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**PROGRESS TOWARD YEAR-ROUND SPAWNING OF SOUTHERN FLOUNDER BROODSTOCK BY MANIPULATION OF PHOTOPERIOD AND TEMPERATURE**

Wade O. Watanabe, Christopher A. Woolridge, Harry V. Daniels-2006

Journal of the World Aquaculture Society 37(3): 256-272

**Abstract:**

Reliable methods have been developed for controlled spawning of captive southern flounder, *Paralichthys lethostigma*, broodstock during their natural winter (December–February) spawning season. From 1999 to 2004, we evaluated the effects of manipulation of photoperiod and temperature on both advance and delay spawning to produce viable embryos throughout the year. Wild-caught adult broodstock were held in 4.8- to 7.0-m<sup>3</sup> controlled-environment tanks at a sex ratio of approximately 12 females to 4 males. Broodstock were subjected to different artificial photothermal conditioning regimes: extended winter (EW), accelerated (A-10-, A-6-, A-4.5-, and A-3.8-mo regimes), and delayed (D-16- and D-14-mo regimes), with gradual and abrupt transitions, respectively, from long to short daylengths. Under an EW cycle, fish were exposed to constant short daylengths (10 L: 14 D) after the winter solstice in January. Eighty-seven natural spawnings from December to April produced  $18.3 \times 10^6$  eggs, with 20.9% hatching successfully (i.e., overall egg viability). Under an A-10-mo cycle, rate of decrease in daylength was accelerated after the summer solstice in July, to reach winter conditions in October. Seven induced spawning trials from October to November produced  $897 \times 10^3$  eggs, with 40.4% viability. Under an A-6-mo cycle, rate of change of photoperiod was accelerated after the winter solstice in January, to reach winter conditions in July. Three induced spawning trials in July produced  $550 \times 10^3$  eggs, with 14.7% viability. Under an A-4.5-mo cycle, broodstock exposed to EW from January through April were exposed to an accelerated cycle to reach winter conditions by October. Four induced spawning trials from September to November produced  $729 \times 10^3$  eggs, with 28.7% viability. Under an A-3.8-mo cycle, broodstock exposed to EW conditions from January through April were exposed to an accelerated cycle to reach winter conditions by September. Five induced spawning trials from September to November produced  $510 \times 10^3$  eggs, with 45.9% viability. Under a D-16-mo cycle, fish were exposed to a decelerated decline in photoperiod after the summer solstice in July, to reach winter conditions in May, when atretic females were observed. Under a D-14-mo cycle, fish were exposed to constant summer conditions from December through mid-June and then to an abrupt decline in photoperiod to winter conditions in late June. Six induced spawning trials from September to November produced  $763 \times 10^3$  eggs, with 13.0% viability. Production of viable embryos was greatest during the extended winter because of abundant natural spawnings. While successful natural spawnings were rare during the fall or summer, viable embryos were produced through induced spawnings during all seasons of the year, with no significant ( $P > 0.05$ ) differences in egg viability. Extended winter conditions prolonged spawning from 3 to 5 mo. Accelerated (3.8–10 mo) regimes were effective in producing viable embryos from summer through fall, but a minimum of 5 mo was required to complete gonadal recrudescence. While constant long daylengths after the summer solstice delayed gonadal recrudescence, with spawning obtained 2.5 mo after an abrupt reduction to short daylengths, a decelerated decline in photoperiod did not. Artificial control of daylength enabled precise control of gonadal recrudescence and year-round spawning in southern flounder without adverse effects on the quality of eggs and larvae and will improve availability of seedstock for commercial aquaculturists. (Center for Marine Science, University of North Carolina Wilmington, 7205 Wrightsville Avenue, Wilmington, North Carolina 28403 USA)

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**EFFECT OF PHOTOPERIOD MANIPULATION ON BROODSTOCK SPAWNING, FERTILIZATION SUCCESS, AND EGG DEVELOPMENTAL ABNORMALITIES IN ATLANTIC COD, GADUS MORHUA**

Randy W. Penney, Pauline L. Lush, A. Joy Wade, Joseph A. Brown, Margaret P. M. Burton-2006

Journal of the World Aquaculture Society 37(3): 273-281

Abstract :

Spawning activity in two captive Atlantic cod broodstock groups previously captured from the wild stock fishery was monitored over three spawning seasons. Both groups spawned under ambient photoperiod (PP) in the first year, after which photomanipulation was applied to compress the PP cycle of one broodstock group, while the second group was maintained on natural PP. Increased total egg volumes, number of egg batches, total egg production, and duration of the spawning season were observed in both ambient PP and advanced PP groups in the second and third spawning seasons. Compared to ambient controls, the photoadvanced group commenced spawning earlier, had a longer spawning season, produced a lower total volume of eggs over the entire spawning season, and had lower mean daily batch volumes of eggs, and the eggs were of smaller mean diameter. Fertilization success was >90% in all years, increased in both groups in the first year after photomanipulation but declined to prior levels in the following year. No consistent difference in fertilization success was observed between advanced PP and ambient PP groups. Rates of cell development abnormalities were low (<10%) in both groups over the three spawning seasons. Abnormalities of cell symmetry and clarity increased in both ambient PP and advanced PP groups over the three spawning seasons. However, no consistent differences in rate of cell abnormalities were observed between advanced PP and ambient PP groups within years. We conclude that PP advancement is an effective technique to compress the time between successive spawning periods in Atlantic cod and does not negatively affect fertilization success or rates of cell developmental abnormalities. However, it has a negative effect on both volume and size of eggs produced.

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EFFECTS OF TANK WALL COLOR AND UP-WELLING WATER FLOW ON GROWTH AND SURVIVAL OF EURASIAN PERCH LARVAE (*PERCA FLUVIATILIS*)

Sissel Jentoft, Sigurd Øxnevad, Are H. Aastveit, Øivind Andersen-2006

Journal of the World Aquaculture Society 37(3): 313-317

Abstract :

The influence of tank wall color and up-welling water flow on growth and survival of Eurasian perch larvae (*Perca fluviatilis*) was tested in an intensive culture system. Newly hatched larvae were fed *Artemia nauplii*, later combined with dry feed, and reared for 5 wk in either black tanks with up-welling water flow or in gray tanks with or without up-welling water flow. The perch larvae grew significantly faster in black tanks than in gray tanks regardless of water flow. Two weeks after hatching, a significantly higher mean weight was shown in larvae reared in black tanks compared to larvae reared in gray tanks with up-welling water flow, and after 4 wk, the mean weight was significantly higher than in both of the other treatments. The difference in growth was further enhanced during the last week of the experiment, and the final mean weights were  $51.1 \pm 1.9$  mg in black tanks with up-welling water flow,  $23.8 \pm 2.1$  mg in gray tanks with up-welling water flow, and  $23.7 \pm 2.2$  mg in gray tanks without up-welling water flow. The cumulative mortality at the end of the experiment averaged 75% in all treatment groups. Taken together, the enhanced growth of Eurasian perch larvae in black tanks could be explained by high prey contrast and increased prey consumption. Up-welling water flow had no impact on growth and survival of the perch larvae in gray tanks, indicating that the availability and consumption of the prey were independent of water movement.

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EFFECT OF DIETARY ADMINISTRATION OF 17 $\alpha$ -METHYLTESTOSTERONE ON THE SEX RATIO OF POSTLARVAL FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII*, DURING THE NURSERY STAGE OF CULTURE

Cortney L. Ohs, Louis R. D'Abramo, Anita M. Kelly-2006

Journal of the World Aquaculture Society 37(3): 328-333

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RELATIONSHIP BETWEEN TIME OF INSEMINATION POSTOVULATION AND FERTILIZATION RATE OF EGGS OF OBSCURE PUFFER FISH, TAKIFUGU OBSCURUS

Zhou Yang, Ya-Fen Chen-2006

Journal of the World Aquaculture Society 37(3): 334-338

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DETECTION OF *V. HARVEYI* IN SHRIMP POSTLARVAE AND HATCHERY TANK WATER BY THE MOST PROBABLE NUMBER TECHNIQUE WITH PCR

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Aquaculture 261(1): 1-9

Abstract:

*V. harveyi* is the cause of serious disease in the shrimp industry in Thailand during cultivation. In this study, the *gyrB* gene of *V. harveyi* NICA, isolated from shrimp in Thailand, was sequenced. A pair of specific primers (A2B3) was designed that allowed amplification of a 363 bp gene fragment of *V. harveyi*. No cross reaction was detected in 17 other *Vibrio* species tested except for *V. carchariae* which is a synonym for *V. harveyi*. The possibility of using A2B3 for confirmation and enumeration of *V. harveyi* by PCR was demonstrated. Of 40 possible *V. harveyi* strains isolated from seafood on the basis of their growth on TCBS plates and biochemical reactions, 36 gave a reaction with the specific primers. The primers could detect *V. harveyi* at a level of as few as 15 cells/ml. The Most Probable Number (MPN) technique was applied to enumerate *V. harveyi*. We have demonstrated that when PCR was applied directly to the enrichment broth of shrimp artificially inoculated with *V. harveyi*, the MPN value was no different from the MPN value obtained using the standard technique with selective agar. This technique was employed to enumerate *V. harveyi* in postlarvae and hatchery tank water. *V. harveyi* were detected in 18 out of 21 postlarval samples and in 14 out of 21 tank water samples. The numbers of *V. harveyi* detected in postlarvae and water were 150–1.1 × 10<sup>8</sup>/g postlarvae and 7–4.6 × 10<sup>4</sup>/ml of water samples, respectively. Screening of postlarvae to reduce the high risk of *V. harveyi* contamination in cultivation ponds is suggested as a measure to prevent the catastrophic losses caused by *V. harveyi* disease.

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DISTRIBUTION OF A FISH PATHOGEN LISTONELLA ANGUILLARUM IN THE JAPANESE FLOUNDER PARALICHTHYS OLIVACEUS HATCHERY

Hiroataka Mizuki, Sayaka Washio, Tetsuo Morita, Shiro Itoi, Haruo Sugita-2006

Aquaculture 261(1): 26-32

Abstract:

The present study was undertaken to investigate the distribution of *Listonella anguillarum* in a Japanese flounder (*Paralichthys olivaceus*) hatchery. A total of 2704 isolates were obtained from the developing fish, live diets and artificial feeds of Japanese flounder and their rearing water, 439 of which were identified as *L. anguillarum* by the combining incubation on thiosulfate-citrate-bile salt-sucrose (TCBS) agar at 35 °C overnight with polymerase chain reaction (PCR) detection for the VAH1 hemolysin gene. *L. anguillarum* was detected in all seven rotifer samples, with densities of 2.5 × 10<sup>3</sup> to 4.6 × 10<sup>6</sup> colony forming units (CFU) g<sup>-1</sup>. Both the analyzed samples of *Nannochloropsis oculata* contained this bacterium at densities of 1.6 × 10<sup>4</sup> to 1.4 × 10<sup>5</sup> CFU g<sup>-1</sup>. *L. anguillarum* was detected in only one of four samples of *Artemia nauplii* with a density of 4.8 × 10<sup>5</sup> CFU g<sup>-1</sup> (35%) and it was not detected in the two analyzed artificial feed samples. *L. anguillarum* was detected in 11

of 18 specimens of larval and juvenile Japanese flounder at densities of  $5.0 \times 10^1$  to  $7.4 \times 10^5$  CFU  $g^{-1}$ , while it was not detected in the two analyzed egg specimens of Japanese flounder. These results indicate that *L. anguillarum* associated with the developing Japanese flounder is likely derived from rearing water and live diets such as rotifers. Further, it is strongly suggested that *L. anguillarum* is a transient bacterium of the intestinal microflora for the Japanese flounder but is a permanently indigenous one for the Japanese flounder hatcheries.

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#### NILE TILAPIA (*OREOCHROMIS NILOTICUS*) SEED PRODUCTION IN IRRIGATED RICE-FIELDS IN NORTHWEST BANGLADESH—AN APPROACH APPROPRIATE FOR POORER FARMERS?

Benoy K. Barman, David C. Little-2006

Aquaculture 261(1): 72-79

Abstract:

Participatory research was conducted with poorer farmers in two communities, Girai (G) and Bahagili (B) in NW Bangladesh to assess the production of Nile tilapia seed in irrigated spring rice-fields. All the selected households (G = 15; B = 4) had previous experience producing common carp (*Cyprinus carpio*) in the rice-fields allocated a separate plot in which a deeper area had been excavated for this trial. Mature GIFT strain Nile tilapia (12 female and 6 male;  $121 \pm 34$  g and  $158 \pm 54$  g size, respectively) were supplied to each household irrespective of the size of their trial plot (mean < 0.15 ha). The trial started in the spring rice season (boro) in February and ended at the end of main season (amon) in December 1999. Management practices, production and sales of fish were monitored weekly.

The majority of farmers succeeded in producing fingerlings in their plots; 11% failed totally but around 70% produced more than 2000 fingerlings from a single plot. Production during boro and fallow period was much higher (> 90% total) than during the subsequent amon crop (< 10% total). Total production was highly variable among households but not different between the two study areas (G =  $4092 \pm 3277$ ; B =  $3730 \pm 4232$  fingerlings household $^{-1}$ ). Daily production of fingerlings per unit area was relatively low (< 1 fish  $m^{-2}$  day $^{-1}$ ) but efficiency of production was high, averaging 17.3 fingerlings.  $kg^{-1}$  female day $^{-1}$ . Mean individual harvest weight was 21 g.

Most fingerlings were sold (43%) and/or stocked for further culture in their own grow-out system (39%), but some were used directly for household consumption (17%).

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#### PERFORMANCE OF DIFFERENT TYPES OF DIETS ON EXPERIMENTAL LARVAL REARING OF ENDANGERED CHITALA CHITALA (HAMILTON) IN RECIRCULATORY SYSTEM

U.K. Sarkar, W.S. Lakra, P.K. Deepak, R.S. Negi, S.K. Paul, A. Srivastava-2006

Aquaculture 261(1):141-150

Abstract:

This is the first report on the successful larval rearing of captive bred population of *Chitala chitala* (Hamilton). *C. chitala* is one of the endangered fresh water fish species in India for which the development of controlled larval rearing procedures are needed for stock enhancement. Fifteen days old post-hatchlings were stocked for 28 d in a 30 L recirculatory tanks using eight different diets i.e. live feed (tubifex worms, chironomous larvae, zooplanktons), dry feed (dry tubifex, spirulina, daphnia) and other non-conventional feed (fish eggs and boiled egg-yolk). Fishes accepted all types of diets. The study revealed that specific growth rate (SGR) was higher in post-hatchlings fed on live tubifex worms ( $2.40 \pm 0.72$ ) followed by fish eggs ( $2.15 \pm 0.71$ ), dry tubifex ( $2.12 \pm 0.40$ ), chironomous larvae ( $1.91 \pm 0.44$ ), spirulina ( $1.79 \pm 0.38$ ), daphnia ( $1.42 \pm 0.79$ ) and planktons ( $1.37 \pm 0.77$ ) whereas minimum SGR was recorded with boiled egg-yolk ( $0.63 \pm 0.5$ ). A highly significant

difference ( $p < 0.01$ ) in SGR was observed in fish fed on live feed (tubifex worms, chironomous larvae, planktons, spirulina), dry tubifex and fish eggs whereas for daphnia and boiled egg-yolk it was only significant ( $p < 0.05$ ). The final mean weight and weight gain showed highly significant difference ( $p < 0.01$ ) in live tubifex, zooplanktons, spirulina, chironomous larvae, dry tubifex and fish eggs, whereas daphnia and boiled egg-yolk fed larvae showed significant difference ( $p < 0.05$ ). Highest mean survival rate on day 28 was observed in live tubifex worms (94%) and chironomous larvae (92%). The post-hatchlings reared with spirulina and daphnia showed same survival rate of 88% whereas the lowest mean survival of 66% was recorded in boiled egg-yolk. The experiments showed that captive bred post-hatchlings of *C. chitala* could be reared in experimental recirculatory system for attaining higher growth and survival during early life stages. However, methods to improve the larval rearing have to be improved further for commercial farming of the species.

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#### COMPOSITION IN ESSENTIAL AND NON-ESSENTIAL ELEMENTS OF EARLY STAGES OF CEPHALOPODS AND DIETARY EFFECTS ON THE ELEMENTAL PROFILES OF OCTOPUS VULGARIS PARALARVAE

Roger Villanueva, Paco Bustamante-2006

Aquaculture 261(1):225-240

Abstract:

During the present study, we aimed at providing a first look at the elemental composition of the early stages of cephalopods as an approach to their elemental requirements in culture. Essential and non-essential elemental profiles of the European cuttlefish *Sepia officinalis*, the European squid *Loligo vulgaris* and the common octopus *Octopus vulgaris* laboratory hatchlings and wild juveniles were analysed. In addition, for *O. vulgaris* we determined elemental profiles of mature ovary, eggs in different stages of development and followed possible effects of four dietary treatments during paralarval rearing, also analyzing elemental content of the live preys *Artemia nauplii* and *Maja brachydactyla* hatchling zoeae. Content was determined for essential (As, Ca, Cr, Co, Cu, Fe, K, Mg, Mn, Na, Ni, P, Rb, S, Sr, Zn) and non-essential (Ag, Al, Ba, Cd, Hg, Pb) elements. The content in non-essential elements found in hatchlings and juveniles of the three species analyzed here seems to be far lower in comparison with subadult and adult stages of coastal cephalopods. In the octopus eggs, the non-essential element concentrations remained globally low compared to hatchlings and juveniles indicating the absorption of these elements along the ontogenetic development. The elemental composition of the octopus ovary and of the eggs, hatchlings and juveniles of the three cephalopod species analyzed here showed a high content in S. As expected, the calcified internal shell of the cuttlefish, rich in Ca and Sr, originates the main difference between species. It is remarkable the richness in Cu of hatchling octopus, that may indicate a particular nutritional requirement for this element during the planktonic life. The reared octopus paralarvae feed on *Artemia nauplii*, a prey with relatively low Cu content, showed nearly half Cu content that the “natural” profile of octopus hatchlings or wild juveniles. This suggests a dietary effect and/or an indication of the poor physiological stage of the *Artemia*-fed paralarvae. At the present, the percentage of essential element absorption by food or seawater is unknown for cephalopods and should be determined in the future to understand their feeding requirements in culture.

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#### ISOLATION OF THE NON-FASTIDIOUS MICROALGA WITH ASTAXANTHIN-ACCUMULATING PROPERTY AND ITS POTENTIAL FOR APPLICATION TO AQUACULTURE

Katsuhiko Fujii, Eri Imazato, Hisatoshi Nakashima, Osamu Ooi, Akihiko Saeki-2006

Aquaculture 261(1): 285-293

Abstract:

Astaxanthin has recently attracted considerable attention for its biological properties such as the antioxidant activity as well as a coloring agent used for farmed fish. However, its biological production, mainly by a green microalgae *Haematococcus pluvialis*, is costly because of its fastidious growth characters. Therefore, for future applications of biological astaxanthin production in aquaculture, non-fastidious microalgal strains were isolated from environmental samples, examined for their astaxanthin-accumulating activity, and characterized phylogenetically. While  $\beta$ -carotene was found in all tested isolates, one isolate, GK12, from activated sludge of a sewage treatment plant accumulated  $2.5 \pm 0.36$  mg/g dry cell of free astaxanthin de novo under photoautotrophic culture condition, which was comparable to photoautotrophic cultures of other known astaxanthin-accumulating microorganisms. Mixotrophic culture conditions increased GK12 biomass, but astaxanthin content was decreased, suggesting that the simple photoautotrophic cultivation is more efficient way for GK12 to produce astaxanthin than mixotrophic cultivation. A phylogenetic study of SSU rDNA strongly suggested that GK12 is a novel species in the genus *Monoraphidium*, Chlorophyta. Besides making efforts to increase astaxanthin-productivity of known astaxanthin accumulators, it is also beneficial to study GK12 from the view point of applications to aquaculture. (Department of Agriculture, Yamaguchi University, Yoshida, Yamaguchi, 753-8515, Japan; email of Katsuhiko Fujii: [kfujii@yamaguchi-u.ac.jp](mailto:kfujii@yamaguchi-u.ac.jp))

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THE EFFECT OF NITRATE SUPPLEMENTATION ON THE BIOCHEMICAL COMPOSITION OF BENTHIC DIATOMS AND THE GROWTH AND SURVIVAL OF POST-LARVAL ABALONE

Iker Uriarte, Rodney Roberts, Ana Farias-2006

Aquaculture 261(1): 423-429

Abstract:

Elevating the nitrogen concentration in microalgal growth media can elevate the protein content of the algae and consequently increase their dietary value to molluscs. This study examined the protein content, and dietary value to abalone post-larvae, of seven benthic diatoms acclimated to high-("H" =  $49.4$  mg  $\text{NO}_3\text{-N l}^{-1}$ ) and standard-nitrate ("S" =  $16.5$  mg  $\text{NO}_3\text{-N l}^{-1}$ ) Walne medium. In Experiment 1, the level of nitrate in the culture medium did not significantly affect the protein content of *Cocconeis* sp., *Cylindrotheca closterium* or *Nitzschia ovalis*, and variation in the growth of post-larval *Haliotis iris* was not associated with nitrate level or the protein content of the diatoms. When the same six diatom diets were added daily as cell suspensions, growth and survival were much lower than for the equivalent strains fed as established films ( $P < 0.0001$ ). In Experiment 2a, the protein content of *Navicula incerta* was elevated by nitrate supplementation, and the high nitrate strain retained a higher protein content ( $P = 0.005$ ) even after 24 days growing in unenriched, flowing seawater. *Haliotis rufescens* post-larvae grew significantly faster when fed *N. incerta* H than when fed *N. incerta* S ( $P = 0.006$ ) and survival was also higher with this diet (67 versus 54%). In Experiment 2b, there was no significant effect of nitrate level on protein content of four diatoms or the performance of post-larval *H. rufescens*. There was, however, a strong positive correlation ( $r = 0.95$ ,  $P < 0.0001$ ) between post-larval survival and the lipid content of the diatom diets. Post-larval growth showed positive, but marginally non-significant, relationships with diatom protein and carbohydrate, but no relationship with lipid content. Overall, only one of seven benthic diatom species acclimated to high-nitrate growth medium displayed significantly elevated protein content. Diatom protein content explained relatively little of the variation in food value of diatom strains for post-larval abalone but lipid content correlated strongly with post-larval survival.

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CROSS-MATING OF EURYHALINE ROTIFER BRACHIONUS PLICATILIS STRAINS AS A MEANS TO DEVELOP USEFUL STRAINS FOR LARVAL FISH FOOD

Tomonari Kotani, Kazuhiko Ihara, Atsushi Hagiwara-2006

Aquaculture 261(2): 495-500

Abstract:

In the field of live food science, newly inbred strains resulting from cross-mating are of interest, especially if these strains have valuable characteristics, such as high fecundity or suitable size for the mouth of larvae. We conducted cross-mating trials using Japanese and German strains of *Brachionus plicatilis* and reproductive parameters were characterized and compared among their progenies. Two hybrid strains A and B were obtained from the cross-mating between a Japanese female and German male, and between a German female and Japanese male, respectively. Percent mictic female production and fertilization in both hybrid strains were lower (0%), compared with the parental strains (16.7–78.4%). Strain A did not reproduce sexually, but was capable of asexual reproduction. Higher population growth was observed in the hybrid strains within crosses relative to parents. The population growth rates of parental strains were 0.31, while those of hybrid strains ranged from 0.35 to 0.37. Lorica length of hybrid strains was intermediate between the two parental strains. Using the cross-mating technique, it may be possible to produce new rotifer strains with phenotypes useful to aquaculturists.

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#### INFLUENCE OF DIETARY SOYBEAN MEAL LEVELS ON GROWTH, FEED UTILIZATION AND GUT HISTOLOGY OF EGYPTIAN SOLE (*SOLEA AEGYPTIACA*) JUVENILES

Alessio Bonaldo, Andries J. Roem, Alessio Pecchini, Ester Grilli, Pier Paolo Gatta-2006

Aquaculture 261(2): 580-586

Abstract:

The present study was undertaken in order to determine the effect of a dietary incorporation of soybean meal (SBM) on growth performance, nutrient utilization and gut histology of Egyptian sole *Solea aegyptiaca*. This species, which is genetically and morphologically close to *Solea solea*, is currently present in the Mediterranean Sea and reared in some Italian farms, using the same techniques for *S. solea* production and it could represent a promising new species for Mediterranean aquaculture.

Three isoproteic and isolipidic extruded diets (crude protein, 47%, crude fat, 20%) were formulated containing 0 (diet 1), 18 (diet 2) and 30% SBM (diet 3). 540 juveniles (initial body weight of  $6.9 \pm 0.2$  g) were equally distributed into nine 150 l squared tanks (bottom surface: 520 cm<sup>2</sup>) connected with a recirculation system (temperature  $20 \pm 1$  °C and dissolved oxygen above 6 ppm). Animals have been hand-fed twice a day (at 9.00 a.m. and 5.00 p.m.) at a daily ratio of 1.5% body weight<sup>-1</sup>. Diets were tested in triplicate for 87 days. The fish were weighed at days 28, 57 and 87, and they were sampled at days 0, 57 and 87 for nutrient retention determination. One-way ANOVA with Newman–Keuls' post-test were used to analyze data ( $P \leq 0.05$ ).

No differences in terms of palatability were observed and any diet has been consumed completely all throughout the experiment. At day 87, fish fed diet 1, 2 and 3 reached weights of  $25.8 \pm 0.8$  g,  $27.5 \pm 0.9$  g and  $26.1 \pm 1.5$  g, respectively and no differences in performances or nutrient retention were found.

Histopathological gut examinations have revealed no noticeable differences in the appearances of the intestines between any of diet groups. Intestinal mucosal cells presented well vacuolated upper, with well defined microvillar brush borders. On the basis of the results of this trial, SBM seems to be a good protein source for Egyptian sole and can be added in the diet up to 30% without any reduction in growth rate and no adverse effect on gut histology.

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#### AMINO ACID PROFILES OF *DIPLodus SARGUS* (L., 1758) LARVAE: IMPLICATIONS FOR FEED FORMULATION

M. Saavedra, L.E.C. Conceição, P. Pousão-Ferreira, M.T. Dinis-2006  
Aquaculture 261(2): 587-593

Abstract:

The indispensable AA profile of fish carcass has been commonly used as a good indicator of fish amino acids requirements. Amino acid composition of the whole body tissue of *Diplodus sargus* was determined for the larval ages of 0, 2, 5, 8, 12, 17, 25, 35 and 45 days after hatching (DAH). No significant differences were found during this species ontogeny, except for phenylalanine. A comparative analysis of amino acid profiles from larvae and respective diet was performed. Low correlation was found to rotifers ( $R^2$  approximately 0.5), while higher correlations were found for *Artemia nauplii*, *metanauplii* ( $R^2$  approximately 0.8) as well as for the dry feed. These results suggest that *D. sargus* are subjected to higher nutritional imbalances during the first 10 days of feeding when larvae are fed on rotifers alone. Arginine, threonine, lysine, cysteine and histidine appeared to be limiting amino acids at 2, 12, 25 and 45 DAH, respectively. Similar results were reported in literature for *Sparus aurata* and *Solea senegalensis*, although *D. sargus* diets seem to have more amino acids in deficiency as well as more severe differences between larval and diet amino acid profiles. To solve these apparent nutritional imbalances, amino acid supplementation should be considered. The use of inert diets in early larvae ages seems to be most adequate as live feed supplementation appears to be more difficult.

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#### SELECTIVE PARTICLE FEEDING BY THE CHILEAN OYSTER, *OSTREA CHILENSIS*; IMPLICATIONS FOR NURSERY CULTURE AND BROODSTOCK CONDITIONING

B.J. Dunphy, J.A. Hall, A.G. Jeffs, R.M.G. Wells-2006

Aquaculture 261(2): 594-602

Abstract:

Hatchery broodstock conditioning and nursery culture of the Chilean flat oyster *Ostrea chilensis* have been hampered by the poor performance of oysters fed typical microalgal hatchery diets. To determine the feeding capabilities of this species the selective removal and consumption of natural planktonic assemblages and artificial inert particles (polystyrene beads) by juvenile and adult oysters were examined experimentally. The arrangement of the eulaterofrontal cirri of the ctenidia was also examined to infer their potential efficiency of particle selection for feeding. Polystyrene beads of 45 and 15  $\mu\text{m}$  in diameter had high rates of removal from suspensions by both juvenile (45  $\mu\text{m}$  = 70%, 15  $\mu\text{m}$  = 73%) and adult (45  $\mu\text{m}$  = 88%, 15  $\mu\text{m}$  = 87%) oysters. In contrast, beads of 6  $\mu\text{m}$  diameter had lower rates of removal (adults = 68%, juveniles = 53%), while 1  $\mu\text{m}$  beads were not removed at all. Both adult and juvenile oysters feeding upon natural plankton assemblages removed only microphytoplankton (20–75  $\mu\text{m}$  in size) despite the presence of nanophytoplankton (2–20  $\mu\text{m}$ ), picophytoplankton (< 2  $\mu\text{m}$ ), cyanobacterium *Synechococcus* spp. (< 2  $\mu\text{m}$ ), and bacterial cells (< 75  $\mu\text{m}$ ) in the experimental suspensions. Eulaterofrontal cirri of both juvenile (15.2  $\mu\text{m} \pm 0.9$  SE) and adult oysters (18.9  $\mu\text{m} \pm 0.3$  SE) are the shortest that have been reported for any ostreid species which helps to explain the inability of this species to retain small food particles. The clearance rates for oysters feeding on microphytoplankton only were 1.49 ( $\pm 0.05$  SE) and 7.1 ( $\pm 1.2$  SE)  $\text{l h}^{-1} \text{g}^{-1}$  for juveniles and adults respectively. These values are much higher than previously reported for this species being fed smaller sized cultured microalgae. Our results strongly suggest that the difficulties in the nursery and broodstock hatchery culture of this oyster may be due to inappropriate phytoplankton diets. We recommend the provision of cultured microalgae of 20–75  $\mu\text{m}$  in diameter for improving the performance of hatchery maintained juvenile and adult *O. chilensis*.

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#### ACTIVITY OF DIGESTIVE ENZYMES ALONG THE GUT OF JUVENILE RED ABALONE, *HALIOTIS RUFESCENS*, FED NATURAL AND BALANCED DIETS



Zaul Garcia-Esquivel, Horst Felbeck-2006

Aquaculture 261(2): 615-625

Abstract:

Two carbohydrases (cellulase, lysozyme), three proteases (trypsin, aminopeptidase and non-specific protease), a non-specific lipase, and semiquantitative tests of 19 digestive enzymes were assayed in different gut sections of juvenile red abalone, *Haliotis rufescens*, in order to identify the regions where digestion takes place and investigate the extent to which diet composition can modify the digestive capacity of abalone. The abalone were fed either fresh kelp (K) or balanced diets containing 25 or 38% crude protein for 6 months. Enzyme assays were carried out on different sections of the abalone's gut at the end of this period. On a weight-specific basis, the digestive gland was the site containing most of the enzymes. On a protein-specific basis, two main digestion regions were identified: the digestive gland-stomach region that is characterized by high activities of cellulase and lysozyme, chymotrypsin and protease, and the mouth-intestine region with a typically high activity of lipase and amino peptidase. Significant dietary effects were observed on the activity of enzymes, especially in the digestive gland. Abalone fed with 25 and 38% crude protein diets exhibited higher cellulase ( $39.8 \pm 4.6$  and  $14.2 \pm 0.8$  mU mg<sup>-1</sup> protein, respectively) and lysozyme activities ( $88.0 \pm 20.4$  and  $56.6 \pm 15.7$  U, respectively) than those fed with fresh kelp ( $5.5 \pm 0.7$  mU mg<sup>-1</sup> protein and  $17.1 \pm 1.8$  U). In contrast, higher protease activity was found in kelp-fed organisms ( $234.1 \pm 20.4$  µg product/mg protein) than those fed the 25 and 38% crude protein diets ( $109.5 \pm 20.7$  and  $119.5 \pm 20.5$  µg product/mg protein, respectively). Semiquantitative API ZYM assays resulted in no clear food-specific effects on the activity of carbohydrases, proteases, ester hydrolases or phosphohydrolases, yet organ-specific differences were conspicuous in various cases, and generally agreed with quantitative results. It is suggested that the increased carbohydrase activity exhibited by organisms fed the balanced diets resulted from a combination of an increased number of resident bacteria in the abalone's gut and facilitated contact between dietary substrates and digestive cells. The present results indicate that *H. rufescens* can adjust their enzyme levels in order to maximize the acquisition of dietary protein and carbohydrates. This characteristic can be advantageously used to search for suitable diets in abalone aquaculture.

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SUITABILITY OF THE COPEPOD, *ACARTIA CLAUSI* AS A LIVE FEED FOR SEABASS LARVAE (*LATES CALCARIFER* BLOCH): COMPARED TO TRADITIONAL LIVE-FOOD ORGANISMS WITH SPECIAL EMPHASIS ON THE NUTRITIONAL VALUE

M. Rajkumar, K.P. Kumaraguru Vasagama-2006

Aquaculture 261(2): 649-658

Abstract:

Though artificial propagation of Asian seabass *Lates calcarifer* (Bloch) in captivity through induced breeding techniques is standardized under Indian conditions, larval and nursery rearing techniques including suitable nursery feeds have to be standardized to obtain better survival and growth. Feeding experiments in triplicate were conducted to evaluate the suitability of the marine copepod *Acartia clausi* as live prey for fourteen day-old seabass larvae ( $6.53 \pm 0.06$  mm;  $8.58 \pm 0.33$  mg) and compared with the traditional live prey, rotifers and *Artemia* nauplii. While *A. clausi* and rotifers were mass produced using algae *Isochrysis galbana*, *Chaetoceros affinis* and *Chlorella marina*, *Artemia* nauplii were produced using cysts. Nutritional quality of cultured copepods was evaluated based on the proximate composition, amino acid and fatty acid composition, and compared with that of rotifers and *Artemia* nauplii. Proximate composition varied significantly ( $P < 0.05$ ) among the different live feeds. *A. clausi* showed higher protein (63.12%) and lipid (16.65%) content than *Artemia* nauplii and rotifers. Total essential amino acids content was 2% lower in *A. clausi* compared to that in *Artemia* nauplii. Fatty acid profiles of the live feed organisms showed that *A. clausi* is a rich source of n – 3 fatty acids. The total n – 3 fatty acid content of *A. clausi* was 33.94%. Length, weight overall weight gain and survivorship were significantly ( $P < 0.05$ ) different among the dietary treatments, and weight gain was comparatively higher in *A. clausi* fed larvae. Survival of seabass larvae fed *A. clausi* was

obtained highest as 58.13% against the lower values of 39.93% and 41.62% in larvae fed rotifer and *Artemia nauplii* respectively. Final carcass composition of the larvae of *L. calcarifer* fed different live-food organisms showed significant differences ( $P < 0.05$ ) among the dietary treatments. The fatty acid composition of the dietary treatments was reflected to a certain extent in the fatty acid composition of the seabass larvae. The present investigation revealed the nutritional value of calanoid copepod and thus underlining its usefulness as a suitable live-food organism for rearing larvae of the commercially valuable Asian seabass.

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#### EFFECTS OF NAUPLIAL DENSITY, PRODUCT CONCENTRATION AND PRODUCT DOSAGE ON THE SURVIVAL OF THE NAUPLII AND EFA INCORPORATION DURING ARTEMIA ENRICHMENT WITH LIPOSOMES

Óscar Monroig, Juan Carlos Navarro, Francisco Amat, Pedro González, Francisco Hontoria-2006  
Aquaculture 261(2): 659-669

Abstract:

In order to determine an optimal enrichment protocol with krill phospholipid liposomes, three different experiments were carried out to evaluate the effects of naupliar density, the concentration of liposome, and the number of doses delivered to the nauplii on the survival and the essential fatty acid (EFA) bioencapsulation in *Artemia nauplii*. No survival differences were found between the different liposome treatments and the control nauplii enriched with a commercial emulsion. This result confirms that previously described mortalities during *Artemia* enrichment with liposomes can be minimized and controlled. On the other hand, the EFA incorporation obtained from the three experiments indicates that maximal bioencapsulation is achieved when incubations are carried out under naupliar densities of 300 nauplii ml<sup>-1</sup>, where liposomes are dispensed in a single dose at the beginning of the incubation yielding a concentration of 0.5 g l<sup>-1</sup>. These findings complete those obtained in two preliminary studies, and propose a well defined enrichment protocol with krill liposomes as an alternative complementary method to improve the nutritional value of the *Artemia nauplii* used as food for marine fish larvae.

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#### SHORT-CHAIN FATTY ACIDS PROTECT GNOTOBIOTIC ARTEMIA FRANCISCANA FROM PATHOGENIC VIBRIO CAMPBELLII

Tom Defoirdt, Dirk Halet, Patrick Sorgeloos, Peter Bossier, Willy Verstraete-2006  
Aquaculture 261(2): 804-808

Abstract:

Infections caused by antibiotic resistant luminescent vibrios can cause considerable losses in aquaculture. In this study, different short-chain fatty acids were investigated as possible alternative biocontrol agents. The addition of 100 mM formic, acetic, propionic, butyric or valeric acid to the growth medium of a pathogenic *Vibrio campbellii* strain completely inhibited its growth at pH 6. At 10 mM, the growth of the pathogen was delayed, whereas at 1 mM, no effect could be observed. The growth-inhibitory effect was clearly pH-dependent and decreased with increasing pH. An in vivo challenge test with gnotobiotic *Artemia franciscana* nauplii revealed that all five short-chain fatty acids protected the shrimp from the pathogenic *V. campbellii* strain. The addition of 20 mM of the short-chain fatty acids to the culture water resulted in a significantly increased survival of infected nauplii, with no difference between the different fatty acids. In conclusion, our data indicate that short-chain fatty acids might be useful as alternative biocontrol agents to treat luminescent vibriosis.

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## SHORT COMMUNICATION

### FIRST FEEDING OF OCTOPUS VULGARIS CUVIER, 1797 PARALARVAE USING ARTEMIA: EFFECT OF PREY SIZE, PREY DENSITY AND FEEDING FREQUENCY

J. Iglesias, L. Fuentes, J. Sánchez, J.J. Otero, C. Moxica, M.J. Lago-2006

Aquaculture 261(2): 817-822

#### Abstract:

Different assays related to the first feeding of *Octopus vulgaris* Cuvier, 1797 are compiled in this paper. They include: age at initial feeding age, prey size selection and optimal density, attack timing after feeding, and effect of dose number on the number of captures. Prey capture and ingestion processes were also analysed. Food supplied was cultured *Artemia* sp. Each assay lasted 15 min.

Although paralarvae already start to feed on the hatching day (day 0), it is during day 2 when a greater number of attacks is recorded ( $81.7 \pm 14.7\%$  paralarvae attack). They mainly prefer (significance level  $\alpha = 0.05$ ) large *Artemia*,  $1.4 \pm 0.4$  mm ( $77.0 \pm 5.6\%$  of the total attacks) than small *Artemia*,  $0.8 \pm 0.1$  mm ( $23.0 \pm 5.6\%$ ). There is also a slight predilection for the lowest *Artemia* concentration ( $33.3 \pm 12.6\%$  paralarvae attack in a 0.1 *Artemia* ml<sup>-1</sup> density, opposite  $16.7 \pm 7.6$  and  $18.3 \pm 7.6\%$  in densities of 0.5 and 1 *Artemia* ml<sup>-1</sup> respectively). The greatest predatory activity is recorded during the first 5 min after food is supplied ( $72.2 \pm 25.5\%$ ). An increase in the predatory activity was also observed when food was distributed in several doses instead of a single dose ( $75.0 \pm 10.0\%$  and  $46.7 \pm 17.6\%$  respectively). It was proved for the first time that paralarvae completely ingest their preys (including their exoskeletons), in this case *Artemia*. Time needed for their total ingestion ranges between 4 and 10 min.

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### DO PROTEIN HYDROLYSATES IMPROVE SURVIVAL AND GROWTH OF NEWLY-HATCHED SPOTTED WOLFFISH (ANARHICHAS MINOR), A NON-METAMORPHIC AQUACULTURE FISH SPECIES?

A. Savoie, N.R. Le François, C. Cahu, P.U. Blier, I. Andreassen-2006

Aquaculture 261(2): 782-788

#### Abstract:

Despite larval robustness characterized by the absence of metamorphosis and readiness for exogenous feeding based on commercial feed, the spotted wolffish (*Anarhichas minor*) displays highly variable survival at first-feeding. In this study, we investigated the use of three dietary concentrations of protein hydrolysates (PH, pre-digested proteins) (0, 10 and 20%) when newly hatched juvenile wolffish were held at three different rearing temperatures namely, 5, 8 and 12 °C to determine whether digestion of protein was a limiting factor for fish growth and survival. Final weights for fish at 5, 8, and 12 °C at day 60 were respectively 0.35, 1.19 and 2.02 g. Mean specific growth rates were 1.97, 4.01 and 4.88%/day and survival rates were 49.8, 53.4 and 33.2% respectively. No significant effects of PH were observed on growth or survival at any time during the experiment. However, as a general trend, fish survival was always higher when the diet contained 20% PH. We suggest that the degree of hydrolysis of the PH used may have been insufficient to induce specific digestive enzyme stimulation for promoting larval growth. Moreover, precocious ontogeny of the digestive system may have precluded any significant effect of using dietary protein hydrolysates. Our results are discussed in comparison with metamorphic species which characteristically display incomplete development of the digestive system.

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### DISRUPTION OF GONADAL MATURATION IN CULTURED SENEGALESE SOLE SOLEA SENEGALENSIS KAUP BY CONTINUOUS LIGHT AND/OR CONSTANT TEMPERATURE REGIMES

Ángel García-López, Emilio Pascual, Carmen Sarasquete, Gonzalo Martínez-Rodríguez-2006

Abstract:

A major problem in the development of Senegalese sole *Solea senegalensis* intensive culture is the poor control on reproduction, in part due to the lack of knowledge on the precise role of photoperiod and temperature. Thus, gonadal maturation was evaluated by assessing the sequential changes in plasma levels of 17 $\beta$ -estradiol (E2), 11-ketotestosterone (11-KT), and testosterone (T) in both female and male cultured Senegalese sole (F1 generation) exposed to various combinations of constant or naturally-fluctuating daylength and water temperature. Under natural photoperiod (NP; 36° N), exposure to constant temperature (t0; 18–20 °C) disrupted gonadal development, as indicated by a lower incidence (in comparison with naturally-fluctuating water temperature; 14–24 °C) of females at advanced maturation (from February to April: 12 vs. 33%) and running males (from February to May: 46% vs. 57%), and the reduced mean ( $\pm$  S.E.M.) sex steroid plasma levels (female peak E2 levels: 2.9  $\pm$  0.28 vs. 1.8  $\pm$  0.3 ng ml<sup>-1</sup>; male peak T levels: 1.5  $\pm$  0.14 vs. 0.9  $\pm$  0.06 ng ml<sup>-1</sup>). Therefore, the onset and progression of gonadal development in this species seem to be strongly (“proximally”) influenced by fluctuating water temperature. When compared to NP and t0, exposure to continuous light (LL) under t0 significantly reduced steroid production (female peak E2 levels: 1.8  $\pm$  0.28 vs. 0.5  $\pm$  0.05 ng ml<sup>-1</sup>; male peak 11-KT levels: 9.4  $\pm$  1.06 vs. 5.4  $\pm$  1.33 ng ml<sup>-1</sup>) and subsequently gonadal development (lower proportions of females at intermediate [46 vs. 6%] and advanced maturation [12 vs. 0%] from February to April and of RM [46 vs. 33%] from February to May). Thus, the seasonal changes of daylength would be crucial for normal gonadal development, being its cueing effects of higher magnitude than those of water temperature. The present report constitutes the first systematic study focused on the environmental control of reproductive events in Senegalese sole.

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