

MICROASSAYS FOR A SET OF ENZYMES IN INDIVIDUAL SMALL MARINE COPEPODS

Susanna Knotz, Maarten Boersma, Reinhard Saborowski-2006

Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 145(3): 406-411

Abstract:

Fluorogenic assays for a set of five hydrolytic enzymes involved in digestion and food utilization (alanine and arginine aminopeptidase, lipase/esterase, chitinase, and beta-glucosidase) were optimized to measure activities of these enzymes in the same extracts of individual small North Sea copepods. The enzyme activities of *Acartia clausi*, *Centropages typicus*, *Corycaeus anglicus*, *Paracalanus parvus*, and *Temora longicornis* showed distinct species specific activity patterns, but also high intra-specific variability. Protein, lipids, carbon and nitrogen (C, N) were determined with micro-scale assays in individual copepods or in batches of 10 to 50 animals. Water soluble protein contents ranged from 16% to 38%, and lipid contents from 2.4% to 5.5% of dry mass. The molar C/N ratios were between 4.1 and 4.5. The presented microassays provide suitable tools for studying physiological reactions of copepods and other small pelagic crustaceans in response to variable environmental conditions.

(Biologische Anstalt Helgoland, Foundation Alfred Wegener Institute for Polar and Marine Research, P. O. Box 180, D-27483 Helgoland, Germany; email of S. Knotz: sknotz@awi-bremerhaven.de)

COMPARISONS OF STRESS PROTEINS AND SOLUBLE CARBOHYDRATE IN ENCYSTED EMBRYOS OF ARTEMIA FRANCISCANA AND TWO SPECIES OF PARARTEMIA

James S. Clegg, Veronica Campagna-2006

Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 145(2): 119-125

Abstract:

We compared stress proteins (p26, artemin, hsp70) and alcohol-soluble carbohydrates (ASC) in cysts of *Artemia franciscana* and two as yet un-named species populations of *Parartemia*, the brine shrimp endemic to Australia. The small stress proteins and molecular chaperones, p26 and artemin, previously thought to be restricted to *Artemia*, and present in very large amounts in its encysted embryos (cysts), were also detected by western blotting in *Parartemia* cysts, even though roughly 85–100 million years have passed since these genera diverged. We interpret this finding as further evidence for the adaptive importance of these proteins in coping with the severe stresses these encysted embryos endure. As expected, hsp70 was present in all three groups of cysts, but apparently at somewhat lower concentrations in those of *Parartemia*. Based on measurements of ASC we propose that the disaccharide trehalose, critical for desiccation tolerance in many animal cells, has probably also been maintained in the metabolic repertoire of *Parartemia* whose cysts have well developed tolerance to severe desiccation.

(Bodega Marine Laboratory and Section of Molecular and Cellular Biology, University of California, Davis, Bodega Bay, CA 94923, USA; email of J. Clegg: jsclegg@ucdavis.edu)

EFFECTS OF ESTRADIOL-17B ON EMBRYOS AND LARVAE OF THE GIANT FRESHWATER PRAWN, MACROBRACHIUM ROSENBERGII (DECAPODA, PALAEMONIDAE)

Pakdeenarong Noppakun, Damrongphol Praneet-2006

Crustaceana 79(5): 563-572

Abstract:

The effects of estradiol-17 β (E2) on embryos and larvae of the giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879), were investigated. Embryos, obtained from brooding females within one day of spawning, were exposed to 1, 10, or 50 μ g/ml estradiol-17 β for 2 days. No significant differences in growth or in survival rate with those of controls were found. However, those

exposed to 10 µg/ml E2 showed an increase in hatching rate, in the number of primordial germ cells (PGCs), and in the rate of incorporation of the PGCs into the developing gonads. Similarly, newly hatched larvae exposed to 1, 5, or 10 µg/ml E2 for 2 days did not show significant differences in growth, survival, or developmental rate from those of the controls. However, larvae exposed to 5 or 10 µg/ml E2 showed slightly more developed gonads.

EFFECTS OF TEMPERATURE AND BODY SIZE ON THE SWIMMING SPEED OF LARVAL AND JUVENILE ATLANTIC COD (GADUS MORHUA): IMPLICATIONS FOR INDIVIDUAL-BASED MODELLING

Myron A. Peck, Lawrence J. Buckley, David A. Bengtson-2006

Journal Environmental Biology of Fishes 75(4): 419-429

Synopsis:

The routine swimming speed (S) of three groups of 4, 9 and 32 cm total length (LT) juvenile cod (*Gadus morhua*) was quantified in the laboratory at 6 – 10 different temperatures (T) between 3.2 and 16.7°C. At temperatures between 5 and 15°C, mean group S increased exponentially with increasing T ($S = a e^{bT}$) and the effect of temperature ($b = 0.082$, $Q_{10} = 2.27$) was not significantly different among the groups (over the 8-fold difference in fish sizes of early- and post-settlement juveniles). Differences in mean S among individuals within each group were quite large (coefficient of variation = 40 – 80%). Swimming data for juveniles and those collected for groups of 0.4, 0.7 and 0.9 cm standard length (LS) larvae were combined to assess the effect of body size on S . At 8°C, S (mm s⁻¹) increased with LS (mm) according to: $S = 0.26LS^{\Phi} - 5.28LS - 1$, where $\Phi = 1.55LS - 0.08$. Relative S (body lengths s⁻¹) was related to LS by a dome-shaped relationship having a maximum value (0.49 body lengths s⁻¹) at 18.5 – 19 mm LS corresponding to the sizes of fish at the end of larval-juvenile metamorphosis. Previous larval cod IBM's using a cruise-predator mode likely overestimated rates of foraging (prey searching and encounters) by a factor of ~2, whereas foraging rates in pause-travel models are closer to estimates of swimming velocities obtained in this and other laboratory studies.

(Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882, USA; email of Myron A. Peck: myron.peck@uni-hamburg.de)

IMPROVEMENT OF THE SURVIVAL IN THE SEVEN-BAND GROUPER EPINEPHELUS SEPTEMFASCIATUS LARVAE BY OPTIMIZING AERATION AND WATER INLET IN THE MASS-SCALE REARING TANK

Yoshitaka Sakakura, Shigeaki Shiotani, Hisashi Chuda, Atsushi Hagiwara-2006

Fisheries 72(5):939-947

Abstract:

The water flow in larval rearing tanks has been indicated to cause mass mortality of the seven-band grouper *Epinephelus septemfasciatus* larvae. Therefore, a new aerating method was tested in an actual scale intensive rearing tank (8.0 m in diameter, 1.87 m of water depth, 100 m³ of volume), in which an aerator was positioned at the center of the rearing tank surrounding cylindrical drain (1.2 m in diameter) to generate the flow field, and seven larval rearing trials were performed. The survival rate with the former aeration methods were compared, in which several aerators were located in the rearing tank. The survival rate at 10 days after hatching with the new aeration method ($61.5 \pm 5.1\%$, $n = 7$) was approximately three times higher than the former methods ($21.2 \pm 13.7\%$, $n = 6$). The flow environment of rearing tanks was also examined by quantifying the flow field, and the relationship between the flow field in the rearing tank, behavior of larvae and survival discussed. It was confirmed that the vertical circulating flow was observed in rearing tanks, and determined effectively the survival and the behavior of grouper larvae in patchiness.

(Faculty of Fisheries, Nagasaki University, Nagasaki 852-8521, Japan; email of Y. Sakakura: sakakura@nagasaki-u.ac.jp)

EVALUATION OF LARVAL QUALITY OF VIVIPAROUS SCORPIONFISH SEBASTISCUS MARMORATUS

Yoko Matsuo, Yasushi Kasahara, Atsushi Hagiwara, Yoshitaka Sakakura, Toshihisa Arakawa-2006
Fisheries Science 72(5): 948-954

Abstract:

The aim of this study was to develop an acute test for larval quality in the viviparous scorpionfish *Sebastes marmoratus*. Rearing experiments until day 13 post parturition were conducted to investigate the survival of larvae for 13 different batches, and tolerance to starvation of larvae was examined and expressed by the survival activity index (SAI). The morphological characters, enzyme activity, and swimming behavior of larvae on day 0 and 1 were also observed, followed by the correlation analysis between SAI. Larvae with high SAI (≥ 26) showed significantly higher survival on day 13 than larvae with low SAI, which confirmed that SAI is a reliable index that can be used to evaluate larval quality, similar to the former findings. The esterase activity ($r = -0.713$, $P < 0.01$), swim frequency ($r = -0.735$, $P < 0.01$) and swimming speed ($r = -0.588$, $P < 0.05$) of larvae on day 0 were significantly and negatively correlated with SAI. It was concluded that enzyme activity and behavioral characters of larvae just after parturition can be a real-time index for evaluating the larval quality of this species.

(Graduate School of Science and Technology, Nagasaki University, Nagasaki 852-8521, Japan; email of Y. Sakakura: sakakura@nagasaki-u.ac.jp)

EFFECTS OF TAURINE LEVELS IN BROODSTOCK DIET ON REPRODUCTIVE PERFORMANCE OF YELLOWTAIL SERIOLA QUINQUERADIATA

Hiroyuki Matsunari, Kazuhisa Hamada, Keiichi Mushiake, Toshio Takeuchi-2006
Fisheries Science 72(5): 955-960

Abstract:

The effect of dietary taurine was investigated on reproductive performance in yellowtail *Seriola quinqueradiata*. Two-year-old fish of average body weight 6.1 kg were fed on diets containing three levels of taurine (T-0, T-0.5 or T-1.0%) for 5 months prior to spawning. For spawning investigations, fish were induced to maturity by human chorionic gonadotropin injection (600 IU/kg-fish) and artificially inseminated. Oocyte growth improved significantly ($P < 0.05$) with the increase of dietary taurine. The collection of eggs from females reared on the T-0% diet was not successful. The success rate of spawning for females fed on T-0.5 and 1.0% diets was one out of six, and six out of seven, respectively. The taurine levels of the liver and serum in the T-0% diet group were much lower than that in the T-0.5 and T-1.0% diet groups ($P < 0.05$). Fish fed the T-0% diet showed higher contents of serine in the liver and serum. The taurine content of the ovary was not significantly different among the different dietary treatments. These results indicate that taurine has a positive effect on the improvement of spawning performance of yellowtail.

(Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Minato, Tokyo 108-8477, Japan; email of T. Takeuchi: take@s.kaiyodai.ac.jp)

EFFECTS OF TEMPERATURE ON VITELLOGENESIS IN JAPANESE EEL ANGUILLA JAPONICA

Narumi Sato, Ichiro Kawazoe, Yuzuru Suzuki, Katsumi Aida-2006
Fisheries Science 72(5): 961-966

Abstract:

Cultured immature female Japanese eels acclimated to sea water at either 10 or 20°C were treated weekly with salmon gonadotropin fraction (sGTH) in order to investigate the effects of water temperature on artificial induction of ovarian maturation. In eels maintained at 20°C, ovulation was induced in 11 of 18 fish during the experimental period of 13 weeks, whereas at 10°C all fish showed a low gonadosomatic index (GSI) at the end of the experiment. Plasma vitellogenin levels were higher in eels kept at 20°C than in eels at 10°C throughout the experiment. However, no significant differences were observed in the plasma testosterone and estradiol-17 β levels between groups. Eels

pretreated with sGTHs at 10°C for 13 weeks were separated to two different temperature (10 and 20°C) groups, and received the same weekly sGTH injections. In eels transferred to 20°C, plasma vitellogenin levels, GSI and oocyte diameter were increased, but these values were maintained at low levels in eels that remained at 10°C. These results clearly indicate that water temperature is an important factor regulating vitellogenesis in the Japanese eel.

(Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, University of Tokyo, Bunkyo, Tokyo 113-8657, Japan; email of N. Sato: narumi@marine.fs.a.u-tokyo.ac.jp)

EFFECT OF LIGHT IRRADIATION ON DYNAMICS OF VITAMIN A COMPOUNDS IN ROTIFERS AND ARTEMIA

Yutaka Haga, Fuminori Taru, Kengo Ohta, Yasuhiro Shima, Toshio Takeuchi-2006

Fisheries Science 72(5): 1020-1026

Abstract:

The dynamics of vitamin A (VA) compounds in live food during enrichment were examined under different light conditions. Rotifers *Brachionus plicatilis* and *Artemia nauplii* (*Artemia*) were enriched with or without 10 mg VA palmitate (VAp) in 1 L of culture medium for 24 h under either bright (2000 lx), or dark (<1 lx) conditions. VAp, retinol (ROH), retinal (RAL) and retinoic acid (RA) contents were analyzed at 0 h (before enrichment) and at 3, 6, 12, 18 and 24 h after the onset of enrichment. Retinoid content in rotifers enriched in darkness was always higher than that enriched under light. VAp content showed two peaks at 3 and 18 h in rotifers enriched in darkness, but it showed one peak at 3 h in rotifers enriched under light. ROH and RA contents increased over the 24-h period in rotifers enriched in darkness, whereas they decreased 12 h onward in rotifers enriched under light. In *Artemia*, VAp and ROH contents were always higher than when enriched under the bright condition, but their dynamics showed a similar pattern in *Artemia* enriched under dark and bright conditions.

(Department of Marine Biosciences, Faculty of Marine Science, Tokyo University of Marine Science and Technology, Konan, Minato 4-5-7, Tokyo 108-8477, Japan; email of T. Takeuchi: take@s.kaiyodai.ac.jp)

SUCCESSFUL FERTILIZATION AND HATCHING OF FOUR EUROPEAN CYPRINID SPECIES USING CRYOPRESERVED SPERM

B. Urbányi, T. Szabó, E. Miskolczi, S. Mihálffy, K. Vranovics, Á. Horváth-2006

Journal of Applied Ichthyology 22(3): 201-204

Summary:

In this study we tried to develop a uniform method of sperm cryopreservation for four cyprinid fish species indigenous to Hungarian waters: the roach (*Rutilus rutilus* L.), the bream (*Abramis brama* L.), the silver bream (*Blicca bjoerkna* L.) and the barbel (*Barbus barbus* L.). The sperm was frozen in liquid nitrogen vapor in the presence of five extenders (350 mm fructose, 30 mm Tris, pH 8.0; 350 mm glucose, 30 mm Tris, pH 8.0; 300 mm sucrose, 30 mm Tris, pH 8.0; 200 mm KCl, 30 mm Tris, pH 8.0 and modified Kurokura's extender) and two cryoprotectants: 10% methanol (MeOH) and 10% dimethyl-sulfoxide. The highest post-thaw motility (roach: 77 ± 6%, bream: 77 ± 6%, silver bream: 67 ± 5%, barbel: 75 ± 6%), fertilization (roach: 84 ± 4%, bream: 83 ± 2%, silver bream: 63 ± 2%, barbel: 70 ± 4%) and hatching (roach: 74 ± 2%, bream: 67 ± 6%, silver bream: 54 ± 2%, barbel: 61 ± 4%) rates were found when either fructose or glucose extenders were used in combination with MeOH as cryoprotectant for all four investigated species. Strong correlations were found between post-thaw motility of the sperm and fertilization or hatching rates, which indicates that motility can be used to predict fertilization success in these species.

(Department of Fish Culture, Szent István University, Páter K. u. 1., Gödöllő, H-2103, Hungary; email of Ákos Horváth: horvath.akos@mkk.szie.hu)

PRE-EXPOSURE TO INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS OR TO INACTIVATED WHITE SPOT SYNDROME VIRUS (WSSV) CONFERS PROTECTION AGAINST WSSV IN PENAEUS VANNAMEI (BOONE) POST-LARVAE

J. Melena, B. Bayot, I. Betancourt, Y. Amano, F. Panchana, V. Alday, J. Calderón, S. Stern, Ph. Roch, J.-R. Bonami-2006

Journal of Fish Diseases 29(10): 589-600

Abstract:

Larvae and post-larvae of *Penaeus vannamei* (Boone) were submitted to primary challenge with infectious hypodermal and haematopoietic necrosis virus (IHHNV) or formalin-inactivated white spot syndrome virus (WSSV). Survival rate and viral load were evaluated after secondary per os challenge with WSSV at post-larval stage 45 (PL45). Only shrimp treated with inactivated WSSV at PL35 or with IHHNV infection at nauplius 5, zoea 1 and PL22 were alive (4.7% and 4%, respectively) at 10 days post-infection (p.i.). Moreover, at 9 days p.i. there was 100% mortality in all remaining treatments, while there was 94% mortality in shrimp treated with inactivated WSSV at PL35 and 95% mortality in shrimp previously treated with IHHNV at N5, Z1 and PL22. Based on viral genome copy quantification by real-time PCR, surviving shrimp previously challenged with IHHNV at PL22 contained the lowest load of WSSV ($0-1 \times 10^3$ copies μg^{-1} of DNA). In addition, surviving shrimp previously exposed to inactivated WSSV at PL35 also contained few WSSV ($0-2 \times 10^3$ copies μg^{-1} of DNA). Consequently, pre-exposure to either IHHNV or inactivated WSSV resulted in slower WSSV replication and delayed mortality. This evidence suggests a protective role of IHHNV as an interfering virus, while protection obtained by inactivated WSSV might result from non-specific antiviral immune response.

(Fundación CENAIM-ESPOL, Guayaquil, Ecuador; email of J-R Bonami: bonami@univ-montp2.fr)

SHORT COMMUNICATION

A NEW PARASITE THAT INFECTS EGGS OF THE MUD CRAB, *SCYLLA SERRATA*, IN AUSTRALIA

Renate Kvingedal, Leigh Owens, Dean R. Jerry-2006

Journal of Invertebrate Pathology 93(1): 54-59

Abstract:

The mud crab, *Scylla serrata*, is currently being evaluated for its feasibility for mass aquaculture in Australia. As part of the evaluation process, pathogens that can affect this species need to be identified. This research note describes a possible new parasite that infects the eggs of *S. serrata*. The parasite was discovered in two separate cases (three months apart) in a broodstock research program and appears to cause 100% egg mortality. The parasite consists of a cluster of cells with rhizoids that appear to function as an anchorage and a feeding organ. The individual cells range from 3 to 6 μm with a single nucleus. The parasite could not be classified to a phylum by morphology alone. However, BLAST analysis of the DNA sequence from a PCR amplicon generated using universal 18S ribosomal RNA gene primers indicated similarity to pathogenic thraustochytrids, *Dermocystidium* sp. and *Rhinosporidium seeberi*. All except *R. seeberi* are protistan parasites of fish and crustaceans. A neighbour-joining phylogenetic tree confirmed the homology with the thraustochytrids; however, further molecular techniques need to be exploited for absolute classification of this new parasite.

(aAquaculture Genetics Group, School of Marine Biology and Aquaculture, James Cook University, Townsville, Queensland 4811, Australia; email of Renate Kvingedal: renate.kvingedal@jcu.edu.au)

PREDATION OF STOCKED HATCHERY-REARED JUVENILES OF OCELLATE PUFFER TAKIFUGU RUBRIPES IN SALT POND MESOCOSM

Daisuke Shimizu, Kazutaka Sakiyama, Yoh-Ichi Takahashi-2006

Nippon Suisan Gakkaishi 72 (5): 886-893

Abstract:

To investigate the effect of predation on the post-release mortality of hatchery-reared juveniles of the ocellate puffer *Takifugu rubripes*, we reared fish in an experimental salt pond (5,300 m²) with or without predators. The pond was divided into halves, one with 30 sea bass, *Lateolabrax* sp. (30 cm in mean total length), which is an experimental section, the other (control section) with no predators. Three hundred juveniles (mean standard length, 30 mm) were released into each section of the pond. The survival rate of the juveniles decreased remarkably in the presence of the bass, indicating that predation immediately after release is an important mortality factor. The size of the juveniles and the degree of loss of caudal fin at the time of release showed no influence on growth of juveniles after release or on their survival.

(Momoshima Station, National Center for Stock Enhancement, Fisheries Research Agency, Onomichi, Hiroshima 722-0061, Japan)

HYDROGEOCHEMISTRY OF SEASONAL VARIATION OF URMIA SALT LAKE, IRAN

Samad Alipour-2006

Saline Systems 2:9

Abstract:

Urmia Lake has been designated as an international park by the United Nations. The lake occupies a 5700 km² depression in northwestern Iran. Thirteen permanent rivers flow into the lake. Water level in the lake has been decreased 3.5 m in the last decade due to a shortage of precipitation and progressively dry climate. Geologically the lake basin is considered to be a graben of tectonic origin. Na, K, Ca, Li and Mg are the main cations with Cl, SO₄, and HCO₃ as the main anions. F & Br are the other main elements in the lake. A causeway crossing the lake is under construction, which may affect the lake's annual geochemistry. The main object of this project is mainly to consider the potential of K-mineral production along with ongoing salt production.

Seven hundred and four samples were taken and partially analyzed for the main cations and anions. Surface water (0.5 m. depth) was analyzed for Na, K, Mg, Ca, Br and Li, and averaged 87.118 g/lit, 1.48 g/lit, 4.82 g/lit, 4.54 g/lit, 1.19 ppm and 12.7 ppm respectively for the western half of the lake. Sodium ranged between 84 to 91.2 g/lit, and showed higher concentrations in the south than in the north. This unexpected result may be caused by shallower depth in the south and a higher net evaporation effect. Calcium ranged between 4.2 to 5 g/lit, apparently slightly higher in the north. K is higher in the south, possibly due to rivers entering from south that may carry slightly higher K in solution.

In the middle-range samples (0.5–5 m.), K averaged 1.43 g/lit and ranged from 1.40 to 1.46 g/lit. At this intermediate depth the distribution of K is clearly higher to the south of the causeway that is currently under construction. It is not clear whether this increase is the effect of the causeway or the effect of the salty Aji-Chay River to the east, and the Khoy salt domes to the north of the lake. At depth (5 m–10 m), K averaged 1.48 g/lit and ranged from 1.4 to 1.49 g/lit, differing only in the second decimal from the average of the middle and surface samples.

Ignoring the small difference between the averages of the three sample depths, the distribution of K is highly homogeneous in the lake water due to the mixing process. Therefore causeway construction has not yet strongly affected K distribution, or it may be at the starting point. Magnesium concentration ranged from 4.6 to 5-g/lit, and was elevated in the south. This differs somewhat compared to calcium. Lithium, with an average of 12–13 ppm, is slightly higher in the south, and has not shown any significant variation in all three seasons. Iodine was below the detection limit in the lake.

Urmia Lake, geochemically, is highly uniform both to the south and north of the causeway, in both the surface and deep brines. K and Mg, which average 1.48 and 6.6 g/lit in order, could be elements worth production in addition to the NaCl currently being produced from the lake. Br, F, Li and B in the limit of <50 ppm don't look to be in the economical range.

(University of Urmia, P.O. Box 165, Urmia, Iran)
