

**CHANGES IN THE DIGESTIVE ENZYMES AND HORMONES OF GILTHEAD SEABREAM LARVAE (SPARUS AURATA, L. 1758) FED ON ARTEMIA NAUPLII ENRICHED WITH FREE LYSINE**

Mehmet Naz, Mustafa Türkmen-2009

Aquaculture International 17(6): 523-535

**Abstract:**

Variations in digestive enzymes and hormones during the larval development of gilthead seabream (*Sparus aurata*) fed on live prey (*Artemia nauplii*) enriched with free lysine were investigated for 16 days (from day 24 to day 40). Prior to initiation of the experiment, newly hatched larvae were transferred from incubators to fiber glass tanks (300 l) with black walls and fed the same diet until day 24. Each experiment was performed in triplicate. The amount of free lysine in the *Artemia nauplii* was increased by adding a 5.3 mM free amino acid solution to the culture water during a 16-h enrichment period. Larvae were sampled four times at four-day intervals, for 16 days. Larvae fed on the control group had lower growth than those fed on the lysine group at the end of the study but the difference was not significant ( $P > 0.05$ ). The highest trypsin activity and lap/leu-ala peptidase ratios were observed in the control group ( $P < 0.05$ ). Significant differences between bombesin activities of the treatment groups were not found in the fifth minute after the start of feeding ( $P > 0.05$ ) but were significant in the fifteenth minute ( $P < 0.05$ ). Significant differences between the CCK levels of the treatment groups were found ( $P < 0.05$ ). In conclusion, three main ideas are presented in this study. First, *Artemia nauplii* can successfully be enriched with free lysine. Second, bombesin (GRP) and cholecystokinin (CCK) activity can be stimulated by free lysine. Third, the mechanisms controlling adaptation of the activity of the trypsin to the amount of dietary protein were not activated until day 40.

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**THE EFFECT OF VARYING DIETARY ENERGY ON GONAD DEVELOPMENT AT FIRST SEXUAL MATURITY OF THE SHARPTOOTH CATFISH (CLARIASGARIEPINUS BURCHELL, 1822)**

Şehriban Çek, Erdal Yılmaz-2009

Aquaculture International 17(6): 553-563

**Abstract:** The objective of this study was to investigate the effects of five isonitrogenous (35% crude protein) diets having different energy contents; 10.85, 11.82, 12.73, 13.69, and 15.06 MJ DE/kg, respectively, on the gonad development and weight gain of Sharptooth catfish fingerlings. Energy contents of the experimental diets were increased with the increasing amount of soy-acid oil (0, 4, 8.5, 13, 18%) and each group of fish was fed on their respective diets (group I was fed diet I) during 180 days. The gonad maturation was significantly affected by dietary energy. In both sexes, the histological inspections of the gonads did reveal differences among the fish fed different energetic diets. Fish fed diets I, II, and III had significantly heavier and more developed gonads compared to those on diets IV and V. Fish fed the higher energetic diets (IV and V) had partially low numbers of yolky oocytes compared to other groups. The mean GSI values of female and male fish fed with diet III was significantly greater than those fed diets with higher dietary energy; however, higher HSI values were recorded for fish fed with the higher energetic diets (IV and V). Also, the best weight gain for both males and females were recorded from the fish fed diet III (12.73 MJ DE/kg). Therefore, on the basis of the present results, among energy levels tested, 12.73 MJ DE/kg was found to be optimal for healthy gonad development and weight gain.

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**REARING JUVENILE TENCH (TINCA TINCA L.) UNDER CONTROLLED CONDITIONS USING ARTEMIA NAUPLII AS SUPPLEMENT TO A DRY DIET**

Jesús D. Celada, Amelia Aguilera, Vanesa García, José M. Carral, María Sáez-Royuela, Rocío González, Álvaro González-2009

Aquaculture International 17(6): 565-570

Abstract:

A 120-day experiment was performed with 4-month-old juvenile tench [initial mean weight: 0.31 g; total length (TL): 32 mm] to evaluate live *Artemia* nauplii as supplement to a dry diet for salmonids. All groups received the dry diet. Five treatments, differing in the amount of supplemented *Artemia*, were tested: without supplement, 450, 900, and 1,800 nauplii g<sup>-1</sup> initial fish biomass, and nauplii in excess. Groups that received *Artemia* supplement had significantly higher survival (between 89.4 and 98.7%) compared to those that received dry diet alone (49.2%), while among them there was no significant difference. Juvenile tench fed the dry diet supplemented with *Artemia* in excess had a specific growth rate (1.98), weight (3.40 g), and TL (63.90 mm) significantly higher than those in the rest of the treatments. The latter three features were not significantly different under limited co-feeding (450, 900, and 1,800 nauplii g<sup>-1</sup> biomass) averaging 1.26 specific growth rate, 1.40 g weight, and 48.50 mm TL. Tench fed the dry diet alone grew significantly less than the rest. Thus, the drawbacks of feeding juvenile tench with dry foods originally formulated for other fish species can be overcome by providing a supplement of *Artemia* nauplii.

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#### EFFECT OF DIETS CONTAINING DIFFERENT LEVELS OF HIGHLY UNSATURATED FATTY ACIDS ON PHYSIOLOGICAL AND IMMUNE RESPONSES IN PACIFIC WHITELEG SHRIMP *LITOPENAEUS VANNAMEI* (BOONE) EXPOSED TO HANDLING STRESS

Laurence Mercier, Ilie S. Racotta, Gloria Yepiz-Plascencia, Adriana Muhlia-Almazán, Roberto Civera, Marcos F Quiñones-Arreola, Mathieu Wille, Patrick Sorgeloos, Elena Palacios-2009

Aquaculture Research 40(16): 1849 – 1863

Abstract:

Juveniles fed a diet containing a low or a high level of highly unsaturated fatty acids (HUFA) for 38 days were exposed to handling stress. In a first experiment, stress was applied daily for 30 days, after which the physiological and immunological variables were measured, whereas in a second experiment, stress was applied once and samples were obtained 1 and 24 h after the stressor event. Shrimp that were stressed for 30 days showed significantly lower survival, final weight and feed consumption compared with unstressed shrimp. The concentration of the high-density lipoprotein  $\beta$ -glucan-binding protein was significantly higher in shrimp fed the high-HUFA diet. The glucose concentration in the haemolymph was significantly higher in long-term stressed shrimp compared to controls. The lactate level in the haemolymph was significantly lower in shrimp fed the high-HUFA diet. Lactate and glucose in the haemolymph increased in the 1-h stressed shrimp, but returned to normal levels in 24-h stressed shrimp. A negative effect of repeated-handling stress applied for 30 days was mainly observed on biological performance, whereas the single-stressor event had a more pronounced effect on shrimp physiological and immune responses measured 1 and 24 h after the stressor. A beneficial role of enrichment with HUFA on tolerance to handling stress was observed on immune response capacity.

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#### PRELIMINARY INVESTIGATIONS ON THE EFFECTS OF DIETARY LIPID ON THE SPAWNING PERFORMANCE AND EGG QUALITY OF BLACK SEA BASS *CENTROPRISTIS STRIATA* L

Christopher D. Bentle, Wade O. Watanabe, Troy C. Rezek, Pamela J. Seaton-2009

Aquaculture Research 40(16): 1873 – 1883

Abstract:

Adult black sea bass *Centropristis striata* broodstock (N=162) were fed three different dietary treatments: two commercially prepared diets with 45% protein and two different lipid levels (12% and 20%) (diets 1 and 2), and a diet of frozen Atlantic silversides *Menidia menidia* (SS, diet 3). Broodstock

were held under controlled photothermal conditions and induced to spawn with an LHRHa pellet (72 µg kg<sup>-1</sup> bw). Dietary lipid had pronounced effects on spawning performance and egg quality. Diet 3 (SS) produced a significantly ( $P < 0.05$ ) higher fertilization success (22.4%) than diets 1 (0.6%) and 2 (4.8%). The hatching success of fertilized eggs was similar in all diets (range=40–58.6%), but only two spawns from diet 1 (12% lipid) yielded viable yolk-sac larvae (YSL). Diet 3 (SS) also produced significantly more YSL per female ( $21.8 \times 10^3$ ) than the diet 1 ( $0.3 \times 10^3$ ). Eggs from diet 3 (SS) contained a significantly greater proportion of n-3 series fatty acids, with docosahexaenoic acid (DHA) as the largest fraction. Eggs from commercially prepared dietary treatments contained significantly more n-6 fatty acids. The poor spawning performance of fish fed diet 1 (12% lipid) may be related to higher levels of linoleic acid and lower levels of DHA in the diet.

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#### HORMONE INJECTIONS ENHANCE THE TOLERANCE OF LAND-LOCKED AYU SPERMATOOZOA TO CRYOPRESERVATION

Ken-ichi Yokoi, Tomonori Kuwada, Koji Yamane, Yoshinori Nishiguchi, Shinichi Yamamoto, Mitsugi Gouda, Kenji Takii, Hiromi Ohta-2009

Aquaculture Research 40(16): 1893 – 1898

Abstract:

We evaluated the effects of maturation-stimulating hormones on the post-thaw motility of land-locked form ayu (*Plecoglossus altivelis*) spermatozoa. Male ayu were administered three intraperitoneal injections of either salmon pituitary extract (SPE; 0.2 or 0.6 mg g<sup>-1</sup> BW day<sup>-1</sup>) or of 17, 20β-dihydroxy-4-pregnen-3-one (DHP; 2 or 10 µg g<sup>-1</sup> BW day<sup>-1</sup>), the maturation-inducing steroid (MIS) in ayu. Before cryopreservation, the motility of spermatozoa of the SPE- and DHP-treated groups was significantly higher than that of the control group. Similarly, the comparative post-thaw motility (presented as a percentage of the motility obtained before cryopreservation) was significantly higher in the SPE group than in the control; however, there was no significant difference between the DHP group and the control. The effect of SPE and DHP on pre- and post-cryopreservation motility was not dose dependent. Our results suggest that the hormone(s) present in salmon pituitary are effective in enhancing the tolerance of ayu sperm cells to cryopreservation and that the MIS (DHP) is not involved in this process.

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#### INFLUENCE OF HATCHERY DIETS ON EARLY GROW-OUT OF THE CORTEZ OYSTER CRASSOSTREA CORTEZIENSIS IN SINALOA, MEXICO

José M. Mazón-Suástegui, Aurora Parres-Haro, Karen M. Ruíz-Ruíz, Carmen Rodríguez-Jaramillo, Pedro E. Saucedo-2009

Aquaculture Research 40(16): 1908 – 1914

Abstract:

We measured the growth and physiological condition of juveniles of the Cortez oyster *Crassostrea corteziensis* during the early grow-out phase in Sinaloa (Mexico) after using three experimental diets during the hatchery period: (1) 1:1 blend of the microalgae *Isochrysis galbana* and *Chaetoceros muelleri* as the control group; (2) mix of the same microalgae replaced by 50% of its wet weight with cornstarch; and (3) mix of the two microalgae replaced by 50% its wet weight with wheat flour. Specimens were cultivated under suspension conditions for 60 days and monitored weekly for growth (shell height and wet and dry weight) and the first 15 days for physiological response (condition index, digestive gland index and muscle index). Juveniles fed exclusively on microalgae attained larger sizes and had higher digestive gland and muscle indices, while those fed microalgae with cornstarch gained more biomass (wet and dry weight) and reached a better condition. The lowest values of these indicators occurred in oysters fed microalgae with wheat flour. Statistical data revealed that differences in the growth and condition of specimens between treatments were slight, confirming that mixed diets (particularly

microalgae/cornstarch) have potential application for reducing hatchery-operating costs without affecting the performance of planted juveniles in the field.

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#### EFFECTS OF SEMINAL PLASMA COMPOSITION ON SPERM MOTILITY IN MIRROR CARP (CYPRINUS CARPIO)

Yusuf Bozkurt, Fatih Ogretmen, Faik Sertel Secer, Ugur Ercin-2009

The Israeli Journal of Aquaculture – Bamidgheh 61(4): 307-314

Abstract:

This research provides data concerning the biochemical (ionic and organic) composition of sperm of the mirror carp (*Cyprinus carpio*) and its relationship to spermatozoa motility. Seminal plasma contained  $67.12 \pm 1.06$  mM/l  $\text{Na}^+$ ,  $105.1 \pm 2.24$  mM/l  $\text{K}^+$ ,  $7.85 \pm 0.67$  mg/dl  $\text{Ca}^{2+}$ ,  $2.61 \pm 0.11$  mEq/l  $\text{Mg}^{2+}$ ,  $0.14 \pm 0.002$  g/dl total protein,  $10.3 \pm 1.01$  mg/dl triglyceride,  $6.83 \pm 0.72$  mg/dl cholesterol, and  $54.72 \pm 3.49$  mg/dl urea. A positive relationship ( $p < 0.05$ ) was determined between  $\text{Na}^+$  and motility ( $r = 0.522$ ). On the other hand,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ , and  $\text{Mg}^{2+}$  ions negatively correlated ( $p > 0.05$ ) with motility ( $r = -0.565$ ,  $r = -0.160$ , and  $r = -0.184$ , respectively). Spermatozoa motility correlated negatively ( $p > 0.05$ ) with protein ( $r = -0.233$ ), triglyceride ( $r = -0.348$ ), and urea ( $r = -0.331$ ) but positively with cholesterol ( $r = 0.012$ ). This information will help to develop cryopreservation procedures, to meet species-specific extender requirements, and to optimize artificial fertilization procedures in mirror carp.

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#### INTERACTION OF SPIRULINA WITH DIFFERENT LEVELS OF VITAMIN E ON GROWTH, REPRODUCTION, AND COLORATION IN GOLDFISH (CARASSIUS AURATUS)

Raja James, Iyyadurai Vasudhevan, Kunchitam Sampath-2009

The Israeli Journal of Aquaculture – Bamidgheh 61(4): 330-338

Abstract:

The interaction between spirulina (30 g/kg diet) and different levels of vitamin E (100, 200, 300, and 600 mg/kg diet) on growth, gonad weight, reproduction, and coloration were studied in goldfish, *Carassius auratus*, for 120 days. The mean body weight increased with time in all experimental diets. Growth, gonad weight, and fecundity in fish fed the diet containing spirulina+ 300 mg vitamin E were significantly ( $p < 0.01$ ) enhanced, compared to other diets. Supplementation of vitamin E beyond this level significantly ( $p < 0.01$ ) reduced gonad weight and, subsequently, fecundity. Control fish spawned only once, with fewer eggs per spawn, than other groups, which spawned twice with a greater number of eggs per spawn. Females fed spirulina without vitamin E laid 703 eggs in two spawnings compared to 1057 eggs in fish fed with the spirulina+300 mg vitamin E diet. Fish treated with other combinations laid fewer eggs. While all combinations of spirulina and vitamin E significantly enhanced coloration, the combination of spirulina+ 300 mg vitamin E was the most influential.

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#### COMPARISON OF THE PROTEIN PROFILE OF THE REPRODUCTIVE SYSTEM BETWEEN BISEXUAL AND PARTHENOGENETIC SPECIES OF ARTEMIA (BRANCHIOPODA, ANOSTRACA)

Farazmand Ali, Inanloo Kolsoum, Motamed Nasrin-2009

Crustaceana (82): 10: 1237-1248

Abstract:

In the present study we investigated the differences in protein profile of the reproductive systems of two species of Iranian *Artemia*, i.e., *Artemia urmiana* and *Artemia parthenogenetica*. The reproductive systems (ovary and uterus filled with cysts) of adult females of *Artemia* were dissected and kept at  $-70^\circ\text{C}$ . These tissues were used for protein extraction and the protein solution was assayed by the Bradford test to evaluate the quantity of the protein. Proteins were next exposed to two-dimensional gel electrophoresis and the protein spots obtained from the gels were compared between the two species. Through a comparative proteomic approach, the protein profiles of the ovary and the uterus with cysts

from the two species of *Artemia* were analysed. Among the spots obtained, 21 differentially expressed proteins were recognized. Following mass spectrometry, three of these proteins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the BRCA1 (breast and ovarian susceptibility protein 1) associated RING domain (BARD1) protein, and tubulin  $\beta$ 3, were found to be expressed differentially between the two species. Also some other proteins, with unknown functions, were found to be over-expressed in the *A. parthenogenetica* gonads.

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EXTERNAL DESCRIPTION OF THE EMBRYONIC DEVELOPMENT OF THE PRAWN, *MACROBRACHIUM AMERICANUM* BATE, 1868 (DECAPODA, PALAEMONIDAE) BASED ON THE STAGING METHOD

García-Guerrero, Marcelo U., Hendrickx, Michel E.-2009

*Crustaceana*, 82(11): 1413-1422

Abstract:

The embryonic changes during the development of the freshwater prawn, *Macrobrachium americanum* are described from observations made on live embryos based on the percentage-staging method. Eggs were observed with a stereomicroscope to obtain descriptions of embryonic periods. This prawn has an incubation time of 18 days at 24°C. Ten periods are described and illustrated. A comparison of this developmental process with those of congeneric species is included.

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FEEDING, MORPHOLOGICAL CHANGES AND ALLOMETRIC GROWTH DURING STARVATION IN MIIUY CROAKER LARVAE

Xiu-Juan Shan, Liang Cao, Wei Huang, Shuo-Zeng Dou-2009

*Environmental Biology of Fishes* 86(1): 121-130

Abstract: We investigated the effects of the timing of first feeding (larvae in F0, F1, F2, F3 and S were first fed on day 3, 4, 5, 6 days after hatching (DAH) and unfed, respectively) on feeding, morphological changes, survival and growth in miiuy croaker larvae at 24°C. The fed larvae initiated feeding on 3 DAH and reached point of no return (PNR) on 6 DAH. Larvae in F0 and F1 groups survived apparently better than F2 group at the end of the experiment on 36 DAH. High larval mortality occurred from 3 to 7 DAH in all feeding groups, accounting for 40% (F0, F1 and F2 groups) to 90% (F3 and S groups) of the total mortality. Larvae in F0 and F1 groups grew better than F2 group throughout the experiment. Eye diameter, body height, head height and mouth gape of the first feeding larvae were more sensitive to starvation than other morphometrics and could be used as indicators for evaluating their nutritional status. Results indicated that delayed first feeding over 1 day after yolk exhaustion could lead to poor larval survival and growth. To avoid starvation and obtain good growth in culturing, larvae feeding should be initiated within 1 day after yolk exhaustion at 24°C.

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SEASONAL FORAGING AND PISCIVORY BY SYMPATRIC WILD AND HATCHERY-REARED STEELHEAD FROM AN INTEGRATED HATCHERY PROGRAM

William G. Simpson, Benjamin M. Kennedy, Kenneth G. Ostrand-2009

*Environmental Biology of Fishes* 86(4): 473-482

Abstract:

We compared the diet of hatchery-reared steelhead produced from an integrated hatchery program as emigrating spring smolts and non-migrating hatchery residuals to their sympatric wild counterparts. Our results suggest that there is a potential for hatchery fish to affect wild steelhead populations due to dietary overlap and subyearling salmonid predation; however, relative ecological risk did not increase as steelhead delayed or forwent emigration. Predation by hatchery smolts was related to release timing, but not experience with native fish. Diet composition appears to be more strongly affected by seasonal and yearly differences in prey abundance and presence rather than differences in rearing environments. Hatchery and wild steelhead showed small but important foraging differences. Hatchery smolts did not consume as many salmonids as wild fish and hatchery residuals showed relatively stronger surface

oriented feeding behavior than wild parr. Because most hatchery smolts emigrated shortly after release and the overall number of residuals in the study creek was low, we speculate that in this case there is low dietary and predatory-based risk of hatchery steelhead in Abernathy Creek negatively impacting wild salmonids.

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ANALYSIS OF EFFECTS INDUCED BY A POLLOCK PROTEIN HYDROLYSATE ON EARLY DEVELOPMENT, INNATE IMMUNITY AND THE BACTERIAL COMMUNITY STRUCTURE OF FIRST FEEDING OF ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.) LARVAE

R. Hermannsdottir, J. Johannsdottir, H. Smaradottir, S. Sigurgisladottir, B.K. Gudmundsdottir, R. Bjornsdottir-2009

Fish & Shellfish Immunology 27(): 595-602

Abstract:

A pollock protein hydrolysate was used for enrichment of the live feed offered to halibut larvae from the onset of exogenous feeding and the effects of treatment on selected innate immune parameters studied. The effects of treatment on the bacterial community structure of larvae were furthermore studied using the PCR–DGGE method. C3 and lysozyme were identified in larvae already at the onset of first feeding and low concentrations of IgM detected at this stage indicate maternal origin. Endogenous production of IgM was validated in the gastrointestinal tract of larvae from 29 days post onset of first feeding, with similar concentrations found in both groups. Feeding the peptide-enriched live feed stimulated production of lysozyme and affected the distribution of C3 in larval tissue but survival and normal development of halibut larvae were not affected by the treatment. *Vibrio* sp. and *Pseudoalteromonas* sp. dominated the bacterial community of larvae from both groups and peptide enrichment of the live feed was not found to affect the bacterial community structure associated with surface sterilized larvae.

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STRUCTURAL ABNORMALITIES OF COMMON CARP *CYPRINUS CARPIO* SPERMATOZOA

Martin Pšenička, Marek Rodina, Martin Flajšhans, Vojtech Kašpar, Otomar Linhart-2009

Fish Physiology and Biochemistry 35(4): 591-597

Abstract:

Spermatozoa of common carp *Cyprinus carpio* are typically consist of a primitive head without acrosome, a midpiece with several mitochondria, a centriolar complex (proximal and distal centriole), and one flagellum. During an evaluation of the motility of common carp spermatozoa, we found spermatozoa with more than one flagellum and/or “double head” in three different individuals. This may be related to abnormal spermatogenesis. Ultrastructure and physiological parameters of spermatozoa were examined using light microscopy (dark field with stroboscopic illumination), transmission and scanning electron microscopy, and flow cytometry. The recorded pictures and videos were evaluated using Olympus MicroImage software. All spermatozoa with more than one flagellum had a larger head and shorter flagella. They occasionally demonstrated several cytoplasmic channels separating the flagella from the midpiece. Each flagellum was based upon its own centriolar complex, with the connection of the flagellum to the head always at a constant angle. The flagella always consisted of nine peripheral pairs and one central doublet of microtubules. Sperm exhibited a relative DNA content similar to that found in sperm from normal males, with higher coefficients of variation. Although similar abnormalities have been found in livestock, where they were described as a defect in spermiogenesis, no comparable results have been reported in fish. The frequency at which these abnormalities occurs, the fertilization ability of males with defects in spermiogenesis, the influence of these abnormalities on progeny in terms of ploidy level, and the occurrence of deformities warrant further investigation.

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#### BIOENERGETICS OF FISH SPERMATOZOA DURING SEMEN STORAGE

M. S. Ziętara, A. Biegniewska, E. Rurangwa, J. Swierczynski, F. Ollevier, E. F. Skorkowski-2009

Fish Physiology and Biochemistry 35(4): 607-614

##### Abstract:

This mini-review focuses on changes in ATP and creatine phosphate concentrations in fish sperm under storage conditions. The storage of catfish sperm at 4°C leads to ATP depletion and decreased sperm motility. The rate of intracellular ATP depletion can be diminished through the addition of energetic substrates to the sperm storage medium, with lactate + pyruvate being the most efficient substrates for maintaining ATP concentrations in catfish sperm. The decrease in ATP concentration is closely associated with increases in AMP and hypoxanthine content. In contrast to catfish sperm, carp sperm is able to maintain intracellular ATP concentration close to the physiological level during storage. Collectively, these results suggest that fish species differ in terms of the energy metabolism of their spermatozoa and that the semen storage medium must be carefully selected for a particular fish species so as to maintain the ATP concentration and adenylate energy charge close to physiological values as long as possible.

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#### THE EFFECTS OF HEAVY METALS ON EMBRYONIC DEVELOPMENT OF FISH (A REVIEW)

Barbara Jezierska, Katarzyna Ługowska, Małgorzata Witeska-2009

Fish Physiology and Biochemistry 35(4): 625-640

##### Abstract:

Early developmental stages of fish are particularly sensitive to water pollution. Heavy metals may affect various developmental processes during the embryonic period, which results in a reduction of offspring quantity and quality. Waterborne metals may accumulate in the gonads of spawners and adversely affect gamete production and viability, or exert direct toxic influence upon developing embryos. The egg shell does not fully protect the embryo against metal penetration, particularly during the swelling phase; thus, metals may accumulate in the egg. The results depend on metal concentration and range from developmental disturbances to death of the embryo. Metals disturb various processes of fish embryonic development and affect the development rate. Early stages just after fertilization are particularly sensitive to metal intoxication, when most disturbances and the highest embryonic mortality occur. Waterborne metals also promote developmental anomalies during organogenesis, including body malformations. Heavy metals often induce a delay in the hatching process, premature hatching, deformations and death of newly hatched larvae. All these disturbances result in reduced numbers and poor quality of the larvae, which show small body size, high frequency of malformations and reduced viability.

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#### PRE-SPAWNING WATER TEMPERATURE AFFECTS SPERM RESPIRATION AND REACTIVATION PARAMETERS IN MALE CARPS

Sergey Boryshpolets, Boris Dzyuba, Sergey Drokin-2009

Fish Physiology and Biochemistry 35(4): 661-668

##### Abstract:

Concentration, ability to motility, motility during the second activation (reactivation), and endogenous respiration were studied in sperm from two experimental groups of carp males. Group 1 was maintained for 7 days at 15°C (cold water (CW) group), whereas the second group was subjected to a temperature of 20°C (warm water (WW) group) before sperm sampling. Reactivation were achieved after incubation of firstly activated sperm in media with osmotic pressure adjusted up to 300 mOsm\*kg<sup>-1</sup> by increasing K<sup>+</sup> concentration. Statistically significant reduction of spermatozoa concentration in CW samples versus WW (from 46.0 ± 12.5 (15°C) to 59.3 ± 7 109 (20°C) spermatozoa /ml) have been observed. The

sperm of the CW group required a significantly longer incubation time (37 min) under isotonic conditions to achieve a maximum percentage of potent motility at repeated activation than the WW group (23 min). After activation of sperm motility, an increase of respiration rate up to maximum level has been found, this level remained the same under condition of recovering the potential to repeated activation. During the sperm movement respiration rate, in CW group (6.1 nmolO<sub>2</sub>/min/10<sup>9</sup>spermatozoa) and WW (3.9 nmolO<sub>2</sub>/min/10<sup>9</sup>spermatozoa), was significant higher compared to nonactivated sperm (2.4 nmolO<sub>2</sub>/min/10<sup>9</sup>spermatozoa for CW and 1.1 nmolO<sub>2</sub>/min/10<sup>9</sup>spermatozoa for WW). And keeping males for 7 days at 15°C increase the respiration rate of sperm.

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#### THE USE OF IMMUNOSTIMULATING HERBS IN FISH. AN OVERVIEW OF RESEARCH

Jeney Galina, G. Yin, L. Ardó, Z. Jeney-2009

Fish Physiology and Biochemistry 35(4): 669-676

Abstract:

The use of immunostimulants as an alternative to the drugs, chemicals and antibiotics currently being used to control fish diseases in fish culture is attracting the attention of many researchers. In this context, many have focused on the use of medicinal plant products as potential therapeutic measures for modulating the immune response and, specifically, on the use of herbs to prevent and control fish diseases. Medicinal plants (plant remedies) are a deeply rooted component of the cultural heritage of many people from diverse cultures and countries and are, as such, closely linked to the maintenance of good health. The aim of this paper is to review research currently being carried out on the herbs and herbal extracts that have been shown to modulate the immune system of fish. Special attention is given to the use of Chinese and Indian herbs.

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#### SPERM QUALITY IN MALE BARBUS BARBUS L. FED DIFFERENT DIETS DURING THE SPAWNING SEASON

Sayyed Mohammad Hadi Alavi, Martin Pšenička, Tomáš Polícar, Marek Rodina, Jitka Hamáčková, Pavel Kozák, Otomar Linhart-2009

Fish Physiology and Biochemistry 35(4): 683-693

Abstract: Sperm quality of *Barbus barbus* L. was compared among the three following dietary regimes: Group A, fed 100% commercial diet (Karpico™ containing 33% crude protein and 6% fat), Group B, fed 78% commercial diet and 22% frozen chironomid (*Chironomus plumosus*) larvae, and Group C, fed 56% commercial diet and 44% frozen chironomid larvae. Concentrations of polyunsaturated fatty acids (PUFAs) in Group A, B, and C were 39.1, 42.0, and 44.6, respectively, as a percentage of total fatty acids. Sperm morphology, volume, concentration and motility, total number of spermatozoa, and osmolality of the seminal plasma were compared during the spawning season. Dietary regime did not influence sperm volume, concentration, or total number of spermatozoa, osmolality of seminal plasma, or the percentage of motile sperm, but significantly affected sperm morphology (except for anterior and posterior parts of the midpiece) and sperm velocity ( $P < 0.05$ ). Groups B and C showed similar sperm characteristics during the spawning season compared to Group A. Almost all parameters changed either among or within groups during the spawning season, suggesting differences in terms of the optimal time for sperm collection. The best time for sperm collection was March for Group A, but April for Groups B and C, when the osmolality of the seminal plasma measured 289 mOsmol kg<sup>-1</sup> and sperm motility was maximal. Spermatogenesis, hydration, and cell decomposition were confirmed as the three major parameters controlling sperm characteristics during the spawning season. The possible correlation between sperm morphology and motility requires further study.

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COMPOSITION OF OVARIAN FLUID IN ENDANGERED CASPIAN BROWN TROUT, *SALMO TRUTTA CASPIUS*, AND ITS EFFECTS ON SPERMATOZOA MOTILITY AND FERTILIZING ABILITY COMPARED TO FRESHWATER AND A SALINE MEDIUM

Azadeh Hatef, Hamid Niksirat, Sayyed Mohammad Hadi Alavi-2009

Fish Physiology and Biochemistry 35(4): 695-700

Abstract:

In this study, ovarian fluid composition and its effects on the motility and fertilizing ability of sperm were studied in endangered Caspian brown trout, *Salmo trutta caspius*, and were compared with a saline activation medium (125 mM NaCl, 30 mM Glycine, 20 mM Tris-HCl, pH = 9.0) and freshwater as the control. The ovarian fluid was composed of sodium  $164.4 \pm 4.4$  mM l<sup>-1</sup>, potassium  $1.8 \pm 0.1$  mM l<sup>-1</sup>, calcium  $0.6 \pm 0.1$  mM l<sup>-1</sup>, magnesium  $0.4 \pm 0.02$  mM l<sup>-1</sup>, chloride  $127.4 \pm 5.9$  mM l<sup>-1</sup>, total protein  $389.5 \pm 89.6$  mg 100 ml<sup>-1</sup>, cholesterol  $9.3 \pm 1.2$  mg dl<sup>-1</sup>, and glucose  $3.3 \pm 0.2$  mM l<sup>-1</sup>. The percentage of motile spermatozoa and the duration of sperm motility were significantly higher in ovarian fluid ( $62 \pm 3\%$ ,  $74.6 \pm 0.8$  s) than freshwater ( $35 \pm 4\%$ ,  $44 \pm 1$  s), but they did not differ significantly from saline medium ( $56 \pm 3\%$ ,  $74.3 \pm 0.7$  s) ( $P > 0.05$ ). Higher eyeing rates were observed after the activation of sperm in ovarian fluid and saline solution than freshwater when 35,000 or 350,000 spermatozoa per egg were added into the activation media. However, no significant differences were observed at higher concentrations of spermatozoa per egg (730,000) ( $P > 0.05$ ). Also, this study showed that the ovarian fluid composition can be considered as a species-specific character among salmonid fishes. As a conclusion, the results of this study recommend the use of ovarian fluid or the saline solution as an activation medium in the artificial reproduction of Caspian brown trout.

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RELATIONSHIP BETWEEN BIOLOGICAL CHARACTERISTICS OF EGGS AND FEMALE BROOD STOCKS OF PERSIAN STURGEON *ACIPENSER PERSICUS*: A COMPARISON WITH OTHER FISH SPECIES

Mohammad Reza Imanpoor, Sayed Hadi Alavi, Jacky Cosson-2009

Fish Physiology and Biochemistry 35(4): 701-707

Abstract:

We have determined a number of biological characteristics of the Persian sturgeon egg (including diameter, surface-to-volume ratio, yolk sphere and perivitelline space) and of female brood stocks (including age, length, weight, condition and absolute fecundity) in 420 specimens of the migratory population of *Acipenser persicus* in Gorgan bay of Iran from 2001 to 2004. In the hydrated egg, the average egg diameter, yolk diameter, surface-to-volume ratio and yolk sphere-to-perivitelline space ratio values were:  $3.64 \pm 0.17$  mm,  $3.26 \pm 0.25$  mm,  $1.65 \pm 0.08$  and  $0.75 \pm 0.01$ , respectively. In this chondrosteian species, the egg surface and volume were found to increase with increasing egg diameter, but the egg surface-to-volume ratio decreased. In Persian sturgeon, the surface-to-volume ratio and yolk sphere-to-perivitelline space ratio is very high; therefore, the metabolic rate is low and female spawning occurs in low-temperature waters. The statistical Pearson correlation between egg diameter relative to a number of brood stock characteristics, including fork length ( $r = 0.059$ ), weight ( $r = 0.140$ ), age ( $r = 0.081$ ) and absolute fecundity ( $r = 0.095$ ), were not significantly different ( $P > 0.05$ ). An extensive comparison of these results with data published on other fish species is presented and discussed.

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