeFer expression was also detected in larvae cultured in artificial seawater with different iron concentrations ranging from 0 to 100  $\mu M$ . These results suggest that ferritin may play a role in the shell formation of mollusks.

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