#### **REVIEW ARTICLE**

PREBIOTICS IN AQUACULTURE: A REVIEW

E. Ringø, R.E. Olsen, T. Ø. Gifstad, R.A. Dalmo, H. Amlund, G.-I. Hemre, A.M. Bakke-2010

Aquaculture Nutrition 16(2): 117 - 136

Abstract:

A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon. Despite the potential benefits to health and performance as noted in various terrestrial animals, the use of prebiotics in the farming of fish and shellfish has been less investigated. The studies of prebiotics in fish and shellfish have investigated the following parameters: effect on growth, feed conversion, gut microbiota, cell damage/morphology, resistance against pathogenic bacteria and innate immune parameters such as alternative complement activity (ACH50), lysozyme activity, natural haemagglutination activity, respiratory burst, superoxide dismutase activity and phagocytic activity. This review discusses the results from these studies and the methods used. If the use of prebiotics leads to health responses becoming more clearly manifested in fish and shellfish, then prebiotics might have the potential to increase the efficiency and sustainability of aquaculture production. However, large gaps of knowledge exist. To fully conclude on the effects of adding prebiotics in fish diets, more research efforts are needed to provide the aquaculture industry, the scientific community, the regulatory bodies and the general public with the necessary information and tools.

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INFLUENCE OF FORAGE FISH AND DIETARY LIPID SUPPLEMENTS ON EGG QUALITY AND FRY PRODUCTION IN CHANNEL CATFISH (ICTALURUS PUNCTATUS)  $\times$  BLUE CATFISH (ICTALURUS FURCATUS) HYBRIDIZATION

E.R. Durland, H.E. Quintero, D.A. Davis, R.A. Dunham-2010

Aquaculture Nutrition 16(2): 153 - 162

Abstract:

Hybrid catfish (channel catfish Ictalurus punctatus  $\times$  blue catfish Ictalurus furcatus) display characteristics that are favourable to aquaculture production. Low hatch percentages are a principal reason this hybrid is not used widely in the catfish industry. This study was conducted to determine whether additional food source rich in lipids may lead to a higher quality egg production. A 10-week feed trial was conducted in ponds in Auburn, AL. A total of 219 female Kansas Select channel catfish were stocked into nine ponds, 0.04 ha in size. Three dietary treatments were randomly allocated to the ponds. Diet-1 was a standard 60 g kg1 lipid floating catfish feed. Diet -2 was the same feed supplemented with forage fish at ~28 kg ha-1. The third diet was the aforementioned catfish feed topcoated with 20 g kg1 lipid [10 g kg-1 menhaden fish oil, 5 g kg-1 high docosahexaenoic aci d (DHA) oil and 5 g kg1 high arachidonic acid oil]. Results indicate that brood fish fed the high lipid diet spawned larger egg masses and had larger eggs both in weight and in diameter, with increased complements of fatty acids such as DHA, eicosapentaenoic acid and total n-3 fatty acids. The neutral and polar lipid fractions are also presented.

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SOYBEANS HEAT-TREATED BY DIFFERENT METHODS IN DIETS FOR GOLDFISH (CARASSIUS AURATUS) FRY

Vivek Rohidas Vartak, Ravendra Kumar Singh-2010 The Israeli Journal of Aquaculture - Bamidgeh 62(2), 2010, 72-77

Abstract:

The objective of this study was to determine the effect of diets supplemented with soybean meal processed by different heat treatments on growth, survival, and body composition of goldfish (Carassius auratus) fry. The fry  $(5.25\pm0.27 \text{ cm})$  were fed isonitrogenous (40%) diets containing soybean meal for 60 days. The first treatment contained soybeans toasted in a pan for 10 min. The second and third treatments contained soybeans oven-heated for 30 and 60 min, respectively. The fourth treatment contained raw soybeans. There were no significant differences (p>0.05) in survival between treatments. There was a significant increase in specific growth rate, protein efficiency ratio, and food conversion ratio with the diet containing soybeans oven-heated for 60 min. No significant differences were detected in body composition among the four treatments.

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#### EFFECT OF LOW SALINITY ON YOLK SAC ABSORPTION AND ALEVIN WET WEIGHT OF RAINBOW TROUT LARVAE (ONCORHYNCHUS MYKISS) Nadir Başçınar-2010

The Israeli Journal of Aquaculture - Bamidgeh 62(2): 116-121 Abstract:

Growth, yolk sac absorption, and maximum wet weight of alevin rainbow trout (Oncorhynchus mykiss) stocked in fresh water (0 salinity), water with 4‰ salinity, or water with 8‰ salinity were determined. Larvae were sampled on the 329th degree-day when 50% of the eggs hatched, and on degree-days 361, 396, 432, 467, 496, 528, 557, and 584. Sampled larvae were anesthetized in benzocaine solution (20 mg/l) and preserved in 10% formaldehyde. At hatching, mean length was  $14.25\pm0.63$  mm and total wet weight was  $58.16\pm9.98$  mg. Larvae reached the swim-up stage at  $96.94\pm8.71$  mg on the 28th day in fresh water, at  $120.29\pm9.26$  mg on 19th day in 4‰ salinity, and at  $102.80\pm5.88$  mg on 22nd day in 8‰ salinity. The total length and dry weight of the larvae at the swim-up stage differed significantly among treatments (p<0.001). There was a significant relationship between larvae dry weight and degree-day. The best growth, yolk sac consumption, and alevin wet weight were obtained in 4‰ salinity.

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THERMAL DYNAMICS OF OVARIAN MATURATION IN ATLANTIC COD (GADUS MORHUA) Olav Sigurd Kjesbu, David Righton, Maria Krüger-Johnsen, Anders Thorsen, Kathrine Michalsen, Merete Fonn, Peter R. Witthames-2010

Can. J. Fish. Aquat. Sci. 67(4): 605–625

Abstract:

The timing and success of spawning in marine fish are of fundamental importance to population persistence and distribution and, for commercial species, sustainability. Their physiological processes of reproduction are regulated, in part, by water temperature, and therefore changes in marine climate may have dramatic effects on spawning performance. Using adult Atlantic cod (Gadus morhua) as a case study, we examined the links between water temperature, body size, vitellogenesis, and spawning time by conducting extensive laboratory and field studies. Our experiments documented that vitellogenesis generally starts at autumnal equinox and that oocyte growth and investment are greater in cod held at warmer temperatures. Furthermore, spawning occurred earlier when oocyte growth was more rapid. Large females spawned earlier than smaller females at warmer temperatures, but this effect vanished at colder temperatures. The experimental results were confirmed by measurements of oocyte growth collected from wild-caught cod in northern (Barents Sea) and southern (Irish Sea and North Sea) populations. The established, general model of oocyte maturation was consistent with published egg production curves of cod from these waters, considering relevant in situ temperatures recorded by individual data-storage tags on cod. These findings have considerable relevance for future studies of fish recruitment in relation to climate change.

# GOBAL BIODIVERSITY AND GEOGRAPHICAL DISTRIBUTION OF DIAPAUSING AQUATIC INVERTEBRATES: THE CASE OF THE COSMOPOLITAN BRINE SHRIMP, ARTEMIA (BRANCHIOPODA, ANOSTRACA).

Muñoz J., Pacios F.-2010

Crustaceana 83(4)

The genus Artemia comprises passively dispersd anostracan species with a distribution all around the world, except in Antarctica. We used both published and personal data to assess and update existing knowledge on the diversity and distribution of Artemia, in particular compiling also genetic and geographic information. Our results indicate there are three Artemia complexes, A. franciscana, A. tibetiana and A. salina, suggesting at least three undescribed, and one unidentified to date, highly isolated lineages, to be re-evaluated taxonomically. Additionally, at a global scale, our data set shows two large, poorly explored geographic regions in Central East Asia, which in future studies could provide interesting information on geographic speciation, the origin of parthenogenesis, and range expansion in this group. We also discuss the implications for conservation as derived from knowledge on the biodiversity (native and invasive species) and geographic distribution (i.e., identification of species/lineages, and regions occupied), which have major relevance for conservation management at the level of wetland ecosystems.

# BEHAVIORAL RESPONSES TO LIGHT GRADIENTS, OLFACTORY CUES, AND PREY IN LARVAE OF TWO NORTH PACIFIC GADIDS (GADUS MACROCEPHALUS AND THERAGRA CHALCOGRAMMA)

Amanda R. Colton, Thomas P. Hurst-2010 Environmental Biology of Fishes 88(1): 39-49 Abstract:

The growth and survival of larvae can be significantly enhanced through close association with patches of high prey concentration. However, the taxis and kinesis responses used by larvae to locate and maintain residence in micro-patches remains poorly understood. In this study, the behavioral responses of Pacific cod (Gadus macrocephalus) and walleye pollock (Theragra chalcogramma) larvae (45–100 dph) to light, prey scent, and prey were examined. Both species displayed an ontogenetic shift in response to a horizontal light gradient, with small larvae (11–13 mm SL) exhibiting a positive phototaxis and large larvae (23–32 mm SL) exhibiting a negative phototaxis. Whether this reversal is related to ontogenetically appropriate foraging cues or some other aspect of the environment remains to be determined. Neither species displayed significant behavioral responses to the introduction of olfactory prey cues at either size. The aggregating (taxis) response of large larvae to introduction of live prey was stronger than that of small larvae, possibly due to increased reaction distances and encounter rates. In addition, both species exhibited a kinesis response of reducing the frequency of swimming bouts in response to introduction of live prey. These results suggest that the scale of prey patchiness and the physical factors that determine patch encounter rates are a significant determinant of larval growth and survival in the early feeding stages of marine fishes.

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#### NOVEL METHODOLOGIES IN MARINE FISH LARVAL NUTRITION

Luis E. C. Conceição, Cláudia Aragão, Nadège Richard, Sofia Engrola, Paulo Gavaia, Sara Mira, Jorge Dias-2010

Fish Physiology and Biochemistry 36(1): 1-16

Abstract:

Major gaps in knowledge on fish larval nutritional requirements still remain. Small larval size, and difficulties in acceptance of inert microdiets, makes progress slow and cumbersome. This lack of knowledge in fish larval nutritional requirements is one of the causes of high mortalities and quality problems commonly observed in marine larviculture. In recent years, several novel methodologies have contributed to significant progress in fish larval nutrition. Others are emerging and are likely to bring further insight into larval nutritional physiology and requirements. This paper reviews a range of new

tools and some examples of their present use, as well as potential future applications in the study of fish larvae nutrition. Tube-feeding and incorporation into Artemia of 14C-amino acids and lipids allowed studying Artemia intake, digestion and absorption and utilisation of these nutrients. Diet selection by fish larvae has been studied with diets containing different natural stable isotope signatures or diets where different rare metal oxides were added. Mechanistic modelling has been used as a tool to integrate existing knowledge and reveal gaps, and also to better understand results obtained in tracer studies. Population genomics may assist in assessing genotype effects on nutritional requirements, by using progeny testing in fish reared in the same tanks, and also in identifying QTLs for larval stages. Functional genomics and proteomics enable the study of gene and protein expression under various dietary conditions, and thereby identify the metabolic pathways which are affected by a given nutrient. Promising results were obtained using the metabolic programming concept in early life to facilitate utilisation of certain nutrients at later stages. All together, these methodologies have made decisive contributions, and are expected to do even more in the near future, to build a knowledge basis for development of optimised diets and feeding regimes for different species of larval fish.

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#### DEVELOPMENT OF DIGESTIVE ENZYME ACTIVITY IN LARVAE OF SPOTTED SAND BASS PARALABRAX MACULATOFASCIATUS II: ELECTROPHORETIC ANALYSIS

C. A. Alvarez-González, F. J. Moyano-López, R. Civera-Cerecedo, V. Carrasco-Chávez, J. L. Ortíz-Galindo, H. Nolasco-Soria, D. Tovar-Ramírez, S. Dumas-2010

Fish Physiology and Biochemistry 36(1): 29-37

Abstract:

The activities of several digestive enzymes during larval development of the spotted sand bass (Paralabrax maculatofasciatus) were evaluated using electrophoretic techniques. The results show the presence of three isoforms of alkaline protease from day 2 after hatching (ah) and the early appearance of one pepsin-like band from day 12 ah onwards. In addition, two lipase bands first appeared on day 2 ah, and there was a change in the molecular weight of one band from day 15 ah onwards. Several  $\alpha$ -amylase isoforms were observed from hatching up to day 5 ah. These results indicate that the important digestive enzymes develop rapidly in these larvae, supporting the possibility of early weaning at day 12 ah using artificial diets.

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# VARIABILITY IN FATTY ACIDS OF TWO MARINE COPEPODS UPON CHANGING FOOD SUPPLY IN THE COASTAL UPWELLING ZONE OFF CHILE: IMPORTANCE OF THE PICOPLANKTON AND NANOPLANKTON FRACTIONS

Ruben Escribano, Claudia S. Péreza-2010

Journal of the Marine Biological Association of the United Kingdom 90:301-313 Abstract :

Fatty acids composition of two marine copepods, Acartia tonsa and Centropages brachiatus, and lipid profiles of natural food assemblages were studied during the austral summer 2006 at three upwelling sites in the coastal upwelling zone off Chile, along with oceanographic conditions. Fatty acids of food supply were assessed for the picoplankton, nanoplankton and microplankton size fractions. There were marked differences in upwelling conditions among locations, as well as in their food supply in terms of quantity and quality. Differences in fatty acid composition were also found, both among food assemblages and between copepod species. Essential polyunsaturated fatty acids (PUFA; linoleic acid) and monounsaturated fatty acids (MUFA; oleic acid) dominated the picoplankton and nanoplankton size fractions of food, and they were highly represented in both species of copepods, indicating these size fractions were the major contributors to their diet. These fatty acids can thus be considered as useful trophic markers for copepods. Variation in lipid profiles between species depended on sampling sites, whereas differences in lipid composition among sampling sites were attributed to distinct upwelling

conditions, which drive the changes in food quality, such that trophic response is highly dependent on food offer. Variation in fatty acids compositions of copepods may thus act as an indicator of upwelling variability. Our findings suggest that lipid transfer from primary producers to primary consumers can have a crucial role for carbon cycling in the marine food web, and that picoplankton and nanoplankton fractions are the key items of copepod diet in this upwelling system.

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ISOLATION AND CHARACTERIZATION OF TAIWANESE HETEROTROPHIC MICROALGAE: SCREENING OF STRAINS FOR DOCOSAHEXAENOIC ACID (DHA) PRODUCTION Huey-Lang Yang, Chung-Kuang Lu, Shu-Fen Chen, Young-Mao Chen, Yi-Min Chen-2010 Journal Marine Biotechnology 12(2): 173-185

Abstract:

Marine heterotrophic microalgal species which are potentially rich in docosahexaenoic acid (DHA, C22:6n-3) have been found in Taiwan; however, there was a lack of detailed analysis and characterization of these indigenous algae which is needed for the development of commercial applications. Hence, the objective of this study was to screen DHA-rich heterotrophic microalgae species indigenous to Taiwan for commercial purposes. Heterotrophic microalgae from a variety of marine habitats were isolated, cultivated, and then identified according to their 18S rRNA gene sequences and morphological characteristics. A comparison was made of their fatty acid profiles, fatty acid content, and amount of biomass. For the strain with highest DHA yield, the optimal growth conditions were determined in order to establish the best fermentation conditions for scale-up. In this study, 25 heterotrophic microalgal strains were successfully isolated from marine habitats around Taiwan. All of the isolated strains showed a close phylogenic relationship with the Thraustochytriaceae family according to their 18S rRNA gene sequences. GC/MS analysis discerned seven distinctive fatty acid profiles of these strains, with the production of eicosapentaenoic acid (C20:53) ranging from 0.02 to 2.61 mg E-1, and DHA ranging from 0.8 to 18.0 mg L-1. An Aurantiochytrium strain BL10 with high DHA production was subsequently chosen for further manipulation. Under optimal growth conditions it could produce up to 59.0 g of dry biomass per liter of culture, with dry biomass containing 73% total fatty acid and 29% DHA, revealing BL10 as an excellent source of microbial DHA. (Institute of Biotechnology, National Cheng Kung University, Tainan, Taiwan; email of Yi-Min Chen: cohens.tw@yahoo.com.tw)

### IDENTIFICATION AND EXPRESSION ANALYSIS OF GENES INVOLVED IN EARLY OVARY DEVELOPMENT IN DIPLOID GYNOGENETIC HYBRIDS OF RED CRUCIAN CARP $\times$ COMMON CARP

Dong Liu, Shaojun Liu, Cuiping You, Lin Chen, Zhen Liu, Liangguo Liu, Jing Wang, Yun Liu-2010 Journal Marine Biotechnology 12(2): 186-194

#### Abstract:

Diploid eggs of allotetraploid hybrids (red crucian carp $\bigcirc \times$  common carp  $\bigcirc$ ), when activated by UV irradiated sperm of scatter scale carp, can develop into diploid progenies without chromosome duplication treatment. Diploid progenies produce diploid eggs, which develop into diploid population by the same way. To understand the molecular mechanism underlying the production of diploid eggs by the diploid fish, we constructed a forward suppression subtractive hybridization complementary DNA (cDNA) library. The cDNAs from the ovary in proliferation phase were employed as the "tester," and those in growth phase were used as the "driver." Seventy-three cDNA clones that are specifically expressed in proliferation phase were detected by dot-blot hybridization. Sequencing analyses revealed that several of these cDNAs have high homologies to the known sequences in the NCBI database. Their encoded proteins include the protein preventing mitosis catastrophe (PMC), the signal recognition particle 9, the ATP-binding cassette transporter, the glucanase–xylanase fusion protein, and others. These genes were confirmed by reverse transcriptase–polymerase chain reaction. The expression profile of the PMC gene at different time points was analyzed by quantitative real-time polymerase chain reaction. The results indicated that the expression of this suppression subtractive hybridizationidentified gene changed during the time course, corresponding with the cellular phenomenon in the ovary development. Our studies provide insights into the molecular mechanism underlying the ovary development of diploid gynogenetic fish.

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EFFECT OF EARLY INTRODUCTION OF MICROENCAPSULATED DIET TO LARVAL ATLANTIC HALIBUT, HIPPOGLOSSUS HIPPOGLOSSUS L. ASSESSED BY MICROARRAY ANALYSIS

H. M. Murray, S. P. Lall, R. Rajaselvam, L. A. Boutilier, R. M. Flight, B. Blanchard, S. Colombo, V. Mohindra, M. Yúfera, S. E. Douglas-2010

Marine Biotechnology 12(2): 214-229

Abstract:

An experimental microdiet prepared using an internal gelation method was used to partially replace the traditional live feed (Artemia) for larval Atlantic halibut, Hippoglossus hippoglossus L. Three trials were conducted with microdiet introduced at 20, 32, and 43 days post first feeding and larvae were sampled at approximately 2, 13, 23, and 33 days after microdiet introduction in each trial. The success of feeding was assessed by morphometrics and histological analysis of gut contents. Microdiet particles were readily consumed after a period of adaptation and provided an adequate source of nutrients with no significant increase in mortality in the microdiet-fed group compared to the control group. However, growth was limited and there was an increased incidence of malpigmentation of the eye and skin. Subtle changes in underlying digestive and developmental physiology were revealed by microarray analysis of RNA from control and experimental fish given microdiet from day 20 post first feeding. Fifty-eight genes were differentially expressed over the four sampling times in the course of the trial and the 28 genes with annotated functions fell into five major categories: metabolism and biosynthesis, cell division and proliferation, protein trafficking, cell structure, and stress. Interestingly, several of these genes were involved in pigmentation and eye development, in agreement with the phenotypic abnormalities seen in the larvae.

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# THE ONTOGENETIC DEVELOPMENT OF THE DIGESTIVE TRACT AND ACCESSORY GLANDS OF STERLET (ACIPENSER RUTHENUS L.) LARVAE DURING ENDOGENOUS FEEDING

Arleta Wegner, Teresa Ostaszewska, Wojciech Rożek-2010

Reviews in Fish Biology and Fisheries 19(4): 431-444

Abstract:

The process of differentiation of digestive tract structures in the sterlet Acipenser ruthenus (L.) larvae was studied from hatching to the beginning of exogenous feeding [9 dph (day post hatching)] using histological procedures. On the day of hatching the digestive tract was closed and completely filled with nutrients (the yolk platelets) that were successively utilized during development. A liver primordium was present in the ventral region of the yolksac. The pancreas was observed on the 2 dph. At the same time, the mouth opening took place. Glandular and nonglandular stomach and anterior and intermediate intestine developed from the yolksac walls. Gastric glands became visible on the 7 dph. The primary intestine developed into the spiral intestine. At the moment of onset of exogenous feeding the yolk material was completely exhausted and there was not mixed feeding observed in sterlet larvae. The fish started exogenous feeding on the 9 dph, which was accompanied with evacuation of melanin plug. At the end of endogenous feeding the digestive tract of sterlet larvae was developed and functional, so they could properly utilize food.

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