

PREFACE LARVI'09 SPECIAL ISSUE

Aquaculture 315(1-2): 1

Peter Bossier, Olav Vadstein, Luis Conceição, guest editors

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

This special volume is comprised of selected invited papers presented at the plenary session of the 5th Fish & Shellfish Larviculture symposium LARVI'09 held at Ghent University, Belgium on September 7–10, 2009. The “LARVI” symposia (1991, 1995, 2001, 2005, and 2009) have now an established international reputation, and bring together researchers who are focussing their work on understanding the biology of larval stages and the challenge of reproducible production of high quality and robust larvae.

Also in LARVI'09 progress was reported, but lots remain to be discovered. This can only be achieved through the support of multi-disciplinary research. This need is reflected in the ongoing trends in larval research. Sophisticated tools are being developed and improved which can help test interesting hypotheses. This special issue brings together interesting research and review papers, which will hopefully contribute to a deepening of our knowledge and support the transfer of knowledge to the sector.

The Symposium contributions were divided into sections on maturation and spawning, developmental biology, nutritional studies, deformities, larviculture, microbiology and health management. The programme consisted of 46 oral and 109 poster presentations, of which 18 oral presentations are included in this issue of Aquaculture. Selected poster presentations are published in Aquaculture Research. The Symposium was preceded by a one day workshop on the outcome of the EU project “FineFish”. During LARVI'09 two workshops were held, namely “Backyard hatchery production of marine fish and shellfish” and “Larviculture — production constraints and research priorities”, the latter co-organised by the EU supported COST action “LARVANET”.

Conference details, including pdf files of most of the oral and the poster presentations can be found at <http://www.aquaculture.ugent.be/larvi/presentations.htm>.

LARVI'09 was co-organised by the Laboratory of Aquaculture & Artemia Reference Center of Ghent University (Belgium), the Norwegian University of Science and Technology (NTNU), Trondheim (Norway) and the COST action “LARVANET”. LARVI'09 was organised under the patronage of His Majesty Albert II, King of Belgium and was sponsored in part by VLIR-UOS (Flemish Interuniversity Council); Research Foundation — Flanders (FWO); Faculty of Bioscience Engineering, (Ghent University); Province of East Flanders (Belgium); and Norwegian University of Science and Technology, NTNU.

We are grateful to the referees who have assisted us in the peer-reviewing and final selection of the papers submitted by the invited speakers.

We like to dedicate this volume to the memory of Magda Vanhooren, who has been strongly involved in the organisation of the LARVI symposia from the very beginning, and unexpectedly passed away in June 2010.

(peter.bossier@ugent.be)

A REVIEW OF THE BROODSTOCK MANAGEMENT AND LARVICULTURE OF THE PACIFIC NORTHERN BLUEFIN TUNA IN JAPAN

Shukei Masuma, Takayuki Takebe, Yoshitaka Sakakura-2011

Aquaculture 315(1-2): 2-8

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

We reviewed research on the broodstock management and larviculture of the Pacific northern bluefin tuna (PBT) *Thunnus orientalis* in Japan. Japan has been at the forefront of PBT research since 1970 due to the participation by federal and prefectural governments and universities in a national project aimed

to optimize productivity of the sea around Japan. In 1979, scientists at Kinki University succeeded in the first spontaneous spawning in captivity by the broodstock of 5 year-old PBT. Successful spawning was also performed in 1980 and 1982, but no spawning then occurred until 1993, when Maruha Nichiro Holdings, Inc. (MNH) and Nippon Formula Feed Manufacturing Company, Ltd. (NFFMC) became involved in tuna farming and succeeded in the spawning of four-year-old broodstock. Since then, successful spawning of PBT in captivity has been reported from several sites as well as spawning in Kinki Univ. since 1994. With the successful spawning of PBT, the Fisheries Research Agency (FRA; formerly, Japan Sea Farming Association) of Kinki University, in conjunction with MNH, NFFMC, and Takuyo Ltd., has actively carried out research on and development of tuna larviculture technology. Thus, knowledge about broodstock management and larviculture has accumulated in Japan, but technical problems with larviculture still remain to be solved. There are 9 sites of successful spawning in net pens in Japan so far. At 4 of these sites in regions around Amami Island, yearly spawning has stably occurred. We have been accumulating data about the period of maturation, environmental key factors triggering the spawning, the age of onset of spawning, and the pattern of spawning through measurement of the ambient environment, gonad morphometry, endocrinology, mitochondrial DNA analysis, and daily careful observation of broodstock. Research on PBT larviculture at Kinki University, FRA, MNH, NFFMC, and Takuyo have succeeded in producing tens of thousands of hatchery-raised juveniles. As a result, Kinki University succeeded in establishing the full life cycle of PBT in captivity, and also achieved its aquaculture life cycle. However, solutions are needed for the remaining technological issues of PBT larviculture, including sinking syndrome, where larvae die on the bottom of the tank during the early phase of larviculture, the search for appropriate food (species, size, and nutrition) around the transition stage from larva to juvenile, cannibalism and collision against walls in the juvenile, and malformation and viral diseases in the young stage.
(Miyazu Station, National Center for Stock Enhancement, Fisheries Research Agency, Kyoto 626-0052, Japan; email of Yoshitaka Sakakura: sakakura@nagasaki-u.ac.jp)

BROODSTOCK MATURATION AND REPRODUCTION OF THE INDIGENOUS PINK SHRIMP *FARFANTEPENAEUS PAULENSIS* IN BRAZIL: AN UPDATED REVIEW ON RESEARCH AND DEVELOPMENT

Silvio Peixoto, Wilson Wasielesky, Ronaldo O. Cavalli-2011

Aquaculture 315(1-2): 9-15

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

The interest in culturing indigenous penaeid species has increased lately due to *Litopenaeus vannamei* disease outbreaks, especially in southern Brazil. Although sustainable production of the pink shrimp *Farfantepenaeus paulensis* is greatly desired, it has been constrained by the current dependency on wild-caught broodstock. To overcome this problem, research efforts have focused on the development of domesticated stocks and optimization of their reproductive performance under laboratory conditions. We present the latest developments on maturation and reproduction of this closed thelycum species, aiming to improve the future outlook for a sustainable nauplii production.

(Universidade Federal Rural de Pernambuco, Departamento de Pesca e Aqüicultura, 52171-900, Recife, PE, Brazil; email of Silvio Peixoto: silvio.peixoto@gmail.com)

LARVICULTURE TECHNIQUES OF CHINESE MITTEN CRAB *ERIOCHEIR SINENSIS*

Liyong Sui, Mathieu Wille, Yongxu Cheng, Xugan Wu, Patrick Sorgeloos-2011

Aquaculture 315(1-2): 16-19

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

Eriocheir sinensis is considered a luxury aquatic food for Chinese people due to its delicate flavor, and it therefore reaches a high market value. Its hatchery production and farming are being performed almost exclusively in China. Although breakthroughs in seed production and larval rearing techniques

of *E. sinensis* have been achieved in the early 1980s, a fast expansion of hatchery production only took off in the 1990s, with the dramatic decline in natural recruitment. Many techniques have only been published as brief descriptions of local farmers' experience and most of these articles were written in Chinese. This paper provides general information on the hatchery techniques in aspects of broodstock maturation, spawning and larval rearing and points out the main bottlenecks of current mitten crab hatchery operations.

(Tianjin Key Laboratory of Marine Resources and Chemistry, College of Marine Science and Engineering, Tianjin University of Science and Technology, Tianjin 300457, China; email of Liying Sui: suily@hotmail.com)

COBIA *RACHYCENTRON CANADUM* AQUACULTURE IN VIETNAM: RECENT DEVELOPMENTS AND PROSPECTS

Van Can Nhu, Huy Quang Nguyen, Thanh Luu Le^a, Mai Thien Tran, Patrick Sorgeloos, Kristof Dierckens, Helge Reinertsen, Elin Kjørsvik, Niels Svennevig-2011

Aquaculture 315(1-2): 20-25

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

The paper presents a review of the recent developments in research and production of cobia in Vietnam in hatching and cage farming, which have made Vietnam the 3rd largest producer of farmed cobia in the world. Conservative estimations for the 2007 production for the Asian-Pacific region exceed 35,000 t, with remaining global production adding an additional 2000 t, while official farm production registered by FAO is considerably lower. Estimated 2008 production in Vietnam was 1500 t, following the major production of PR China and Taiwan Province of China. This review reports on the various aspects of hatchery technology such as broodstock management, intensive and semi-intensive larval rearing, fry transportation as well as small-scale grow-out in wooden raft cages and large-scale in Norwegian style circular HDPE cages. Some of the prospects for accelerating future development of this species in aquaculture and challenges to be solved are also identified.

(Research Institute for Aquaculture No1. Dinh-bang, Tu-son, Bac-ninh, Vietnam; email of Patrick Sorgeloos: patrick.sorgeloos@UGent.be)

ANALYSIS OF THE MECHANISM OF SKELETAL DEFORMITY IN FISH LARVAE USING A VITAMIN A-INDUCED BONE DEFORMITY MODEL

Yutaka Haga, Shao-Jun Du, Shuichi Satoh, Tomonari Kotani, Hiroshi Fushimi, Toshio Takeuchi-2011

Aquaculture 315(1-2): 26-33

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

Vitamin A (VA) is an essential nutrient in fish. VA is involved in a large spectrum of biological processes. One of the most important functions of VA is to control embryonic development in animals. In mammals, organogenesis is completed during embryogenesis. In contrast, most marine fish larvae are in a comparatively immature state at hatching and undergo organogenesis during the exogenous feeding stage. This developmental feature of marine fish larvae requires appropriate control of the nutritional composition of the diet to support normal skeleton development. Nutrient deficiency or imbalance results in skeletal deformities that are often recognized in hatchery-reared fish. However, the etiology and precise mechanism of such skeletal deformities are unknown, which makes it difficult to achieve an effective prevention protocol in hatcheries. Skeletal deformities induced by excess VA are a popular model for studying the development of skeletons in fish larvae. Several studies suggest the importance of retinoic acid receptor (RAR) and retinoid X receptor (RXR) pathways in skeletogenesis in fish. This paper reviews the current understanding of VA-induced skeletal deformities and recent progress in this area and proposes future perspectives for model studies.

(Tokyo University of Marine Science and Technology, Minato, Tokyo 108-8477, Japan; email of Yutaka Haga: haga@kaiyodai.ac.jp)

THE EFFECT OF VITAMIN A ON FLATFISH DEVELOPMENT AND SKELETOGENESIS: A REVIEW

Ignacio Fernández, Enric Gisbert-2011

Aquaculture 315(1-2): 34-48

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

Flatfish can provide an excellent model to study developmental alterations of cartilage and bone tissues in response to vitamin A (VA), and therefore, with respect to the underlying morphogenesis, cell differentiation and proliferation processes, and gene expression regulation. Bone and cartilage development and homeostasis at different life stages (embryonic, larval, juvenile or adult stages) are primarily regulated by retinoic acid, the active metabolite of VA. Since fish are not able to synthesize VA, they have to obtain it from the diet. Thus, dietary VA imbalance may modify VA homeostasis, resulting in a disruption of gene expression, and cell proliferation and differentiation of skeletal tissues (bone and cartilage), leading to the appearance of skeletal deformities. These skeletal deformities are one of the most important factors that affect production efficiency and quality of flatfish aquaculture. However, different flatfish species present different developmental timing in skeletogenesis and consequently, different species may present different VA requirements. In the present work, special attention has been made to different experimental approaches to evaluate the effects of this morphogenetic nutrient on early morphogenesis (bath exposure vs. dietary dose-response nutritional essays), as well as the different retinoid forms in which VA was administered to larvae. We review the current state of knowledge on the effects of dietary VA content in flatfish development with an emphasis on the skeletal deformities induced by dietary VA imbalances.

(IRTA, Centre de Sant Carles de la Ràpita (IRTA-SCR), Unitat de Cultius Experimentals, Crta. del Poble Nou s/n, 43540-Sant Carles de la Ràpita, Spain; email of Ignacio Fernández: Ignacio.fernandez@irta.es)

REVIEW

OVERVIEW OF VITAMIN D AND C REQUIREMENTS IN FISH AND THEIR INFLUENCE ON THE SKELETAL SYSTEM

M.J. Darias, D. Mazurais, G. Koumoundouros, C.L. Cahu, J.L. Zambonino-Infante-2011

Aquaculture 315(1-2): 49-60

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

Vitamins D and C are essential in many physiological functions. Vitamin D, a fat soluble vitamin, is crucial to preserve calcium and phosphate homeostasis and to protect the skeletal integrity. This hormone functions through the vitamin D receptor (VDR) inducing the expression of various calcium binding and transport proteins in the intestine to stimulate active calcium uptake, thus preserving normocalcemia and, indirectly, maintaining bone mineralization. Besides, vitamin D also acts directly on osteoblasts, the resident bone-forming cells of the skeleton, to inhibit proliferation, modulate differentiation, and regulate mineralization of the extracellular matrix. Vitamin C, a water soluble vitamin, acts as a co-substrate for hydroxylase and oxygenase enzymes involved in the biosynthesis of pro-collagen, carnitine and neurotransmitters, among other numerous physiological functions such as antioxidant or pro-oxidant. Both vitamins should be supplied by the diet because fish are unable to synthesize them. However, their wide range of action makes it difficult to adjust the adequate amount of these vitamins to achieve an optimal fish performance. Besides, the dietary vitamin needs of fish depend on several factors such as developmental stage, physiological, environmental/ecological and genetic conditions. In this sense, vitamin requirements of flatfish do not necessarily meet those of pelagic fish and depends also on their feeding habits (carnivorous, planktivorous or detritivorous); the dietary vitamin demands of an adult fish differ from those of a larva; and even within the same fish species and developmental stage, the environmental conditions would also influence the vitamin needs

(i.e., under stress conditions, high vitamin C levels have been demonstrated to improve stress resistance and, consequently, growth).

The present paper gives a general overview about the requirements of vitamins D and C in fish and specifically reviews the role of these vitamins in fish skeletogenesis and their influence in the development of skeletal deformities. In addition, new insights on the molecular pathways involving these vitamins in the skeletal ossification process are provided.

(Ifremer Marine Fish Nutrition Team, Nutrition Aquaculture and Genomics Research Unit, UMR 1067, Ifremer, Technopole Brest-Iroise, BP 70, 29280 Plouzané, France; email of M.J. Darias: maria.darias@irta.cat)

PLOIDY EFFECTS ON HATCHERY SURVIVAL, DEFORMITIES, AND PERFORMANCE IN ATLANTIC SALMON (*SALMO SALAR*)

J.F. Taylor, A.C. Preston^a, D. Guy, H. Migaud-2011

Aquaculture 315(1-2): 61-68

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

The production of sterile triploid Atlantic salmon (*Salmo salar*) may help address the increasing pressure on the industry to reduce potential breeding between farmed escapees and wild fish populations. However, many previous studies have observed poor performance at sea in triploid stocks (growth, survival, and deformity). This may result from poor early hatchery performance and a strong parental effect. Therefore, in the present study, two year classes (2007 and 2008) of mixed sex fish were created (10 males:10 females) to examine ploidy interactions on hatchery performance. Egg batches were divided in two at fertilisation with one group subjected to a hydrostatic pressure shock to induce triploidy. Triploid rate was confirmed at 100% in all groups, verified by red blood cell nucleus length measurements. Survival to hatch did not differ between ploidy. However, reduced survival was found to strongly correlate with gamete quality. During the hatchery phase ploidy significantly affected size at hatch, with diploids generally larger than triploids. Growth advantage of diploids over triploids was only maintained for 6 weeks post-first feeding, with triploids generally out-growing their diploid siblings by the end of the hatchery phase. Deformity prevalence in first feeding stages was generally low (mean < 2%), with no overall effect of ploidy. Our findings show that triploid salmon can perform as well if not better than their diploid siblings. The low incidence of deformity during the hatchery and freshwater phases is also a significant improvement over previous reports in triploid salmon stocks.

(Institute of Aquaculture, University of Stirling, Stirling, Scotland, UK; email of J.F. Taylor: jft2@stir.ac.uk)

APPLICATION OF THE SELF-ORGANIZING MAP TO THE STUDY OF SKELETAL ANOMALIES IN AQUACULTURE: THE CASE OF DUSKY GROUPER (*EPINEPHELUS MARGINATUS* LOWE, 1834) JUVENILES REARED UNDER DIFFERENT REARING CONDITIONS

T. Russo, M. Scardi, C. Boglione, S. Cataudella-2011

Aquaculture 315(1-2): 69-77

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

The setting up of effective rearing protocols for domestication of new candidate species for aquaculture and/or to enhance quality in widely reared species requires the availability of appropriate tools to detect patterns of covariation among rearing parameters and fish quality. In this framework, the pattern of occurrence of skeletal anomalies (SAs) in reared lots represents a proxy for quality, since the presence of SAs is associated with both a general lowering of performance and a negative image of aquaculture products in consumers. In this paper we explore the advantages of using Self-Organized Maps (SOMs) when dealing with the analysis of correlations between the pattern of SAs presence and rearing parameters in dusky grouper (*Epinephelus marginatus*, Lowe 1834) lots following two different

experimental rearing approaches. SOMs were tested because the classic multivariate approach failed to produce meaningful results in the same dataset. A SOM was trained on a dataset containing the mean frequencies of 43 SAs occurring in 20 lots of dusky grouper sampled during three larval rearing cycles carried out in 2001, 2002 and 2004 in Italy, using two rearing approach: Green Water and Large Volume. A series of well-defined patterns were detected in SAs occurrence with respect to body regions. When SOM units were grouped into three clusters, a significant relationship was detected between lot origin (in terms of rearing approach) and SAs occurrence: The Large Volume methodology is to be considered more effective in enhancing the quality of dusky grouper larvae. This finding was independently validated by the superimposition of Hellinger distances obtained from the analysis of meristic counts. Finally, SOM visualized coherent and clear patterns of covariation between SAs occurrence and two crucial aspects of rearing: initial rearing density and final survival rate. We concluded that as a new ordination method SOMs afford effective representations of information gathered from patterns of SAs occurrence in aquaculture lots. Furthermore, SOM appears able to detect subtle but meaningful relationships between quality, as measured by independent descriptors such as SAs and meristic counts, and rearing parameters. It could be useful for quality assessment in both experimental and productive contexts, ultimately helping to reduce the incidence of SAs in aquaculture products and facilitating the identification of more effective approaches to the domestication of new species.

(Experimental Ecology and Aquaculture Laboratory — Department of Biology, University of Rome 'Tor Vergata', Via della Ricerca Scientifica, 00133 Rome (RM), Italy; email of T. Russo: tommaso.russo@uniroma2.it)

THE INFLUENCE OF INITIAL FEEDING ON MUSCLE DEVELOPMENT AND GROWTH IN PACU *PIARACTUS MESOPOTAMICUS* LARVAE

Natalia de Jesus Leitão, Maeli Dal Pai-Silva, Fernanda Losi Alves de Almeida, Maria Célia Portella-2011

Aquaculture 315(1-2): 78-85

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

The effects of different feeding schemes on pacu *Piaractus mesopotamicus* early development were evaluated with respect to growth, survival, muscle development, and differential gene expression of MyoD and myogenin. The pacu larvae (4 days post hatch-dph, 0.77 mg wet weight) were given six feeding treatments intentionally designed to cause variations in the larvae growth rate: (A) only artemia nauplii; (CD) only a commercial diet; (ED) only a semi-purified experimental diet; (ACD) and (AED) two treatments that involved weaning; and (S) starvation. Early weaning from artemia nauplii to the formulated diets (ACD and AED) affected growth and survival of the pacu larvae compared with the exclusive use of artemia (A). Starvation (S) and the commercial diet (CD) caused total mortality in pacu larvae at 18 dph. The experimental diet (ED) assured low fish survival and growth. The skeletal muscle morphology was not affected by the delay in somatic growth from early weaning onto the formulated diets. Three distinct muscle compartments were observed throughout the larval development in treatments A, ACD and AED: superficial, deep and intermediate, accompanied by muscle thickening. Severe undernourishment caused drastic differences in growth and in the morphology of the muscle fibers. Pacu larvae fed only formulated diets (CD and ED) showed muscle characteristics similar to the larvae in starvation (S) during the first 15 dph. At 27 and 35 dph, a slight increase in epaxial muscle mass was noted in larvae fed only the experimental diet (ED). At 35 dph, we observed a high frequency of fibers $\geq 40 \mu\text{m}$ in the larvae that were weaned onto the formulated diets (ACD and AED), indicative of hypertrophy. In contrast, the larvae fed only artemia nauplii (A) displayed a larger number of fibers with diameters $\leq 20 \mu\text{m}$, which is indicative of hyperplasia. The expression of the MyoD and myogenin genes in pacu larvae at 35 dph was not affected by initial feeding ($p > 0.05$). In conclusion, the formulated diets used impaired pacu larvae growth and survival; therefore, they were inadequate for pacu, at least at the times they were introduced. Artemia nauplii were the most adequate food source during first feeding of the pacu, and they produced bigger fish upon completion of the experiment.

Moreover, the contribution of hyperplasia to the skeletal muscle growth appeared higher in fast- than in slow-growing pacu larvae.

(São Paulo State University, Faculty of Agricultural and Veterinarian Sciences, 14884-900 Jaboticabal, SP, Brazil; email of Natalia de Jesus Leitão: nati_leitao@yahoo.com.br)

EFFECTS OF LIGHT DURING EARLY LARVAL DEVELOPMENT OF SOME AQUACULTURED TELEOSTS: A REVIEW

Aquaculture 315(1-2): 86-94

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

N. Villamizar, B. Blanco-Vives, H. Migaud, A. Davie, S. Carboni, F.J. Sánchez-Vázquez-2011

Abstract:

Light is a key environmental factor that synchronizes all life-stages of fish, from embryo development to sexual maturation. The underwater photo-environment is complex since light characteristics (i.e. intensity, photoperiod and spectrum) depend on the absorbance properties of the water column. The aim of this paper is to review the effects of artificial lighting conditions on the performance, development and welfare of some fish larvae of commercial interest. Reviewed results show that larvae were significantly affected by light characteristics. For example, European sea bass and sole larvae achieved the best performance, and showed fastest development and lowest degree of deformity under a light/dark cycle using blue light (half-peak bandwidth = 435–500 nm), conditions which were the closest to their natural aquatic environment. However, constant light (LL) or constant darkness (DD) was shown to negatively affect normal larval development and resulted in increased malformations and poor survival in most of the studied species. Similar results have been observed in other fish larvae such as Atlantic cod, which performed better under short wavelengths (blue and green). These findings highlight the role of lighting conditions during the early development of fish larvae and should be taken into account for the optimization of rearing protocols in fish hatcheries as juvenile supply is one of the main production bottlenecks.

(Department of Physiology, Faculty of Biology, University of Murcia, Campus Espinardo, 30100-Murcia, Spain; email of F.J. Sánchez-Vázquez: javisan@um.es)

NATURALLY-OCCURRING STABLE ISOTOPES AS DIRECT MEASURES OF LARVAL FEEDING EFFICIENCY, NUTRIENT INCORPORATION AND TURNOVER

Lewis Le Vay, Julián Gamboa-Delgado-2011

Aquaculture 315(1-2): 95-103

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

Stable isotopes are non-hazardous markers that have been widely-used in assessing energy flow within aquatic ecosystems. Hatchery systems are also highly amenable to this approach, as they represent controlled mesocosms with a limited number of food sources and short planktonic food chains with rapid and measurable bioaccumulation of the heavier stable isotopes of carbon and nitrogen at each trophic step. Differences in the natural isotopic composition of dietary components may be used to provide direct integrated measures of ingestion, nutrient incorporation and growth through development under normal feeding and environmental conditions, in either the laboratory or the hatchery. Simple isotopic mixing models allow estimation of relative utilisation of inert diets and live feeds, and individual components of compound feeds. Such experiments have investigated the effectiveness of co-feeding regimes, optimal timing of live food transitions (e.g. from rotifers to *Artemia*), presentation of inert diets, optimal size/age for weaning and incorporation of specific dietary components. Furthermore, time series measurement of changes in tissue isotopic signature ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) enables modelling of growth dilution and tissue turnover components of isotopic change driven by nutritional sources. These measures need to take into account the difference in isotope values that is typically observed between the diet and consumer (isotopic discrimination factor, Δ). In marine larvae and early postlarvae, $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ have been found to range widely, from 0.4–4.1‰ and 0.1–5.3‰ respectively. The observation

of such a high level of variation within species and life stages indicates a strong effect of diet quality on isotopic discrimination. Elucidating mechanisms underlying such observations, and much greater resolution in larval nutritional studies, can be achieved by application of rapidly-developing techniques for compound specific stable isotope analysis in tracing the transfer of dietary sources of carbon and nitrogen into tissue components. Fast growing aquatic larvae represent excellent model organisms exhibiting rapid transitions in isotopic composition in response to diet, rapidly-changing feeding behaviour and transitions in trophic level with ready ingestion of modifiable experimental diets in short and controlled food chains. Thus results of studies of the effects of diet composition, developmental stage, growth rates or environmental conditions on stable isotope incorporation will be of broad relevance not only in terms of larval nutrition but can also more broadly inform the design and interpretation of ecological studies.

(School of Ocean Sciences, College of Natural Sciences, Bangor University, Menai Bridge, Anglesey, Wales, LL59 5AB, United Kingdom; email of Lewis Le Vay: llevay@bangor.ac.uk)

REVIEW

DETERMINATION OF APPROPRIATE FEEDING SCHEDULES FROM DIEL FEEDING RHYTHMS IN FINFISH LARVICULTURE

Tomonari Kotani, Hiroshi Fushimi-2011

Aquaculture 315(1-2): 104-113

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

In finfish larviculture, feeding regimes and schedules vary with hatchery and species. They have no biological or technological foundation and are dependent of operator expediency and previous experience. Inadequate feeding regimes and/or inappropriate food intake, especially during early larval stages, may result in a decline in health and/or quality and high mortalities. Fish have species-specific diel feeding rhythms; therefore, feeding schedules in larviculture cannot be determined uniformly among target species. To improve survival and quality, it is important to establish feeding schedules corresponding to the diel feeding rhythms of larval fish species. In fish, the feeding and circadian rhythms are linked; most species have diurnal feeding peaks, especially at dusk and dawn, such as flat fishes, groupers, sparids, devil stinger *Inimicus japonicas* and ayu *Plecoglossus altivelis*. These fish do not ingest food at night and the larvae actively feed at dawn and dusk. Differences in this trend have not been reported during the co-feeding period of rotifer and *Artemia* nauplii. Although ocellate puffer larvae also have diurnal peaks of food intake, they commenced ingestion before day break.

The delay in first feeding causes serious problems in finfish larviculture and in many cases, it is performed to prevent this delay. After mouth opening, larvae do not have a well-developed functional jaw and, thus, rotifers or *Artemia* nauplii remain in the rearing water. They metabolize the enriched nutrients, resulting in deterioration in the nutritional quality of the residual live food. Therefore, the timing of first feeding is important.

In conclusion, the feeding schedule of a particular fish species should be determined on active diel ingestion. Delays in active ingestion result in un-ingested live food remaining in the rearing water and consequent nutritional deterioration. Therefore, feeding schedules in finfish larvae and juveniles should be matched to the peak of food intake.

(Department of Marine Biotechnology, Faculty of Life Science and Biotechnology, Fukuyama University, 452-10 Innoshima-Ohama, Onomichi, Hiroshima 722-2101, Japan; email of Tomonari Kotani: tkotani@ma.fuma.fukuyama-u.ac.jp)

THE INFLUENCE OF DIETARY CONSTITUENTS ON THE MOLECULAR ONTOGENY OF DIGESTIVE CAPABILITY AND EFFECTS ON GROWTH AND APPETITE IN ATLANTIC COD LARVAE (*GADUS MORHUA*)

Trond M. Kortner, Ingrid Overrein, Gunvor Øie, Elin Kjørsvik, Augustine Arukwe-2011

Aquaculture 315(1-2): 114-120

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

Development of optimal nutrition feeding protocols is considered a major challenge in intensive marine fish larval rearing. There is a general lack of knowledge on the molecular basis underlying larval digestive capability and endocrine function, and relatively few studies have employed molecular biological techniques to study the ontogeny of digestive capability and effects of nutrients on growth and appetite of marine fish larvae. In this study, Atlantic cod larvae were fed either *Acartia tonsa* nauplii, rotifers grown on *Rhodomonas baltica* or a control rotifer diet, before weaning to formulated diet. Larvae were sampled at 5, 8, 16, 29 and 38 days post hatch. Larval dry weight and specific growth rate were measured, and gene expression patterns of trypsin, amylase, bile salt-activated lipase, phospholipase A2, Acyl CoA dehydrogenase, neuropeptide Y, cholecystokinin, growth hormone and thyroid hormone receptors α and β determined by quantitative PCR. We show that transcripts involved in appetite regulation and digestion were differentially expressed after different feeding protocols. Interestingly, several similar transcript profiles for genes coding for digestive enzymes and neural controlling factors were demonstrated. In addition, these relationships were partially reflected in larval specific growth rates, demonstrating for the first time in cod, that the ontogeny of digestive capability and its hormonal components may be tied directly to the type and quality of initial and early dietary constituents.

(Department of Biology, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway; email of Augustine Arukwe: arukwe@bio.ntnu.no)

OBSERVATIONS ON FEEDING AND BIOCHEMICAL CHARACTERISTICS TO IMPROVE LARVICULTURE OF ROBSONELLA FONTANIANA (CEPHALOPODA: OCTOPODIDAE)

Iker Uriarte, Ana Fariás, Kurt Paschke, Juan Carlos Navarro, Carlos Rosas-2011

Aquaculture 315(1-2): 121-124

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

At present, the production of merobenthic octopuses with a post-eclosion planktonic phase is a great challenge for global aquaculture. So far, juveniles from only four species have been reported to have been obtained under controlled rearing conditions: *Octopus vulgaris*, *Octopus joubini*, *Enteroctopus dofleini* and *Robsonella fontaniana*, the only Chilean species. *R. fontaniana* (Orbigny, 1834) is a small-sized octopus traded in international markets as “baby octopus”. It is distributed over nearly the entire southern coast of South America, both in the Pacific and Atlantic coasts. This study shows new results regarding the biochemical characteristics in embryonic and paralarval stages, as well as the characteristics of food consumption during the paralarval stage that allow us to discuss a baseline on which to define a strategy for the larviculture of this species.

(Instituto de Acuicultura, Universidad Austral de Chile, P.O. Box 1327, Puerto Montt, Chile; email of Iker Uriarte: juriarte@uach.cl)

ONSET OF THE PRIMARY STRESS IN EUROPEAN SEA BASS *DICENTRARCHUS LABRAX*, AS INDICATED BY WHOLE BODY CORTISOL IN RELATION TO GLUCOCORTICOID RECEPTOR DURING EARLY DEVELOPMENT

M. Pavlidis, E. Karantzali, E. Fanouraki, C. Barsakis, S. Kollias, N. Papandroulakis-2011

Aquaculture 315(1-2): 125-130

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

The temporal patterns of whole body cortisol, the onset of the primary stress response and mRNA abundance of the two glucocorticoid receptors isoforms during early ontogeny was investigated for the first time in the European sea bass, *Dicentrarchus labrax*. The ontogenetic profile of basal cortisol

content was similar to that observed in other pelagic marine species, with minimum values at hatching ($0.12 \pm 0.01 \text{ ng g}^{-1}$), a slight increase at first feeding ($2.44 \pm 0.71 \text{ ng g}^{-1}$) and a first peak at flexion ($47.91 \pm 6.72 \text{ ng g}^{-1}$). However, basal cortisol content at flexion and at the more advanced developmental stages was one of the highest reported for fish. Exposure to a physical stressor (high currents) increased whole body cortisol above control levels at all developmental stages examined (first feeding, pre-larvae, flexion, post flexion, end of metamorphosis and juveniles). However, the magnitude of the cortisol response was low at first feeding and high in juvenile fish. There was a greater number of GR-1 than GR-2 transcripts levels in embryos, pre-larvae and larvae of European sea bass. GR-1 transcripts showed minimum mRNA abundance in embryos and at post flexion and maximum at the end of metamorphosis and in juveniles. The mRNA transcript levels of GR-2 showed a slight increase at hatch to reach a peak in post flexion larvae and in juveniles but these differences were not statistically significant. These results indicate that the onset of cortisol production in European sea bass is near the transition from endogenous to exogenous feeding and show that fish are capable of a stress-induced stimulation of cortisol production even at first feeding.

(University of Crete, Department of Biology P.O. Box 2208, GR-714 09, Heraklion, Crete, Greece; email of M. Pavlidis: pavlidis@biology.uoc.gr)

CHARACTERIZATION AND MODULATION OF GENE EXPRESSION AND ENZYMATIC ACTIVITY OF DELTA-6 DESATURASE IN TELEOSTS: A REVIEW

M. Vagner, E. Santigosa-2011

Aquaculture 315(1-2): 131-143

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

There is currently considerable interest in understanding how the biosynthetic pathways of highly unsaturated fatty acids (HUFA) are regulated in fish. The aim is to know if it is possible to replace fish oils (FO), rich in HUFA, by vegetable oils (VO), poor in HUFA and rich in their 18 carbon fatty acid precursors, in the feed of cultured fish species of commercial importance. Thus many studies have focussed on delta-6 desaturase ($\Delta 6D$) since it is the rate-limiting enzyme involved in HUFA biosynthesis from precursors. The aims of this paper were (i) to review and compare the structure, function, and tissue distribution of the $\Delta 6D$ gene in teleosts and (ii) to review the effect of nutrition and environment on the modulation of $\Delta 6D$ gene expression and on the activity of this enzyme in teleosts. Most existing studies have clearly shown that $\Delta 6D$ is modulated by nutrition and environment in freshwater fish. This modulation allows the control of lipid metabolism and the maintenance of cell membrane functionality. $\Delta 6D$ gene expression and enzymatic activity were higher in fish fed VO diets than in those fed FO diets, irrespective of their life cycle in seawater or freshwater; this concurs with expectations regarding the compensation for HUFA deficiency in VO. However, the magnitude of these increases was not great enough to maintain HUFA tissue content. Such a decrease in tissue content may result either from competition between substrates for $\Delta 6D$ or from the inhibition of subsequent steps in HUFA biosynthesis, such as elongation or delta-5 desaturation activity. Other studies showed that $\Delta 6D$ enzyme activity is stimulated at low temperatures and low salinities, which keep cell membranes fluid. In salmonids, $\Delta 6D$ would then play an important role in the adaptation to salinity changes at parr-smolt transformation. In marine fish, similar nutritional and environmental modulations may occur, although conflicting data exist. A low expression of the $\Delta 6D$ gene or the involvement of genetic, environmental, or hormonal factors could explain why $\Delta 6D$ appears to be barely functional in marine fish. This review shows that, despite the large number of studies investigating the regulation of $\Delta 6D$, little is known about the molecular mechanisms involved. Furthermore, the nutritional and environmental regulation of other enzymes involved in HUFA biosynthesis still need to be investigated to obtain a better understanding of the regulation of HUFA biosynthetic pathways in teleosts, ultimately leading to improvements in fish production.

(UMR 1067 INRA-Ifremer-Bordeaux 1, Ifremer Centre de Brest, BP 70, 29280 Plouzané, France ; email of M. Vagner : marie.vagner@hotmail.fr)

A NEW METHOD TO INCREASE AND MAINTAIN THE CONCENTRATION OF SELENIUM IN ROTIFERS (BRACHIONUS SPP.)

S. Penglase, K. Hamre, J.W. Sweetman, A. Nordgreen-2011

Aquaculture 315(1-2): 144-153

Larvi 2009 - Proceedings of the 5th Fish and Shellfish Larviculture Symposium, Gent, Belgium, September 2009

Abstract:

Rotifers are used as the first feeding diet for the larvae of many commercially produced marine fish species. However, the nutritional requirements of marine fish larvae appear to be better fulfilled by their wild feed, copepods. Consequently, rotifers are fed diets that alter their body composition to better imitate copepod composition and this results in greater success when rearing rotifer fed marine fish larvae. Despite this, copepod fed fish larvae still have higher growth rates and survival than rotifer fed larvae. This may occur because of the less investigated mineral differences that exist between rotifers and copepods. The concentration of selenium (Se) in rotifers *Brachionus* sp. (0.08–0.09 mg Se kg⁻¹ dry weight) can be over 30 fold lower than the concentration found in copepods (3–5 mg Se kg⁻¹ dry weight). In this study, the enrichment and retention of Se in rotifers fed Se enriched yeast (Se-yeast) were investigated. Rotifer Se concentration increased linearly with increasing levels of Se-yeast, with rotifers reaching a maximum of 138 mg Se kg⁻¹ dry weight. The use of Se-yeast was highly effective, with only 1% of the batch culture or short term enrichment diet needed to be replaced with Se-yeast to achieve copepod Se levels. At this feeding level there were no negative effects on rotifer egg ratio or population growth. Uptake of Se from Se-yeast was over 40 fold higher than obtained from using sodium selenite in short term enrichments (3 h). Se-yeast enriched rotifers had a high retention (100–85%) of Se for up to 10 h storage in clear water at cold (10 °C) or warm (20 °C) temperatures, while storage in green water (with algae) resulted in a slightly lower Se retention (65%) in a similar time period (8 h). Overall, rotifers enrichment with Se-yeast resulted in reproducible concentrations of Se that were then retained for extended periods of time. This will allow Se-yeast enriched rotifers to be used as a Se delivery method for fish larvae nutritional requirement or toxicological studies.

(National Institute of Nutrition and Seafood Research (NIFES), PO Box 2029, NO-5817 Bergen, Norway; email of S. Penglase: spe@nifes.no)
