
INDUCTION OF OVULATION AND SPAWNING IN THE MEDITERRANEAN RED PORGY, *PAGRUS PAGRUS*, BY CONTROLLED DELIVERY AND ACUTE INJECTION OF GnRH α

L. Kokokiris, A. Canario, C. Mylonas, M. Pavlidis, M. Kentouri, P. Divanach-2005

The Israeli Journal of Aquaculture – Bamidgeh 57(4): 223-230

Abstract:

Gonadotropin-releasing hormone analogue (GnRH α) in the form of saline injections or sustained-release microspheres was used to induce oocyte maturation, ovulation, and spawning in captive red porgy (*Pagrus pagrus*). Individually tagged vitellogenic females (n = 9 or 10) were treated at the beginning of the spawning season (March) with 20 μ g/kg body weight (bw) GnRH α -loaded microspheres, a single injection of 20 μ g/kg bw dissolved in saline, or physiological saline (control). Females were placed in tanks (one tank per treatment) connected to overflow egg collectors and monitored for 11 days. In addition to the eggs collected from the tank overflow, eggs were stripped from the fish on a daily basis. Only one spawn was obtained from the control fish, probably from a single female, given the small relative fecundity (700 eggs/kg bw). On the contrary, treatment with a GnRH α injection produced two spawns (9 and 11 days after treatment) and 50% of the fish ovulated. Treatment with GnRH α microspheres induced seven spawns (3 and 6-11 days after treatment) and 100% of the females ovulated. Females did not spawn all the eggs ovulated on a particular day, evident from the significant number of eggs obtained by manual stripping. Egg quality did not significantly differ among treatments, whereas number of spawned eggs and total relative fecundity were significantly higher in fish treated with GnRH α microspheres (ANOVA, $p < 0.05$). The results demonstrate the potential of GnRH α -loaded microspheres to induce spawning in red porgy, as a method of overcoming spawning failures in commercial hatcheries.

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SHORT COMMUNICATION

EFFECT OF LOW SALINITY ON THE SURVIVAL OF POSTLARVAE OF THE BLUE SHRIMP, *LITOPENAEUS STYLIROSTRIS*, AT DIFFERENT STAGES

Su Xinhong, Shen Changchun, Yang Zhangwu, Zheng Yayou-2005

The Israeli Journal of Aquaculture – Bamidgeh 57(4): 271-277

Abstract:

The development of low salinity culture techniques is the major factor that facilitated the widespread cultivation of the white shrimp *Litopenaeus vannamei* in China. Similarly, adaptation to fresh or low salt water is critical for the commercial farming of a related species, the blue shrimp *L. stylirostris*. To determine the effects of low salinity on *L. stylirostris* development, the survival of postlarvae was investigated at different levels of salinity at the Haichang Jinhaiian Shrimp Breeding Farm and Gulangyu Breeding Farm for Aquatic Animals of the Fisheries Research Institute of Fujian during 2002-2003. Results showed that both salinity and the age of the postlarvae significantly affected survival ($p < 0.01$). The minimum seawater salinity for young postlarvae was 10-14 ppt and survival rates increased with age. When the post-larvae were 9 days old or older ($\geq P9$), survival did not significantly differ in the low salinity treatments from that of the control (P12). The first hour was the most critical period. If larvae survived the first hour, they usually survived the whole study period (24 h) or longer.

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NOTES ON THE DISTRIBUTION OF THE GENUS ARTEMIA IN THE FORMER USSR COUNTRIES (RUSSIA AND ADJACENT REGIONS)

Graziella Mura, Liubov Nagorskaya-2005

Journal of Biological Research 4: 139-150

Abstract:

In this study we review the Russian literature on Artemia distribution in several saline lakes and ponds of former USSR, and report on the results of our studies for a number of populations, aiming to update available information and to identify species, often erroneously designated as *A. salina* in the existing literature. Most of the populations recorded /examined belong to parthenogenetic Artemia while a number of bisexual populations were also found. The morphology of the basal part of penis, investigated by means of SEM revealed that not all of these bisexuals belong to the species *A. salina*, known to occur only in the Mediterranean area. The males of some populations are characterized by the presence of a spine-like outgrowth at the basal part of the penis, a character shared by all bisexuals but Mediterranean. More detailed SEM morphological studies of the frontal knobs and the eversible penis reinforced biomolecular and morphometrical data will certainly contribute to species identification. The need for extensive sampling of the territory is stressed to bridge the gap about Artemia distribution and status in the countries of former USSR.

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REPRODUCTIVE PERFORMANCE OF THREE ARTEMIA FRANCISCANA KELLOGG (CRUSTACEA, ANOSTRACA) POPULATIONS IN NORTH-EASTERN BRAZIL POND CULTURE CONDITIONS

Marcos R. Camara, Ligia G. Reis, Marcos F. Costa-2005

Journal of Biological Research 4: 173-179

Artemia franciscana Kellogg (Crustacea, Anostraca) is found on a year-round and permanent basis in the State of Rio Grande do Norte (RN) in north-eastern Brazil as a result of inoculations made in Macau saltworks in 1977 with cysts from San Francisco Bay (California, USA). Inoculation of Artemia in the saltworks of RN was initially followed by high cyst yields. However, recent data indicate that these feral brine shrimp populations reproduce predominantly ovoviviparously. In the present study, the reproductive performance of three populations from Macau (5°06'S; 36°38'W), Areia Branca (4°57'S; 37°08'W), and Grossos (4°58' S; 37°09'W) in RN was examined under similar pond culture conditions. A higher incidence of ovoviviparous than oviparous females for all experimental populations was found throughout then culture cycles carried out in ponds of 0.72 ha. Furthermore, no significant variability in reproductive mode (ovoviviparity versus oviparity) or in fecundity (brood size) was observed ($p > 0.05$). These results presumably reflect the homogeneity of local populations and are consistent with the suspected decrease of genotypic diversity of feral *Artemia franciscana* in the saltworks of RN in north-eastern Brazil.

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GERM CELL DIFFERENTIATION AND SEXUAL MATURATION OF THE HANGING CULTURED FEMALE SCALLOP PATINOPECTEN YESSOENSIS ON THE EAST COAST OF KOREA

EE-Yung Chung, Young-JE Park, Jeong-Yong Lee , Dong-KI Ryu-2006

Journal of Shellfish Research 24(4): 913-922

PROPHYLACTIC USE OF ANTIBIOTICS IN LARVAL CULTURE OF ARGOPECTEN VENTRICOSUS (SOWERBY, 1835)

Campa-Cordova, A. Luna-Gonzalez, M. Zarain-Herzberg, C. J. Caceres-Martinez-2006

CAN SELECTIVE BREEDING REDUCE THE HEAVY METALS CONTENT OF PACIFIC OYSTERS (*CRASSOSTREA GIGAS*), AND ARE THERE TRADE-OFFS WITH GROWTH OR SURVIVAL?

Mark D. Camara, Stephen M. Griffith, Sanford Evans III-2006
Journal of Shellfish Research 24(4): 979-986

THE SUSCEPTIBILITY OF YOUNG PRESPAWNING OYSTERS, *OSTREA EDULIS*, TO *BONAMIA OSTREAE*

S. A. Lynch, D. V. Armitage, S. Wylde, M. F. Mulcahy, S. C. Culloty-2006
Journal of Shellfish Research 24(4): 1019-1026

THE EVALUATION OF SPRAY-DRIED MICROALGAE IN DIETS FOR JUVENILE MANILA CLAMS, *TAPES PHILIPPINARUM*

Ebru Onal, Chris Langdon, Umur Onal-2006
Journal of Shellfish Research 24(4): 1061-1066

HEAT SHOCK INDUCED METAMORPHOSIS OF THE QUEEN CONCH, *STROMBUS GIGAS*: COMPARISON WITH INDUCTION BY ALGAL ASSOCIATED CUES

Anne A. Boettcher-2006
Journal of Shellfish Research 24(4): 1123-1126

THE EFFECT OF EGG QUALITY ON LARVAL PERIOD AND POSTLARVAL SURVIVAL OF AN ABALONE *HALIOTIS DISCUS HANNAI*

Hirotsu Fukazawa, Hideki Takami, Tomohiko Kawamura, Yoshiro Watanabe-2006
Journal of Shellfish Research 24(4): 1141-1148

GROWTH AND ENERGY UTILIZATION OF JUVENILE PINK ABALONE *HALIOTIS CORRUGATA* FED DIETS CONTAINING DIFFERENT LEVELS OF PROTEIN AND TWO STARCH:LIPID RATIOS

Jessica Montano-Vargas, Maria Teresa Viana, Louis R. D'Abramo, Armando Shimada, Carlos Vasquez-Pelaez-2006
Journal of Shellfish Research 24(4): 1179-1186

CRYOPRESERVATION OF BLACK-LIP PEARL OYSTER (*PINCTATA MARGARITIFERA*, L.) SPERMATOZOA: EFFECTS OF CRYOPROTECTANTS ON SPERMATOZOA MOTILITY

Lauren Lyons, Dean R. Jerry, Paul S. Southgate-2006
Journal of Shellfish Research 24(4): 1187-1190

DIGITAL IMAGE ANALYSIS OF LIPID AND PROTEIN HISTOCHEMICAL MARKERS FOR MEASURING OOCYTE DEVELOPMENT AND QUALITY IN PEARL OYSTER PINCTADA MAZATLANICA (HANLEY,1856)

Eliana Gomez-Robles, Carmen Rodriguez-Jaramillo, Pedro E. Saucedo-2006

Journal of Shellfish Research 24(4): 1197-1202

EFFECTS OF SALINITY FLUCTUATION PATTERN ON GROWTH AND ENERGY BUDGET OF JUVENILE FENNEROPENAEUS CHINENSIS

Yingchun Mu, Fang Want, Shuanglin Dong, Guoqiang Huang, Shaoshuai Dong-2006

Journal of Shellfish Research 24(4): 1217-1222

USE OF METABOLISM TO EVALUATE THE SUBLETHAL TOXICITY OF MERCURY ON FARFANTEPANEUS BRASILIENSIS LARVAE (LATREILLE 1817, CRUSTACEAN)

Edison Barbieri, Elisangela A. Passos, Carlos A. B. Garcia-2006

Journal of Shellfish Research 24(4): 1229-1234

ROE ENHANCEMENT IN SEA URCHIN: EFFECTS OF HANDLING DURING HARVEST AND TRANSPORT ON MORTALITY AND GONAD GROWTH IN STRONGYLOCENTROTUS DROEBACHIENSIS

Trine Dale, Sten Ivar Siikavuopio, Kåre Aas-2006

Journal of Shellfish Research 24(4): 1235-1240

GROWTH, SURVIVAL AND REPRODUCTIVE PERFORMANCE OF DOMESTICATED AUSTRALIAN STOCKS OF THE GIANT TIGER PRAWN, PENAEUS MONODON, REARED IN TANKS AND RACEWAYS

Coman, G.J., Crocos, P.J., Arnold, S.J. et al-2005

J. World Aquacult Soc. 36 (4): 464-479

EFFECT OF A POVIDONE-IODINE TREATMENT ON LITOPENAEUS VANNAMEI LARVAE IN A COMMERCIAL HATCHERY SETTING

Cooney, M.J., Bienfang, P., Alvarez, A.M. et al.-2005

J. World Aquacult Soc. 36 (4): 546-553

NATURAL SPAWNING OF GREATER AMBERJACK (SERIOLA DUMERILI) KEPT IN CAPTIVITY IN THE CANARY ISLANDS

S. Jerez, M. Samper, F.J. Santamaría, J.E. Villamandos, J.R. Cejas, B.C. Felipe-2006

Aquaculture 252 (2-4): 199-207

Abstract:

This paper describes results on sexual maturation and natural spawning of *Seriola dumerili* kept in captivity on Tenerife (Canary Islands). Wild fish captured at an average weight of 8 kg, spawned naturally and reaching an average weight of 25 kg, after 6 years in captivity in 500 m³ raceway tanks, under natural conditions of light and temperature and fed on low-value fish. Spawning took place between April and October, coinciding with the increase in temperature from 19 to 24 °C. A total of

14 million eggs were collected in 38 spawns, with an average of 61.75% fertilization and 16.49% hatching. The greatest number and highest fertilization and hatching rates of eggs were obtained in the month of July. The greater amberjack eggs with 1.121 ± 0.032 mm diameter, hatch within 34 to 45 h, producing larvae with an average length of 3.639 ± 0.012 mm, yolk sac volume of 0.097 ± 0.015 mm³, and oil globule diameter of 0.243 ± 0.001 mm.

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EFFECT OF DIATOM DIETS ON GROWTH AND SURVIVAL OF THE ABALONE HALIOTIS DISCUS HANNAI POSTLARVAE

Nurit Gordon, Amir Neori, Muki Shpigel, John Lee, Sheenan Harpaz-2006

Aquaculture 252 (2-4): 225-233

Abstract:

Growth and survival of postlarval abalone *Haliotis discus hannai* Ino fed different diatom diets were examined for one month from settlement. Two diatoms, *Amphora luciae* Cholnoky and *Navicula cf. lenzii* Hustedt, supported high postlarval growth and survival, especially when supplied in combination. A third species, *Nitzschia laevis* Hustedt, did not support survival for more than two weeks as a unialgal diet and had limited value in mixed diets.

Diatom mixtures were superior to single-species diets as of the first week after settlement. The mixture of *N. cf. lenzii* and *A. lucia* supported the highest survival, up to 50%, and growth rate up to 36µm of shell length per day, reaching a size of 1.4mm 30 days after settlement. The three diatom species contained high levels of total lipids (6.4%–14.5% of dry weight) and fatty acids (16%–22% of lipids); from 39% to 48% of fatty acids were polyunsaturated (PUFA). The three diatoms were richer in n-3 PUFA than in n-6 PUFA. The content of the essential fatty acid 20:5n-3 (EPA) was highest among the PUFAs and higher, though not significantly, in the two diatom species *A. luciae* and *N. cf. lenzii* that produced the better results. Among the free amino acids, arginine was dominant in *N. laevis*, proline in *N. cf. lenzii*, and both free amino acids plus glutamic acid were equally dominant in *A. luciae*. The suitability of *A. luciae* and *N. cf. lenzii* for enhancing growth and survival of postlarvae was attributed to their complementary balanced nutritional properties.

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REPRODUCTIVE EFFICIENCY OF THE SIGNAL CRAYFISH (*PACIFASTACUS LENIUSCULUS* DANA, ASTACIDAE) AT DIFFERENT DENSITIES UNDER BOTH CULTURE AND LABORATORY CONDITIONS

J.D. Celada, J.I. Antolín, J.M. Carral, J.R. Pérez, M. Sáez-Royuela-2006

Aquaculture 252 (2-4): 298-304

Abstract:

Studies carried out to evaluate reproductive efficiency from maturing up to juvenile stage 2 production in astacid crayfish are scarce. Research with a view to determining total losses over the whole reproductive cycle is required. Two experiments were carried out with *Pacifastacus leniusculus* to obtain data on reproductive efficiency in earthen ponds and under laboratory conditions, in each case with two different densities of animals. All the parameters recorded were related to the initial number of females. In earthen ponds, numbers of egg-bearing females close to hatching (April) showed no significant differences between the two densities (76.1% with 3.4 breeders/m² and 72.5% with 6.6 breeders/m²), with an estimated mean of 228 eggs per female. In the laboratory, 81.3% and 75% of females yielded stage 2 juveniles at densities of 6 and 12 breeders/m², respectively, with an average of 135.4 juveniles per initial female, achieving an efficiency from first to final count around 70%. Productivity results are discussed with relation to those attained in other astacid species.

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THE REPRODUCTIVE CYCLE AND POTENTIAL PROTANDRIC DEVELOPMENT OF THE NOAH'S ARK SHELL, *ARCA NOAE* L.: IMPLICATIONS FOR AQUACULTURE

Melita Peharda, Ivona Mladineo, Jaks̃a Bolotin, Lovorka Kekez, Bos̃ko Skaramuc-2006

Aquaculture 252 (2-4): 317-327

Abstract:

The reproductive biology of *Arca noae* (L.) was investigated to assess its aquaculture potential and exploitation as a sustainable fishery. *A. noae* was sampled monthly from Mali Ston Bay, south Adriatic Sea, from November 2001 to October 2002 and from January to December 2004. A total of 590 individuals ranging in shell length from 9.7 to 80.8 mm was analyzed using standard histological techniques.

Results indicate that males dominate small shell length categories with females becoming more predominant as size increases suggesting that some individuals may undergo protandric development. The overall male to female ratio was 1.0 : 1.3 ($\chi^2 = 9.529$, $p = 0.002$). A number (2.2%) of hermaphroditic animals were also identified. The smallest sexually mature *A. noae* had a shell length of 12 mm. Gametogenic development began in October (female) and November (male), with spawning occurring during summer (July to August). Results of a size frequency analysis of oocyte diameters agreed with a qualitative analysis of gonadal development stages, confirming the occurrence of one annual spawning peak. There was no correlation between mean gonad index and environmental conditions. Data on the reproductive characteristics of *A. noae* obtained in this study give an insight into the biology of this species and are crucial for initiating its aquaculture.

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EVALUATION OF FAST GREEN UPTAKE AS A SIMPLE FITNESS TEST FOR SPAT OF *PERNA CANALICULUS* (GMELIN, 1791)

S.C. Webb, K.G. Heasman-2006

Aquaculture 252 (2-4): 305-316

Abstract:

The method described here gauges marine mussel (*Perna canaliculus*) spat health by observing their declining ability to isolate themselves from hyposmotic water after exposure to apparently deleterious agents such as exposure to air, elevated temperature, and ethanol. This inward movement of water is disclosed by Fast Green stain. Although individual spat were found to stain either markedly or not at all, test groups showed variation in proportions staining with different treatments thus giving an indication of group ($n = 50$) fitness. Control and lethally stressed spat groups showed low and high staining proportions, respectively, that corresponded with group differences ($P < 0.001$) in physical activity and valve closure in freshwater. Furthermore, staining and activity levels in a range of spat samples from controls to lethal exposures show a highly significant correlation ($r = -0.967$, $P < 0.001$). Thus staining is a good surrogate for activity as an indicator of group health. The advantage of staining over activity assessment is its ease and brevity. A range of conditions caused by exposure to air, ethanol and nutrient loaded (hypoxic) water were detectable ($P < 0.05$): normal health, sublethal and lethal conditions were statistically distinguishable. Such a test may have application in the mussel industry as currently there is no quick means of testing spat viability. This is needed because spat at this stage are commonly distributed from nursery or wild settlement site to grow-out locations; different handling and transport regimes may impact on spat viability. Successful grading and ensuing pricing based on viability will encourage best practice in maximizing spat quality thereby extending a currently limited spat supply.

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BIOACCUMULATION OF COPPER IN POST-LARVAE AND JUVENILES OF FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII* (DE MAN) EXPOSED TO SUB-LETHAL LEVELS OF COPPER SULFATE

Rameswara Reddy, Bindu R. Pillai, Subhendu Adhikari-2006

Aquaculture 252 (2-4): 356-360

Abstract:

The culture of giant freshwater prawn *Macrobrachium rosenbergii* (de Man) is fast expanding in Asia including India. Copper sulfate is extensively used in freshwater prawn culture ponds to control diseases and filamentous algae. In the present investigation, bioaccumulation of copper was studied in post-larvae (10 ± 1 mm; 0.008 ± 0.002 g) and juvenile (58 ± 5 mm; 1.25 ± 0.28 g) *M. rosenbergii*. The post-larvae and juveniles were exposed to three sub-lethal levels (0.001, 0.01, 0.1 of the 96 h LC50 value; i.e., 0.0652, 0.652, 6.52 $\mu\text{g l}^{-1}$ for post-larvae, 0.39, 3.9 and 39.0 $\mu\text{g l}^{-1}$ for juveniles) for a period of 30 and 60 days, respectively. Copper accumulation was analyzed in post-larvae (whole animal) and gill, hepatopancreas and muscle tissue of juveniles. Results revealed that accumulation of copper in both post-larvae and juveniles increased with increasing test concentration ($p < 0.05$). Among tissues, hepatopancreas accumulated maximum levels of copper followed by gills; while muscle tissue accumulated less copper. There were significant differences ($p < 0.05$) in accumulation of copper in various tissues among treatments. In the case of post-larvae, significant differences ($p < 0.05$) in accumulation of copper were observed in the 6.52 $\mu\text{g l}^{-1}$ treatment. Prawn muscle tissue accumulating copper is important from the point of human food safety as constant exposure to sub-lethal levels of copper for 6–8 months (culture period) may result in accumulation of higher levels of copper in muscle tissue.

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THE EFFECTS OF WAVE AND FEEDING DISTURBANCE ON ROE ENHANCEMENT OF THE SEA URCHIN *EVECHINUS CHLOROTICUS* HELD IN SEA-CAGES

Philip J. James-2006

Aquaculture 252 (2-4): 361-371

Abstract:

An experiment was conducted over a 10-week period to test the effects of wave and feeding disturbance on the gonad index (GI) and gonad color of sea urchins fed an artificial diet. The sea urchins were held in sea-cages at a depth of 6 m. Eight cages were suspended from a surface line (wave disturbed) and eight were subsurface buoyed from a bottom line (not wave disturbed). These two treatments were further divided into four replicates that were fed and cleaned in situ underwater (no feed disturbance) and four replicates that were removed from the water three times per week for feeding and cleaning (feed disturbance). Increased water movement, probably caused by the vertical motion in the wave disturbed cages resulted in a higher GI in these urchins compared to urchins in cages that were subsurface buoyed and did not experience any vertical movement. Feeding disturbance had no effect on the GI values or color quality of the urchin gonads, regardless of whether the urchins were held in sea-cages that were wave disturbed or not wave disturbed.

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REPRODUCTIVE PERFORMANCE OF RECIPROCALLY CROSSED WILD-CAUGHT AND TANK-REARED *PENAEUS MONODON* BROODSTOCK

G.J. Comana, S.J. Arnold, S. Peixoto, P.J. Crocos, F.E. Coman, N.P. Preston-2006

Aquaculture 252 (2-4): 372-384

Abstract:

The reproductive performance of reciprocally crossed wild-caught (W) and domesticated lines (L1

and L2) of *Penaeus monodon* broodstock was assessed over two moult periods under standardised conditions. Significant variations in the reproductive performance of the broodstock from the three sources (W, L1 and L2) were found. Reproductive performance was more dependent on the source of the female broodstock than the male broodstock. W females matured and spawned more rapidly after ablation and more often than L1 and L2 females. The percentage of W females spawning (86.8%) was significantly greater than that for both L1 (38.9%) and L2 females (23.7%). W females had significantly more spawnings (1.99 spawnings female⁻¹) than the L1 (0.62 spawnings female⁻¹) and L2 females (0.37 spawnings female⁻¹), and spawned within a shorter period after ablation (9.2 days compared with 15.3 days for the L1 and 18.7 days for the L2 females). There was no difference in the numbers of eggs per spawning, percentage of spawnings that hatched, hatch rates and protozoal metamorphosis rates produced from the W and the L1 tank-reared females. However, the L2 females had significantly lower numbers of eggs per spawning, percentage of spawnings that hatched and protozoal metamorphosis rates per spawning than the W and L1 females. The lower performance of the L2 stocks, compared with the L1 stocks, demonstrates the variability in reproductive performance that can exist between different domesticated stocks. Our results suggest that the largest improvements in the reproductive output of these tank-reared *P. monodon* stocks will likely be found through improvements in the quality of the female broodstock.

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INFLUENCE OF PHOTOPERIOD ON REPRODUCTIVE PERFORMANCES IN EURASIAN PERCH *PERCA FLUVIATILIS*

Hervé Migaud, Neil Wang, Jean-Noel Gardeur, Pascal Fontaine-2006

Aquaculture 252 (2-4): 385-393

Abstract:

The aim of the study was to determine the effects of photoperiod regimes under natural temperature conditions on reproductive performances (gametogenesis, spawning quality) in Eurasian perch *Perca fluviatilis*. During a 10-month experiment, fish were reared in 12 tanks (3000 L, 88 fish/tank, initial mean weight of 300 g, age of 2+) in an outdoor water-recirculating system and subjected to 4 photoperiod regimes in triplicate: continuous lighting (24L:0D), constant photoperiod (16L:8D), simulated natural (SNP) and natural (NP) photoperiods. Gametogenesis, spawning time and egg quality were studied. No gonadal development was observed in males and females under 24L:0D treatment. The 16L:8D treatment resulted in heterogeneous gametogenesis with only 54% of gravid females and less than 30% of maturing males. A normal gametogenesis was observed under NP and SNP conditions with 100% of gravid females and spermiating males during the spawning period. Under SNP and 16L:8D regimes, spawning period was delayed in comparison to NP regime and 45% and 65% of the maturing females did not spawn, respectively. All spawning were released and harvested between dawn and 4 p.m., independently of the photoperiodic regime applied. These results show that daily lighting variations are important in the control of Eurasian perch spawning. Significantly higher fecundities were observed under SNP and NP regimes compared to 16L:8D. The photoperiod regimes and artificial lighting significantly affected egg quality, as fertilization rates in 16L:8D and SNP groups were very low (7.7% and 3.3% respectively) compared to the NP treatment (57.2%). Hatching rates were null in SNP and 16L:8D regimes, whereas a 54% hatching rate was estimated in NP treatment. Finally, high mortality rates were recorded during the spawning period. These mortality rates were significantly higher in 16L:8D and SNP treatments (63–72%) than in NP and 24L:0D treatments (36–40%). Photoperiod regimes thus strongly influenced Eurasian perch gametogenesis, spawning time, spawning rate, eggs quality and broodstock mortality.

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CLONING AND EXPRESSION ANALYSIS OF THREE DIGESTIVE ENZYMES FROM

ATLANTIC HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS) DURING EARLY DEVELOPMENT: PREDICTING GASTROINTESTINAL FUNCTIONALITY

H.M. Murray, J.W. Gallant, S.C. Johnson, S.E. Douglas-2006

Aquaculture 252 (2-4): 394-408

Abstract:

The objective of the present study was to describe the histological and physiological development of the gastrointestinal system in Atlantic halibut from the time of first-feeding until metamorphosis. At first-feeding, the gastrointestinal (GI) tract is divided into anterior, mid and hindgut regions. The liver is present, as is the pancreas. During development the pancreas changes from a compact organ to a diffuse tissue interspersed through much of the mesentery surrounding the GI tract. Functional gastric glands are not present until approximately 66 days post-hatch (dph). Using primers based upon winter flounder digestive enzyme gene sequences, we were able to amplify partial sequences for bile salt-activated lipase (BAL), trypsinogen (TRP), and pepsinogen (PEP) from RNA extracted from whole larvae and juveniles using Reverse Transcription-PCR (RT-PCR). PCR products were sequenced and the sequences used to design halibut gene-specific primers for BAL and PEP. RT-PCR analysis revealed that BAL and TRP gene expression was evident at least from the time of first-feeding but PEP gene expression was not detectable until 80 dph. In situ hybridization using molecular probes from winter flounder sequences localized expression of BAL and TRP to the exocrine pancreas. PEP expression was only localized to the glandular regions of the stomach. These data provide a first step toward understanding the molecular events governing the ontogeny of digestive capacity in Atlantic halibut.

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DOURADO (SALMINUS BRASILIENSIS) LARVICULTURE: WEANING AND ONTOGENETIC DEVELOPMENT OF DIGESTIVE PROTEINASES

Orestes Manuel Vega-Orellana, Débora Machado Fracalossi, Juliet Kiyoko Sugai-2006

Aquaculture 252 (2-4): 484-493

Abstract:

This study examines the optimal weaning period in rearing larval dourado. This aim is achieved by comparing a series of differential weaning schedules, and by measuring the ontogeny of proteinase activities. Weaning from live food (*curimba*, *Prochilodus lineatus*, larvae) was carried out at the third, fifth or seventh day AH by gradual or total transition to formulated feed. Live food was either kept for two days after feeding the test diet (GW) or removed at the same day (TW). Acceptance of test diet started at the fifth day after hatching (AH) and larvae total length was higher ($P < 0.05$) when feed transition occurred at the third (TW) (20.93 ± 1.33 mm, mean \pm SEM) or seventh day (GW) AH (20.41 ± 0.80 mm); however, higher ($P < 0.05$) wet weight was observed in larvae submitted to feed transition at the third (TW) (0.067 ± 0.014 g) or seventh day (GW) AH (0.078 ± 0.009 g). Total length and wet weight were not affected by the form of alimentary transition ($P > 0.05$). Survival was significantly lower ($P < 0.05$) when weaning occurred at the third or fifth day AH when compared to weaning at the seventh day AH. Additionally, survival was superior when transition to formulated feed was gradual. The ontogenetic development of proteinases was measured in the larval gut after hatching by sampling every 12 h AH, during seven days. Acid protease activity (0.0004 ± 0.0 U mg⁻¹ protein, mean \pm SD) was detected at the third day AH and increased subsequently until the seventh day AH (7T), indicating stomach functionality at this time. Intestine proteinases activities (trypsin and chymotrypsin, 0.088 ± 0.0 and 0.018 ± 0.0 U mg⁻¹ protein, respectively) were detected as early as 12 h AH, before the onset of exogenous feeding. Digestive enzymatic contribution of live food was not clearly shown. Our findings corroborate the hypothesis that fish larvae will accept dry feed only when it is physiologically able to digest it, which happens when acid protease activity is detected, indicating stomach functionality. Our findings also demonstrate the importance of live food soon after hatching for dourado larvae and suggest that formulated feed could be offered, in a gradual form, as early as at the fifth day AH.

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DIGESTIVE ENZYMES ACTIVITY DURING ONTOGENETIC DEVELOPMENT AND EFFECT OF STARVATION IN JAPANESE FLOUNDER, *PARALICHTHYS OLIVACEUS*

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Abstract:

Japanese flounder larvae and early juveniles were reared for 39 days after hatching (DAH) in order to determine pancreatic enzymes activities (trypsin and neutral lipase) using fluorogenic substrates during ontogeny and to compare enzymes activities of starved fish at different developmental stages.

Larvae were fed L-type rotifers *Brachionus plicatilis* from 3 to 28 DAH, *Artemia nauplii* from 14 to 39 DAH and then gradually switched to pelleted feed from 25 DAH. Temperature was kept at 18 °C and photoperiod was 12L : 12D. Three starvation trials were made: from 2 to 8 DAH (premetamorphic larvae), 23–29 DAH (metamorphic) and 31–39 DAH (metamorphic and postmetamorphic). Fluorometric determinations on individual larvae were made using specific substrates for trypsin and lipase activity.

Trypsin and neutral lipase activities were found in 2 DAH larvae (0.18 ± 0.09 U $\mu\text{g protein}^{-1}$ and 25.12 ± 7.36 nmoles 10^{-3} 4MU $\mu\text{g protein}^{-1} \text{ min}^{-1}$, mean \pm SD, respectively), just at the time of first feeding. The evolution of both enzymes activities showed a profile marked by decreases during settlement (27–36 DAH). There is a significant increment ($P < 0.05$) on specific trypsin activity at 15 DAH, corresponding with the beginning of *Artemia* feeding and a significant decrease of lipase activity at 6 DAH.

In the first starvation experiment, larval mortality was 100% after 6 days. In the second trial a starvation-feeding experiment was done with two and four days starved larvae. Survival at day 6 was 50% and 25%, respectively. On the third trial post-metamorphic larvae were starved 5 days, survival at day 6 was 81%.

As a general trend, enzymes activities get significantly lower ($P < 0.05$) in starved fish. On two day starved–fed fish, lipase and trypsin activities levels took two days to reach control levels; on the other hand four days starved–fed fish showed significant differences two days after feeding again. During the last trial (31–39 DAH) lipase and trypsin activities went down, coinciding with juvenile settlement. These results show that trypsin and also neutral lipase activity measurements could be used for determining the critical periods during ontogeny and the nutritional status of larvae

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LARVICULTURE OUTPUT AND STRESS TOLERANCE OF FARFANTEPENAEUS PAULENSIS POSTLARVAE FED ARTEMIA CONTAINING DIFFERENT FATTY ACIDS

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Abstract:

Fatty acid nutrition is known to play a vital role on survival, growth and stress tolerance of aquatic animals. In this study, we assessed whether the enrichment of *Artemia franciscana* (GSL strain, USA) with emulsions containing different fatty acid profiles would affect larviculture output and tolerance to salinity, temperature and total ammonia (TAN) of the shrimp *Farfantepenaeus paulensis*. From mysis I up to postlarvae 10 (PL10), three replicate groups of shrimp were reared in 40-l tanks at an initial density of 60 l⁻¹. They were fed one of the following diets: (1) newly hatched *Artemia nauplii*; (2) *Artemia metanauplii* enriched with a saturated fatty acid (SFA) emulsion (ICES enrichment emulsion 0/–/C); and (3) *Artemia metanauplii* enriched with an emulsion containing 50% n – 3 highly unsaturated fatty acids (n – 3 HUFA) (ICES emulsion 50/0.6/C). Newly hatched *Artemia*

nauplii were enriched for 24 h with two doses of 0.3-g emulsion per liter added at 12 h intervals. Larval feeding was carried out daily at 10:00 and 22:00 hours. Survival and total length (TL; from the tip of rostrum to the tip of the telson) were estimated. Tolerance to salinity, temperature and total ammonia (TAN; $\text{NH}_4^+ + \text{NH}_3$) were assessed by exposing PL10 to 10‰ salinity for 1 h, 16–17 °C for 1 h and increasing total ammonia levels (0, 15, 30, 45 60 and 90 mg TAN l⁻¹) for 24 h, respectively. Tolerance to salinity and temperature were expressed as cumulative stress indexes (CSI; sum of cumulative mortalities over 1 h), while tolerance to ammonia was estimated as the median lethal concentration for 50% of the population during 24 h (LC50). All data were subjected to ANOVA and Tukey's test, except LC50 values, which were compared graphically. Survival of shrimp fed n – 3 HUFA-enriched Artemia was significantly higher than in the other treatments. TL of PL1 was not significantly different, but PL10 fed Artemia nauplii were significantly longer. No differences were found in terms of tolerance to salinity and temperature. PL10 fed n – 3 HUFA enriched Artemia presented a higher tolerance to ammonia. Results indicate that feeding n – 3 HUFA-enriched Artemia to *F. paulensis* larvae increases survival and tolerance to ammonia, which may be advantageous under adverse rearing conditions.

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