

## EGG AND LARVAL QUALITY ASSESSMENT IN THE ARGENTINEAN RED PORGY (PAGRUS PAGRUS)

Eddie Aristizabal, Julieta Suárez, Adrián Vega, Rita Bargas-2009

Aquaculture 287(3-4): 329-334

## Abstract:

The egg and larval quality of red porgy (*Pagrus pagrus*) during six consecutive spawning seasons was analyzed. The spawning season of red porgy in captivity lasted between 2 and 4 months, showing wide daily variations in relative fecundity and floating rate. Annual fecundity showed a minimum of 331,900 eggs kg<sup>-1</sup> in 2003 and a peak of 958,200 eggs kg<sup>-1</sup> female wet weight in 2002. Floating rate differed significantly among seasons, with an average gross value of 52.7%. Annual production of floating eggs ranged between 131,500 eggs kg<sup>-1</sup> (2003) and 646,300 eggs kg<sup>-1</sup> female wet weight (2002), representing a 39.6% and a 67.4% of the annual fecundity, respectively. A multiple regression model using hatching and floating rates was formulated, which correlated well with larval survival as dependent variable. Our results indicated that hatching and floating rates could be used as potential markers to predict survival of red porgy larvae with certain degree of accuracy very early during hatchery activities.

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## EFFECTS OF FATTY ACID COMPOSITION AND SPAWNING SEASON PATTERNS ON EGG QUALITY AND LARVAL SURVIVAL IN COMMON SNOOK (CENTROPOMUS UNDECIMALIS)

Carlos Yanes-Roca, Nicole Rhody, Michael Nystrom, Kevan L. Main-2009

Aquaculture 287(3-4): 335-340

## Abstract:

Common snook (*Centropomus undecimalis*) is a new candidate species for aquaculture. Its reproductive cycle has not been completed in farmed fish since knowledge of their behaviour in the wild and its reproductive physiology remains incomplete, and the only source of seeds comes from wild broodstock. This study was undertaken to examine the fatty acid profile of common snook eggs throughout the spawning season (May to September) in relation to egg quality and larval survival. The fatty acid (FA) composition of eggs collected from wild broodstock stripped on the field (2002–2005), was determined over the spawning season. In general the FA profile observed was consistent with that observed in marine fish apart from a high level of arachidonic acid (ARA) (3.68% of Total FA). The profile of polyunsaturated fatty acids (PUFA) changed over the spawning season (37.5%–29.4% Total FA) and egg quality was best in May, June and July. Eggs with higher concentration (13% of Total FA) of docosahexaenoic acid (DHA) were found to have higher fertilization, hatching and larval survival rate.

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## PATTERNS OF LARVAL GROWTH AND CHEMICAL COMPOSITION IN THE AMAZON RIVER PRAWN, MACROBRACHIUM AMAZONICUM

Klaus Anger, Liliam Hayd, Jan Knott, Uwe Nettelmann-2009

Aquaculture 287(3-4): 341-348

## Abstract:

The Amazon River prawn, *Macrobrachium amazonicum* (Heller, 1862), is a target species for regional fisheries in Brazil and a candidate for aquaculture. Under controlled laboratory conditions (29 °C, 10‰), the larval phase of this species shows variability in the morphology and number of successive stages (mostly 9–10, occasionally 8 to > 12). In the most commonly observed developmental pathway (9 stages, taking approximately 20–22 days from hatching to the first juvenile

stage), we studied patterns of larval growth in terms of total body length (TL), carapace length (CL), dry mass (W), and elemental composition (carbon, hydrogen, nitrogen; collectively CHN). At hatching, about 12% of late embryonic W, 15–18% of C and H, but only 7% of N were lost, indicating higher losses of lipids and/or carbohydrates than proteins. Significant variability was observed in the initial biomass and elemental composition of newly hatched larvae from 20 different egg batches. This may cause variation in the endotrophic potential of the early stages, as the zoea I of this species is a non-feeding stage, and also the zoea II may still utilize internal energy stores remaining from the egg yolk. Lacking or low larval feeding activity from hatching through stage II coincided with low initial growth. Concomitantly, the proportions of C and H (in % of W) as well as the C:N ratio decreased from hatching through stage IV, indicating a utilization of stored lipids. The percentage of N showed an opposite pattern, reflecting protein synthesis associated with morphogenesis. Size growth showed maximum increments per moult in the late zoeal stages (III–VI), followed by lower increments in the subsequent decapodid stages (VII–IX). This sigmoidal growth pattern may reflect ontogenetic changes in morphometric relationships. Biomass showed exponential patterns of increase from zoeal stage III throughout later larval development and in the first two juvenile stages. Furthermore, patterns of larval growth in *M. amazonicum* are characterized as linear relationships between larval W in stage *n* and that in stage *n* + 1 (Hiatt diagram), between larval size (CL) and biomass (W, C), and between W and either C or N. Using CHN data, we also provide estimates of the protein and lipid contents of larval biomass (ca. 38–46% and 10–12% of W, respectively). High survival, rapid development, and predictable patterns of larval growth support the assumption that *M. amazonicum* should be a suitable species for production in aquaculture.

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#### EFFECT OF PROBIOTIC ON LARVAE SHRIMP (*PENAEUS VANNAMEI*) BASED ON WATER QUALITY, SURVIVAL RATE AND DIGESTIVE ENZYME ACTIVITIES

Xu-xia Zhou, Yan-bo Wang, Wei-fen Li-2009

Aquaculture 287(3-4): 349-353

##### Abstract:

The effect of probiotic, *B. coagulans* SC8168, as water additive on larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities was investigated at ontogenetic stages (Z3, M3, PL1–2 and PL7–8). Twelve tanks with three replicates for each treatment group and control group were used. The treatments consisted of three SC8168 levels at an initial concentration of  $1.0 \times 10^5$  cfu ml<sup>-1</sup> (T-1),  $5.0 \times 10^5$  cfu ml<sup>-1</sup> (T-2) and  $1.0 \times 10^6$  cfu ml<sup>-1</sup> (T-3) and one control (without any probiotic), and were conducted every day. Addition of the probiotic significantly increased survival rate ( $P < 0.05$ ) for all treatments over controls. However, no significant difference was found between T-2 and T-3. At early larval stages (Z3 and M3), protease activity in shrimp was not significantly different among probiotic treatments and control. At the subsequent ontogenetic stages (PL1–2 and PL7–8), the highest protease activity was observed in T-2 and there was a significant difference ( $P < 0.05$ ) between the treatment and the control. Similar results were observed in T-3 at PL7–8 stage ( $P < 0.05$ ). Amylase activity in T-2 at Z3, M3, PL1–2 and PL7–8 stages was significantly higher ( $P < 0.05$ ) than that in the control. The amylase activity was also increased significantly ( $P < 0.05$ ) in T-3 than the control except the M3 stage. As for the lipase activity, assays showed a significant difference ( $P < 0.05$ ) in groups treated with SC8168 as compared with the control except the initial stage (Z3). However, a concentration response of probiotic strains in T-1, T-2 and T-3 was not observed in the present research. The results from this study suggest that *B. coagulans* SC8168 supplemented at a certain concentration could significantly increase survival rate and some digestive enzyme activities of *P. vannamei* larvae.

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#### INTEGRATED DYNAMIC AQUACULTURE AND WASTEWATER TREATMENT MODELLING FOR RECIRCULATING AQUACULTURE SYSTEMS

Torsten E.I. Wik, Björn T. Lindén, Per I. Wramner-2009  
Aquaculture 287(3-4): 361-370

Abstract:

Recirculating aquaculture systems (RAS) in land based fish tanks, where the fish tank effluent is biologically treated and then recirculated back to the fish tanks, offers a possibility for large scale ecologically sustainable fish production. In order to fully exploit the advantages of RAS, however, the water exchange should be as small as possible. This implies strong demands on the water treatment, e.g. the maintenance of an efficient nitrification, denitrification and organic removal. Because of the RAS complexity, though, dynamic simulations are required to analyze and optimize a plant with respect to effluent water quality, production and robustness. Here, we present a framework for integrated dynamic aquaculture and wastewater treatment modelling, where fish growth and evacuation rate models can be linked to state of the art dynamic wastewater treatment models through massbalance based waste characterization. It provides means to analyze, predict and explain RAS performance. Using this framework we demonstrate how a new and improved RAS configurations is identified.

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#### DEVELOPMENT OF DIGESTIVE ENZYMES IN COMMON DENTEX DENTEX DENTEX DURING EARLY ONTOGENY

Enric Gisbert, Gemma Giménez, Ignacio Fernández, Yannis Kotzamanis, Alicia Estévez-2009  
Aquaculture 287(3-4): 381-387

Abstract:

The digestive physiology of common dentex was studied by assessing the activity of different pancreatic (trypsin, chymotrypsin, amylase and lipase), intestinal (alkaline phosphatase, aminopeptidase N, maltase and leucine–alanine peptidase) and gastric (pepsin) enzymes from hatching until the juvenile stage (50 dph at 19 °C). Enzymes involved in the digestion of protein, lipid and carbohydrate were present in common dentex larvae at hatching and before the onset of exogenous feeding. The specific activity of trypsin and chymotrypsin in newly hatched larvae was higher than that of amylase and lipase, indicating the importance of these enzymes in the cleavage of yolk proteins and hatching, while after hatching, the activity of these serine proteases dramatically decreased, whereas lipase showed the opposite trend. A progressive shift in activity from alkaline to acid proteases was observed during larval development, reflecting that alkaline proteases were not longer the main digestive enzymes involved in protein digestion after the development of gastric glands and onset of acidic digestion. Lipase total activity in common dentex peaked at 35 dph and decreased after weaning. Diet change due to weaning might partially explain the decrease in lipase activity, although this change might be also indicative of a change in the nutritional requirements of this species, since the juveniles prefer diets with high protein levels than those with lower protein and higher lipid content. Regarding intestinal enzymes, leucine–alanine peptidase and alkaline phosphatase and aminopeptidase N were found in newly hatched larvae, while maltase was detected after the onset of exogenous feeding. The achievement of an efficient brush border membrane digestion takes place much earlier (6–12 dph) than in any of the studied species which might reflect the different metabolic profile and rapid growth of this species.

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#### INFLUENCE OF FOOD DENSITY AND TEMPERATURE ON INGESTION, GROWTH AND SETTLEMENT OF PACIFIC OYSTER LARVAE, CRASSOSTREA GIGAS

B. Rico-Villa, S. Pouvreau, R. Robert-2009  
Aquaculture 287(3-4): 395-401

Abstract:

Ingestion, growth and metamorphosis of Pacific oyster, *Crassostrea gigas*, larvae were studied under controlled conditions of food density and temperature using a combination of a flow-through rearing

system and a hydrobiological monitoring device. In a first experiment larvae were exposed to three different phytoplankton densities (12, 20 and 40 cells  $\mu\text{l}^{-1}$ ) while in a second trial larvae were reared at five different temperatures (17, 22, 25, 27 and 32 °C). Both food concentration and temperature significantly affected the larval physiology throughout the entire development from D-veliger to young spat. Larvae survived over a wide range of both environmental parameters with high survival at the end of experiments. The feeding functional response provided the maximal ingestion rate (50 000 cells larva<sup>-1</sup> day<sup>-1</sup>) which occurred at an algal density of 20 cells  $\mu\text{l}^{-1}$  surrounding the larvae and 25 °C. At the highest temperature (32 °C), maximal growth and metamorphosis performances were reached in less than 2 weeks while the lowest temperature (17 °C) consistently inhibited ingestion and growth over the entire larval period. The estimate of the Arrhenius temperature (TA) was 11 000 K for *C. gigas* larvae. Larval development could be divided on the basis of feeding activity into an initial mixotrophic period with a lower and constant ingestion over the first days (from D-stage to early umbonate larva of  $\approx 110 \mu\text{m}$  length) followed by an exotrophic phase characterized by a sharp increase in ingestion (umbonate to eyed of  $\approx 300 \mu\text{m}$  length) and, finally, a third period for larvae  $\geq 300 \mu\text{m}$  during which ingestion decreased suddenly because of metamorphosis. Optimum larval development and settlement of the oyster *C. gigas* occurred at 27 °C and an increasing food supply as the larvae were growing. A food density of  $\geq 20$  cells  $\mu\text{l}^{-1}$  of T-ISO + CP or CG (1:1 cells number) in the culture water was required to maximise growth and metamorphosis success.

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#### EARLY DEVELOPMENT OF THE SILVER CATFISH RHAMDIA QUELEN (QUOY & GAIMARD, 1824) (PISCES:HEPTAPTERIDAE) FROM THE SÃO FRANCISCO RIVER BASIN, BRAZIL

Marcelo Pimenta de Amorim, Bruno Vilaça Campos Gomes, Yuri Simões Martins, Yoshimi Sato, Elizete Rizzo, Nilo Bazzoli-2009

Aquaculture Research 40(2): 172 – 180

Abstract:

The silver catfish, *Rhamdia quelen*, is endemic to North, Central and South America with high aquaculture potential and wide acceptance in the market. Breeder fish were subjected to induced reproduction through hypophysation using a crude common carp pituitary extract. Egg characteristics, oocyte surface ultrastructure and histology of larval ontogenesis until whole yolk resorption were described for the first time for this species. Oocytes and semen were obtained by manual extrusion, and fertilization was conducted using the dry method. After fertilization, eggs were kept in incubators at 24 °C. The embryonic development was monitored using a stereomicroscope every 10 min until hatching. To analyse the larval development, larvae samples were collected from incubators daily until the fifth day, fixed in Bouin's fluid and subjected to routine histological techniques. The oocyte extrusion occurred 8 h after the second hormone dose at 26 °C. The oocytes were spherical, non-adhesive and yellow, with a diameter of  $1471.75 \pm 47.63 \mu\text{m}$ . Scanning electron microscopy revealed a thin jelly coat covering the zona radiata in the animal pole around the micropyle. The blastopore closure occurred within 8 h after fertilization, and the fertilization rate was  $79.9 \pm 5.2\%$  at 24 °C. Embryonic development was completed within 25 h 30 min after fertilization. The complete resorption of the yolk and the formation of the digestive system organs and the mouth opening occurred on the fifth day, indicating a need for exogenous feeding. The results of this study provide information important for improvement in *R. quelen* culture and management.

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#### DEVELOPMENT AND DISTRIBUTION OF INTESTINAL ENZYMATIC ACTIVITY IN PARALABRAX MACULATOFASCIATUS (STEINDACHNER, 1868) LARVAE FED WITH LIVE PREY

Renato Peña, Silvie Dumas, Carmen Rodríguez-Jaramillo-2009

Aquaculture Research 40(2): 218 – 224

Abstract:

We describe the development and distribution of intestinal aminopeptidase M, dipeptidyl aminopeptidase IV, non-specific esterase, alkaline phosphatase and acid phosphatase, using enzyme histochemistry techniques, in the spotted sand bass larvae (*Paralabrax maculatofasciatus*) under culture conditions. All digestive enzymes tested showed a positive reaction from first feeding (day 2) and throughout the study period (day 30). At first feeding, the main enzymatic activity was in the mucosa throughout the intestines. Later, enzymatic activity occurred in the liver, kidney and stomach. All enzymatic activities increased from days 15 to 20, remaining constant until the end of the study. This enzymatic activity suggests the onset of maturation of the digestive tract. After day 20, a positive reaction was recorded in the pyloric caeca for all tested enzymatic activities. Our study confirms the digestive and absorptive functions in the intestines in spotted sand bass larvae from first feeding. It also brings new insight to establish an early weaning strategy during cultivation of spotted sand bass larvae.

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#### SHORT COMMUNICATION

#### EFFECTS OF PHOTOPERIOD ON SURVIVAL AND GROWTH OF THE MARINE PEJERREY ODONTESTHES ARGENTINENSIS LARVAE

Luciano Siqueira Freitas, Ricardo Vieira Rodrigues, Marcelo Hideo Okamoto, Luiz Roberto Louzada, Luís André Sampaio-2009

Aquaculture Research 40(2): 252 – 255

(Laboratório de Maricultura, Departamento de Oceanografia, Fundação Universidade Federal do Rio Grande, CP 474, 96.201-900, Rio Grande-RS, Brazil; email of L. A. Sampaio: [sampaio@mikrus.com.br](mailto:sampaio@mikrus.com.br))

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#### FERTILIZATION EFFICIENCY OF CRYOPRESERVED SPERM FROM STRIPED CATFISH, PANGASIU HYPOPHthalmus (SAUVAGE)

Samorn Kwantong, Amrit N. Bart-2009

Aquaculture Research 40(3): 292 – 297

Abstract:

The fertilization efficiency of cryopreserved sperm was compared with fresh sperm from striped catfish, *Pangasius hypophthalmus*. Of the two sets of experiments carried out, the first compared four sperm doses using fresh sperm and fresh eggs. The second experiment compared six concentrations of cryopreserved sperm ranging from  $6.94 \times 10^7$  to  $6.94 \times 10^{10}$  to fertilize 100 eggs per batch. Fertilization, hatch and survival rates were compared between cryopreserved and fresh sperm. The highest fertilization rate ( $53.75 \pm 1.62\%$ ) was achieved with a sperm dose of  $6.94 \times 10^8$ . Increasing the sperm dose to  $3.47 \times 10^9$  did not increase the fertilization rate, indicating that the optimum sperm:egg ratio lies between  $6.94 \times 10^6$  and  $3.47 \times 10^7$  sperm per egg. Both highest ( $6.94 \times 10^{10}$ ) and the lowest ( $6.94 \times 10^7$ ) sperm doses resulted in lower fertilization rates (2.04% and 16.90% respectively). No significant differences were found among four fresh sperm doses compared. Mean hatch and survival rates resulting from fresh and cryopreserved sperm were similar. The experiment shows that while only  $1.89 \times 10^6$  fresh spermatozoa was required to fertilize a fresh egg,  $6.94 \times 10^6$  (or 3.67 times more) cryopreserved sperm was required to achieve the same level of fertilization. This provides important information for making decision to cryopreserve sperm for commercial and/or conservation purposes.

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EFFECT OF DIFFERENT SYNTHETIC GONADOTROP-RELEASING HORMONE ANALOGUES AND THEIR COMBINATIONS WITH AN ANTI-DOPAMINERGIC COMPOUND ON THE REPRODUCTION PERFORMANCE OF STERLET (*ACIPENSER RUTHENUS* L.)

András Rónyai-2009

Aquaculture Research 40(3): 315 – 321

Abstract:

In this study, three synthetic gonadotrop-releasing hormones (GnRH) (azagly-nafarelin; des-Gly10-(d-Ala6)-LH-RH; and des-Gly10-(d-Phe6)-LH-RH) either alone or in combination with metoclopramide were used to induce reproduction of sterlet. The GnRH analogues were applied in a single dose of 40 µg kg<sup>-1</sup> of female and 20 µg kg<sup>-1</sup> of male body weight. Metoclopramid was administered in a simultaneous injection of 10 and 5 mg kg<sup>-1</sup> of body weight for females and males respectively. There were no significant differences in the ovulatory responses of females; ovulation rates varied between 57% and 80%, and at the temperature of 15.5–16.0 °C about 30–34 h were required for final maturation, when eggs of 17.3±1.3% of body weight were stripped. However, the fertilization rates of the des-Gly10-(d-Phe6)-LH-RH-treated groups were significantly lower than that in the other treatment. In males, the combination of the above peptidergic hormones with metoclopramide gave significantly better results than their single application. The results demonstrate that the final stage of gamete maturation in sterlet may be achieved by several hormonal means. The possibility of using new GnRH analogues without dopamine antagonists yields new perspectives for induced breeding of sturgeons, which have particular importance in the light of meat and roe (caviar) production for human consumption.

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IMMUNOLocalIZATION OF Na<sup>+</sup>, K<sup>+</sup>-ATPase-RICH CELLS IN THE GILL AND URINARY SYSTEM OF PERSIAN STURGEON, *ACIPENSER PERSICUS*, FRY

Saber Khodabandeh, Zahra Khoshnood, Saeide Mosafer-2009

Aquaculture Research 40(3): 329 – 336

Abstract:

Localization of Na<sup>+</sup>, K<sup>+</sup>-ATPase-rich cells in the gill and urinary system of *Acipenser persicus* fry was performed through immunofluorescence light microscopy using a mouse monoclonal antibody IgGα5 raised against the α-subunit of chicken Na<sup>+</sup>, K<sup>+</sup>-ATPase. Different types of epithelia were clearly identified in the gill epithelium: epithelia of branchial arch, interbranchial septum, filament and lamellar epithelium. The Na<sup>+</sup>, K<sup>+</sup>-ATPase-rich cells were found in the epithelia of branchial arch, interbranchial septum, filament, interlamellar region and also in the lamellae. Histologically, the urinary system is divided into head kidney, trunk kidney and short caudal kidney. The head kidney is composed of the pronephric tubules and the haemopoietic tissues, while the trunk kidney is composed of a large number of glomeruli and convoluted nephrons. Each nephron consisted of a large glomerulus and tubules (neck, proximal, distal and collecting tubules) which connected to ureters. Posteriorly, ureters extended and joined together to form a small urinary bladder. In the urinary system, no specific fluorescence staining was observed in the glomerulus, neck segment and proximal tubules. The distal tubule cells and collecting tubule cells showed a strong immunostaining of Na<sup>+</sup>, K<sup>+</sup>-ATPase. Epithelia of ureters and urinary bladder also showed several isolated immunofluorescent cells. Immunofluorescent cells were rich in Na<sup>+</sup>, K<sup>+</sup>-ATPase enzyme which is very important for osmoregulation.

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SURVIVAL AND GROWTH OF HATCHERY-PRODUCED POSTLARVAE AND SPAT OF THE CARIBBEAN SCALLOPS *ARGOPECTEN NUCLEUS* AND *NODIPECTEN NODOSUS*

Luz Adriana Velasco, Judith Barros-2009

Aquaculture Research 40(3): 362 – 375

Abstract:

A study was made of the growth and survival of postlarvae and spat of *Argopecten nucleus* and *Nodipecten nodosus* produced in the laboratory and exposed to different conditions of settlement and culture. An evaluation was made of the different types of collection substrates (onion-bags, artificial-grass, fibreglass-plates), two types of settlement inducers (epinephrine, low temperature), and two culture environments (sea, laboratory). The results showed that larger numbers of postlarvae were obtained in artificial-grass collectors than on fibreglass-plates or in the onion-bags. The growth of the *A. nucleus* postlarvae was not affected by the collector type, while that of *N. nodosus* was higher on fibreglass-plates, and lesser in the onion-bags. Both thermal and chemical stimulation of the competent larvae resulted in recovery of a greater percentage of postlarvae. The growth of postlarvae of *A. nucleus* was better in groups exposed to the two methods of settlement induction, while those of *N. nodosus* remained unaffected by this treatment. The survival of the postlarvae and spat was greater in the laboratory than at sea except in the case of *A. nucleus* postlarvae attached to collectors, whose survival was similar in both environments. The growth of postlarvae and spat was greater in the sea than in the laboratory.

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#### EFFECT OF PARTIAL OR TOTAL REPLACEMENT OF FORAGE FISH BY A DRY DIET ON THE QUALITY OF REPRODUCTION IN PIKEPERCH, SANDER LUCIOPERCA

Neil Wang, S. N. M. Mandiki, Emilie Henrotte, Abdel-Ghany Bouyahia, Guillaume Mairesse, Carole Rougeot, Charles Melard, Patrick Kestemont-2009

Aquaculture Research 40(3): 376 – 383

Abstract:

The aim of this preliminary study was to evaluate the influence of different feeding regimes on the quality of reproduction in pikeperch. Three diets were tested: forage fish (FF), a commercial dry feed (DD) and a mix of both (FD). The diets were given to fish throughout a complete reproductive cycle. During the spawning season, couples were injected with human chorionic gonadotropin (hCG) and let to spawn on nests. Proportion of running males, spawning and hatching success and larval quality (weight, length, body protein, total lipid, fatty acid and lipid class compositions and resistances to osmotic shock and starvation stress tests at hatching) were evaluated. The proportion of running male was lower in the DD group than in the FF and FD groups (54% for DD against 76–89% for FF and FD). In addition, 25%, 62.5% and 75% of injected couples gave spawning that hatched in DD, FF and FD groups respectively. Larval quality parameters were not significantly different between treatments. The results indicate that overall quality of reproduction was higher in FF and FD treatments than in DD. It suggests that the dry feed used was not totally adequate for pikeperch reproduction. Relations between breeder reproductive performances and the feed compositions are discussed.

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#### LIPID CHARACTERIZATION OF BOTH WILD AND CULTURED EGGS OF CUTTLEFISH (*SEPIA OFFICINALIS* L.) THROUGHOUT THE EMBRYONIC DEVELOPMENT

A.V. Sykes, E. Almansa, A. Lorenzo, J.P. Andrade-2009

Aquaculture Nutrition 15(1): 38 – 53

Abstract:

The present work reports a characterization of mean wet weight and moisture, the lipid class and fatty acid (FA) composition from the total lipids (TL), of both culture and wild eggs of the cuttlefish, *Sepia officinalis*, throughout the embryonic development. Additionally, reproductive data, such as the number of spawnings, number and mean weight of eggs and duration of spawning period of cultured cuttlefish is provided. Both types of eggs were similar in mean wet weight, moisture content, TL content and lipid composition throughout embryonic development. Females from the cultured group spawned 13 times and laid 8654 eggs in 64 days, with a mean weight of  $0.607 \pm 0.179$  g. A sex ratio of 1.57 (11♀ for 7♂) promoted an individual fecundity of 787 eggs/♀ (the biggest until now on our culture facilities), which might be related to increased bottom areas. The TL increased with day/stage

of embryonic development ( $P < 0.05$ ) only in the cultured egg group. However, no differences were found on TL between culture and wild eggs at the same day/stage ( $P > 0.05$ ). Eggs displayed predominant levels of phosphatidylcholine, phosphatidylethanolamine (PE), cholesterol and triacylglycerol at the end of embryonic development. Polar and neutral lipids of both eggs groups remained consistently proportional (~50% for each lipid fraction) and a significant increase ( $P < 0.05$ ) was observed in phosphatidylserine, PE and free FA throughout the embryonic development. In either egg type and day, 16:0, 18:0, 20:5n-3 and 22:6n-3 accounted for approximately 70 g Kg<sup>-1</sup> of all FA and saturated and n-3 totals seemed to have the same proportion in the cuttlefish eggs. The present results suggest that lipids are not used as energetic substrate but as structural components in cuttlefish egg.

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#### BODY LIPID AND FATTY ACID COMPOSITION IN MALE GILT HEAD SEABREAM BROODSTOCK AT DIFFERENT STAGES OF THE REPRODUCTIVE CYCLE: EFFECTS OF A DIET LACKING N-3 AND N-6 HUFA

M.V. Martin, C. Rodriguez, J.R. Cejas, Ma J. Pérez, S. Jerez, A. Lorenzo-2009

Aquaculture Nutrition 15(1): 60 – 72

##### Abstract:

Total lipid (TL), lipid classes and fatty acid composition of neutral (NL) and polar (PL) lipids were studied in the gonads, liver and muscle of gilthead seabream males (*Sparus aurata*) fed a control diet (diet C) or an n-3 and n-6 highly unsaturated fatty acids (HUFA)-deficient diet (diet D), at different stages of the reproductive cycle. Between pre-spermatogenesis (November) and spermatogenesis (March), the lipid content was high and particularly rich in cholesterol, phosphatidylcholine and phosphatidylethanolamine in gonads from both dietary groups. At post-spermatogenesis (June), TL and especially PL dramatically decreased in the gonads from both groups. However, at this period diet C fish gonads were richer in triacylglycerides (TAG) than those from diet D fish. The liver lipid contents and particularly TAG were over 200% lower in June than in March for both groups. Nevertheless, the most noteworthy depletion of lipids during this period was achieved by the n-3 HUFA in diet D fish. Conversely, arachidonic acid (20:4n-6) did not decrease in NL or PL from gonads and liver in groups C and D. Muscle lipids from diet C fish were relatively insensitive to seasonal influences. However, in June, the muscle TAG content was significantly reduced in diet D fish.

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#### COMPARISON OF DIETARY PHOSPHOLIPIDS AND NEUTRAL LIPIDS: EFFECTS ON GUT, LIVER AND PANCREAS HISTOLOGY IN ATLANTIC COD (*GADUS MORHA L.*) LARVAE

P.-A. Wold, K. Hoehne-Reitan, C. L. Cahu, J. Z. Infante, J. Rainuzzo, E. Kjørsvik-2009

Aquaculture Nutrition 15(1):73 – 84

##### Abstract:

The aim of the present study was to compare effects of dietary n-3 highly unsaturated fatty acids (HUFA) being incorporated in the phospholipid (PL) or in the neutral lipid (NL) fraction of the larval feed, on larval growth and histology of digestive organs in Atlantic cod (*Gadus morhua L.*) larvae. Three isoproteic and isolipidic diets, labelled according to the percentage of n-3 docosahexaenoic acid and eicosapentaenoic acid contained in NL1 or in PL1 and PL3 of the diets, were fed to cod larvae from 17 days post hatching (dph) to 45 dph.

In the liver, hepatocytes and their nuclei were smaller in NL1 larvae compared with the PL larvae; the mitochondrial membrane structures were less dense and the amount of lipids observed in the liver was significantly higher in NL1 larvae compared with the PL3 larvae. The liver and gut size was related to larval size, with no differences between the larval groups. The results demonstrated that the essential fatty acids were more beneficial for cod larvae when they were incorporated in the dietary polar PL



rather than in the NL, and that the n-3 HUFA requirements in cod larvae is possibly higher than that in the PL1 diet.

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RELATIONSHIP BETWEEN SPAT DENSITY, FOOD AVAILABILITY, AND GROWTH OF SPAWNERS IN CULTURED MIZUHOPECTEN YESSOENSIS IN FUNKA BAY: CONCURRENCE WITH EL NIÑO SOUTHERN OSCILLATION

Katsuhisa Baba, Rieko Sugawara, Hisashi Nitta, Kiyoshi Endou, and Akira Miyazono-2009

Can. J. Fish. Aquat. Sci. 66(1): 6–17

Abstract:

To elucidate the factors that influence the interannual variation in the density of cultured Japanese scallop (*Mizuhopecten yessoensis*) spat, we analyzed the relationship between spat density (Ds), monthly chlorophyll a (chl a) concentration, water temperature, and adductor muscle weight of spawners over 15 years (1992–2006) in Funka Bay (Japan) on the western North Pacific Ocean. The interannual variation of spat density was best explained by a campaniform model that used chl a concentration in February ([chl]Feb) and a categorical variable that indicates whether growth of spawners in a year is low or not as independent variables ( $R^2 = 0.91$ ). The gonadosomatic index increased fastest in February. Low growth years were detected as outliers in the Ds – [chl]Feb relationship and were characterized by an average weight of adductor muscles in February of <12 g. Therefore, food availability during gonadal development and growth conditions of spawners were the main factors determining spat density. The proportion of ovary necrosis was high in the years of low [chl]Feb and low growth. Those years corresponded with El Niño and La Niña years, respectively. Thus, global climatic anomalies apparently affect reproduction of the scallop in Funka Bay.

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EFFECTS OF SALINITY ON RATES OF PROTEIN SYNTHESIS AND OXYGEN UPTAKE IN THE POST-LARVAE AND JUVENILES OF THE TROPICAL PRAWN *MACROBRACHIUM ROSENBERGII* (DE MAN)

I. Intanai, E.W. Taylor, N.M. Whiteley-2009

Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 152(3): 372-378

Abstract:

Protein synthesis is a major determinant of growth and yet little is known about the environmental factors that influence protein synthesis rates in farmed freshwater prawns. To this end, post-larvae and juveniles of *Macrobrachium rosenbergii* were exposed to various salinities (0, 14, 30‰) to determine whole-animal rates of fractional protein synthesis (ks) and oxygen uptake. In the post-larvae that migrate upstream from brackish to freshwater areas, whole-animal ks was unaffected by salinity, but rates of oxygen uptake were significantly lower at 14‰. In the freshwater juveniles, a different response was observed, as mean ks was significantly higher at 14‰ compared with 0‰, but rates of oxygen uptake remained unchanged. Such differences are thought to be related to the energetic costs of osmoregulation and to the ability to maintain osmotic gradients in freshwater. In an additional experiment, acclimation temperature (20, 26, 30 °C) had a direct effect on ks in juveniles held at 0‰. In all cases, changes in ks resulted from alterations in RNA activity at constant RNA capacity. In juveniles at least, whole-animal rates of protein synthesis were highest at 14‰ and 30 °C which corresponds to the optimal salinity and temperature recommended for the growth and culture of *M. rosenbergii*.

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EVALUATION OF CANDIDATE REFERENCE GENES IN Q-PCR STUDIES OF ATLANTIC COD (*GADUS MORHUA*) ONTOGENY, WITH EMPHASIS ON THE GASTROINTESTINAL TRACT

Øystein Sæle, Andreas Nordgreen, Kristin Hamre, Pål A. Olsvik-2009

Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 152(1) 94-101

Abstract:

To obtain reliable relative qPCR data in developing fish larvae, stable reference genes have to be found. This study is focused on finding good candidates for normalization of qPCR data for ontogenetic studies of Atlantic cod. Ten commonly used reference genes; Acidic ribosomal protein, Actin-related protein 2,  $\beta$ -actin, Elongation factor 1 A, Glyceraldehyde-3-phosphate dehydrogenase, Ribosomal protein 37, Ribosomal protein 4, Ribosomal protein S9,  $\beta$  2-Tubulin and Ubiquitin were analyzed in developing larvae from 3 to 97 day post hatch (DPH). Two different tools were used to evaluate the stabilities of these genes; the geNorm software ranks the most stable genes based on a pair-wise analysis whereas NormFinder uses a model-based approach. The same genes were also analyzed in GI tract homogenates and compared to whole larvae homogenates. During Atlantic cod larval development there are several strong candidates with Ubiquitin as the most stable. The ribosomal proteins RPL4 and RPS9 are also strong candidates. RPL37 may be used but only when normalizing qRT-PCR results from one type of tissue. We also suggest the use of multiple genes for normalization of qRT-PCR. Our study suggests that whole-larvae samples can be used to study relative expression of genes that are expressed only in certain tissues.

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#### IDENTIFICATION AND CHARACTERIZATION OF HYPOXIA-INDUCED GENES IN CARASSIUS AURATUS BLASTULAE EMBRYONIC CELLS USING SUPPRESSION SUBTRACTIVE HYBRIDIZATION

Xue-Ping Zhong, Dan Wang, Yi-Bing Zhang, Jian-Fang Gui-2009

Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 152(2): 161-170

Abstract:

Organisms living in water are inevitably exposed to periods of hypoxia. Environmental hypoxia has been an important stressor having manifold effects on aquatic life. Many fish species have evolved behavioral, physiological, biochemical and molecular adaptations that enable them to cope with hypoxia. However, the molecular mechanisms of hypoxia tolerance in fish, remain unknown. In this study, we used suppression subtractive hybridization to examine the differential gene expression in CAB cells (*Carassius auratus* blastulae embryonic cells) exposed to hypoxia for 24 h. We isolated 2100 clones and identified 211 differentially expressed genes (e-value  $\leq 5e^{-3}$ ; Identity > 45%). Among the genes whose expression is modified in cells, a vast majority involved in metabolism, signal transduction, cell defense, angiogenesis, cell growth and proliferation. Twelve genes encoding for ERO1-L, p53, CPO, HO-1, MKP2, PFK-2, cystatin B, GLUT1, BTG1, TGF $\beta$ 1, PGAM1, hypothetical protein F1508, were selected and identified to be hypoxia-induced using semi-quantitative RT-PCR and real-time PCR. Among the identified genes, two open reading frames (ORFs) encoding for CaBTG1 and Cacystatin B were obtained. The deduced amino acid sequence of CaBTG1 had 94.1%, 72.8%, 72.8%, 72.8%, 68.6% identity with that of DrBTG1, HsBTG1, BtBTG1, MmBTG1 and XIBTG1. Comparison of Cacystatin B with known cystatin B, the molecules exhibited 49.5 to 76.0% identity overall. These results may provide significant information for further understanding of the adaptive mechanism by which *C. auratus* responds to hypoxia.

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#### ON THE ROLE OF CDC2 KINASE DURING MEIOTIC MATURATION OF OOCYTE IN THE CHINESE MITTEN CRAB, ERIOCHEIR SINENSIS

Gao-Feng Qiu, Ping Liu-2009

Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 152(3): 243-248

Abstract:

Cdc2 kinase is a catalytic subunit of maturation-promoting factor (MPF), a central factor for inducing the meiotic maturation of oocyte. To understand the role of Cdc2 kinase on the oocyte maturation in crustacean, a complete cDNA sequence of Cdc2 kinase was cloned from Chinese mitten crab *Eriocheir sinensis* and its spatial-temporal expression profiles were analyzed during oogenesis at RNA and protein levels. The crab Cdc2 cDNA (1364 bp) encodes for a 299 amino acids protein with calculated molecular weight of 34.7 kDa. The Cdc2 mRNAs level showed no significant change in the ovary during oogenesis, whereas higher protein level was found at previtellogenesis, late vitellogenesis and germinal vesicle breakdown (GVBD) stages. Two forms (35 kDa and 34 kDa) of Cdc2 proteins were simultaneously identified in ovary at all stages. Immunocytochemistry analysis revealed that Cdc2 proteins locate exclusively in ooplasm of previtellogenic oocyte, and then relocate into germinal vesicle at vitellogenesis stage and accumulate on meiotic spindle at oocyte maturation. These findings suggest that Cdc2 kinase has essential roles in inducing GVBD and generating meiotic apparatus during the crab oocyte maturation.

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LIPID, FATTY ACID AND PROTEIN CONTENT OF LATE LARVAL TO EARLY JUVENILE STAGES OF THE WESTERN ROCK LOBSTER, PANULIRUS CYGNUS

Andrew J. Limbourn, Peter D. Nichols-2009

Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 152(3): 292-298

Abstract:

Lipid, fatty acid and protein content were determined individually on 7 phyllosomata, 69 clear pueruli, 286 pre-moult pueruli, and 86 juvenile western rock lobster (WRL) collected from four locations between the settlement seasons 2000 to 2006 to evaluate compositional changes during the non-feeding puerulus stage. Only the lipid content, particularly the phospholipids, decreased significantly with development. Protein declined sharply following moult to the juvenile. PL comprised between 86–94% of total lipid in all animals, and declined most between phyllosomata and clear pueruli (238.5 to 121.4 mg g<sup>-1</sup> DW) ( $p < 0.001$ ). Triacylglycerols were the only lipid to increase in absolute amounts with development, but declined 53% on average following moult to juvenile. This increase in TAG is likely due to the conversion of phospholipids to triacylglycerols. Monounsaturated fatty acids were the main energy form utilised during benthic development while polyunsaturated fatty acids showed a high degree of sparing. The n-3:n-6 fatty acid ratio of juveniles indicates that they may be approaching critically low levels of stored lipid energy reserves. Both protein, and lipid, declined sharply from the final puerulus phase to the juvenile confirming that a high energetic demand is required to fuel the moulting process.

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A REVIEW OF SPAWNING INDUCTION, LARVICULTURE, AND JUVENILE REARING OF THE FAT SNOOK, CENTROPOMUS PARALLELUS

V. R. Cerqueira, M. Y. Tsuzuki-2009

Journal Fish Physiology and Biochemistry 35(1): 17-28

Abstract:

The fat snook, *Centropomus parallelus*, is a commercially valuable marine fish species with potential for aquaculture. This paper describes the development of technology for mass production of fat snook juveniles at the Experimental Fish Hatchery of the Universidade Federal de Santa Catarina, focusing on research about reproduction, larviculture, and juvenile rearing. Induced spawning of wild fat snook

was first achieved in 1991 with a single injection of human chorionic gonadotropin (hCG). There was a substantial increase in egg quality when broodstock was conditioned in maturation rooms and induced to spawn. Different dosages of luteinizing hormone-releasing hormone analogue (LHRHa) with saline injection and cholesterol implant were also tested. As fat snook exhibits group-synchronous oocyte development, females could be induced to spawn (with 35–50  $\mu\text{g kg}^{-1}$  of LHRHa) once a month, resulting in up to four consecutive spawnings. Results of larval culture were highly variable at the beginning; survival rates were frequently around 1% until the juvenile stage. Several experiments were conducted to evaluate the effect of environmental factors and feeding quality on survival and growth. With the improvement of the spawning induction technique and better larviculture practices, survival rates increased to 10–30%. Studies on the particular requirements of juveniles in terms of stocking density, feeding, nutrition, and environmental factors were also performed in order to improve growth rates and feed utilization. The present study demonstrates the feasibility of mass production of fat snook juveniles. However, further research is needed to develop cost-effective grow-out technology.

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#### NUTRITION AND FEEDING RESEARCH IN THE SPOTTED ROSE SNAPPER ( LUTJANUS GUTTATUS ) AND BULLSEYE PUFFER (SPHOEROIDES ANNULATUS), NEW SPECIES FOR MARINE AQUACULTURE

Armando García-Ortega-2009

Journal Fish Physiology and Biochemistry 35(1): 69-80

Abstract:

The spotted rose snapper (*Lutjanus guttatus*) and bullseye puffer (*Sphoeroides annulatus*) are fish species from the tropical Eastern Pacific for which controlled production of larvae and juveniles has been accomplished in recent years. Diverse topics relating to their biology and aquaculture production are currently under study, in particular the nutrition and feeding aspects required to formulate practical feeds and rearing protocols. Improvements in larval growth and survival are possible by feeding live food organisms with natural or enhanced essential fatty acids content and highly digestible artificial microdiets. The ontogeny of the digestive tract and the expression and activity of digestive enzymes have been described for *S. annulatus* larvae. The effect of various protein and lipid levels on growth and feed utilization has been studied in juvenile and on-growing fish. Both species have carnivorous feeding habits and require high levels of protein in their diets, from 40% to 45% (dry weight) in spotted rose snapper and above 50% in bullseye puffer, with the younger stages requiring the highest protein levels. Encouraging results have been obtained in feeding experiments with different sources of dietary protein from animal and plant origin to evaluate their suitability as feed ingredients in practical diets. Optimization of fish culture practices through feeding management has also been investigated. Trials with various fish densities and feeding frequencies in intensive culture systems are providing information to improve feed utilization and growth in on-growing fish. Further research is underway to evaluate factors in broodstock nutrition which have an impact on egg and larval quality, and into the use of various commercially available oil sources in on-growing diets. In this paper, the results on nutrition and feeding research with both species are reviewed and research needs to support their commercial production in the region are discussed.

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#### COMBINED EFFECTS OF IRRADIANCE LEVEL AND CARBON SOURCE ON FATTY ACID AND LIPID CLASS COMPOSITION IN THE MICROALGA PAVLOVA LUTHERI COMMONLY USED IN MARICULTURE

Freddy Guihéneuf, Virginie Mimouni, Lionel Ulmann, Gérard Tremblin-2009

Abstract:

*Pavlova lutheri*, a marine Pavlovophyceae, has been well documented as it is commonly used as a food source in mariculture. In this study, we investigated the combined effects of carbon sources and irradiance levels on the growth, lipid classes and fatty acid profiles of this microalga. The microalga was cultured at 15 °C with a 14 h photoperiod in artificial seawater containing bicarbonate or acetate as carbon source.

The growth and lipid composition of *P. lutheri* were more sensitive to variations in light intensity than in carbon source. However, *P. lutheri* seems to be able to use acetate to growth cell and lipid metabolism. With the both carbon source, the lowest cellular lipid contents were obtained under low light intensity. The proportions of PUFAs, especially EPA, were significantly higher under low light, and saturating fatty acids and DHA levels were significantly higher under high light. In *P. lutheri*, galactolipids, a major component of chloroplast lipid membranes, made up approximately 54-66% of total lipids. The highest PUFA levels, such as those of EPA, were predominantly found in the galactolipid fraction when the cells were grown at low light, regardless of the carbon source. The consequent accumulation of n-3 fatty acids in the galactolipids could facilitate thylakoid membrane fluidity, and therefore the velocity of electron flow involved in photosynthesis during light acclimatization. These results could be used to optimize the culture conditions and the nutritional value of this microalga, which is used to feed marine invertebrates and fish larvae in mariculture hatcheries, and to produce n-3 fatty acids for human health care and nutrition.

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THE RELATIONSHIP OF OOCYTE DIAMETER AND INCUBATION TEMPERATURE TO INCUBATION TIME IN TEMPERATE FRESHWATER FISH SPECIES

F. Teletchea, J.-N. Gardeur, E. Kamler, P. Fontaine-2009

Journal of Fish Biology 74(3): 652 – 668

Abstract:

Based on the analysis of six egg variables and incubation temperature of 65 temperate freshwater fish species, the possible relationships between oocyte diameter, incubation time and incubation temperature were reassessed and compared to the results obtained from marine fishes. Most freshwater species have eggs (mean  $\pm$ s.d.  $2.19 \pm 1.52$  mm) larger than marine species, that are chiefly demersal and develop stuck to various substrata, such as plants or rocks. A strong negative relationship was found between incubation time (t, days) and incubation temperature (T, ° C):  $t = 186.23e^{-0.197T}$  ( $r^2 = 0.87$ ). A strong dependence of incubation time on oocyte diameter ( $\emptyset$ , mm) and incubation temperature was also found and was defined as:  $\log_{10}t = 3.002 + 0.599 \log_{10}\emptyset - 1.91 \log_{10}(T + 2)$ , which explained 92% of the variance of the data set. Five major groups of species were defined based on the principal component analysis (PCA) of four quantitative variables. There were two distinct groups of salmonids, displaying demersal and non-adhesive eggs with a long incubation time at low temperature, the eggs of which required a high number of degree-days. There was a large group of species possessing small, mostly demersal and adhesive eggs developing at high temperature during a short period of time, and requiring a low number of degree-days. Between these two extremes, there was a fourth group displaying intermediate values and a fifth group including three species with large, adhesive and demersal eggs incubating at high temperatures during a short period of time. The burbot *Lota lota* displayed an unusual combination of variables compared to the remaining species in the data set.

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EFFECT OF INCUBATION TEMPERATURE ON MUSCLE GROWTH OF BARRAMUNDI LATES CALCARIFER AT HATCH AND POST-EXOGENOUS FEEDING

G. R. Carey, P. G. Kraft, R. L. Cramp, C. E. Franklin-2009

Abstract:

Muscle morphology was investigated in newly hatched barramundi *Lates calcarifer* larvae incubated at set temperatures (26, 29 and 31° C) prior to hatching. Three days after hatching (the start of exogenous feeding), larvae from the 26 and 31° C treatments were each divided into two groups and reared at that temperature or transferred over the period of several hours to 29° C (control temperature). Incubation temperature significantly affected muscle cellularity in the developing embryo, with larvae incubated at 26° C (mean  $\pm$ s.e.  $223.3 \pm 7.9$ ) having on average 14.4% more inner muscle fibres than those incubated at 31° C ( $195.2 \pm 8.8$ ) and 4.8% more than those incubated at 29° C ( $213.5 \pm 4.7$ ). Conversely, inner muscle fibre cross-sectional area significantly increased at the warm incubation temperature in *L. calcarifer*, so that the total cross-sectional muscle area was not different between treatment groups. The total cross-sectional area of superficial muscle fibres and the proportion of superficial to total fibre cross-sectional area in just hatched *L. calcarifer* were also affected by incubation temperature, with incubation at the cool temperature (26° C) increasing both the total cross-sectional area and proportion of superficial muscle fibres. By 9 days post-hatch, the aforementioned differences were no longer significant. Similarly, there was no difference in total superficial fibre cross-sectional area between any treatment groups of *L. calcarifer*, whereas incubation temperature still significantly affected the proportion of superficial to total muscle fibre cross-sectional area. Larvae hatched and grown at 31° C had a significantly reduced percentage of superficial muscle cross-sectional area (mean  $\pm$ s.e.  $5.11 \pm 0.66\%$ ) compared with those incubated and grown at 29° C ( $8.04 \pm 0.77\%$ ) and 26° C ( $9.32 \pm 0.56\%$ ) and those incubated at 26° C and transferred to 29° C ( $7.52 \pm 0.53\%$ ), and incubated at 31° C and transferred to 29° C ( $6.28 \pm 0.69\%$ ). These results indicate that changes in muscle cellularity induced by raising or lowering the incubation temperature of *L. calcarifer* display varying degrees of persistence over developmental time. The significance of these findings to the culture of *L. calcarifer* is discussed.

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A FATTY ACID FROM THE DIATOM PHAEODACTYLUM TRICORNUTUM IS ANTIBACTERIAL AGAINST DIVERSE BACTERIA INCLUDING MULTI-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

Andrew P. Desbois, Andrew Mearns-Spragg, Valerie J. Smith-2009

Journal Marine Biotechnology 11(1): 45-52

Abstract:

Pathogenic bacteria, such as multidrug-resistant *Staphylococcus aureus* (MRSA), which are not susceptible to most conventional antibiotics, are causing increased concern in healthcare institutions worldwide. The discovery of novel antibacterial compounds for biomedical exploitation is one avenue that is being pursued to combat these problematic bacteria. Marine eukaryotic microalgae are known to produce numerous useful products but have attracted little attention in the search for novel antibiotic compounds. Cell lysates of the marine diatom, *Phaeodactylum tricorutum* Bohlin, have been reported to display antibacterial activity in vitro, but the compounds responsible have not been fully identified. In this paper, using column chromatography and reversed-phase high-performance liquid chromatography, we report the isolation of an antibacterial fatty acid. Mass spectrometry and <sup>1</sup>H-nuclear magnetic resonance spectroscopy revealed it to be the polyunsaturated fatty acid, eicosapentaenoic acid (EPA). We show that EPA is active against a range of both Gram-positive and Gram-negative bacteria, including MRSA, at micromolar concentrations. These data indicate that it could find application in the topical and systemic treatment of drug-resistant bacterial infections.

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A MULTIPLEX PCR METHOD FOR RAPID IDENTIFICATION OF BRACHIONUS ROTIFERS

Kalliopi Vasileiadou, Spiros Papakostas, Alexander Triantafyllidis, Ilias Kappas, Theodore J. Abatzopoulos-2009

Journal Marine Biotechnology 11(1): 53-61

Abstract:

Cryptic species are increasingly being recognized in many organisms. In *Brachionus* rotifers, many morphologically similar yet genetically distinct species/biotypes have been described. A number of *Brachionus* cryptic species have been recognized among hatchery strains. In this study, we present a simple, one-step genetic method to detect the presence of those *Brachionus* sp. rotifers that have been found in hatcheries. With the proposed technique, each of the *B. plicatilis* sensu stricto, *B. ibericus*, *Brachionus* sp. Nevada, *Brachionus* sp. Austria, *Brachionus* sp. Manjavacas, and *Brachionus* sp. Cayman species and/or biotypes can be identified with polymerase chain reaction (PCR) analysis. Based on 233 cytochrome c oxidase subunit I sequences, we reviewed all the available cryptic *Brachionus* sp. genetic polymorphisms, and we designed six nested primers. With these primers, a specific amplicon of distinct size is produced for every one of the involved species/biotypes. Two highly sensitive protocols were developed for using the primers. Many of the primers can be combined in the same PCR. The proposed method has been found to be an effective and practical tool to investigate the presence of the above six cryptic species/biotypes in both individual and communal (bulk) rotifer deoxyribonucleic acid extractions from hatcheries. With this technique, hatchery managers could easily determine their rotifer composition at the level of cryptic species and monitor their cultures more efficiently.

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## FACTORS THAT CONTRIBUTE TO THE ECOLOGICAL RISKS OF SALMON AND STEELHEAD HATCHERY PROGRAMS AND SOME MITIGATING STRATEGIES

Kathryn Kostow-2009

Journal Reviews in Fish Biology and Fisheries 19(1): 9-31

Abstract:

State and federal agencies in the United States annually release millions of hatchery salmon and steelhead into public waters. Many of the hatchery programs are located in areas where the wild populations are now listed under the U.S. Endangered Species Act (ESA) (16 U.S.C. §§ 1531–1544). These hatchery programs pose genetic and ecological risks to wild fish populations. Genetic risks occur when hatchery and wild fish interbreed and usually occur within a taxonomic species. Ecological risks occur when the presence of hatchery fish affects how wild fish interact with their environment or with other species and may affect whole species assemblages. This paper reviews some of the factors that contribute to ecological risks. Important contributing factors include the relative abundance of hatchery and wild fish in natural production areas, hatchery programs that increase density-dependant mortality, residual hatchery fish, some physical advantages that hatchery fish can have over wild fish, and life history characteristics that may make some species especially vulnerable to the effects of ecological risks. Many of these risk factors can be mitigated by management activities that reduce the level of interactions between hatchery and wild fish. This paper concludes by recommending twelve mitigation strategies that may be useful when agencies need to bring hatchery programs into compliance with the take provisions of the ESA.

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