

INTERNATIONAL WORKSHOP ON ADVANCED TECHNIQUES IN STURGEON FISH LARVICULTURE

March 12-14, 2007

Artemia & Aquatic Animals Research Institute, Urmia University, Urmia, Iran

Aim of the workshop will be to learn more about the advanced techniques used in Larviculture and Aquaculture and how they can be applied to improve common practice in sturgeon rearing.

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DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) AS A TOOL FOR THE CHARACTERISATION OF BRACHIONUS SP. STRAINS

Stefania Doods, Spiros Papakostas, Stefan Hoffman, Daan Delbare, Kristof Dierckens, Alexander Triantafyllidis, Tania De Wolf, Olav Vadstein, Theodore J. Abatzopoulos, Patrick Sorgeloos, Peter Bossier-2007

Aquaculture 262(1): 29-40

Abstract:

Many zooplanktonic organisms, like the cyclic parthenogenetic rotifer *Brachionus plicatilis* (Rotifera: Monogononta), are actually a complex of species and biotypes with a high degree of morphological similarity (i.e. cryptic species). Various phylogenetic studies with molecular markers (e.g. ITS1 and COI) on wild *Brachionus* populations described the presence of at least nine genetically divergent *Brachionus* species and biotypes. Because different studies found evidence that these cryptic species and biotypes differ significantly in ecological preferences and thus probably behave differently in response to rearing conditions in the hatchery, questions rise on the actual identity of the rotifer strains used in aquaculture, where *Brachionus* discrimination is still based on morphology. This study is a part of an investigation of the genetic make-up of strains used in hatcheries, aquaculture research institutes and laboratories, and describes the rapid and sensitive PCR–DGGE method for the detection of *Brachionus* species and biotypes based on nucleotide sequence variation within the mitochondrial 16S rRNA gene. Considerable genetic diversity was found, albeit smaller within hatcheries than within laboratories and aquaculture research institutes. All 16S haplotypes produced an unambiguous DGGE fingerprint out of which a database was constructed.

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SUMMER MORTALITY OF HATCHERY-PRODUCED PACIFIC OYSTER SPAT (CRASSOSTREA GIGAS). I. ESTIMATION OF GENETIC PARAMETERS FOR SURVIVAL AND GROWTH

Lionel Dégremont, Bruno Ernande, Edouard Bédier, Pierre Boudry-2007

Aquaculture 262(1): 41-53

Abstract:

The multidisciplinary project "MOREST" aims to improve our understanding of causes of summer mortality in *Crassostrea gigas* juveniles in France and to reduce its impact on oyster production. As part of the MOREST project, 43 full-sib families nested within 17 half-sib families were produced, planted out and tested in 3 sites during summer 2001 to assess to what extent genetic variability exists for this trait. A strong genetic basis was found for survival as narrow-sense heritability estimates ranged from 0.47 to 1.08, with higher values in sites where summer mortality was higher. Genetic correlations across sites were positive and very high for survival, indicating no genotype by environment interaction. In contrast, lower genetic variation was observed for growth in all sites. Finally, genetic correlations between growth and survival were low, in all sites. Selective breeding in a single site should therefore be an efficient means of improving survival in oysters less than one year old along the French Atlantic and Channel coastlines with only very limited effects on growth. As yield mostly depends on survival and growth, this approach should significantly improve harvestable yield. Possible reasons why a high genetic variance for survival appears to be maintained in wild populations are discussed.

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REPRODUCTIVE BEHAVIOUR AND EARLY DEVELOPMENT IN YELLOWTAIL KINGFISH (SERIOLA LALANDI VALENCIENNES 1833

Damian Moran, Cea K. Smith, Brendan Gara, Carolyn W. Poortenaar-2007

Aquaculture 262(1): 95-104

Abstract:

The spawning behaviour of wild caught brood stock as well as early egg and larval development were studied in yellowtail kingfish (*Seriola lalandi*). Spawning occurred naturally in the austral spring/summer (November–February) when the seawater temperature was above 17 °C. Courtship behaviour involved one male and female, and consisted of a high-speed pursuit punctuated by stalling, nipping and touching. This lasted for approximately 0.5–1.5 h until, immediately prior to spawning, the male would nip at the female gonoduct, presumably to induce spawning. At this stage, in 50% of spawns, a second male would become involved. The release of gametes involved frenzied circling behaviour near the bottom of the tank and lasted approximately 22 s. Spawning occurred in the early daylight hours at the start of the spawning season, but shifted to around dusk in the latter part. Spawning eggs were positively buoyant, had a high fertilisation rate (> 99%), ranged 1.33–1.50 mm in diameter with a single oil droplet 0.30–0.33 mm diameter, and developed in a similar manner to that described in congenics. Egg viability within the floating fraction was visually determined to be 74% ± 17% over the entire reproductive season. Indistinct cell margins and asymmetrical cleavage were the most common blastomere deformities observed. Egg and oil droplet volume were found to decrease by 15–20% over the spawning season, though no relationship was found between visually assessed egg viability and date. Egg incubation trials between 16 and 24 °C indicated that temperature accelerated the time to hatch by a Q10 of 5.0. While larvae were found to hatch at a smaller length with a larger yolk sac and oil droplet at warmer incubation temperatures, there was little difference in the maximum larval length reached at the onset of first feeding among the rearing temperatures used. It is proposed that the reason for this was that higher incubation temperatures accelerated the hatching process faster than the rate of tissue deposition. The findings from this study are discussed in terms of the biological significance and implications for the larviculture of this species.

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EFFECTS OF MODERATELY OXIDIZED DIETARY LIPID AND THE ROLE OF VITAMIN E ON THE DEVELOPMENT OF SKELETAL ABNORMALITIES IN JUVENILE ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*)

Leah M. Lewis-McCrea, Santosh P. Lall-2007

Aquaculture 262(1): 142-155

Abstract:

A study was conducted to characterize the effects of oxidized marine fish oil (MFO) on skeletal development in juvenile Atlantic halibut (*Hippoglossus hippoglossus*) and to determine the role of vitamin E on their bone health and antioxidant defense mechanisms. Juvenile halibut (4.5 ± 0.1 g) were fed six experimental diets containing untreated (peroxide value (POV) = 0.6 meq kg^{-1}), moderately oxidized (POV = 7.5 meq kg^{-1}) and highly oxidized (POV = 15 meq kg^{-1}) MFO either with or without α -tocopherol acetate (0 or 300 IU kg^{-1}) supplementation for 14 weeks. No significant effects on growth, survival, hepatosomatic indices, or hematocrit were observed among the dietary treatments. Fish fed diets without vitamin E and highly oxidized dietary lipids showed increased hepatic malonaldehyde concentrations indicating a response to oxidative stress. Both muscle and liver α -tocopherol concentrations were significantly lower in fish fed diets without vitamin E supplementation. Alkaline phosphatase levels in serum and bone were increased when vitamin E was present within the diet indicating higher bone formation activity by osteoblasts. Oxidized lipids and lack of dietary vitamin E significantly increased saturated and decreased polyunsaturated hepatic fatty acids. Liver lipids of fish fed diets without vitamin E also exhibited a lower ratio of 22:6n-3 to 22:5n-3 and n-3 fatty acids. The most frequent skeletal deformity observed was scoliosis, spanning the cephalic/prehemal regions, as well as the anterior hemal region of the vertebral column, which increased the frequency according to elevated levels of oxidized dietary lipid. Lordosis was also observed, with no specific pattern along the vertebral column. The pattern and type of abnormalities observed were similar to those reported in an earlier study in halibut from a commercial hatchery.

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QUANTIFICATION BY FLUORESCENT IN SITU HYBRIDIZATION OF BACTERIA ASSOCIATED WITH *LITOPENAEUS VANNAMEI* LARVAE IN MEXICAN SHRIMP HATCHERY

Antonio T. Garcia, Jorge S. Olmos-2007

Aquaculture 262(2-4): 211-218

Abstract:

The bacterial composition in the hatchery at Unidad Experimental Peñasco (UEP) of the Sonora University, Mexico, was studied by using Fluorescent In Situ Hybridization (FISH) with rRNA-specific oligonucleotide probes. We applied fluorochrome-labeled polyribonucleotide probes to identify and enumerate marine shrimp culture hatchery related bacteria. Quantitative whole-cell hybridization experiments using α -, γ - and δ -Proteobacteria, and high and low G + C Gram-positive bacteria accounted for $20.8 \pm 3.4\%$ to $69.3 \pm 3.3\%$ of the total 4',6-diamidino-2-phenylindole (DAPI)-stained cells in most samples. As predicted in a previous study, marine high G + C and γ -Proteobacteria predominated in different shrimp life sub-stages. The elevated percent of high G + C and γ -Proteobacteria, extending from nauplii to mysis stages, suggest that they represent a large and significant fraction of the total picoplankton biomass in *Litopenaeus vannamei* larval culture.

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GROWTH, SURVIVAL AND FEED EFFICIENCY FOR POST-METAMORPHOSED ATLANTIC COD (*GADUS MORHUA*) REARED AT DIFFERENT TEMPERATURES

L.J. Kling, J. Muscato Hansen, A. Jordaan-2007

Aquaculture 262(2-4): 281-288

Abstract:

Two growth trials were completed on post-metamorphosed Atlantic cod (*Gadus morhua*) for a period of four weeks to determine the optimal temperature for best growth and feed efficiency. The same experiment was repeated twice under similar conditions to determine the effect of four temperature regimes (10, 12, 14 and 16 °C) on randomly selected juvenile cod with an initial weight ranging from 0.34 to 0.51 g. Post-metamorphosed cod grown at 14 and 16 °C were significantly larger at the end of the experiments than the fish grown at 10 or 12 °C, with specific growth rates following a similar trend ($p < 0.05$). Fish held at 16 °C utilized feed less efficiently than those held at the lower temperatures in experiment II and the highest feed efficiency was observed in fish held at 10 °C ($p < 0.05$). The results of the feed efficiency and the maximum growth per degree (dG / dT_{max}) in experiment II suggest that the feed efficiency was likely maximized at a lower temperature than those used in this study. By using data collected from sub-samples of fish in experiment II, maximum growth (G_{max}) was estimated at 14.5 °C using a growth temperature model. Cannibalism was a problem in the first experiment but was greatly reduced in experiment II by feeding a larger size feed pellet (2 mm).

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EFFECT OF RHODOMONAS SALINA ADDITION TO A STANDARD HATCHERY DIET DURING THE EARLY ONTOGENY OF THE SCALLOP *PECTEN MAXIMUS*

Réjean Tremblay, Simon Cartier, Philippe Miner, Fabrice Pernet, Claudie Quéré, Jeanne Moal, Marie-Louise Muzelle, Michel Mazuret, Jean-François Samain-2007

Aquaculture 262(2-4): 410-418

Abstract:

The main objective of this study was to identify algal diets that maximize the survival and growth and alter the biochemical content of *Pecten maximus* larvae with the aim of improving metamorphosis. We also evaluated the potential of the cryptophyceae *Rhodomonas salina* as a food source for these larvae. Two flagellates, *Isochrysis* aff. *galbana* (T) and *Pavlova lutheri* (P), and two diatoms, *Chaetoceros gracilis* (C) or *Skeletonema costatum* (S), were tested as two ternary diets, namely PTC and PTS. PTC and PTS were compared with diets that also included *R. salina* (R). The addition of *R. salina* and the replacement of *C. gracilis* by *S. costatum* in the traditional hatchery diet seem to be two interesting alternatives for increasing the productivity of larval scallop culture and improving the metamorphosis rate. With these two diets, larval growth increased and metamorphosis was observed to occur more rapidly. Moreover, our results showed that the addition of *R. salina* significantly improved the overall condition of the larvae by promoting an increase in organic matter and total lipids. This accumulation of lipids during ontogeny seems to promote larvae to grow and to complete metamorphosis more rapidly than with the other diets. The level of polyunsaturated fatty acids in the algae could also at least partially explain the results obtained, since the PUFA level of *C. gracilis* was about half those of *S. costatum* and *R. salina*.

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IN VITRO DIGESTIBILITY OF WATER-SOLUBLE AND WATER-INSOLUBLE PROTEIN FRACTIONS OF SOME COMMON FISH LARVAL FEEDS AND FEED INGREDIENTS

S.K. Tonheim, A. Nordgreen, I. Høgøy, K. Hamre, I. Rønnestad-2007

Aquaculture 262(2-4): 426-435

Abstract:

In vitro methods have previously been utilised for the rapid and reliable evaluation of protein digestibility in fish. In this study we used in vitro methods to compare the digestibility of various live and artificial larval feeds and feed ingredients. Given previous suggestions that water-soluble dietary proteins are efficiently digested and utilised by stomachless fish larvae, we also analysed the content of water-soluble nitrogen in the feeds and feed ingredients and then measured the specific in vitro digestibility (simulated midgut conditions) of the water-soluble and insoluble fractions. The soluble nitrogen fractions were generally more digestible than the insoluble nitrogen fractions ($P < 0.05$). A soluble reference protein (Na⁺-caseinate) was digested faster than the similar but insoluble reference protein (casein) although their final digestibility was the same (94%). Frozen live feeds (*Artemia franciscana* and *Calanus finmarchicus*) contained high fractions of soluble nitrogen (54–67%) and also had high digestibility in vitro (84 and 87%, respectively). The in vitro digestibility of two formulated larval feeds tested was lower (53 and 70%) than the frozen live feeds. The digestibility of the ingredients of the protein-encapsulated feeds particles was reduced as a result of the production process (from 71 to 53%, respectively). Three meals of marine origin (fish meal, squid meal and fish roe meal) all had low contents of water-soluble nitrogen (11–17%) but showed different degrees of digestibility (77, 77 and 49%, respectively). The results also demonstrated that while pre-hydrolysis of a feed ingredient (fresh frozen cod fillet) almost doubled the water-soluble nitrogen fraction (from 34 to 65%) the positive effect of pre-hydrolysis on in vitro digestibility was much lower (from 80 to 86%). This demonstrates the complexity of assessment of bioavailability of dietary protein sources in larval fish; a number of factors such as leaching rates from feed particles, digestibility, digestion rates and absorption rates all need to be taken into account.

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THE EFFECTS OF FOOD AND THE SUM OF EFFECTIVE TEMPERATURE ON THE EMBRYONIC DEVELOPMENT OF THE SEAHORSE, HIPPOCAMPUS KUDA BLEEKER

Qiang Lin, Yongli Gao, Junqing Sheng, Qingxiang Chen, Bin Zhang, Junyi Lu-2007

Aquaculture 262(2-4): 481-492

Abstract:

The effect of different food (live *Acetes* spp., live *Mysis* spp., frozen *Mysis* spp., and mixed food of 50% live *Acetes* spp. and 50% frozen *Mysis* spp.) on gonad development of seahorses, *Hippocampus kuda* Bleeker, was evaluated in this experiment. The developmental durations of testes and ovary of seahorses were significantly different among the four experimental treatments. The live *Acetes* spp. treatment presented the shortest developmental durations to stage V, which were 87.6 ± 3.84 days and 89.2 ± 3.71 days, respectively, for ovary and testes. The frozen *Mysis* spp. treatment had the longest developmental durations ($F_{3,15} = 13.284$, $P < 0.05$). The relationship between developmental duration of the ovary and gonad developmental stages could be formulated: $Y_1 = 12.04x + 24.36$ ($r^2 = 0.9722$, $n = 16$, $P < 0.05$). The gonadosomatic index (GSI) of parent seahorses among the four treatments differed significantly ($F_{3,15} = 18.364$, $P < 0.05$). The standard GSI of seahorses fed live *Acetes* spp. was $15.64 \pm 1.65\%$, which was the highest. Feeding live food had a significant effect on the fecundity and spawning of seahorses. The fecundity and spawning number of the live *Acetes* spp. treatment were 598 ± 45.49 and 552 ± 49.19 individuals, respectively, which were dramatically higher than those of frozen treatment ($F_{3,19} = 34.152$, $P < 0.05$). Live food also had a similar effect on the fertilization and hatching rate during the embryonic development of seahorses ($F_{3,19} = 11.386$, $P < 0.05$). Food treatment could also induce an indirect effect on survival rate of juveniles through gonadal and embryonic development of the parents ($F_{3,15} = 14.519$, $P < 0.05$). In this experiment, the mortality within parent seahorses in the frozen *Mysis* spp. treatment was the highest ($15.1 \pm 6.55\%$), and the survival of juveniles was the highest in the live *Acetes* spp. treatment ($90.4 \pm 2.26\%$ at 10 days). In conclusion, feeding live *Acetes* spp. significantly benefited the gonadal and embryonic development of *H. kuda*.

The effect of temperature (22 °C, 24 °C, 26 °C, 28 °C, 30 °C and 32 °C) on the hatching time of *H. kuda* was also studied. We demonstrated that the higher the temperature, the shorter the hatching time, as well as the higher the hatching speed. The relationship between hatching time and temperature could be expressed: $T = -39.337 t + 677.75$ ($r^2 = 0.9755$, $n = 30$, $P < 0.05$). In this finding, we

provided the sum of effective temperature (SET) and threshold temperature of embryonic development of *H. kuda* (14066.9 °C h⁻¹ and 13.7 °C, respectively). This new information on the effect of feeding type and culture temperature is beneficial for the commercial rearing and breeding industry of this species.

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TEMPERATURE TOLERANCE OF YOUNG SPOROPHYTES FROM TWO POPULATIONS OF LAMINARIA JAPONICA REVEALED BY CHLOROPHYLL FLUORESCENCE MEASUREMENTS AND SHORT-TERM GROWTH AND SURVIVAL PERFORMANCES IN TANK CULTURE

Shao Jun Pang, Zhen Hui Jin, Jian Zhang Sun, Su Qin Gao-2007

Aquaculture 262(2-4): 493-503

Abstract:

The cold-water subtidal brown alga *Laminaria japonica* has been commercially farmed in the Far East and has been on top of all marine-farmed species in terms of farming area and annual output worldwide. The successful trials of transplantation of young sporophytes from the north to the south in winter along the Chinese coast in the 1950s led to the spreading of cultivation activities down to a latitude of 25–26°N. Up to today, nearly 50% of the annual output of this farmed alga, as a cold-water species, comes from the sub-tropical south in China. The demand to have high-temperature-tolerant strains/ecotypes in farming area calls for a practical method to judge and select the desired parental plants for breeding programs and for seedling production. In this paper, we report our results on using chlorophyll fluorescence measurement and short-term growth performance in tank culture to estimate the temperature tolerance of offspring from two populations, Fujian Farmed Population (FFP) sampled from Fujian province (latitude: 25–26°N) in subtropical area and Qingdao Wild Population (QWP) sampled from Qingdao (latitude: 36°N). Contrary to what has been usually thought, the results revealed that offspring from Qingdao wild population in the north showed better performance both in short-term growth and survival rates and in optimal quantum efficiency (F_v / F_m) when exposed to higher temperature (20–25 °C). This result was further confirmed by fluorescence quenching analysis. QWP distributed along the southern distribution limit at a latitude of 36°N in the Pacific west coast is thus taken as a more ideal one than the farmed population in subtropical region as a source of parental plants for breeding high-temperature-tolerant varieties.

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EVALUATION OF METHODS FOR ASSESSING THE RETENTION OF SEED MUSSELS (*PERNA CANALICULUS*) PRIOR TO SEEDING FOR GROW-OUT

A.G. Carton, A.G. Jeffs, G. Foote, H. Palmer, J. Bilton-2007

Aquaculture 262(2-4): 521-527

Abstract:

The process of catching and relaying wild seed green-lipped mussels, *Perna canaliculus*, onto grow-out farms in New Zealand is extremely inefficient with the majority of the seed mussels being lost after transfer, mostly due to secondary settlement behaviour of the seed. In an attempt to identify the causes of this behaviour the retention of seed mussels was experimentally determined after exposure to three sub-lethal stressors, desiccation, starvation, and temperature fluctuation. In addition, two types of mussel seed behaviour were tested for their potential for developing a commercially useful method for identifying seed mussels with high retention properties prior to seeding. The two behaviours were the ability of seed mussels to attach by byssus threads to the substrate in slow flowing water, and the ability of seed mussels to pedal walk vertically up through a substrate filled column. Of the three stressors tested only desiccation and starvation significantly reduced mussel seed

retention compared to “unstressed” controls by 38% ($P < 0.05$) and 42% ($P < 0.05$) respectively. Of the two types of mussel seed behaviour used for attempting to sort seed mussels, only the ability of seed mussels to attach by byssus threads to the substrate in slow flowing water showed any sensitivity for identifying individuals with higher retention properties ($P < 0.05$). Seed mussels that failed to byssal attach had a 23% lower mean retention, after 10 d experimental grow-out compared to seed that successfully attached. Although significant, this difference was relatively small and is unlikely to provide the basis for the development of a useful commercial seed mussel sorting method. Desiccation and starvation are both stressors that seed mussels are likely to experience during their commercial harvesting, transportation and seeding process. Consequently, seed mussels need to be relayed quickly and under moist conditions to reduce the potential for starvation and desiccation stress. Identifying seed mussels in poor nutritional condition and feeding them prior to seeding out on farms could also help to prevent the intermittent large scale seed mussel losses currently experienced. The application of these findings will assist in making more efficient use of the wild seed mussel resource and significantly lower financial losses to growers in New Zealand. (Leigh Marine Laboratory, University of Auckland, P.O. Box 349, Warkworth, New Zealand, 1241; email of A.G. Carton: a.carton@auckland.ac.nz)

STUDIES ON CHORION HARDENING INHIBITION AND DECHORIONIZATION IN TURBOT EMBRYOS

V. Robles, E. Cabrita, P. de Paz, M.P. Herráez-2007
Aquaculture 262(2-4): 535-540

Abstract:

Embryo dechorionization is a common practice used in certain fish species for different purposes. It facilitates techniques like microinjection, transfection or electroporation in embryos. Dechorionization is easily achieved in some fish species but is a more complex problem in species that have very thick chorions. In this study, we address this problem in turbot embryos, where chorion removal is practically unachievable post-chorion hardening. For this purpose, different solutions that lacked ions required for the hardening of this envelope or contained inhibitors of enzymes involved in the process were used during egg fertilization. The toxicity of the solutions was assessed, and their effect on embryo cleavage and on chorion structure was studied by light and scanning electron microscopy (SEM). The results demonstrated that embryos are very sensitive to these solutions and that first cellular cleavages are affected with most of them. This study also provides the first report on turbot chorion structure, analyzed by SEM. The chorion is a very thick envelope in this species, and its total removal was not observed with the employed treatments. Nevertheless, partial dechorionization was achieved when embryos were fertilized in some of the tested solutions and later treated with pronase (3 mg/ml).

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REVIEW ARTICLE

NUTRITIONAL REQUIREMENTS AND FEEDS DEVELOPMENT FOR POST-LARVAL SPINY LOBSTER: A REVIEW

Kevin C. Williams-2007

Aquaculture 263(1-4): 1-14

Abstract:

On-growing of wild-caught juvenile spiny (Palinuridae) lobsters to market size is an emerging aquaculture industry in Asia, Oceania and Central America but most notably in Vietnam where a US\$90 million per annum industry flourishes. The sustainability of the supply of wild juveniles is an ever present threat to industry expansion, which ultimately may be overcome through successful life cycle closure. However, another and perhaps more imminent impediment is the present reliance on fresh fishery by-catch as a source of food for lobster culture. The decreasing availability and increasing cost of the by-catch, together with the downstream environmental impacts caused by this

type of feeding, has focused attention on the need for more sustainable and environmentally-friendly pelleted feeds. Efforts to develop palatable and high performance pelleted dry feeds for spiny lobster grow-out have not met with a lot of success until recently. This paper reviews the progress that has been made and identifies where further research effort is needed.

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USE OF COMPUTER-ASSISTED SPERM ANALYSIS (CASA) TO EVALUATE THE QUALITY OF CRYOPRESERVED SPERM IN RED SEABREAM (PAGRUS MAJOR)

Qing Hua Liu, Jun Li, Zhi Zhong Xiao, Fu Hong Ding, Dao De Yu, Xi Zhang Xu-2007

Aquaculture 263(1-4): 20-25

Abstract:

In the present study, the quality of post-thaw sperm of red seabream *Pagrus major* frozen with 6–24% DMSO was investigated. The motility, average path velocity and fertilizing capacity of fresh and their corresponding post-thaw sperm were examined for evaluation of the post-thaw sperm motion characteristics and its association with fertilizing capacity. An analysis of sperm motility before and after cryopreservation has been performed using computer-assisted sperm analysis (CASA). For post-thaw sperm frozen with 12–21% DMSO, the percentages of motile sperm were not significantly ($P > 0.05$) changed 10 s after activation. Moreover, the main motility pattern and swimming velocity of the motile post-thaw sperm were not significantly ($P > 0.05$) changed and the progressive linear motion was still the dominant pattern. However, the total motility of post-thaw sperm ($72.3 \pm 6.3\%$) 30 s after activation was ($P < 0.05$) lower than the corresponding fresh sperm ($82.7 \pm 7.2\%$). Additionally, the fertilizing capacity of post-thaw sperm was investigated with a standardized sperm to egg ratio 500:1. There is a linear regression relationship between the percentage of motile post-thaw sperm and fertilizing capability. These data demonstrate that 12–21% DMSO can provide good protection to the sperm during the freezing–thawing process.

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MATERNAL AND PATERNAL INFLUENCE ON EARLY EMBRYONIC SURVIVAL OF ANDROGENETIC RAINBOW TROUT (ONCORHYNCHUS MYKISS): IMPLICATIONS FOR MEASURING EGG QUALITY

Steven J. Patton, Stephanie L. Kane, Paul A. Wheeler, Gary H. Thorgaard-2007

Aquaculture 263(1-4): 26-34

Abstract:

Breeding programs may have the unintended consequence of elevating levels of inbreeding; mutations unmasked through inbreeding may have deleterious effects on salmonid population viability. We used induced all-paternal inheritance (androgenesis) to assess the incidence of mutations affecting early development in rainbow trout (*Oncorhynchus mykiss*) and to provide insights into the genetic basis for inbreeding depression. Assays conducted on androgenetic haploid embryos derived from four rainbow trout populations allowed us to test whether a homozygous clonal line produced in our laboratory had been purged of deleterious alleles affecting early embryonic development. Assays showed no significant difference in viability of androgenetic haploids produced from clonal or outbred lines of rainbow trout during early development. These results are not consistent with the dominance hypothesis of inbreeding depression, which attributes inbreeding depression primarily to expression of deleterious recessive alleles. However, significant maternal effects on haploid embryo viability were identified, implying that egg quality may have a larger effect than paternally inherited deleterious recessive alleles on early haploid embryonic development. Androgenetic haploid assays may thus be useful as a stringent measure of egg quality in aquaculture breeding programs.

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EVALUATION OF NEW MICROPARTICULATE DIETS FOR EARLY WEANING OF ATLANTIC COD (*GADUS MORHUA*): IMPLICATIONS ON LARVAL PERFORMANCES AND TANK HYGIENE

R.C. Fletcher, Jr., W. Roy, A. Davie, J. Taylor, D. Robertson, H. Migaud-2007

Aquaculture 263(1-4): 35-51

Abstract:

In recent years, interest in the intensive culture of Atlantic cod (*Gadus morhua*) has increased dramatically due to several factors including a reduced supply of cod from capture fisheries, a high market price for wild cod and the suitability of the species for culture. One of the major problems facing the industry has been the high cost and unreliability of live feeds, specifically the live feed *Artemia*. The main objective of this project was to determine whether *Artemia* use could be reduced or replaced completely with two novel microparticulate diets (MPD's), without negatively compromising growth, survival performance and tank hygiene under simulated commercial conditions.

The experiment consisted of four treatments, a live feed control treatment (group A), a 50% *Artemia* replacement treatment with MPD-1 (group B), a 100% *Artemia* replacement treatment with MPD-1 (group C) and a 100% *Artemia* replacement treatment with MPD-2 (group D). All treatments were run in triplicate. Growth performances, development (standard length, eye diameter, myotome height and wet weight), water quality (bacteriology and spectrophotometry) and survival were measured throughout the duration of the trial.

The results of the experiment indicate that the treatments containing *Artemia* (groups A and B) both achieved significantly higher growth rates than treatments that did not contain *Artemia* (groups C and D). The highest survival rates achieved at 70 dph were in treatments A and D ($13.8\% \pm 0.7\%$ and $14.2\% \pm 2.1\%$ respectively) when compared to treatments B and C ($11.8\% \pm 0.3\%$ and $5.5\% \pm 1.3\%$ respectively). Survival was also significantly higher in treatment B than in treatment C. This study demonstrates that while the best growth and survival rates are still achieved when cod larvae are fed *Artemia*, combining live feeds and commercially available MPD's (co-feeding) can produce comparable growth and survival rates thus potentially reducing the reliance on live feeds. However the complete replacement of *Artemia* with MPD's still significantly reduced growth potential suggesting that the nutritional composition of MPD's, requires further investigation.

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A RECIRCULATED MATURATION SYSTEM FOR MARINE ORNAMENTAL DECAPODS

Ricardo Calado, António Vitorino, Gisela Dionísio, Maria Teresa Dinis-2007

Aquaculture 263(1-4): 68-74

Abstract:

The present work describes an easy to operate recirculated maturation system for different types of marine ornamental decapods that: i) demands shorter periods of time to perform routine tasks, while allowing better water quality for broodstock keeping, ii) eliminates the need to capture ovigerous females (or euhermaphrodites) before larval release, minimizing the risks of disrupting reproductive pairs, iii) separates newly hatched larvae from the reproductive pair, impairing adults from preying on larvae and iv) allows live prey to be provided to larvae immediately after hatching if needed. Breeding pairs of the following species were used to test the maturation system: cleaner shrimp *Lysmata amboinensis*, fire shrimp *L. debelius*, Monaco shrimp *L. seticaudata*, peppermint shrimp *L. boggesii*, cleaning rock pool shrimp *Urocaridella antonbruunii*, sexy shrimp *Thor amboinensis*, dancing shrimp *Rhynchocinetes durbanensis*, boxer shrimp *Stenopus cyanoscelis*, *S. hispidus*, hermit crabs *Clibanarius tricolor*, *C. erythropus* and the emerald crab *Mithraculus sculptus*. Larger species displaying strong agonistic behavior toward conspecifics (*L. debelius* and *S. hispidus*) had to be kept in larger divisions (0.20 m × 0.30 m × 0.15 m), while all other species were successfully kept in smaller divisions (0.20 m × 0.15 m × 0.15 m). All tested species were able to successfully mate and produce consecutive larval batches during the experimental period, and routine tasks (e.g. checking for and collecting newly hatched larvae, monitoring molts of breeding pairs, recording the presence of

specimens carrying embryos about to hatch, tank siphoning, filters cleaning and water changes) were daily performed in about 1 h. The unidirectional water flow inside each maturation tank, as well as the presence of actinic light in the front glass, allowed newly hatched caridean, stenopodid, anomuran and brachyuran larvae to be successfully removed from the chamber containing the reproductive pair. The simple use of 150 µm mesh size screens inside maturation tanks allowed larval prey (*Artemia nauplii*) to be provided to larvae immediately after hatching, avoiding the negative effects of early larval starvation. The use of suitable maturation and larviculture systems will play a vital role for the successful development of profitable commercial scale culture protocols for the most heavily collected marine ornamental decapod species.

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EFFECT OF TWO MATURATION DIET COMBINATIONS ON REPRODUCTIVE PERFORMANCE OF DOMESTICATED *PENAEUS MONODON*

G.J. Coman, S.J. Arnold, T.R. Callaghan, N.P. Preston-2007

Aquaculture 263(1-4): 75-83

Abstract:

The reproductive performance of tank-reared, 2nd generation domesticated *Penaeus monodon* was compared when fed on two experimental maturation diet combinations; a control diet (CD) containing a treatment portion of 32.5% squid (*Photololigo* sp.) and 32.5% bivalves (*Plebidonax* sp.); and a shrimp-supplemented diet (SSD) containing a treatment portion of 21.6% squid (*Photololigo* sp.), 21.6% bivalves (*Plebidonax* sp.) and 21.6% shrimp (sexually mature *Penaeus* sp. and *Metapenaeus* sp.). The remaining portion of both diets consisted of 5% polychaetes and 30% commercial shrimp pellets. Broodstock were fed on the diets from approximately 10 months of age until commencement of reproductive assessment at 11 months, and until completion of the assessment when females had completed two moult periods post-ablation. No significant difference in growth, survival, ovarian maturation, spawning and egg production was found between diet treatments ($P > 0.05$). However, the percentage of spawnings that hatched (mean \pm standard error) (CD $77.5 \pm 6.7\%$; SSD $41.2 \pm 8.6\%$) ($P < 0.01$), egg fertility rates per spawning (CD $60.2 \pm 6.1\%$; SSD $34.4 \pm 8.4\%$) ($P < 0.05$), hatch rates per spawning (including both unhatched and hatched spawnings) (CD $23.3 \pm 4.2\%$; SSD $5.2 \pm 1.7\%$) ($P < 0.01$) and nauplii per spawning ($\times 10^3$) (CD 41 ± 9 ; SSD 6 ± 2) ($P < 0.01$) were significantly lower for broodstock fed the SSD than the CD. No difference in spermatophore weight or sperm quantity was found between diet treatments ($P > 0.05$). These results indicated that partial replacement of squid and bivalves with sexually mature shrimp in the maturation diet of tank-domesticated *P. monodon* had a negative effect on egg fertility and hatching. Furthermore, these results highlight the large effect that the final maturation diet can have on reproductive output of domesticated *P. monodon* broodstock.

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PRODUCTION AND BIOCHEMICAL COMPOSITION OF EGGS FROM NERITIC CALANOID COPEPODS REARED IN LARGE OUTDOOR TANKS (LIMFJORD, DENMARK)

Thomas F. Sørensen, Guillaume Drillet, Kirsten Engell-Sørensen, Benni W. Hansen, Hans Ramløv-2007

Aquaculture 263(1-4): 84-96

Abstract:

Populations of *Acartia tonsa* (Dana) and *Centropages hamatus* (Lilljeborg) were monitored in outdoor enclosures imitating the natural estuarine environment in northern Denmark from August 2003 to February 2004. *A. tonsa* was predominant in summer while *C. hamatus* dominated from October and onwards. Mean egg production was normally higher in *C. hamatus* as compared to *A. tonsa* and continued until late December before a decrease was observed. Hatching success remained high even in February at temperatures below zero degree indicating that no diapause eggs were produced.

Further, the egg quality evaluated from the fatty acids (AA, EPA and DHA) and amino acid contents did not change markedly during the study period. From this observation it is strongly suggested that nauplii originated from copepod eggs produced throughout the monitored sampling period constitute an adequate live feed for fish larvae probably even year round.

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IMMOBILIZATION OF TWELVE BENTHIC DIATOM SPECIES FOR LONG-TERM STORAGE AND AS FEED FOR POST-LARVAL ABALONE HALIOTIS DIVERSICOLOR

Yean-Chang Chen-2007

Aquaculture 263(1-4): 97-106

Abstract:

In a survey of diatom species in hatcheries for small abalone *Haliotis diversicolor*, 33 species of diatom were identified. Among them 12 species of benthic diatom were isolated to form monospecific cultures that were successfully entrapped in alginate beads for long-term storage. The immobilized diatoms remained alive and maintained their capacity to multiply after 1 year of storage in absolute darkness at 4 °C without liquid media. These diatoms were subsequently applied as feed to cultivate post-larvae of *H. diversicolor*. Growth and survival of the post-larval abalone were examined when they were fed different species of diatoms. *Nitzschia grossestriata* yielded the best post-larvae harvest (average 112.6 individuals/1000 cm²), in contrast to *Cocconeis scutellum*, which produced the lowest harvest with an average of 7.7 individuals/1000 cm² only. Diatom components analysis showed that *Caloneis platycephala* had the highest protein content (17.4% dw). Lipid and bound EPS were the highest in *Seminavis gracilentia* (20.3% dw and 1188.24 pg/100 μm², respectively). *N. grossestriata* had the highest soluble EPS at 212.31 pg/100 μm². Diatom species with a combination of sufficient lipid (7–13.7% dw) and soluble EPS (ca. 50–212 pg/100 μm²) contents produced the best survival (harvest) rate of post-larvae.

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ONTOGENETIC VARIATIONS IN LIPID CLASS AND FATTY ACID COMPOSITION OF HADDOCK LARVAE MELANOGRAMMUS AEGLEFINUS IN RELATION TO CHANGES IN DIET AND MICROBIAL ENVIRONMENT

Sébastien Plante, Fabrice Pernet, Rémy Haché, Rachael Ritchie, Baijing Ji, Douglas McIntosh-2007

Aquaculture 263(1-4): 107-121

Abstract:

This study examined the ontogenetic variations in lipid class composition and fatty acid partitioning between neutral and polar lipids of haddock (*Melanogrammus aeglefinus*) larvae in relation to changes in diet and microbial environment. Three groups of larvae were exposed to the candidate probiotic bacterium *Arthrobacter* sp. (RSXII) and three equivalent control groups were left unexposed. Larvae were fed rotifers between 2 and 31 days post hatch (dph), which were gradually replaced by *Artemia* until 45 dph and progressively weaned from live feed to a dry diet between 45 and 56 dph. Overall, the larvae grew exponentially during the course of the experiment as reflected by rates of increase in dry mass and accumulation of lipid. Proportions of arachidonic acid (20:4n-6, ARA) in haddock larvae were 3–7× higher than observed in the diets, suggesting the selective incorporation of this fatty acid in both lipid reserves and membranes. The initial proportion of docosahexaenoic acid (22:6n-3, DHA) in haddock larvae was 4.6× higher than the dietary values. However, DHA in the neutral lipids decreased markedly during larval ontogeny to proportions that reflected the dietary values. Regarding polar lipids, the proportions of DHA were markedly less variable than those recorded for neutral lipids, which supports the notion that phospholipids in fish are generally higher in polyunsaturated fatty acids (PUFA) and more resistant to changes in diet than are neutral lipids. During the transition from a diet of rotifer to a diet of *Artemia*, haddock larvae showed an arrested accumulation of triacylglycerols and a marked increase in ARA, presumably attributable

to stimulation of immune functions due to an increase in the bacterial load in the rearing system. Finally, larvae exposed to RSXII showed a superior rate of increase in dry mass and lipid accumulation compared to control groups, suggesting that RSXII enhanced the general health of haddock larvae by modifying their bacterial microflora.

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THE EFFECT OF PREDATOR/PREY DENSITY AND WATER DYNAMICS ON FEED INTAKE AND GROWTH IN SPINY LOBSTER LARVAE (*JASUS EDWARDSII*)

Greg G. Smith, Luisa Lyall, Arthur J. Ritar-2007

Aquaculture 263(1-4): 122-129

Abstract:

Consumption of *Artemia* by phyllosoma of the spiny lobster *Jasus edwardsii* was examined under static and turbulent conditions. Phyllosoma larvae were stocked at two densities (2 or 4 phyllosoma container⁻¹; 2P or 4P) and fed juvenile *Artemia* (1.6 mm total length) at two feed rates (1.5 or 0.75 *Artemia* ml⁻¹; 1.5A or 0.75A) in 50 ml of seawater. This provided a combination of 4 treatments (2P/1.5A, 2P/0.75A, 4P/1.5A, 4P/0.75A). Daily intake of *Artemia* by phyllosoma was monitored and assessed relative to moult size and intermoult duration at differing predator-prey densities. Phyllosoma numbers were held constant; in the event of mortality, animals were replaced with others from the same cohort cultured under similar conditions. Phyllosoma endogenous reserves at hatch combined with the lowest ration of 0.75 A were sufficient to ensure normal growth and intermoult duration until Stage II. When phyllosoma were fed for an extended period (several moults), low ration and high phyllosoma density resulted in smaller size and extended intermoult duration. Fluctuations in the feeding pattern of phyllosoma were evident between treatments during the experiment. Phyllosoma with access to more *Artemia* consumed more, and consumption was reduced prior to a moult, even during early stages of development. The second phase of the study examined the intake of 1.7 mm juvenile *Artemia* by phyllosoma and their response to flow-induced turbulence with a combination of two water exchange rates (2.5 or 5 times h⁻¹) and two inlet positions (2 cm above the water surface or 0.5 cm above the culture vessel bottom and parallel to it). Larvae exposed to slow flow were larger and had a shorter intermoult duration associated with an increased ability to capture and consume more *Artemia*. Similar numbers of *Artemia* were consumed during the intermoult in high flow treatments compared to the low flow, albeit over a longer duration. Phyllosoma development between moults required less *Artemia* to be consumed under static conditions.

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THE POTENTIAL VALUE OF DIFFERENT SPECIES OF BENTHIC DIATOMS AS FOOD FOR NEWLY METAMORPHOSED SEA URCHIN STRONGYLOCENTROTUS INTERMEDIUS

Rong-lian Xing, Chang-hai Wang, Xue-bin Cao, Ya-qing Chang-2007

Aquaculture 263(1-4): 142-149

Abstract:

Eight benthic diatom species were isolated from coastal seawater of Dalian, Liaoning, China and their potential as food for aquaculture was evaluated based on their cell characteristics and effect on settlement, growth and survival of newly metamorphosed *S. intermedius*. All species were within the size range ingested by juvenile sea urchins, i.e. 6.74–35.89 µm. Cellular growth rates ranged from 0.44 divisions day⁻¹ (*Rhaphoneis surirella*) to 0.73 divisions day⁻¹ (*Navicula seminulum*). Eight monocultures of benthic diatoms, mixed diatoms (an equal mixture of the eight species), natural occurring diatoms, and a control (no diatoms) were used as substrata and food to evaluate larval settlement and juvenile growth of *S. intermedius*. Larval settlement rates differed significantly between experimental substrata after 24 h ($p < 0.05$) and 48 h ($p < 0.01$). A significantly higher number of larvae settled on *R. surirella* (53.34% ± 2.22 SE) and *Nitzschia* sp. (58.32% ± 5.00 SE) compared to *N. corymbosa* (15.00% ± 0.56 SE), *A. coffeaeformis* (8.34% ± 2.78 SE) and the control

(5.00% ± 1.67 SE) ($p < 0.05$) plates. Juveniles could detach benthic diatoms with both weak and strong adhesion on the sixth day after settlement when the mouth opened and the teeth could be seen. Greatest growth (test diameter) occurred on *N. corymbosa* (631.64 $\mu\text{m} \pm 1.14$ SE). Survival of juvenile was also greatest on *N. corymbosa* (95.83% ± 1.05 SE). These results indicate the effectiveness of *Nitzschia* sp., *R. surirella*, *A. proteus* var. *oculata* and *N. corymbosa* as single species over mixed diatoms and natural diatoms in larval settlement and juvenile growth of *S. intermedius*. Thus, *Nitzschia* sp. has the best potential diet for larval settlement and juvenile growth of *S. intermedius*. *R. surirella* can be used as cue to induce larvae settlement, and *A. proteus* var. *oculata* and *N. corymbosa* can be used as food for growing juveniles. *Nitzschia* sp., *R. surirella*, *A. proteus* var. *oculata* and *N. corymbosa* can be used as good food by sea urchin hatcheries or nurseries, which have traditionally operated with natural diatom films in China, to improve growth and survival of post-larvae, and increase the consistency of production.

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EVALUATION OF BIOAVAILABILITY OF INDIVIDUAL AMINO ACIDS IN DIPLodus PUNTAZZO LARVAE: TOWARDS THE IDEAL DIETARY AMINO ACID PROFILE

M. Saavedra, M. Beltran, P. Pousão-Ferreira, M.T. Dinisb, J. Blasco, L.E.C. Conceição-2007

Aquaculture 263(1-4): 192-198

Abstract:

The indispensable AA profile of fish carcass has been commonly used as a good indicator of fish amino acids requirements. Amino acids composition of the whole body tissue of *Diplodus puntazzo* was determined for the larval ages of 5, 9, 12, 17, 25 and 35 days after hatching (DAH).

No significant differences were found during this species ontogeny for any indispensable amino acid although for dispensable amino acids, significant differences were found for glutamate and glutamine ($p = 0.003$), aspartate and asparagine ($p = 0.027$) and serine ($p = 0.027$).

In order to obtain the ideal dietary amino acid profile, bioavailability was determined using ^{15}N -labelled rotifers together with GC-C-IRMS. High relative bioavailabilities were found for isoleucine, leucine and valine, meaning these amino acids are retained more efficiently. On the contrary, alanine, glutamate and aspartate presented a lower bioavailability when compared to lysine.

Histidine appeared to be the first limiting amino acid at 4 days after hatching (DAH) when larvae were fed rotifers. At 12, 25 and 35 DAH the first limiting amino acid seemed to be threonine.

Comparing amino acid profiles, with and without taking into account the relative bioavailability data, accentuated differences were found for methionine and leucine.

Using larval amino acid profiles corrected with relative amino acid bioavailability enables an estimation of the ideal dietary amino acid profile for *Diplodus puntazzo*.

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THE EFFECT OF MICROALGAL DIETS ON GROWTH, BIOCHEMICAL COMPOSITION, AND FATTY ACID PROFILE OF CRASSOSTREA CORTEZIENSIS (HERTLEIN) JUVENILES

Susana Rivero-Rodríguez, Andy R. Beaumont, María Concepción Lora-Vilchis-2007

Aquaculture 263(1-4): 199-210

Abstract:

The culture of the mangrove oyster *Crassostrea corteziensis* is a promising enterprise on the Pacific coast of Mexico, yet little is known about the diet and essential nutrients required to maximize growth during culture. *C. corteziensis* juveniles were grown for 22 days under hatchery conditions and using monospecies or binary diets of the microalgal species *Chaetoceros calcitrans*, *Chaetoceros muelleri*, *Isochrysis galbana* clone T-iso, *Phaeodactylum tricornutum*, and *Tetraselmis suecica*. The monospecies diet of *C. calcitrans* provided a superior diet for *C. corteziensis* juveniles, yielding a growth rate up to 272- μm shell length day⁻¹. The nutritional value of the microalgae tested was in the order *C. calcitrans* > *C. muelleri* > *I. galbana* clone T-iso > *P. tricornutum* > *T. suecica*. The

reasons for the different nutritional values of the algae investigated were not related to carbohydrate, protein, or lipid content. The HUFA composition of oysters was related to their diets. *C. calcitrans* is characterized by high levels of arachidonic acid (ARA; 20:4n-6). The monospecies diet of *T. suecica*, which yielded the lowest growth rate (107- μ m shell length day⁻¹) lacked docosahexaenoic acid (DHA; 22:6n-3) together with having low levels of ARA and eicosapentaenoic acid (EPA; 20:5n-3) that could explain the poor performance of this diet. There were significant differences in the fatty acid composition of the oysters fed different diets but the only correlation with growth was in the case of ARA. We conclude that *C. calcitrans* supplies the appropriate nutrient balance that *C. corteziensis* needs at this juvenile stage.

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USE OF TROPICAL MICROALGAE AS FOOD FOR LARVAE OF THE BLACK-LIP PEARL OYSTER *PINCTADA MARGARITIFERA*

Erika Martínez-Fernández, Paul C. Southgate-2007

Aquaculture 263(1-4): 220-226

Abstract:

This study determined the nutritional value of tropical microalgae for black-lip pearl oyster (*Pinctada margaritifera*, L.) larvae. One-day old larvae were fed the flagellates *Pavlova salina*, *Pavlova* sp., TISO and *Micromonas pusilla* in binary and ternary combinations in Experiment 1. In a second experiment, umbo-stage larvae were fed the best binary combinations from Experiment 1 with the addition of one diatom species (*C. muelleri*, *Chaetoceros* sp. or *Skeletonema* sp.) per combination. The best two ternary combinations (flagellates only) from Experiment 1, *Pav. salina*/*Pavlova* sp./TISO and TISO/*M. pusilla*/*Pavlova* sp., were also assessed in Experiment 2. In Experiment 1, greater growth rate was shown by larvae fed the ternary combination of *Pavlova* sp./*Pav. salina*/TISO followed for the binary combination of *Pavlova* sp./*M. pusilla*; however, larvae fed *Pavlova* sp. as a mono-specific diet performed as well as those fed the ternary combination of *Pavlova* sp./*Pav. salina*/*M. pusilla* ($P = 0.001$). The addition of a diatom to microalgae diets composed of flagellates resulted in increased growth rates and survival of umbo-stage *P. margaritifera* larvae when compared to combinations without a diatom (Experiment 2). The results showed that *Pavlova* sp. supported a high growth rate of D-stage *P. margaritifera* larvae which was equivalent to that of larvae fed plurispecific diets. For umbo-stage *P. margaritifera* larvae, the best growth rate was achieved when the binary combination of *Pavlova* sp. and *C. muelleri* was used. Based on these results, *Pavlova* sp. and *Pavlova* sp./*C. muelleri* are recommended as diets for D-stage and umbo-stage *P. margaritifera* larvae, respectively.

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LARVAL ONTOGENY OF REDBANDED SEABREAM *PAGRUS AURIGA VALENCIENNES*, 1843 WITH SPECIAL REFERENCE TO THE DIGESTIVE SYSTEM. A HISTOLOGICAL AND HISTOCHEMICAL APPROACH

M.I. Sánchez-Amaya, J.B. Ortiz-Delgado, Á. García-López, S. Cárdenas, C. Sarasquete-2007

Aquaculture 263(1-4): 259-279

Abstract:

The larval ontogeny of redbanded seabream *Pagrus auriga* was studied histologically and histochemically from 0 until 30 days after hatching (DAH). According to the source of food and the structural changes in the digestive tract, larval development was divided into four stages: (1) lecithotrophic (0–2 DAH), (2) lecithotrophic (3–4 DAH), (3) exotrophic I (5–15 DAH), and (4) exotrophic II (16–30 DAH). During the first three stages, larvae underwent an intense organogenesis, this being particularly intense from stage 2 (mouth and anus opening) to early stage 4 (appearance of gastric glands). Subsequent development during stage 4 was characterized by the proliferation and

growth of pre-existing structures. During stage 1, the mouth and the anus were closed and the digestive tract undifferentiated (straight tubular segment), as well as the majority of the organs (observed as undifferentiated cells groups or primordial structures). As resorption of endogenous reserves proceeded (3–4 DAH) larvae acquired initial absorptive and digestive equipments necessary for first feeding (enterocytes brush border, zymogen pancreatic granules, and ducts connecting accessory glands to the gut). During stage 2, the digestive tract started to differentiate and buccopharyngeal cavity, oesophagus, incipient stomach, and anterior, mid and posterior intestine were distinguished. During stages 2 and 3, prey capture became guaranteed (early development of jaw, fins, teeth, and taste buds) and the digestive and absorptive processes continued developing (appearance of the gut mucosa folds and protein supranuclear inclusions and lipid infranuclear vesicles in enterocytes). The endocrine elements (Langerhans islets and thyroid follicles) except corpuscles of Stannius appeared from 3 to 5 DAH (mouth opening and total yolk resorption). During stage 2 and early stage 3 (3–7 DAH), the circulatory and excretory systems became functional, with the compartmentalization of the heart and the development of renal corpuscles, tubules, and collecting ducts. The beginning of stage 4 was marked by the appearance of gastric glands (16 DAH), which subsequently proliferated in association with the increase in size of the accessory glands. Such event ensured the development of gastric digestion, which around 30 DAH became fully guaranteed (transition from larval to juvenile stage). During stage 4, gill filaments and lamellae proliferated, the heart completed its compartmentalization, the pronephric and mesonephric regions in the kidney developed, and endocrine elements proliferated. The structural information presented here constitutes an initial step towards the determination of the functional systemic capabilities of *P. auriga* larvae, and thus the physiological requirements needed for optimal welfare and growth.

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