

**A MODULAR, MECHANICAL ROTARY DEVICE FOR THE CLEANING OF COMMERCIAL-SCALE, CIRCULAR TANKS USED IN AQUACULTURE**

Andrew S. McRobbie, Andrew P. Shinn-2011

Aquaculture 317(1-4): 16-19

**Abstract:**

The increased use of large tanks for fish cultivation has led to the need for considerations towards hydrodynamic design. Tank velocities must be optimal for maintaining fish health, muscle tone, and respiration. Velocities must also provide adequate biosolid removal to prevent oxygen depletion and the spread of pathogens. As it is not always possible to reach equilibrium of these requirements, regular house-keeping must be maintained to keep culture tanks clean. In larger tank systems, the task of cleaning may be highly labour-intensive or even impractical due to access issues. A bespoke automated mechanical cleaning device was designed and constructed to aid the removal of biosolids. An unpaired t-test confirmed that tanks with the cleaning device showed a significant ( $P < 0.0005$ ) improvement in cleaning rates than tanks without.

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**NOTOCHORD DEFORMITIES IN REARED JAPANESE EEL *ANGUILLA JAPONICA* LARVAE**  
Akihiro Okamura, Yoshiaki Yamada, Naomi Mikawa, Noriyuki Horie, Satoru Tanaka, Katsumi Tsukamoto-2011

Aquaculture 317(1-4): 37-41

**Abstract:**

Technologies for rearing Japanese eel *Anguilla japonica* larvae (leptocephali) have improved markedly over the past decades. Notochord deformities, however, have been observed frequently in reared leptocephali. To learn more about these deformities, we studied notochord alterations in the leptocephalus stage of Japanese eels in captivity. Of 277 *A. japonica* leptocephali examined 170 to 307 days post hatching, 140 (50.5%) had kyphosis, a dorsal curvature of the notochord column; 4 (1.4%) had kyphosis-lordosis, a complication of the dorsal and ventral curvatures; and 21 (7.6%) had scoliosis, a lateral zig-zag curvature. Kyphotic leptocephali had various degrees of body curvature and often showed abnormal swimming behavior, continually whirling in the water column. The frequency of this whirling motion increased with increasing degree of body curvature. Even in the absence of whirling, the bodies of all reared larvae showed significantly greater curvature when compared with wild caught leptocephali, indicating that all reared larvae were potentially abnormal. Unlike leptocephali with scoliosis, those with kyphosis and kyphosis-lordosis often metamorphosed into glass eels, but retained their deformities, which would affect subsequent feeding behavior. Possible causes of these deformities, including nutritional imbalance, salinity, water temperature, and water current, are discussed.

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**SCREENING THE POST-LARVAE OF *MACROBRACHIUM ROSENBERGII* FOR EARLY DETECTION OF *MACROBRACHIUM ROSENBERGII* NODAVIRUS (MRNV) AND EXTRA SMALL VIRUS (XSV) BY RT-PCR AND IMMUNOLOGICAL TECHNIQUES**

A.S. Sahul Hameed, M. Ravi, M.A. Farook, G. Taju, R.I. Hernandez-Herrera, J.R. Bonami-2011

Aquaculture 317(1-4): 42-47

**Abstract:**

White tail disease (WTD) was found to be a serious problem in hatcheries and nursery ponds of freshwater prawn (*Macrobrachium rosenbergii*). The causative organisms have been identified as *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV). RT-PCR and immunological techniques such as Western blot and ELISA were used for early detection of MrNV and XSV in post-larval samples obtained from time-course experiments at different time intervals. Two

viruses were purified from diseased post-larvae of *M. rosenbergii* by a combination of low and high speed centrifugation using sucrose and CsCl gradients to raise the antisera separately. One structural protein with molecular weight of 43 kDa (CP-43) was identified from the purified preparation of *MrNV*, and two overlapping polypeptides of about 17 kDa (CP-17) and 16 kDa (CP-16) were found in XSV particles by SDS-PAGE. The antisera raised against CP-43 of *MrNV*, CP-16 and CP17 of XSV in mice were used to detect *MrNV* and XSV by Western blot and ELISA. Published primers specific to *MrNV* and XSV were used for the early detection of these viruses by RT-PCR and nested RT-PCR. The post-larval samples collected at 3 h post infection (h p.i.) showed positive for both viruses by nested RT-PCR and negative by RT-PCR, Western blot and ELISA techniques. The samples collected at 24 h p.i. and thereafter were found to be positive for *MrNV* and XSV by RT-PCR, ELISA and Western blot analyses.

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#### EFFECTS OF MICRO-ALGAE COMMONLY USED IN AQUACULTURE ON ACYL-HOMOSERINE LACTONE QUORUM SENSING

F.M.I. Natrah, Mireille Mardel Kenmegne, Wiyoto Wiyoto, Patrick Sorgeloos, Peter Bossier, Tom Defoirdt-2011

Aquaculture 317(1-4): 53-57

Abstract:

Quorum sensing, bacterial cell-to-cell communication with small signal molecules such as acyl-homoserine lactones, regulates the virulence of many pathogenic bacteria. Therefore, interfering with quorum sensing is currently being explored as a novel biocontrol strategy to fight bacterial infections. In this study, the effects of 19 micro-algal strains on acyl-homoserine lactone-regulated phenotypes of three reporter strains were investigated. Two freshwater micro-algae inhibited violacein production of quorum sensing reporter strain *Chromobacterium violaceum* CV026. Further tests using *Escherichia coli* JB523 showed that micro-algal extracts inhibited or stimulated quorum sensing, depending on the algal strain. One freshwater and five marine algae showed quorum sensing inhibitory activity, whereas two algae stimulated quorum sensing-regulated gene expression. Micro-algal strains that showed inhibitory activity in the previous assays also inhibited acyl-homoserine lactone-regulated bioluminescence in the aquaculture pathogen *Vibrio harveyi*. The growth of all reporter strains was found to be unaffected by the micro-algal samples. The most promising micro-algal strain was found to be *Chlorella saccharophila* CCAP211/48, as its extracts inhibited quorum sensing-regulated gene expression in all three reporter strains.

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#### BEHAVIORAL RESPONSES OF EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*) LARVAE AND *ARTEMIA* SP. EXPOSED TO CONSTANT LIGHT OR DARKNESS VS. LIGHT/DARK CYCLES OF WHITE, RED OR BLUE WAVELENGTHS

N. Villamizar, G. García-Mateos, F.J. Sánchez-Vázquez-2011

Aquaculture 317(1-4): 197-202

Abstract:

The performance and survival of fish larvae are strongly influenced by their surrounding photic environment. The aim of this study was to investigate the effect of light characteristics (spectrum and photoperiod) on the feeding and locomotor behaviors of European sea bass larvae and its prey (*Artemia* sp.). To this end, constant light (LL), constant darkness (DD) and 12:12 h LD cycles of red, blue or white LED lights were applied from 1 to 30 days post-hatching. The Modal Action Patterns (swimming duration, orientation, capture, miss and pass frequencies) of larvae and *Artemia* distribution in the tank were video recorded and analyzed using newly developed tracking software. The results showed that under LD<sub>B</sub> the phototactic response of sea bass larvae led to a significantly homogeneous distribution in the tanks and aquaria, while under LD<sub>W</sub> and LL the highest larvae density (52%) was seen on the tank walls. LD<sub>B</sub> and LD<sub>W</sub> resulted in longer swimming duration and earlier weaning. Larvae exposed to

darkness and red light showed the lowest swimming and feeding activity, and a higher aggregation tendency of both fish larvae and the live prey. White light exposure resulted in a strong phototactic response from fish larvae and *Artemia*, which consisted of a tendency to congregate at the corners or close to the walls of the tank/aquaria. *Artemia* hatching rate under blue light was highest ( $56.5 \pm 2.9\%$ ) in contrast with red light ( $26.3 \pm 1.4\%$ ) and total darkness ( $27.9 \pm 3.9$ ). These results showed that the relationship between the behavioral responses of sea bass larvae and *Artemia* is strongly affected by lighting conditions, which has both basic and practical implications for understanding their behavioral ecology and for improving culture protocols.

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#### PREDATORY BEHAVIOR OF THE BACKSWIMMER (*ANISOPS OGASAWARENSIS*) ON LARVAL MEDAKA (*ORYZIAS LATIPES*)

Kosuke Sano, Hisashi Kurokura-2011

Aquaculture 317(1-4): 210-213

Abstract:

To establish ecologically and environmentally safe countermeasures to prevent the predation of aquatic insects on larval fish in aquaculture systems, the predatory behavior of a backswimmer (*Anisops ogasawarensis*) on larval Medaka (*Oryzias latipes*) was observed under laboratory conditions. Observations were conducted to describe the vertical distribution and predatory behaviors of *A. ogasawarensis*. Additionally, a predation experiment evaluated the effect of various light levels (0, 3, 30, 300, and 3000 lx) on predation. Both the vertical distribution of *A. ogasawarensis* and the occurrences of predatory behavior was the highest near the water surface. *A. ogasawarensis* showed ambush predatory behavior and initiated chases when the larval *O. latipes* were within 5 cm of the predator. In the experiment, the predation rate increased with an increase in light intensity from 0 to 300 lx and decreased at 3000 lx. The present study indicates that predation by *A. ogasawarensis* on larval *O. latipes* changes under conditions of visual obstruction for the larvae such as decreasing of light intensity in the fish pond. Encounters with predators during the seed production may be higher in shallower.

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#### ARTIFICIAL SPAWNING OF COMMON TENCH TINCA TINCA (LINNAEUS, 1758), OBTAINED FROM WILD AND DOMESTIC STOCKS

Roman Kujawa, Dariusz Kucharczyk, Andrzej Mamcarz, Daniel Źarski, Katarzyna Targońska-2011

Aquaculture International 19(3): 513-521

Abstract:

The study encompasses three reproduction seasons. Tench spawners caught during the spawning season originated from carp ponds (domestic stock) and a lake (wild stock). Fish were reproduced under controlled conditions after hormonal stimulation with GnRH $\alpha$ -containing pellets combined with metoclopramide (Ovopel) or carp pituitary homogenate (CPH). As a result of hormonal stimulation, eggs were obtained from a larger number of females originating from the lake (71.7%) than those originating from the pond (58.3%), although no other statistical differences were found. A similar relationship was recorded for the spermatozoa motilities (range from 72 to 76%). The obtained results indicate that both investigated reservoirs are suitable for tench broodstock management due to the fact that synchronization of ovulation among different stocks is easy to achieve. For this purpose, among the tested spawning agents, Ovopel could be recommended as being slightly more effective.

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GENETIC VARIATION OF HATCHERY AND WILD STOCKS OF THE PEARL OYSTER  
*Pinctada fucata martensii* (DUNKER, 1872), ASSESSED BY MITOCHONDRIAL DNA  
ANALYSIS

W. S. Gwak, K. Nakayama-2011

Aquaculture International 19(3): 585-591

Abstract:

In order to provide baseline information for the genetic resources, genetic variation in wild and cultured *Pinctada fucata martensii* from southern Korea and Japan was studied using nucleotide sequence analysis of 379 base pairs (bp) in the mitochondrial cytochrome oxidase subunit I gene (COI). The study included three hatchery stocks from Korea (Tongyeong) and Japan (Mie and Tsushima) and one wild stock from Korea (Geoje). A total of 3 haplotypes were identified in hatchery stocks of 78 individuals, of which 63 individuals shared 1 haplotype. Overall, nucleotide diversity ( $\pi$ ) was low, ranging from 0.000 to 0.002, and haplotype diversity ( $h$ ) ranged from 0.000 to 0.541. Considerably low haplotype and nucleotide diversities in hatchery stock indicated that low effective population size and consecutive selective breeding of *P. fucata martensii* could be responsible for the reduction in genetic variation. The wild stock exhibited low haplotype diversity ( $0.507 \pm 0.039$ ) with two shared haplotypes. The results of the present study with first record of wild pearl oyster in Korea support the possibility that the transplanted pearl oyster for overwintering experiments could have survived in winter. In order to enhance and/or maintain genetic diversity in the hatchery stock, further research should be directed toward genetic monitoring and evaluation of the hatchery and wild pearl oysters.

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CRYOPRESERVATION EFFECTS ON THE SPERM QUALITY OF CACHAMA BLANCA  
*Piaractus brachyomus* (CUVIER 1818)

Juan Antonio Ramirez-Merlano, Yohana María Velasco-Santamaría, Víctor Mauricio Medina-Robles,  
Pablo Emilio Cruz-Casallas-2011

Aquaculture Research 42(6): 738–745

Abstract:

The effects of straws volume, cryoprotectants and thawing temperatures were evaluated on the sperm quality of cachama blanca *Piaractus brachyomus* (Cuvier), an important Colombian fish species. Sexually mature fish were induced to ovulation or spermiation with a carp pituitary extract. A pool of suitable sperm samples was diluted in glucose, egg yolk, dimethyl sulphoxide (DMSO-10%), methanol (MET-10%) or ethylene glycol (ETG-5%) and packed in 0.5, 2.5 or 5.0 mL straws and frozen in nitrogen vapour. The thawing process was performed in a 35 or an 80 °C water bath. The fertility was evaluated after 6 h post fertilization. The highest motility percentage ( $33 \pm 3\%$ ) was observed with sperm cryopreserved with DMSO, packed in 5 mL straws and thawed at 35 °C. The treatments with DMSO and MET packed in 0.5 and 5.0 mL straws and thawed at 35 °C showed the highest fertility (higher than 71%) and the lowest fertility was obtained with MET-2.5 mL ( $9 \pm 5\%$ ). In all the treatments, a significant decrease in the sperm quality was observed at 80 °C. Sperm cryopreserved with DMSO-10% or MET-10%, packed in 2.5 or 5.0 mL straws are suitable to achieve acceptable fertilization and to fertilize high amounts of eggs.

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HATCHING RATE AND LARVAL GROWTH VARIATIONS IN PSEUDOPLATYSTOMA  
PUNCTIFER : MATERNAL AND PATERNAL EFFECTS

Jesús Núñez, Diana Castro, Christian Fernández, Rémi Dugué, Fred Chu-Koo, Fabrice Duponchelle,  
Carmen García, Jean-François Renno-2011

Aquaculture Research 42(6): 764–775

Abstract:

In *Pseudoplatystoma punctifer* (e.g. *Pseudoplatystoma fasciatum*) larvae, parental effects on hatching, growth of initial stages and dry feed adaptation were evaluated as they could influence fry heterogeneity, which is responsible for the enhancement of cannibalism, and which remains one of the main factors of mortality during larval stages. A full factorial experiment was carried out with 3 females  $\times$  3 males producing nine families of full siblings, raised separately in triplicates into 30 L tanks at  $28 \pm 0.5$  °C in a water recirculating system. Paternal and maternal effects were observed on hatching success, yolk utilization efficiency and growth until 26 days post fertilization. Hatching success was generally over 80% except for one male  $\times$  female combination (25%). Total length (TL) at hatching and during the first 4 weeks of exogenous feeding on live *Artemia* nauplii and dry feeds was determined in each family using digital photographs of larvae and nih image j analysis freeware. Mean TL was calculated for each family at each sampling time and analysed using multifactorial analysis of variance tests. These results indicate not only dam but also sire effects at very early developmental stages as well as in subsequent stages of *P. punctifer*.

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#### REPRODUCTIVE SUCCESS AND FRY PRODUCTION OF THE PAICHE OR PIRARUCU, ARAPAIMA GIGAS (SCHINZ), IN THE REGION OF IQUITOS, PERÚ

Jesús Núñez, Fred Chu-Koo, Magali Berland, Lamberto Arévalo, Olaff Ribeyro, Fabrice Duponchelle, Jean François Renno-2011

Aquaculture Research 42(6): 815–822

Abstract:

*Arapaima gigas* (paiche) is the largest scaled fish species living in the Amazon basin. Its biology is both fascinating and misunderstood. In a context of overfishing, hence reduced natural populations, aquaculture of a fish with such interesting characteristics (large size, high growth rate, no intramuscular spines) is an important issue. The development of farming production would also reduce the fishing pressure on natural populations and allow re-stocking programmes in certain areas. To determine what factors may influence the reproductive success in captivity, data from breeding reports for 2007–2010 were collected among fish farmers in the region of Iquitos. In parallel, we carried out physicochemical measurements in different ponds where these paiches breed, and conducted personal interviews about the general fish management conditions. The results show that reproduction occurs throughout the year but with a higher intensity during the rainy season. It also highlights farms that have performed much better than others, but no single factor except feeding level has been clearly associated with reproductive success. The environmental control of reproduction in paiche, therefore, remains partly mysterious. To deepen this study, we recommend the systematic sexing of breeders, extending reproductive behavioural studies, and examining the limnological factors involved in fry mortality.

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#### DEMOGRAPHIC PARAMETERS OF ADULTS OF *PSEUDODIAPTOMUS ANNANDALEI* (COPEPODA: CALANOIDA): TEMPERATURE–SALINITY AND GENERATION EFFECTS

Delphine Beyrend-Dur, Ram Kumar, T. Ramakrishna Rao, Sami Souissi, Shin-Hong Cheng, Jiang-Shiou Hwang-2011

Journal of Experimental Marine Biology and Ecology 404(1-2): 1-14

Abstract:

The calanoid copepod *Pseudodiaptomus annandalei* is distributed exclusively in the Indo-Pacific regions from subtropical to tropical estuaries and shallow coastal waters. Its population dynamics is not well understood despite its ecological importance to natural ecosystems and potential applications to aquaculture. We studied the combined effects of temperature and salinity on survivorship and reproduction of *P. annandalei*. The experimental protocol included adult cohort life table analysis and observations on reproductive parameters of individual females (paired with males) in relation to 9 different temperature–salinity combinations. At salinity level 10–20, the average survival ( $l_x = 0.5$ ) and life expectancy at moulting was significantly higher at 18 °C (38 days); however, at salinity 30, the survival was significantly higher at 25 °C (22.17 days) than either of those at 18 or 32 °C. The gross

and net reproductive rates were higher (267 and 176 nauplii female<sup>-1</sup> respectively) at salinity 10 and temperature 25 °C. Neither interclutch duration nor embryonic development time was affected by salinity; whereas, temperature had a significant effect on both parameters. The clutch size was significantly affected by salinity, but not by temperature. Across temperature–salinity combinations tested in the present study, the total lifetime fecundity was significantly correlated with adult generation time, but not with longevity. The population growth rate (Euler's  $r$ ) was negatively related to the adult generation time across temperature–salinity levels tested. Continued production of viable fertile clutches by the female required remating with the male at frequent intervals. Our results suggest that the most appropriate temperature–salinity combination for this species is 25 °C at salinities 10–15 for reproduction, and 20 °C at salinity 10 for survival.

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#### THE RELATIVE IMPORTANCE OF SLOPPY FEEDING, EXCRETION, AND FECAL PELLET LEACHING IN THE RELEASE OF DISSOLVED CARBON AND NITROGEN BY *ACARTIA TONSA* COPEPODS

Grace K. Saba, Deborah K. Steinberg, Deborah A. Bronk-2011

Journal of Experimental Marine Biology and Ecology 404(1-2): 47-56

Abstract:

Crustacean zooplankton produce dissolved organic matter (DOM) and inorganic nutrients via sloppy feeding, excretion, and fecal pellet leaching. These different mechanisms of the release of metabolic products, however, have never been individually isolated. Our study was designed to determine the relative importance of these different modes on release of dissolved organic carbon (DOC), ammonium (NH<sub>4</sub><sup>+</sup>), and urea from *Acartia tonsa* calanoid copepods feeding on the diatom *Thalassiosira weissflogii*. Excretion and sloppy feeding were the dominant modes of DOC production (80 and 20% of total DOC release, respectively) and NH<sub>4</sub><sup>+</sup> release (93 and 7% of total NH<sub>4</sub><sup>+</sup> release, respectively). Urea, however, was predominately produced via sloppy feeding and fecal pellet leaching (25% and 62% of total urea release, respectively). Urea contributed 20% of total measured nitrogen (TMN; NH<sub>4</sub><sup>+</sup> + urea) released from copepods, and constituted 100% of TMN released via fecal pellet leaching, 47% of TMN released via sloppy feeding, and only 3.5% of TMN released via excretion. TMN release was > 100% of copepod body N d<sup>-1</sup>, resulting in low DOC:TMN release ratios (4.1 for sloppy feeding, 2.1 for cumulative release of sloppy feeding, excretion, and fecal pellet leaching). Our results suggest that the mechanism of release plays an important role in the amount of different forms of DOM, NH<sub>4</sub><sup>+</sup>, and urea available to bacteria and phytoplankton.

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#### ULTRASTRUCTURE AND OSMOREGULATORY FUNCTION OF THE KIDNEY IN LARVAE OF THE PERSIAN STURGEON *ACIPENSER PERSICUS*

Z. Taghizadeh Rahmat Abadi, S. Khodabandeh, B. Abtahi, G. Charmantier, M. Charmantier-Daures-2011

Journal of Fish Biology 78(5): 1359–1374

Abstract:

The localization of Na<sup>+</sup>, K<sup>+</sup>-ATPase (NKA) and the ultrastructural features of kidney were examined in larvae of the Persian sturgeon *Acipenser persicus* (L 31–41 mm total length and 182.3–417.3 mg). Investigations were conducted through light and electron microscopy and through immunofluorescence for NKA detection. The kidney nephrons consisted of a large glomerulus and tubules (neck, proximal, distal and collecting), which connected to the ureters. Posteriorly, ureters extended and joined together into a thin-walled ureter terminal sac. Ultrastructurally, the glomerular cells (podocytes) possessed distinctive pedicels that extended to the basal membrane. The proximal tubule (PT) showed two different cells. The cells lining the anterior part of PT possessed apical tall microvilli (c. 2–7 μm), a sub-apical tubular system, a basal nucleus and dense granules. Posteriorly in the cells, the sub-apical tubular

system and granules were absent and round mitochondria associated with basolateral infoldings were found; the apical microvilli were reduced. Distal and collecting tubular cells showed the typical features of osmoregulatory cells, i.e. well-developed basolateral infoldings associated with numerous mitochondria. No immunofluorescence of NKA was detected in the glomeruli. A weak immunostaining was observed at the basolateral side of the cells lining the neck and PT. A strong immunostaining of NKA was observed in the entire cells of the distal tubules, collecting tubules and in some isolated cells of the ureters. In all immunostained cells, the basolateral region showed a much higher fluorescence and nuclei were immunonegative. In conclusion, the epithelial cells of kidney tubules had morphological and enzymatic features of ionocytes, particularly in the distal and collecting tubules. Thus, the kidney of *A. persicus* larvae possesses active ion exchange capabilities and, beside its implication in excretion, participates in osmoregulation.

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#### POST-RELEASE FEEDING AND GROWTH OF HATCHERY-REARED JAPANESE FLOUNDER *PARALICHTHYS OLIVACEUS*: RELEVANCE TO STOCKING EFFECTIVENESS

T. Tomiyama, M. Watanabe, G. Kawata, K. Ebe-2011

Journal of Fish Biology 78(5): 1423–1436

Abstract:

The feeding and growth of hatchery-reared (HR) Japanese flounder *Paralichthys olivaceus* of c. 100 mm total length (LT) released off the coast of Fukushima, Japan, were investigated. From 2 to 15 days after release, the HR *P. olivaceus* frequently exhibited high empty-stomach frequency (>40%), low stomach-content mass (<1% of body mass), reduced somatic condition from release (c. -10%) and negligible growth. Thereafter, empty-stomach frequency decreased, the stomach-content mass of HR fish increased to 2–8% of body mass, the somatic condition recovered and growth rate increased to 0.5–1.5 mm day<sup>-1</sup>. Prey items were initially mysids, shifting thereafter to fishes such as the Japanese anchovy *Engraulis japonica*, as observed similarly in wild counterparts. The proportion of mysids decreased with time after release irrespective of size at release, indicating the importance of mysids for adaptation to natural food. Recapture rates at age 1 year, derived from fish market surveys, varied greatly among release years (4–11%). The variation in the recapture rates was largely accounted for by the post-release growth rates ( $r^2 = 0.5$ ), suggesting a relationship between the post-release growth of HR fish and their survival and subsequent stocking effectiveness.

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#### BEHAVIOURAL RESPONSES OF HATCHERY-REARED AND WILD COD *GADUS MORHUA* TO MECHANO-ACOUSTIC PREDATOR SIGNALS

J. J. Meager, P. Rodewald, P. Domenici, A. Fernö, T. Jrvi, J. E. Skjæraasen, G. K. Sverdrup-2011

Journal of Fish Biology 78(5): 1437–1450

Abstract:

The behavioural responses of wild (predator-experienced) and hatchery-reared (predator-naive) cod *Gadus morhua* to standardized mechano-acoustic (MA) stimuli were compared in the laboratory. Wild fish responded mainly with freezing and fast-start escapes away from the stimulus, whereas hatchery-reared fish often ignored or approached the stimulus. Wild fish also had stronger responses, turning faster during escapes and reducing activity immediately after the stimulus. Both fish types were less active on a 'risky' bare substratum after the stimulus. The antipredator responses of wild fish were consistent to repeated stimuli, whereas hatchery-reared fish that had generally only encountered harmless stimuli showed more variable responses with lower repeatability. This suggests that experience plays a role in shaping the behavioural response of fishes to MA stimuli.

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MORPHOLOGICAL DEVELOPMENT OF LARVAL COBIA RACHYCENTRON CANADUM AND THE INFLUENCE OF DIETARY TAURINE SUPPLEMENTATION

G. Salze, S. R. Craig, B. H. Smith, E. P. Smith, E. McLean-2011

Journal of Fish Biology 78(5): 1470–1491

Abstract:

The morphological development of larval cobia *Rachycentron canadum* from 3 days post hatch (dph) until weaning (27 dph) was examined using S.E.M. Two groups of fish were studied: a control group (CF), reared under standard feeding protocol, and a group in which prey items were enriched with supplemental taurine (4 g l<sup>-1</sup> day<sup>-1</sup>; TF). TF fish grew faster ( $P < 0.001$ ), attained greater size (mean  $\pm$ s.e.  $55.1 \pm 1.5$  v.  $33.9 \pm 1.0$  mm total length) and had better survival (mean  $\pm$ s.e.  $29.3 \pm 0.4$  v.  $7.1 \pm 1.2$  %) than CF fish. Canonical variance analysis confirmed findings with respect to differences in growth between the treatment groups with separation being explained by two cranial measurements. S.E.M. revealed that 3 dph larvae of *R. canadum* (in both groups) possess preopercular spines, superficial neuromasts on the head and body, taste buds in the mouth, an olfactory epithelium which takes the form of simple concave depressions, and primordial gill arches. Gill filaments start to form as early as 6 dph and lamellae buds are visible at 8 dph in both groups. In CF fish, the cephalic lateral line system continues its development at 12–14 dph with invagination of both supra- and infraorbital canals. At the same time, a thorn-like or acanthoid crest forms above the eye. At 14 dph, invaginations of the mandibular and preopercular canals are visible and around 22 dph enclosure of all cranial canals nears completion. In CF larvae, however, completely enclosed cranial canals were not observed within the course of the trial, i.e. 27 dph. In TF larvae, grooves of the cephalic lateral line system form 4 days earlier than observed in CF larvae of *R. canadum* (i.e. at 8 dph), with enclosure commencing at 16 dph, and completed by 27 dph. Along the flanks of 6 dph larvae of either treatment, four to five equally spaced neuromasts delineate the future position of the trunk lateral line. As myomeres are added to the growing larvae, new neuromasts appear such that at 16 dph a neuromast is associated with each myomere. By 27 dph, the trunk lateral line starts to invaginate in CF larvae, while it initiates closure in TF larvae. These findings elucidate important features of the larval development of *R. canadum* and show that dietary taurine supplementation benefits larval development, growth and survival in this species. Moreover, they suggest a conditional requirement for taurine in larval *R. canadum*.

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A NOVEL GROWTH-PROMOTING PROTEIN IN THE CONDITIONED MEDIA FROM THE ROTIFER BRACHIONUS PLICATILIS AT AN EARLY EXPONENTIAL GROWTH PHASE

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Hydrobiologia 667(1): 101-117

Abstract:

We confirmed the existence of growth-promoting substances in the conditioned media (CM) from the rotifer *Brachionus plicatilis* at an early exponential growth phase and isolated a novel protein with a growth-promoting activity from the crude extract (CE) of rotifer cells. CM was prepared from the culture media where rotifers had been cultured at an early exponential growth phase and filtered through a 0.22- $\mu$ m filter membrane. The growth-promoting activity was determined using rotifers in CM for 5 days. As a result, the increase of rotifers added with CM was significantly higher than that of the control in artificial seawater ( $P < 0.001$ ). Moreover, the growth-promoting activity of CM was dose-dependent and inactivated by heat treatment at 80°C for 60 min. Meanwhile, CM filtered through a <10 kDa ultrafiltration membrane showed a low activity, whereas proteinase K treatment resulted in a complete inactivation. These results suggest that the rotifer secretes growth-promoting proteins into CM. CE also contained a protein with the activity and properties similar to those found in CM. Then, CE was subjected to purification of a growth-promoting protein for convenience using various types of chromatography after fractionation with 30–80% saturated ammonium sulfate. Subsequently, a protein with an approximate molecular weight of 25000 was isolated, and its N-terminal amino acid sequence was determined to be PAVVDFTAVWFGPLQMIKP. An orthologue was found in the EST database of *B. plicatilis*, the full sequence of which showed about 50% identity to the corresponding regions of thioredoxins from other organisms.



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EXTRACELLULAR MATRIX PEPTIDES OF *ARTEMIA* CYST SHELL PARTICIPATE IN PROTECTING ENCYSTED EMBRYOS FROM EXTREME ENVIRONMENTS

Li Dai, Dian-Fu Chen, Yu-Lei Liu, Yang Zhao, Fan Yang, Jin-Shu Yang, Wei-Jun Yang-2011

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Background

Many species of the brine shrimp *Artemia* are found in various severe environments in many parts of the world where extreme salinity, high UV radiation levels, high pH, anoxia, large temperature fluctuations, and intermittent dry conditions are often recorded. To withstand adverse environments, *Artemia* undergoes an oviparous developmental pathway to release cysts whereas, under favorable conditions, swimming nauplius larvae are formed directly via an ovoviviparous pathway. In the former case these cysts have an extraordinary ability to keep the embryos protected from the harsh environment for long periods. This is achieved through the protection by a complex out-wrapping cyst shell. However, the formation and function of the cyst shell is complex; the details remain largely unclear.

Principal Finding

A shell gland-specific gene (SGEG2) was cloned and identified from a suppression subtractive hybridization library. Western blot analysis showed that SGEG2 presumably requires post-translational proteolysis in order to be processed into two mature peptides (SGEG2a and 2b). The three matrix peptides (SGEG1 reported previously, 2a, and 2b) were found to distribute throughout the cyst shell. The results of gene knockdown by RNAi and subsequent resistance to environmental stresses assays indicated that these matrix peptides are required for cyst shell formation and are involved in protecting the encysted embryos from environmental stress.

Conclusions/Significance

This study revealed that extracellular matrix peptides participate in protecting embryos from extreme salinity, UV radiation, large temperature fluctuations and dry environments, thereby facilitating their survival. The cyst shell provides an excellent opportunity to link the ecological setting of an organism to the underlying physiological and biochemical processes enabling its survival. The cyst shell material has also a high potential to become an excellent new biomaterial with a high number of prospective uses due, specifically, to such biological characteristics.

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