

**ESTABLISHMENT AND MAINTENANCE OF THREATENED LONG-SNOURED SEAHORSE, HIPPOCAMPUS GUTTULATUS, BROODSTOCK IN CAPTIVITY**

Miquel Planas, Alexandro Chamorro, Patricia Quintas, Antonio Vilar-2008

Aquaculture 283(1-4): 19-28

**Abstract:**

Knowledge on seahorses is generally scarce but has been increasing in recent years due to their conservation status. Seahorse culture is a quite recent activity in most countries attempting it, and captive breeding techniques are available only for some species. With the aim of contributing to the development of breeding in captivity for conservative purposes, captive broodstock of the European long-snouted seahorse (*Hippocampus guttulatus*) was established with 32 wild seahorses captured in Galicia (NW Spain). This study describes the methodologies applied to the maintenance of the broodstocks, with special reference to aquaria design, feeding, growth and breeding. Procedures of seahorse identification (morphologically and genetically) as a tool for broodstock management are also considered. The results achieved during the first year demonstrate a rapid adaptation of wild seahorses to captive conditions. Seahorses were fed exclusively on enriched adult *Artemia* and displayed high growth rates. However, fatty acid analyses performed on unfertilised eggs of captive broodstock showed a progressive decrease in the content of essential fatty acids (DHA, EPA) with time in captivity, suggesting the need for improvement in the nutritional quality of broodstock feed.

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**PRODUCTIVITY AND PROFITABILITY OF MITHRACULUS FORCEPS AQUACULTURE**

Joana Figueiredo, Gil Penha-Lopes, Junda Lin, Luís Narciso-2008

Aquaculture 283 (1-4): 43-49

**Abstract:**

Red-clinging crabs *Mithraculus forceps* are used for their ability to control nuisance algae in marine aquariums. Their larval and juvenile culture protocol has already been successfully developed. The objective of this work is to build a model that integrates the effect of different abiotic and biotic factors (temperature, stocking density, prey density), as well as space (number and volume of tanks) in production and economic parameters (costs of feed, labour and maintenance, market price and profit). The model aimed to be used as a tool to support management decisions. Overall, the model was able to integrate previously collected data and produce expected forecasts of *M. forceps* larval and juvenile culture reared under different combinations of temperature, stocking density, and prey density. Sensitivity analysis revealed that temperature was the most important factor regulating survival and growth, and consequently profit. According to the model, a batch of 1500 larvae reared in ten 10 L tanks in optimal conditions (10 prey mL<sup>-1</sup> and 28 °C) and then, as juveniles, in a 3 m<sup>2</sup> water table at 28 °C, is expected to reach commercial size in 225 days.

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**IMPORTANCE OF LIGHT AND LARVAL MORPHOLOGY IN STARVATION RESISTANCE AND FEEDING ABILITY OF NEWLY HATCHED MARINE ORNAMENTAL SHRIMPS *LYSMATA* SPP. (DECAPODA: HIPPOLYTIDAE)**

Ricardo Calado, Gisela Dionísio, Cátia Bartilotti, Cristovão Nunes, Antonina dos Santos, Maria Teresa Dinis-2008

Aquaculture 283(1-4): 56-63

**Abstract:**

The present work evaluates the resistance of newly hatched *Lysmataamboinensis*, *Lysmata ankeri*, *L. debelius* and *Lysmata seticaudata* larvae to 24, 48, 72, 96 and 120 h of starvation after hatching, as

well as their ability to capture newly hatched *Artemia* nauplii and enriched metanauplii, under different light regimes (24, 12 and 0 h of light). Additionally, it analyses the feeding and swimming behavior of newly hatched larvae and compares their morphological and biometrical features. *L. ankeri* and *L. seticaudata* displayed higher resistance to starvation than *L. amboinensis* and *L. debelius*, molting to zoea II in the absence of food. Larvae starved for longer periods and kept in darkness displayed higher survival rates, probably due to a lower energy consumption induced by reduced swimming activity. Light regimes did not influence *Lysmata* ability to capture larval preys, reinforcing the idea that these larvae do not display true hunting behaviors, rather relying on chance encounters with dietary preys. All *Lysmata* larvae consumed similar levels of *Artemia* nauplii, while *L. ankeri* and *L. seticaudata* were able to consume a significantly higher amount of enriched metanauplii than *L. amboinensis* and *L. debelius*. Interspecific larval size variability, similar swimming ability and the inexistence of morphological features more specialized for prey capture in *L. ankeri* or *L. seticaudata* larvae exclude larval morphology and biometry as explanations for the different consumption rates recorded. The ability of *Lysmata* larvae to capture large dietary preys opens good perspectives for the use of inert diets at an early stage.

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#### EFFECTS OF DIETARY LIPID SOURCE AND CONCENTRATION ON CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) EGG BIOCHEMICAL COMPOSITION, EGG AND FRY PRODUCTION, AND EGG AND FRY QUALITY

Todd D. Sink, Rebecca T. Lochmann-2008

Aquaculture 283(1-4): 68-76

Abstract:

We conducted a study to determine the effects of lipid source (poultry fat, PF or menhaden fish oil, FO) and concentration (4 or 10% lipid supplementation) in channel catfish broodstock diets on subsequent egg biochemical composition, egg production, and egg and fry quality. Three female and one male USDA-103 strain three-year-old broodfish were stocked into 16 3860-L outdoor tanks in January and randomly assigned one of the four diets (four replicates per diet). Fish were fed based on a water-temperature-dependant schedule. Spawning cans were added to tanks when water temperature reached 21 °C, and the cans were examined three times weekly for egg masses. Masses were enumerated, weighed, measured, analyzed for proximate and fatty acid composition, and incubated. Date to hatch, hatching success, fry growth and survival, and fingerling growth and survival were monitored. Supplementation of catfish broodstock diets with 10% fish oil increased spawning success, fecundity, total egg volume (matrix removed), individual egg weight, eggs-spawn<sup>-1</sup> (volumetric), total egg lipid concentration, hatching success, and fry survival compared to a control diet with 4% fish oil. The 10PF diet performed similarly to the 10FO diet in all measured parameters except egg fatty acid composition, although results for the 10PF diet did not always differ from those of the 4FO or 4PF diets. The 10FO diet produced significant decreases in egg saturates and MUFA, and increases in n-3 HUFAs and the ratio of n-3:n-6 fatty acids compared to all other diets. However, in fish fed the 10FO or 10PF diets the differences in egg fatty acid composition did not result in different rates of hatching success, fry survival, or fingerling production. Improved spawning performance of catfish fed 10%-lipid diets may be due partly to increased dietary energy relative to the 4%-lipid diets. Although the dietary n-3:n-6 fatty acid ratios of the 10PF (0.3) and 10FO (5.6) diets were quite different, similar concentrations of HUFAs such as DHA and AA were present in the eggs of fish fed either diet. This indicates that catfish can synthesize HUFAs from 18-carbon precursors and deposit the HUFAs in the egg, and that a variety of lipid sources may be suitable for use in catfish broodstock diets. Current methods of channel catfish egg and fry production using growout diets with 28–32% protein and 6–7% lipid are suboptimal based on the results of this study.

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PRODUCING JUVENILE ARTEMIA AS PREY FOR OCTOPUS VULGARIS PARALARVAE WITH DIFFERENT MICROALGAL SPECIES OF CONTROLLED BIOCHEMICAL COMPOSITION

Pedro Seixas, Manuel Rey-Méndez, Luísa M.P. Valente, Ana Otero-2008

Aquaculture 283(1-4): 83-91

Abstract:

The major bottleneck of *Octopus vulgaris* culture is the rearing of its paralarval life stage, being the obtainment of adequate live prey to feed paralarvae one of the key issues for the success of the culture of this valuable species. *Artemia* has been widely used as a single prey or in combination with crustacean zoeae as food items for paralarvae, but few works have reported the biochemical composition of these prey. The gross biochemical composition and fatty acid profile of *Artemia* juveniles enriched with four marine microalgae of controlled biochemical composition was assessed, as well as the fatty acid composition of newly hatched *O. vulgaris* paralarvae, in order to estimate which prey would be more suitable to meet the nutritional requirements of *O. vulgaris* paralarvae. Microalgae were cultured semi-continuously with a daily renewal rate of 30% of the volume of the cultures in nutrient saturated conditions, in order to achieve biomass of constant and optimal biochemical composition. *Artemia* juveniles of two different sizes (1.5-2.0 mm and 3.0-3.5 mm), appropriate to feed *O. vulgaris* paralarvae, were obtained by growing *Artemia* nauplii with *Tetraselmis suecica* for 2 and 4 days and then enriched for 26 h with four marine microalgae: *T. suecica*, *Isochrysis galbana*, *Isochrysis* aff. *galbana* (T-ISO) and *Rhodomonas lens*. The protein content of *R. lens* (62% of dry weight) was considerably higher than that of the remaining microalgae (42-44%) ( $P < 0.001$ ), whereas lipid and carbohydrate were significantly higher in both T-ISO and *I. galbana* ( $P < 0.05$ ). Small juvenile *Artemia* (3-day old, 1.5-2.0 mm) contained nearly 51% protein (of dry weight) regardless the enrichment diet used, with the exception of individuals enriched with *I. galbana* (AISO) (41%) ( $P < 0.01$ ). In these juveniles, lipid percentages were higher when enriched with T-ISO (group AT-ISO) or *R. lens* (ARHO), both with circa 16% ( $P < 0.05$ ); whereas carbohydrate was higher in juveniles from groups AISO or AT-ISO (11%) ( $P < 0.05$ ). Large juvenile *Artemia* (5-day old, 3.0-3.5 mm) had higher protein percentages than small juveniles with values ranging between 64 and 68% for all treatments, whereas the lipid fraction among groups increased in the order: ARHO (10%) < ATET = AT-ISO (16%) < AISO (18%) ( $P < 0.05$ ). The lowest percentage of carbohydrate was found in group ARHO (6%) ( $P < 0.01$ ). Maximum protein/energy ratio was observed in 5-day old juveniles from group ARHO (P/E ratio = 31). The highest percentage (% total fatty acids) of eicosapentaenoic acid (20:5n-3) in small juvenile *Artemia* was found in individuals from groups AISO or ARHO (circa 9%), whereas in 5-day old juveniles the highest value was found in group AISO (14.6%) ( $P < 0.05$ ). Regarding docosahexaenoic acid (22:6n-3), small juveniles from groups AT-ISO or AISO had higher values (1.9 and 1.5%, respectively) than juveniles from group ARHO (1.0%) ( $P < 0.05$ ), whereas in 5-day old *Artemia* maximum percentage of 22:6n-3 was found in group AT-ISO (3.9%) ( $P < 0.05$ ). None of the *Artemia* juveniles enriched with *T. suecica* contained 22:6n-3. The fatty acid composition of *O. vulgaris* paralarvae revealed a much higher percentage of 22:6n-3 (18.3%) than the values found in the enriched *Artemia* juveniles, suggesting a deficit of this fatty acid in *Artemia*. Even though the highest sum of 20:5n-3 and 22:6n-3 was found in *Artemia* juveniles enriched with *I. galbana*, if the general biochemical composition of *Artemia* juveniles is taken into consideration, the enrichment with *R. lens* would provide the best composition to meet the possible nutritional requirements of *O. vulgaris* paralarvae.

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BREEDING AND REARING THE LONGSNOUT SEAHORSE HIPPOCAMPUS REIDI: REARING AND FEEDING STUDIES

I. Olivotto, M.A. Avella, G. Sampaolesi, C.C. Piccinetti, P. Navarro Ruiz, O. Carnevali-2008

Aquaculture 283(1-4): 92-96

Abstract:

The aim of this study was to evaluate the potential use of the harpacticoid copepod *Tisbe* spp as prey in *Hippocampus reidi* culture together with the effect of natural (14 L/10D) or prolonged (24 L/0D) photoperiod. After release, the juveniles were divided into four experimental groups as follows: group A (control group) fed rotifers (*Brachionus plicatilis*) followed by *Artemia* nauplii subjected to 14 L/10D photoperiod; group B fed a mixed diet of rotifers and *Tisbe* spp nauplii followed by a combination of *Artemia* nauplii and *Tisbe* spp copepodites/copepods subjected to 14 L/10D photoperiod; group C fed rotifers (*B. plicatilis*) followed by *Artemia* nauplii subjected to 24 L/0D photoperiod and group D fed a mixed diet of rotifers and *Tisbe* spp nauplii followed by a combination of *Artemia* nauplii and *Tisbe* spp copepodites/copepods subjected to constant light. We observed higher survival and better growth performance in juveniles fed a combined diet and subjected to an extended photoperiod respect to those fed a standard diet or exposed to natural photoperiod. In conclusion, we found that the harpacticoid copepod *Tisbe* spp may be considered a valuable live prey for *H. reidi* culture when used as a supplement to the traditional diet based on rotifers and *Artemia* nauplii and that photoperiod may play an important role in the successful culture of this species. (Department of Marine Sciences, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy; email of I. Olivotto: [i.olivotto@univpm.it](mailto:i.olivotto@univpm.it))

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#### THE IMPACT OF DIETARY SUPPLEMENTATION WITH ASTAXANTHIN ON EGG QUALITY IN ATLANTIC COD BROODSTOCK (*GADUS MORHUA*, L.)

Jarin Sawanboonchun, William J. Roy, Derek A. Robertson, J. Gordon Bell-2008  
Aquaculture 283(1-4): 97-101

Abstract:

This study investigated the effect on egg quality of dietary supplementation of Atlantic cod broodstock with the carotenoid astaxanthin (ASTA). Duplicate groups of farm-reared Atlantic cod broodstock were fed either a control diet with no added ASTA, or an ASTA supplemented diet (73.7 mg/kg dry weight; Carophyll Pink®) for 2 months prior to peak spawning. The results indicated that ASTA uptake into eggs from the broodstock diet was highly efficient. Fish fed the diet supplemented with ASTA produced fewer batches of eggs, but the mean number per batch of eggs spawned/kg female was higher, and numbers of floating eggs and numbers of fertilised eggs per kg female in each batch were also significantly improved. A correlation between the egg ASTA content and fertilisation success of individual batches was identified. This improvement in egg quality demonstrated the potential value of ASTA supplementation of broodstock diets for cod. ASTA supplementation produced a 20% increase in the number of eggs per batch spawned, a 37% increase in the number per batch of floating eggs per kg female and a 47% increase in the number per batch of fertilised eggs per kg female. These results clearly demonstrate significant benefits of ASTA supplementation of cod broodstock feeds in terms of improved egg quality and larval production.

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#### LARVAL PERFORMANCE AND SKELETAL DEFORMITIES IN FARMED GILTHEAD SEA BREEM (*SPARUS AURATA*) FED WITH GRADED LEVELS OF VITAMIN A ENRICHED ROTIFERS (*BRACHIONUS PLICATILIS*)

Ignacio Fernández, Francisco Hontoria, Juan B. Ortiz-Delgado, Yannis Kotzamanis, Alicia Estévez, Jose Luis Zambonino-Infante, Enric Gisbert-2008

Aquaculture 283(1-4): 102-115

Abstract:

Several nutritional studies have found a direct effect of several vitamins in chondrogenic and osteogenic development during early life stages of marine fish species. In the present study, the effect of vitamin A (VA) in gilthead sea bream skeletogenesis was evaluated by means of four different dietary regimes (enriched rotifers) containing increasing levels of total VA (75, 109, 188 and 723 ng total VA mg<sup>-1</sup> DW). Dietary treatments were offered to larvae during the rotifer-feeding phase (4–20 days after hatching), while later all groups were fed with *Artemia* nauplii and weaned onto the same inert diet. Different dietary doses of VA affected gilthead sea bream larval growth, survival,

performance (maturation of the digestive system) and quality (incidence of skeletal deformities). Higher levels of dietary VA than those included in the commercial emulsion for rotifer enrichment led to different levels and typologies of skeletal deformities, indicating that gilthead sea bream larvae were very sensitive to small increases in dietary VA. The degree of ossification was affected by the level of VA in enriched rotifers: the higher amount of VA in the diet, the higher number of skeletal pieces ossified ( $R = 0.585$ ,  $P = 0.04$ ). Dietary VA affected the normal process of bone formation and skeletogenesis, the skeletal structures mostly affected by high amounts of dietary VA were those from the cranial skeleton (splanchnocranium), vertebral centrums and caudal fin complex. The premaxilla, maxilla and dentary bones were the cranial structures affected by dietary VA levels, resulting in a large incidence of animals with compressed snout. Dietary VA also affected the normal development of the opercular complex, and a dose–response dependant effect was observed in relation to the incidence of specimens with incomplete operculum. Body shape was also affected by the level of dietary VA, increasing the incidence of specimens with lordosis, kyphosis and/or scoliosis with the dose of VA, being the prehaemal and caudal vertebrae the most affected regions of the vertebral column with this kind of abnormalities. The caudal fin complex was the most affected region of the skeleton affected by dietary treatments as seen by the high incidence of skeletal deformities in fish fed different doses of dietary VA. Deformities affected all skeletal elements composing the caudal fin, although the most affected ones were, in order of importance, the epurals, hypurals, parahypural, neural arch and uroneurals. Differences in sensitivity to dietary VA amongst caudal fin skeletal elements might be due to their differential ontogenetic development and differences in the exposure time to VA.

An excess of dietary VA also accelerated the intramembranous ossification process of vertebral centrums leading to one or two supranumerary vertebrae, and a high incidence of fused and compressed vertebral centrums. The sensibility of the developing skeletal structures to dietary VA levels should incline us to test lower doses of VA in live preys enrichments during early larval stages and higher doses afterwards.

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#### RIBOFLAVIN ENRICHMENT THROUGHOUT THE FOOD CHAIN FROM THE MARINE MICROALGA TETRASELMIS SUECICA TO THE ROTIFER BRACHIONUS PLICATILIS AND TO WHITE SEA BREAM (DIPLODUS SARGUS) AND GILTHEAD SEA BREAM (SPARUS AURATA) LARVAE

M. Souto, M. Saavedra, P. Pousão-Ferreira, C. Herrero-2008

Aquaculture 283(1-4): 128-133

Abstract:

The riboflavin enrichment of the marine microalga *Tetraselmis suecica* and the transfer of this vitamin to higher trophic levels of the aquatic food chain such as the rotifer *Brachionus plicatilis* and the larvae of two species of sparids: white sea bream and gilthead sea bream were studied. The preliminary experiment consisted of determining the concentration of riboflavin added to *T. suecica* cultures to achieve a maximum quantity of this vitamin in the microalgal cells. Seven concentrations were tested in triplicate: 0 (control), 2.5, 5, 10, 20, 40 and 80 ng ml<sup>-1</sup>; the results showed that the 10 ng ml<sup>-1</sup> was the optimum concentration that the microalgae accumulated  $4.29 \pm 0.19$  pg cell<sup>-1</sup>,  $21.2 \pm 0.35$  ng ml<sup>-1</sup> and  $19.4 \pm 0.56$  µg g<sup>-1</sup> (dry weight) of riboflavin. Control and enriched microalgal cultures were used for feeding the rotifer *B. plicatilis*. Control and enriched rotifers were used for feeding white sea bream and gilthead sea bream larvae. Rotifers fed on enriched microalgal cultures accumulated significantly ( $P < 0.05$ ) more riboflavin than those fed the control culture after 24 h of enrichment ( $17.7 \pm 1.3$  and  $13.7 \pm 1.2$  µg g<sup>-1</sup> (dw), respectively) and after 24 h of starvation ( $10.2 \pm 4.1$  and  $5.6 \pm 0.4$  µg g<sup>-1</sup> (dw), respectively). In both species of sparids, those larvae fed enriched rotifers contained significantly more riboflavin than those fed control rotifers, the vitamin B2 content in control and enriched white sea bream larvae was  $21.7 \pm 2.7$  and  $29.2 \pm 1.3$  µg g<sup>-1</sup> (dw), respectively, and in control and enriched gilthead sea bream larvae it was  $5.5 \pm 1.0$  and  $7.3 \pm 0.05$  µg g<sup>-1</sup> (dw), respectively. Significant differences in length and survival of white sea bream larvae were

observed. In the present study, riboflavin enrichment of microalgal cultures resulted in higher levels of this vitamin in both rotifers and fish larvae.

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#### EFFECTS OF WATER DEPTH ON SURVIVAL AND GROWTH OF ARGOPECTEN PURPURATUS (LAMARCK, 1819) SPAT IN NORTHERN CHILE

Miguel Avendaño, Marcela Cantillán, Gérard Thouzeau-2008

Aquaculture International 16(5): 377-391

Abstract:

Collection and suspended culture of *Argopecten purpuratus* spat in Japanese-type collectors was undertaken in the Rinconada Marine Reserve (Antofagasta, Chile) to determine growth variations between surface and bottom waters. Scallop spat was collected at 16-m depth and grown at 1- and 16-m depths. An initial settlement of ca. 13,000 post-larvae per bottom collector (2 cohorts) was observed on 3 February 2001. Two new cohorts settled in bottom collectors on 3 March, while no spat settlement occurred in surface collectors. The four cohorts exhibited substantial and different mortality rates over the study period, depending on the cohort and on-growing depth. The first and second cohorts exhibited mortality rates of 80.3 and 53.1% in bottom collectors, respectively, versus 29 and 43.6% in surface structures. The third and fourth cohorts exhibited mortality rates of 21.4 and 59.7% in bottom collectors, respectively. Mean shell height of scallop spat after 88 days of culture was 6.46 mm for the first cohort and 4.37 mm for the second cohort in bottom collectors, versus 10.33 and 7.31 mm in surface collectors, respectively. Mean post-larval growth rate in bottom collectors (86  $\mu\text{m day}^{-1}$ ) was significantly lower than growth rate in surface collectors (146  $\mu\text{m day}^{-1}$ ). Factors explaining the depth-related mortality and growth rates are discussed to improve scallop culture in the Reserve. It was concluded from the results that culture improvement in Antofagasta Bay would require collecting spat in bottom waters and raising it near the surface during initial cultivation stages. (Dpto de Acuicultura, Universidad de Antofagasta, Angamos 601, Antofagasta, Chile; email of Miguel Avendaño: [mavendano@uantof.cl](mailto:mavendano@uantof.cl))

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#### PREVALENCE OF WHITE SPOT SYNDROME VIRUS INFECTION DETECTED BY ONE-STEP AND NESTED PCR IN SELECTED TIGER SHRIMP (*PENAEUS MONODON*) HATCHERIES

Farhana Ayub, Md. Yusuf Sarker, Md. Samsul Alam-2008

Aquaculture International 16(5): 405-415

Abstract:

White spot syndrome (WSS) is considered as a great threat to commercial farming of the tiger shrimp (*Penaeus monodon*). The causal agent of WSS is a DNA virus called white spot syndrome virus (WSSV). The prevalence of this dreadful virus infection has been studied in five randomly selected hatcheries located in the Cox's Bazar district of Bangladesh. Both one-step and nested polymerase chain reaction (PCR) involving two pairs of primers, namely, 146F1/146R1 and 146F2/146R2, amplifying the 1447 bp and 941 bp fragments, respectively, were conducted to detect the WSSV. Out of 60 randomly collected shrimps, 12 (20%) were found to be positive by one-step PCR, while 18 (30%) were found to be positive by nested PCR. The nested PCR was found to be much more sensitive than the one-step PCR. The shrimp specimens showing clinical signs of WSS were positive for WSSV by both one-step and nested PCR. Some of the apparently healthy samples were also found to be positive for WSSV by nested PCR. Among the two primer-pairs, the inner pair amplifying the 941 bp fragment was more sensitive than the outer primer pair amplifying the 1447 bp fragment when used in one-step PCR.

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#### EFFECTS OF DIFFERENT NATURAL OR PREPARED DIETS ON GROWTH AND SURVIVAL OF JUVENILE SPIDER CRABS, *MAJA BRACHYDACTYLA* (BALSS, 1922)

J. Alaminos, P. Domingues-2008  
Aquaculture International 16(5): 417-425

Abstract:

The effects of different diets (natural or pellets) on growth, survival, and moulting interval of juvenile spider crabs, weighing between 0.011–1.56 g and up to 17.6 mm in carapace length, were tested over a period of 90 days. During experiment I, five diets were tested: (1) frozen shrimp—*Paleomonetes* sp., (2) fresh mussels—*Mytilus* sp., (3) white fish fillets—*Merluccius merluccius*, (4) blue fish fillets—*Sardina pilchardus*, and (5) commercial crustacean pellets. Spider crabs fed fresh mussels grew larger ( $0.98 \pm 0.69$  g) and had higher growth rates ( $4.0 \pm 0.7$  %BWd<sup>-1</sup>) compared to the other four diets. The crabs fed shrimp pellet and frozen shrimp grew to intermediate sizes and were smaller than the ones fed fresh mussels, but they were larger than spider crabs fed either blue or white fish fillets ( $0.46 \pm 0.63$  and  $0.26 \pm 0.13$  g, respectively) compared to the ones fed white fish fillets ( $0.12 \pm 0.04$ ) and blue fish fillets ( $0.04 \pm 0.02$  g). The spider crabs fed blue fish fillets only lasted until day 60 of the experiment, after this day none of the 20 fed this diet were left. During experiment II, two diets were tested: (1) white and blue fish fillets and (2) commercial fish pellet. There were no differences in growth both in weight or carapace length ( $2.9 \pm 1.8$  and  $2.1 \pm 1.5$  g in weight, and  $18.9 \pm 5.0$  and  $17.7 \pm 3.3$  mm, respectively) at the end of the experiment. Similarly, there were no differences in growth rates in weight between the two diets ( $1.2 \pm 0.4$  and  $0.9 \pm 0.3$  %BWd<sup>-1</sup>, respectively) or in carapace length ( $0.4 \pm 0.1$  and  $0.4 \pm 0.2$  %BWd<sup>-1</sup>, respectively). Fresh mussel appears to be a very good diet to culture the early stages of this species, while shrimp pellets also deliver acceptable results. On the contrary, frozen shrimp, fish fillets either from blue or white species (much higher lipid content in the blue species), and fish pellets were found to be bad diets for the culture of the early stages of *M. brachydactyla*.

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#### ASSESSING THE SPAWNING SEASON IN COMMON DENTEX (*DENTEX DENTEX*) USING MICROSATELLITES

Yaisel J Borrell, Gloria Blanco, Emilia Vázquez, Jorge A Piñera, Gemma Gimenez, Alicia Estévez, Jose A Sánchez-2008

Aquaculture Research 39(12):1258-1267

Abstract:

A set of five variable microsatellite markers was used for the genetic characterization of two common dentex (*Dentex dentex*) broodstocks from an experimental hatchery and for the screening of the egg batches spawned during the complete spawn season in 2006. After parentage assignment, simulations were performed for the hatchery 1 broodstocks; the microsatellite set correctly assigned over 93% of descendants to parents when one parent was known and 80% when neither parent was known. Of the 261 eggs that the DNA was correctly amplified from, 254 were successfully assigned to a parental couple. More than one female or male took part (at the same point in time) in the spawning season. However, we observed 'dominant' individuals that produced high proportions of the descendants (unequal reproductive success) in all the sampling periods, although the contributions of these dominant individuals could change over time. This phenomenon reduces the effective breeding numbers, and could lead to inbreeding if this factor is not taken into account when the next parental generation is obtained. This factor should also be considered in aquaculture selection programmes because the dominant breeders may not be the most interesting individuals for commercial or productive schemes.

(Laboratorio de Genética Acuícola, Departamento de Biología Funcional, Universidad de Oviedo, IUBA, Oviedo, Spain)

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#### NUTRITIONAL REGULATION OF INTESTINE MORPHOLOGY IN LARVAL CYPRINID FISH, SILVER BREAM (*VIMBA VIMBA*)

Teresa Ostaszewska, Konrad Dabrowski, Piotr Hliwa, Piotr Gomółka, Karolina Kwasek -2008

Aquaculture Research 39(12): 1268-1278

Abstract:

The present study includes the evaluation of morphological changes in the digestive tract of larval, stomachless fish silver bream (*Vimba vimba*) fed with various diets – live *Artemia* nauplii, commercial feed Aglo Norse (NOR) and semi-purified formulated diets: casein–gelatin (CG), dipeptide-protein (50P), dipeptide (100P), no-arginine dipeptide diet (100Pw/oArg) and a free amino acid (FAA) mixture diet. The supranuclear area of enterocytes in the posterior intestine contained enlarged absorptive vacuoles in the FAA, 100P and 100Pw/oArg groups, compared with the remaining groups. Hepatocytes' cytoplasm in fish fed with FAA, 100P and 100Pw/oArg contained mainly glycogen, and no lipid vacuoles were found. Fish fed with 100Pw/oArg showed the lowest hepatocyte surface areas while in those fed with 50P, the largest nuclei diameters were observed. Fish fed with *Artemia*, NOR and CG diets showed significantly ( $P < 0.05$ ) higher number of proliferating cells compared with the remaining groups. Chromogranin A staining showed endocrine-immunoreactive cells (CgA-IR) in the taste buds in the oral cavity and in the enterocytes' supranuclear areas of the anterior and posterior intestine. We conclude that the growth rate and histological examination of the digestive tract in the 50P group of silver bream showed no nutritional deficiency. (Division of Ichthyobiology and Fisheries, Warsaw Agricultural University, 02-786 Warsaw, Ciszewskiego 8, Poland; email of T. Ostaszewska: [teresa\\_ostaszewska@sggw.pl](mailto:teresa_ostaszewska@sggw.pl))

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OUT-OF-SEASON SPAWNING OF PIKE PERCH (*SANDER LUCIOPERCA L.*) WITHOUT THE NEED FOR HORMONAL TREATMENTS

Andreas Müller-Belecke, Steffen Zienert-2008

Aquaculture Research 39(12): 1279-1285

Abstract:

The provision of fry and fingerlings, independent of the natural spawning season, can facilitate the implementation of innovative rearing strategies also in pike perch (*Sander lucioperca L.*). As strict pharmaceutical acts or codes of conduct for organic aquaculture can constrain fish farmers in inducing spawning with hormonal applications, this study intended to develop protocols for advanced and postponed spawning just by simple photo-thermal treatments. After spending between 31 and 61 days at temperatures below 10 °C, different groups of pike perch spawners were treated with light and temperature programmes to advance spawning. Reproduction could be induced successfully 2 months before the natural spawning season when the mating pairs spent 43 or more days below 10 °C, followed by a maturation phase of 44–68 days at 15 °C and 16 h illumination per day. Advanced spawning could be documented for 32 out of 35 females (91%) that underwent photo-thermal treatments. Mean commercial fecundities up to 24% and average rates of developing eggs of 65% were observed in advanced spawning groups. Coldbanking of mature females allowed to postpone spawnings for 2 and 3 months. However, no egg development could be recorded in these treatment groups.

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EFFECT OF VITAMIN E AND HIGHLY UNSATURATED FATTY ACID-ENRICHED ARTEMIA URMIANA ON GROWTH PERFORMANCE, SURVIVAL AND STRESS RESISTANCE OF BELUGA (*HUSO HUSO*) LARVAE

Mohammad Ali Jalali, Seyed Abbas Hosseini, Mohammad Reza Imanpour-2008

Aquaculture Research 39(12): 1286-1291

Abstract:

A 44-day rearing trial was conducted to examine the enrichment of *Artemia urmiana* nauplii with vitamin E and highly unsaturated fatty acid (HUFA) and its effects on the growth performance, survival and stress resistance of great sturgeon, *Huso huso*, larvae. Cod liver oil (EPA 18% and DHA 12%) and  $\alpha$ -tocopherol acetate were used as lipid and vitamin E sources. Beluga larvae at the first exogenous feeding with  $69 \pm 5.9$  mg body weight were randomly distributed into four treatments and three tanks were assigned to each diet. The test treatments were as follows: larvae fed with HUFA+20% and HUFA+50% (w/w) vitamin E-enriched *Artemia* nauplii (E1 and E2 groups,

respectively), HUFA without vitamin E (HUFA group) and non-enriched Artemia (control group). All treatments fed non-enriched Artemia for the initial 5 days after first feeding and then fed enriched Artemia for 7 days. After the period of enrichment, larvae were fed with daphnia from the 13th to the 40th day. At day 40, submersion in salt water (6 ppt for 4 days and 12 ppt for 2 days) and warm water (33 °C for 2 days) was performed to evaluate larvae resistance to salinity and temperature stress. Final weight, daily growth rate, specific growth rate and weight gain were higher in beluga fed with enriched Artemia. The highest growth rates were observed in E1, whereas survival was not significantly different between groups. Use of vitamin E and HUFA significantly increased fish resistance to a salinity of 12 ppt and the lowest stress resistance was observed in the control group. Stress tolerance was not significantly different at 6 ppt and 33 °C between groups. There was no comparable difference in the haematocrit index under stress conditions. These results indicated that the enrichment of Artemia with essential fatty acids and vitamin E can affect some growth and stress tolerance factors in great sturgeon, *Huso huso*, larvae.

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#### INFLUENCE OF SEVERAL NON-NUTRIENT ADDITIVES ON NONSPECIFIC IMMUNITY AND GROWTH OF JUVENILE TURBOT, *SCOPHTHALMUS MAXIMUS* L.

Y. Li, Y.J. Wang, L. Wang, K.Y. Jiang

Aquaculture Nutrition 14(5): 387-395

##### Abstract:

The effects of three non-nutrient additives on nonspecific immunity and growth of juvenile turbot (*Scophthalmus maximus* L.) were studied in this feeding experiment. The five treatments are basal diet alone, basal diets containing three different additives [0.4 g kg<sup>-1</sup> of xylo-oligosaccharides (XOS), 1.3 g kg<sup>-1</sup> of yeast cell wall and 0.8 g kg<sup>-1</sup> of bile acids] individually or in combination. Two hundred and twenty-five turbot (average initial weight 151.3 ± 11.3 g) were randomly allotted in five treatments with three replicates within each treatment in a 72-day period. Comparing with basal diet group, activities of C3, C4, phagocyte, lysozyme, specific growth rate and feed conversion rate in yeast cell wall, XOS and the combined groups was enhanced significantly ( $P < 0.05$ ); however, these parameters in bile acid groups were increased slightly ( $P > 0.05$ ) except for phagocyte ( $P < 0.05$ ); superoxide dismutase activity in additive groups was not significantly increased ( $P > 0.05$ ) except for the combined group ( $P < 0.05$ ). In conclusion, supplementation of yeast cell wall and XOS enhanced the nonspecific immunity of juvenile turbot. Synergistic or additive effect of the three additives was not observed.

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#### USE OF DIFFERENTLY ENRICHED ROTIFERS, *BRACHIONUS PLICATILIS*, DURING LARVICULTURE OF HADDOCK, *MELANOGRAMMUS AEGLEFINUS*: EFFECTS ON EARLY GROWTH, SURVIVAL AND BODY LIPID COMPOSITION

A.S. Garcia, C.C. Parrish, J.A. Brown, S.C. Johnson, S. Leadbeater-2008

Aquaculture Nutrition 14(5): 431-444

##### Abstract:

We evaluated the effects of enriched rotifers on growth, survival and on the lipid composition of haddock larvae. The treatments tested were (1) AlgaMac 2000®, (2) AquaGrow® Advantage and (3) Pavlova sp. paste and AlgaMac 2000®. The treatments did not influence larval growth rate throughout the experimental period ( $P = 0.70$ ). Larvae from all treatments grew approximately 8% of their dry weight per day between 1 and 29 days post hatch (dph). Treatment 3 resulted in the best survival, estimated to be 3 on a scale from 0 to 5, whereas for the two other groups the survival estimates were 0 and 2. Rotifers from treatment 1 had low sterol concentrations, high eicosapentaenoic acid/arachidonic acid ratio and their feeding resulted in high larval mortality. Rotifers enriched with Pavlova sp. had the lowest proportions of the sum of saturated fatty acids, docosahexaenoic acid and sum of  $\omega 3$  and the highest proportions of the sum of monounsaturated fatty

acids ( $\Sigma$ MUFA). This was partially reflected in larvae from treatment 3 in that they had the highest proportions of  $\Sigma$ MUFA and the lowest proportions of  $\Sigma\omega 3$  ( $P < 0.0001$  for both analyses). In addition, these larvae had the highest and lowest  $\Sigma C20$  and  $\Sigma C22$  polyunsaturated fatty acids (PUFA) respectively ( $P < 0.0001$  for both analyses). We suggest that more research with  $\omega 3$  and  $\omega 6$  PUFA can lead to improvements in the rearing of haddock larvae produced in hatcheries.

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EFFECT OF DIETARY PHOSPHORUS SOURCES AND VARYING LEVELS OF SUPPLEMENTAL PHOSPHORUS ON SURVIVAL, GROWTH AND BODY COMPOSITION OF POSTLARVAL SHRIMP (*LITOPENAEUS VANNAMEI*)

J. Niu, Y.-J. Liu, L.-X. Tian, K.-S. Mai, H.-J. Yang, C.-X. Ye, W. Gao-2008

Aquaculture Nutrition 14(5): 472-479

Abstract:

Two experiments were conducted for 30 days each to investigate the effective phosphorus source and supplemental phosphorus levels for postlarval *Litopenaeus vannamei*. The first experiment was performed in postlarval shrimp (mean initial wet weight 2 mg) fed four isoenergetic and isonitrogenous diets containing three supplemented inorganic phosphorus sources [D1: no supplemental phosphorus, D2:  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ , D3:  $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ , D4:  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$ ]. The quantities of the three supplemental  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$  were 11.6, 12.8 and 10 g  $\text{kg}^{-1}$  of the diet, respectively in order to make the three diets have the same total phosphorus. Growth performance (final mean body weight, FBW; weight gain, WG; specific growth ratio, SGR) of shrimp in D3 treatment was the highest and had significant difference with the D1 treatment. The survival of shrimp in D3 treatment was the highest and had significant difference with the other treatments. The mineral concentration and body composition of shrimp were not significantly different among treatments. We could conclude that  $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  was the optimal phosphorus source for postlarval *L. vannamei* from the growth performance and survival. The second experiment was performed in postlarval shrimp (mean initial wet weight 0.88 mg) fed four isoenergetic and isonitrogenous diets containing four supplemental  $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  levels (d1, d2, d3 and d4 with 0, 5, 10 and 20 g  $\text{kg}^{-1}$ , respectively). Shrimp in d2 treatment showed the highest growth performance and survival and also showed significant difference with other diet treatments. The whole body content of zinc (Zn) increased with the increase of dietary  $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  and significant differences were observed when dietary  $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  reached 5 g  $\text{kg}^{-1}$ , excess  $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  supplementation (10 and 20 g  $\text{kg}^{-1}$ ) had a negative effect on Zn content, the Zn content significantly decreased when  $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  was 20 g  $\text{kg}^{-1}$ . We can conclude that the amount of total phosphorus in the diet should be maintained between 20.9 and 22.0 g  $\text{kg}^{-1}$ , the amount of supplemental  $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  in the diet is less than 10 g  $\text{kg}^{-1}$ .

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INDUCED SPAWNING OF *CLARIAS BATRACHUS* (LINN.): EFFECT OF OVAPRIM DOSES AND LATENCY PERIODS ON THE WEIGHT OF STRIPPED EGGS AND OVARY

S.K. Sahoo, S.S. Giri, S. Chandra-2008

Asian Fisheries Science 21 (3):257-368

Abstract:

The breeding performance of *Clarias batrachus* was evaluated at four doses of Ovaprim (0.5, 1.0, 1.5 and 2.0 ml  $\text{kg}^{-1}$  body weight of female) in combination with five latency periods (11, 14, 17, 20 and 23 h). The breeding performance was judged by the total weight of stripped eggs and ovary. The lowest ( $P < 0.05$ ) weight of stripped eggs was obtained from the females injected with 0.5 ml Ovaprim and stripped at 11-17 h. A steady increase in stripped egg weight was observed by the use of hormone doses beyond 0.5 ml with 11 h latency combinations. Free flow of eggs was observed at 1.0 and 1.5 ml dose, when stripped respectively at 14-23 h and 14-17 h post-injection. These doses and

latency period treatments produced the significantly ( $P < 0.05$ ) highest weight of stripped eggs compared to other treatments. The stripped ovary weight was significantly ( $P < 0.05$ ) lowest when the females were injected with 1 ml dose and stripped at 14-23 h, or 1.5 ml dose and stripped at 14-17 h. The injection of 1.0-1.5 ml Ovaprim dose per kg female weight in combination with 14-17 h latency was suitable for obtaining the highest weight of stripped eggs in *C. batrachus* during induced breeding operation.

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#### TEMPORAL AND SPATIAL EXPRESSION PATTERN OF THE MYOSTATIN GENE DURING LARVAL AND JUVENILE STAGES OF THE CHILEAN FLOUNDER (*PARALICHTHYS ADSPERSUS*)

Iselys Delgado, Eduardo Fuentes, Sebastián Escobar, Cristina Navarro, Tatiana Corbeaux, Ariel E. Reyes, María Inés Vera, Marco Álvarez, Alfredo Molina-2008

Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 151(2): 197-202

Abstract:

The full length cDNA sequence of the myostatin gene was cloned from a teleostean fish, the Chilean flounder (*Paralichthys adspersus*) through RT-PCR amplification coupled with the RACE approach to complete the 5'- and 3'-region. The deduced amino acid sequence encodes a protein of 377 amino acid residues, including the structural domains responsible for its biological activity. Amino acid sequence comparison revealed high sequence conservation, and confirmed that the isolated sequence corresponds to the MSTN1 gene. Gene expression analysis showed that cfMSTN mRNA is present in a wide variety of tissues in juvenile fish. In addition, we assessed the spatial expression pattern of the MSTN mRNA during embryos and larval stages through whole mount in situ hybridization. No expression was observed in embryos, whereas in larvae of 8 and 9 days post fertilization, the notochord, somites, intestine and some discrete territories in the head, such as brain and eye, were positive for MSTN mRNA. Our results contribute to the knowledge of the MSTN system in larval and juvenile stages; in particular the strong expression observed in the notochord suggests that MSTN, in synchronization with positive growth signals, may play an important role in the control of the development of larvae somites.

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#### DIETARY SODIUM ALGINATE ADMINISTRATION AFFECTS FINGERLING GROWTH AND RESISTANCE TO STREPTOCOCCUS SP. AND IRIDOVIRUS, AND JUVENILE NON-SPECIFIC IMMUNE RESPONSES OF THE ORANGE-SPOTTED GROUPER, *EPINEPHELUS COIOIDES*

Shinn-Pyng Yeh, Chen-An Chang, Chi-Yao Chang, Chun-Hung Liu, Winton Cheng-2008

Fish & Shellfish Immunology 25(1-2): 19-27

Abstract:

The percent weight gain (PWG) and feeding efficiency (FE) of fingerling orange-spotted grouper, *Epinephelus coioides*, fed diets containing sodium alginate at 1.0 and 2.0 g kg<sup>-1</sup> were calculated on the 2nd, 4th, 6th, and 8th weeks after feeding. Survival rates of the fingerling grouper against *Streptococcus* sp. and an iridovirus, and non-specific immune parameters such as alternative complement activity (ACH50), lysozyme activity, natural haemagglutination activity, respiratory bursts, superoxide dismutase (SOD) activity, and phagocytic activity of juvenile grouper were also determined when the fish were fed diets containing sodium alginate at 0.5, 1.0, or 2.0 g kg<sup>-1</sup>. The PWG and FE of fish were better when the fish were fed diets containing sodium alginate at 1.0, and 1.0 and 2.0 g kg<sup>-1</sup>, respectively. The PWG and FE of fish fed the 0, 1.0 and 2.0 g kg<sup>-1</sup> sodium alginate-containing diets after 8 weeks were 271.0%, 454.4% and 327.8%, and 0.61, 0.72 and 0.68, respectively. Fish fed a diet containing sodium alginate at the level of 2.0 g kg<sup>-1</sup> had a significantly higher survival rate than those fed the control diet after challenge with *Streptococcus* sp. and an iridovirus causing an increase of survival rate by 25.0% and 16.7%, respectively, compared to the control group. The ACH50 level of fish fed the sodium alginate-containing diets at 2.0 g kg<sup>-1</sup> was significantly higher than those fed the 1.0 g kg<sup>-1</sup> sodium alginate diet and control diet after 12 days,

and had increased to 1.9-fold, compared to those fed the control diet. The lysozyme activity, phagocytic activity, respiratory bursts, and SOD level of fish fed the sodium alginate-containing diets at 1.0 and 2.0 g kg<sup>-1</sup> were significantly higher than those fed the control diet after 12 days, and had increased to 1.97- and 1.68-fold, 1.35- and 1.50-fold, 1.63- and 1.81-fold, and 1.23- and 1.31-fold, respectively, compared to those fed the control diet. We therefore recommend dietary sodium alginate administration at 1.0 and 2.0 g kg<sup>-1</sup>, respectively, to promote growth and enhance immunity and resistance against *Streptococcus* sp. and an iridovirus.

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#### THE EFFECTS OF A HARMFUL ALGA ON BIVALVE LARVAL LIPID STORES

R. Przeslawski, P.E. Bourdeau, M.H. Doall, J. Pan, L. Perino, D.K. Padilla-2008

Harmful Algae 7(6): 802-807

##### Abstract:

Marine invertebrates often have complex life histories that include a swimming planktivorous larval stage, at which time they are vulnerable to a variety of stressors, including those associated with nutritional stress and harmful algal blooms. Lipid stores have been shown to be especially important for post-metamorphic survivorship and growth in a variety of marine invertebrates. We investigated the effects of the harmful brown tide alga *Aureococcus anophagefferens* on the lipid stores and growth of larvae of the hard clam (northern quahog, *Mercenaria mercenaria*), a dominant bivalve in many western Atlantic bays and estuaries. *M. mercenaria* was the dominant bivalve in Great South Bay, Long Island, until the mid-1970s, but very few larvae are presently found in these waters. Recent brown tide blooms have been hypothesized to pose a barrier to recovery of *M. mercenaria* populations and hinder recent restoration efforts by negatively affecting clam larvae. To test whether a diet of the brown tide alga affects the accumulation of beneficial lipid stores, we fed larvae one of three diets representing equal biovolumes of *Isochrysis galbana*, a nutritious control alga; *A. anophagefferens*, the brown tide alga for which nutritional quality is not presently known; or a mixture of the two. Larvae fed only brown tide had significantly less lipid stores than those in the other dietary treatments. In addition, brown tide negatively affected larval size. We also tested for evidence of tradeoffs between larval growth and lipid stores, predicting that when the diet was less nutritious as in the brown tide treatments, larval size and lipids would be negatively correlated. In contrast, we found that larvae fed a mixed algal diet or only *A. anophagefferens* showed a significant positive correlation between lipid stores and size, suggesting that some larvae were simply better at obtaining food and associated nutrients. Larval success likely depends on a complex interplay between genetic and environmental factors. Our study suggests that poor nutrition associated with a harmful alga can have negative effects on larval size and lipids stores, which in turn are mediated by the inter-individual variability in the ability to grow and accumulate necessary lipid stores. Phytoplankton quality is likely to be important for the sustainability of bivalve populations even when it primarily impacts the larval phase; and a diet of brown tide algae may have lasting legacies for juveniles and adults.

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#### A NOVEL FEEDING METHOD IN COMMERCIAL BAKER'S YEAST PRODUCTION

J. Zamani, P. Pournia and H.A. Seirafi-2008

Journal of Applied Microbiology 105 (3): 674 – 680

##### Abstract:

Aims: The aim of this investigation was to determine a better leavening ability and shelf life for the same biomass yield of final product.

Methods and Results: A commercial fed-batch bioreactor equipped with circulation loop was used to study the effect of carbon source, molasses, profile on dough-leavening ability, shelf life and biomass yield of Baker's yeast, *Saccharomyces cerevisiae*. A set of 32 commercial batches were performed to investigate the effect of sugar concentration and compare with 32 control experiments.

Conclusions: Higher local sugar concentration in circulation loop resulted in a better leavening ability and shelf life for the same biomass yield of the final product. In addition, this method improved nitrogen assimilation which resulted in higher protein content. Increase in leavening ability and protein content could be a result of the higher levels of glycolytic enzymes.

Significance and Impact of the Study: It was observed that this change resulted in considerable improvement in leavening ability and shelf life at a commercial scale. It must be emphasized that to improve product quality, it is not necessary to pursue classical mutagenesis and selection strategies. A high-quality product can be achieved only by optimizing the feeding profile and strategy.

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