海洋生物工学科 2007 年研究業績

A. 研究発表

1. 論文

(1) Development of rotifer strains with useful traits for rearing fish larvae
Atsushi Hagiwara, Koushiro Suga, Atsushi Akazawa, Tomonari Kotani, and Yoshitaka Sakakura
Aquaculture, 268, 44-52 (2007)

The euryhaline rotifer *Brachionus plicatilis* is a species complex, which is commonly used for rearing marine fish larvae. Providing cultures with an appropriate size of rotifers facilitates size dependent selectivity of the feeding larvae and results in larvae with higher survival, growth and stability. It is also important to obtain rotifers with higher growth rate and tolerance against environmental stress and better nutritional quality after enrichment. This paper reviews the importance of feeding rotifers with appropriate size for fish larvae, as well as how much variation of rotifer traits can be expected from natural population of rotifers, as well as from artificial manipulation. These artificial manipulations include (1) manipulation of culture history to establish descendant rotifer clones with different reproductive traits, (2) manipulation of culture environmental conditions and chemical treatments on rotifer traits, such as size and reproduction, (3) cross-mating and a molecular approach for obtaining rotifer strains with useful traits in clones of their descendants. Recent progress on the production of rotifer strains with ideal characteristics in terms of size, population growth, tolerance against external conditions and resting egg formation is discussed.

(2) Quality of hatchery-reared juveniles for marine fisheries stock enhancement
Lewis Le Vay, Gary R. Carvalho, Emilia T. Quinitio, Junemie H. Lebata, Vu Ngoc Ut, and Hiroshi Fushimi
Aquaculture, 268, 169-180 (2007)

The potential for stock enhancement by release of hatchery-reared juveniles continues to be a topic of interest to researchers and fisheries managers. While, in many studies, the

focus has tended to be on the technology for production of juveniles, the need for a more multidisciplinary approach is now becoming accepted. Ideally, this includes studies of population dynamics and recruitment-limitation of wild stocks, environment–stock interactions, habitat availability, genetic studies of wild and released stocks and integration with appropriate fisheries management. While it may be relatively straightforward to culture large numbers of seed animals, the quality of hatchery-reared juveniles may limit the effectiveness of any release programme. The quality of juveniles may be defined either by their ability to attain the age and size to recruit to a commercial fishery or their fitness to survive to contribute to the spawning stock. Many factors will inevitably influence batch–batch variability in the viability of hatchery-reared juveniles and their ability to recruit and compete in the wild. Some effects of nutrition and environment in the hatchery are well-known or at least recognised and their manipulation offers the potential for improvement of survivorship of juveniles post-release. The choice and utilisation of broodstock also represent a crucial stage in enhancement programmes, and considerations of bottleneck effects arising from reduced effective population size due to skewed parental and family contributions must be given careful consideration. A broodstock design that encompasses sufficient numbers of animals that reflect the genetic, and preferably ecological, identity of the stocks to be enhanced should be adopted. In addition, environmental conditions and husbandry practices within the hatchery as well as broodstock and larval nutrition can all influence the quality of offspring. Further conditioning and/or selection during nursery culture may also be critical in maximising the physiological and behavioural fitness of hatchery juveniles post-release. Although evaluation of long-term performance of individual batches of juveniles requires considerable effort or may be impossible in some cases, this type of quantification is likely to be an important component in the determination of the effectiveness of release programmes. This paper reviews the effects of hatchery and nursery practice on larval and juvenile fitness for stock enhancement and presents examples of comparisons of the quality of wild and hatchery-reared juveniles and the effect of pre-release conditioning on subsequent survival and growth.

(3) Cloning and Characterization of A Gene Encoding Algicidal Serine Protease from Pseudoalteromonas sp. Strain A28

Daiki Kohno, Yuichi Sakiyama, Sun-Og Lee, Moboru Takiguchi, Atsushi Mitsutani, Hirotaka Kitaguchi, and Junichi Kato

Journal of Environmental Biotechnology, 7, 99-102 (2007)

The gene (*espI*) encoding an algicidal extracellular serine protease was isolated from *Pseudoalteromonas* sp. strain A28. DNA sequence analysis revealed that EspI is synthesized as a 711-amino-acid prepropeptide and is further processed to the 50-kDa matured protease after cleavage of a 148-amino-acid N-terminal domain and 6.7-kDa C-terminal domain. The Concerved Domain Search program found that EspI belongs to the subtilisin-like serine protease family. The program also found two PPC domains in the C-terminal region. The PPC domain is normally found at the C-terminus of secreted bacterial peptidases and they are not present in the active peptidase. However, the molecular size of purified EspI predicts that it contains one and half PPC domains in the matured EspI protease.

(4) Elimination of Micro-organisms by Singlet Oxygen Generated from Sensitized Rose Bengal and Monitored by Near Infrared Emission Spectroscopy
Nobutaka Suzuki, Y. Mese, A. Ono, A. Fujimura, Y. Toshitoku, Takeshi Nagai, Iwao Mizumoto, Atsushi Mitsutani, Hirotaka Kitaguchi, Yuzaburo Ishida, A. Go, K. Nakaguchi, S. Moriya, T. Sugiura, Tateo Nomoto, and Binko Yoda
Near Infrared Spectroscopy: NIR in Action - Making Difference (G. R. Burling-Clandge, S. E. Holroyd, R. M. W. Sumner, eds) pp. 523-526. Printer House, Ltd., Hamilton, NZ, (2007)

Control of micro-orbanisms has increased importance, because food poisoning and infection desease by micro-organisims happen often. So we tried to eliminate micro-organisims by using singlet oxygen generated by irradiation of dyes by visible light. In this paper we describe the elimination of *Microcystis aeruginosa* that causes a bad taste and smell in drinking water and is toxic to some freshwater fish. We also present the elimination of *Chattonella* spp. That causes cultured fish and shrimps to die in large quantities in fish farms off the coast of Japan.

(5) 特集 水産業と外来生物　海面養殖種苗導入のリスク管理ータイリクスズキ
谷口　順彦
日本水産学会誌, 73, 1125-1128 (2007)

近年，海水魚養殖の対象種として外国から導入される魚種が増えている。1990年以降，養殖用種苗として導入された魚種は，カンパチ，イサキ，マダイ，クロ

ダイ，スズキ，キジハタ，メバルなど10数種にのぼる。これら種苗の輸入元は中国，韓国，香港，ベトナムなどである。これらの魚種の大半は輸入されはしたものの，養殖漁業として産業的に成立しない場合が多く，一時的輸入に止まっている。しかし，ブリ養殖の補完的位置をしめるカンパチ養殖は，ベトナムや中国南部から輸入される種苗により，ブリ類養殖部門のおよそ30%を占めるところまで成長した。

従来，外国産魚類の導入に対する規制はゆるく，これまで多数の魚種が外国から養殖用種苗として輸入されてきたが，導入前のリスク評価が実施された事例はほとんどない。あるとすれば，生産調整を目的として輸入された種苗のサイズと量に関する規制のみである。2005年には，特定生物種の導入を禁止する「特定外来生物による生態系等に関わる被害の防止に関する法律」が制定され，これに違反すると一定の罰則が課される事になったが，その特定外来生物リストには海産の養殖魚種は含まれていない。ここでは，これまで養殖種苗として輸入されてきた魚種について，そのリスク評価と管理のあり方について考察したい。

（６）Studies on the genetic diversity of wild populations of masu salmon, *Oncorhynchus masou masou*, by microsatellite DNA markers

Daiki Noguchi, and Nobuhiko Taniguchi

Aquaculture Science, 55, 521-527 (2007)

A large number of hatchery masu salmon, *Oncorhynchus masou masou*, have been released into the rivers in Japan in order to enhance the fisheries resources of this species. Unconscious genetic changes that may occur during artificial seed production should be prevented if we are to perform effective and responsible resource enhancement. In this paper, the genetic variability and population structure of masu salmon were estimated using five microsatellite DNA markers (msDNA). Masu salmon showed a high level of genetic variability at the msDNA loci. Average allele numbers ranged between 10.40 and 15.00, average numbers of allele richness between 9.74 and 11.72, and expected heterozygosities between 0.824 and 0.874. Genetic differentiation was not observed between different year classes. Meanwhile, genetic differentiation was detected among captured locations, but distinct correlation between genetic distance and geographic distance was not observed. Finally, methods of brood stock management in artificial propagation to prevent genetic change and loss of variation for conservation of genetic diversity in wild populations are discussed.

(7) Phylogenetic relationships among nine scallop species (Bivalvia:Pectinidae) inferred from nucleotide sequences of one mitochondrial and three nucear gene regions

Chulabhorn Mahidol, Uthairat Na-Nakorn, Srijanya Sukmanomon, Wantana Yoosuk, Nobuhiko Taniguchi, and Thuy T. T. Nguyen

Journal of Shellfish Research, 26, 25-32 (2007)

Current knowledge of the evolutionary relationships among scallop species (Mollusca: Bivalvia: Pectinidae) in the Indo-Pacific region is rather scanty. To enhance the understanding of the relationships within this group, phylogenies of nine species of scallops with the majority from coastal regions of Thailand, were reconstructed by maximum parsimony, maximum likelihood, and Bayesian methods using sequences of the 16S rRNA of the mitochondrial genome, and a fragment containing the ITS1, 5.8S and ITS2 genes of the nuclear DNA. The trees that resulted from the three methods of analysis were topologically identical, however, gained different levels of support at some nodes. Nine species were clustered into two major clades, corresponding to two subfamilies (Pectininae and Chlamydinae) of the three currently recognized subfamilies within Pectinidae. Overall, the relationships reported herein are mostly in accordance with the previous molecular studies that used sequences of the mtDNA cytochrome oxidase subunit I, and the classification system based on microsculpture of shell features and morphological characteristics of juveniles. Levels of divergences were different among genes (i.e., the 5.8S gene showed the lowest levels of nucleotide divergence at all levels, whereas the 16S rRNA showed the highest level of variation within species, and ITS2 gene revealed the highest level of divergence at higher levels).

(8) Mitochondrial DNA Diversity of the Asian Moon Scallop, *Amusium pleuronectes* (Pectinidae), in Thailand

Chulabhorn Mahidol, Uthairat Na-Nakorn, Srijanya Sukmanomon, and Nobuhiko Taniguchi, and Thuy T. T. Nguyen

Marine Biotechnology, 9, 352-359 (2007)

Sequence variation of the mitochondrial DNA 16S rRNA region of the Asian moon scallop, *Amusium pleuronectes*, was surveyed in seven populations along the coast of Thailand. A total of 16 unique haplotypes were detected among 174 individuals with a total 27 variable sites out of 534 bp sequenced. The mitochondrial haplotypes grouped

into two distinct arrays (estimated to differ by about 2.62% to 2.99% nucleotide divergence) that characterized samples collected from the Gulf of Thailand versus the Andaman Sea. Low levels of intrapopulation variation were observed, while in contrast, significant divergence was observed between populations from the Gulf of Thailand and Andaman Sea. Results of AMOVA reveal a high F_{ST} value (0.765) and showed that the majority of the total genetic variance (76.03%) occurred among groups (i.e., Andaman Sea and the Gulf of Thailand) and little among populations within the group (0.52%) and within populations (23.45%). The genetic differentiation between the populations recorded in the present study is similar to that observed in a variety of marine species in the Indo-Pacific. The implications of the findings for management of *A. pleuronectes* genetic resources in Thailand are discussed.

(9) Evaluation of genetic diversity of eight grouper species *Epinephelus* spp. based on microsatellite variations

Worawut Koedprang, Uthairat Na-Nakorn, Masamichi Nakajima, and Nobuhiko Taniguchi

Fisheries Science, 73, 227-236 (2007)

Genetic diversity among eight species of grouper, *Epinephelus bleekeri*, *E. coioides*, *E. malabaricus*, *E. ongus*, *E. akaara*, *E. maculatus*, *E. merra* and *E. fuscoguttatus*, was studied using six microsatellite loci, *Em-01**, *Em-03**, *Em-07**, *Em-08**, *Em-10**, and *CA-07**, with the aim of exploring the feasibility of using microsatellite data for species identification. The results showed high levels of genetic differentiation among species (F_{ST} = 0.4403 and R_{ST} = 0.4954). Species identification based on fixed allelic differences was possible between *E. coioides*, *E. ongus*, and *E. fuscoguttatus* at *Em-01** and between *E. fuscoguttatus* and *E. ongus* at *Em-08**. Private alleles were found in all species, except for *E. ongus*. Pairwise F_{ST} ranged 0.238–0.578 (P < 0.008 Bonferroni correction), and Nei's genetic distance ranged 0.433–2.710. Size homoplasy was observed at *Em-03*157* allele, which was characterized by a T–C transition at the 119th nucleotide site of PCR products. The genetic assignment test unambiguously assigned each individual to the correct species. Thus, this test can be used for species identification of unknown individuals when the multilocus genotypes of the six microsatellite loci are available. The phylogenetic (neighbor-joining) tree, which was constructed based on the genetic distance matrix, separated the eight grouper species into two main groups.

(10) Genetic diversity of wild Mekong giant catfish *Pangasianodon gigas* collected from Thailand and Cambodia

Thawatchai Ngamsiri, Masamichi Nakajima, Srijanya Sukmanomon, Naruepon Sukumasavin, Wongphatom Kamonrat, Uthairat Na-Nakorn, and Nobuhiko Taniguchi

Fisheries Science, 73, 792-799 (2007)

The Mekong giant catfish *Pangasianodon gigas* is endemic to the Mekong River and is a critically endangered species. The genotypes of the microsatellite DNA (msDNA) and mitochondrial DNA (mtDNA) markers (right domain of the control region) were detected to evaluate the present status of genetic divergence of this species from the Mekong River in Thailand and Cambodia. The observed and expected heterozygosity values of Mekong giant catfish in Thailand and Cambodia were relatively low in comparison with those of other nonendangered freshwater fish species. These two populations from Thailand and Cambodia showed similar levels of genetic diversity, as evaluated by the 384 nucleotides of the mtDNA control region with 13 haplotypes. The pairwise F_{ST} value between the two populations based on the genotype frequencies of msDNA and mtDNA markers suggested a close genetic relationship between the populations in Thailand and Cambodia. The results of this study support the conclusion that the Mekong giant catfish is critically endangered. Care should be taken to sustain the genetic diversity of this species, as the level of genetic variability has already decreased in the wild population. This species is a target species for an ongoing stock enhancement program in the Mekong River in Thailand. It is proposed to apply these markers for proper broodstock management, such as for minimal kinship selective breeding in the hatchery.

(11) 魚類集団の遺伝的多様性の保全と利用に関する研究

谷口　順彦

日本水産学会誌, 73, 408-420 (2007)

2. 報文

(1) シオミズツボワムシの栄養強化成績に対する一次培養の方法と個体群増殖フェーズの影響

小谷知也、源河輝久、伏見浩、林雅弘

植え継ぎ及び連続方式で培養したシオミズツボワムシ *Brachionus plicatilis*(以下ワムシ) を栄養強化後の脂肪酸の取り込みにより質的に比較した。植え継ぎ培養は3日周期で植え継ぎを行い、初期収容密度を800個体 /mL とした。粗放連続培養法を改変した方法で連続培養を実施し、培養を行う水槽 (以下培養槽) と培養槽から流出した培養を収容する水槽 (以下収穫槽) を使用した。培養密度が 1,000 個体 /mL を維持するように設定した。植え継ぎ培養からはワムシ接種後1時間後、24時間後、48時間後のワムシを栄養強化に用いた。連続培養では培養槽及び収穫槽からワムシを収穫し二次培養(栄養強化)に用いた。栄養強化は、*Nannochloropsis oculata* を給餌して 24 時間培養する区と、市販栄養強化剤 (以下栄養強化剤) を用いて 8 時間培養する区を設定した。栄養強化を行わない区も設定した。栄養強化後、脂質を抽出し、ガスクロマトグラフィーにより脂肪酸組成を分析した。栄養強化を行わなかった区では連続培養で生産したワムシの方が脂質含量が多くなった。*N. oculata* で栄養強化した区では、ARA および EPA 含量が連続培養からの試料で多くなる傾向があった。一方、栄養強化剤で栄養強化した区では、ARA、EPA および DHA 含量が、植え継ぎ培養の接種 24hr 後及び連続培養収穫槽からの試料で多くなる傾向があった。したがって、脂肪酸取り込みの効率は、連続培養で生産したワムシの方が植え継ぎ培養のワムシよりも高かった。

(2) Neural-tube specific paralogous genes and their upstream regulatory sequences.

Yuki Kitaura, Haruyuki Sonobe, Osamu Tanaka, Daisuke Honda, Youhei Watanabe, Kazuhiro W. Makabe, Katsumi Takamura, and Takahito Nishikata

Memoirs of Konan University, Science and Engineering Series (54), 75-88 (2007)

Ascidians are primitive chordates, and their critical evolutionary position expected to offer an excellent experimental system to understand the origin and the evolution of vertebrates (Satoh, 1994). Especially, neural tube is one of the characteristic features of chordates. In this study, we focused on the mechanism of the neural tube formation, and described the detailed expression profiles of two neural tube specific genes, *CiNut1* and *CiNut2*. Although the amount of *CiNut2* expression is about 1/1000 compared to that of *CiNut1*, both two genes are expressed in the entire neural plate and neural tube during the course of the development. Both two genes are situated in an adjacent position of the

chromosome in the same direction. Comparative analysis of the upstream regulatory sequence of two genes revealed the conserved sequences, which suggested having a role for the neural tube specific expression.

3. 口頭発表

(1) Effect of primary cultivation method and population growth phase on nutritional enrichment of euryhaline rotifer *Brachionus plicatilis*
Tomonari Kotani, Teruhisa Genka, Hiroshi Fushimi, Masahiro Hayashi, Kristof Dierckens, and Patrick Sorgeloos
Asian-Pacific Aquaculture 2007, Hanoi, Vietnam, Abstracts, p.142 (2007-8)

It is important to evaluate the effect of primary cultivation method of rotifer after the secondary cultivation as nutritional enrichment. So far, various methods of rotifer cultivation have been developed. Recently two methods are performed mainly, batch culture and continuous one. This study aimed to clarify the fatty acid contents after the secondary cultivation as nutritional enrichment in order to evaluate the quality of rotifer cultured with different methods.

Two primary rotifer cultures were performed with butch and continuous methods. From the batch culture, three experimental populations were used; they were from the culture one, 24 and 48 hour after inoculation of rotifer. The continuous culture was performed with two tanks; one was for just cultivation with continuous feeding and water supply (cultivation tank), and another was for stocking from cultivation tank by over flow (harvest tank). From the continuous culture, two experimental populations were used from the cultivation and harvest tanks. Secondary cultures were performed after each primary culture and each rotifer population was enriched nutritionally with *Nannochloropsis oculata* and commercial nutritional enrichment diet. Each population was applied to GC after secondary culture and their fatty acid contents were analyzed.

Although there was no significant difference of lipid quantity among primary rotifer cultures in both cases of secondary culture, total n-3 HUFA quantity from both continuous culture populations was higher than that from batch culture population 1 and 48 hour after inoculation. When the enrichment was performed with *N. oculata*, rotifer

populations from two tanks of continuous culture and the batch culture tank 24 hour after inoculation contained higher quantity of ARA and EPA than those from two other tanks of batch culture. When the enrichment was performed with enrichment diet, populations from two tanks of continuous culture and the batch culture tank 24 hour after inoculation contained higher quantity of ARA, EPA and DHA than those from two other tanks of batch culture.

（2） Effect of zinc and manganese enriched *Artemia* on growth and vertebral deformity of red sea bream *Pagrus major* larvae

Tien Nguyen Van, Shuichi Satoh, Yuzuru Haga, Hiroshi Fushimi, Tomonari Kotani

2007 年度日本水産学会春季大会（東京）、講演要旨集、p. 27 (2007-3)

Objective: This study aimed to investigate the effects of zinc (Zn) and manganese (Mn) enriched *Artemia* on growth and skeletal deformity of red sea bream larvae.

Methods: Four treatments of Zn and Mn enrichment for *Artemia* were designated and used for feeding trial of red sea bream larvae. In treatment ZM *Artemia* nauplii were enriched with both Zn and Mn, treatment Z with Zn only, treatment M with Mn only and control treatment without zinc nor Mn. Fish were fed on enriched *Artemia* thrice a day from 15 to 30 day post-hatching (dph). At 30 dph, fish were subjected for growth, mineral contents, survival, skeletal deformity analysis and air-dive challenge test. Six hundred juveniles were cleared and double stained for skeletal analysis using light microscope.

Results: There was no significant difference in survival rate among all groups. A significant higher growth performance of larvae were recorded in M treatment ($P<0.05$). In air-dive challenge test, survival rate of ZM group was significantly higher than Z, M and control groups. Significantly higher skeletal deformity was observed in the control group compared to the others ($P<0.05$). The major skeletal deformities were observed in vertebral column, neural and hemal spines. The results of the present study demonstrated that the supplementation of Zn and Mn in *Artemia* enhance performance of red sea bream larvae.

（3）各種製剤添加配合飼料の給餌はヒラメ、トラフグの免疫能を向上させるか？

北本恵理、平山敬大、小谷知也、倉田　修、伏見　浩、畑井喜司雄

2007 年度日本水産学会春季大会（東京）、講演要旨集、p. 31 (2007-3)

【目的】乳酸菌加熱製剤 *Enterococcus faecalis* FK-23（以下 FK-23）およびポリヒ

ドロキシ酪酸（以下 PHB）の含量を 5 段階に調整した配合飼料をヒラメとトラフグに給餌してそれぞれの免疫能に対する効果を検討した。

【方法】平均全長 15.4cm のヒラメおよび平均全長 15.5cm のトラフグを供試魚とした。自家調製した配合飼料を用いて 2 週間の馴致飼育後、9 週間の飼育実験を行った。朝昼夕の 1 日 3 回給餌を行った。FK-23 の含量を 0～1.25% の間で 5 段階に調整した配合飼料を給餌した。PHB の含量を 0～2.0%の間で 5 段階に調整した配合飼料を給餌した。各製剤による免疫能への影響を検討するため *Vibrio anguillarum* ホルマリン不活化ワクチン接種区と非接種区を設けた。飼育実験開始から 3 週目と 6 週目にワクチンを接種した。細菌凝集法と白血球の異物包囲による *in vitro* の包囲化試験を行った。*in vitro* の包囲化試験には、異物として *Ichthyophonus hoferi* を用いた。

【結果】トラフグでは 1.25%の FK-23 を含有した配合飼料を給餌した飼育区の凝集能が有意に向上した。ヒラメでは 0.05%以上の FK-23 配合を含有した配合飼料を給餌した飼育区の特異免疫が有意に向上した。非特異免疫は 0.01%の FK-23 配合量で効果が現れた。PHB を配合した場合、特異免疫も非特異免疫も 0.5%以上の配合量で向上した。したがって、ヒラメ、トラフグでは FK-23 および PHB を配合飼料に添加すると免疫能の向上が認められた。

（4）連続培養及び植え継ぎ培養法で生産されたシオミズツボワムシの栄養強化成績

小谷知也、源河輝久、伏見　浩、林　雅弘

2007 年度日本水産学会春季大会（東京）、講演要旨集、p. 83（2007-3）

【目的】植え継ぎ及び連続方式で培養したシオミズツボワムシ *Brachionus plicatilis*（以下ワムシ）を質的に比較した。本研究では栄養強化後の脂肪酸の取り込みにより両者を比較した。

【方法】粗放連続培養法を改変した方法で連続培養を実施し、培養を行う水槽（以下培養槽）と培養槽から流出した培養を収容する水槽（以下収穫槽）を使用した。培養密度が 1,000 個体 /mL を維持するように設定した。植え継ぎ培養は 3 日周期で植え継ぎを行い、初期収容密度を 800 個体 /mL とした。連続培養では培養槽及び収穫槽からワムシを収穫し二次培養（栄養強化）に用いた。植え継ぎ培養からはワムシ接種後 1hr 後、24hr 後、48hr 後のワムシを栄養強化に用いた。栄養強化は、*Nannochloropsis oculata* を給餌して 24 時間培養する区と、市販栄養強化剤を用いて 8 時間培養する区を設定した。栄養強化を行わない区も設定した。栄養強化後、脂質を抽出し、ガスクロマトグラフィーにより脂肪酸組成を分析した。

【結果】栄養強化を行わなかった区では連続培養で生産したワムシの方が脂質含

量が多くなった。*N. oculata* で栄養強化した区では、ARA 及び EPA 含量が連続培養からの試料で多くなる傾向があった。一方、栄養強化剤で栄養強化した区では、ARA、EPA 及び DHA 含量が、植え継ぎ培養の接種 24hr 後及び連続培養収穫槽からの試料で多くなる傾向があった。したがって、脂肪酸取り込みの効率は、連続培養で生産したワムシの方が植え継ぎ培養のワムシよりも高いと考えられた。

（5）魚類仔稚魚期の酸素消費量の変化と成長及び生残との関係

宮嶋　暁、多和寛人、小谷知也、伏見　浩、半田岳志、難波憲二

2007 年度日本水産学会春季大会（東京）、講演要旨集、p. 89（2007-3）

[目的] 魚類の種苗生産では健苗性の高い種苗を生産する技術や生産効率の向上が重要であり、それには対象魚種それぞれの生理活性を解明し技術改良の基礎とすることが必要である。本研究では４魚種の成長に伴う生理活性の変化を明らかにすることを目的として、仔稚魚の安静時の酸素消費量を調べ、酸素消費量と成長および生残との関係を検討した。

[方法] 供試個体は実験室で飼育したトラフグ、マダイ、オニオコゼおよびヒラメ仔稚魚（孵化１日前～最大 96 日齢）を用いた。酸素消費量の測定は注射筒を用いた密閉式測定方法を用い、生化学用高感度溶存酸素計で測定した。酸素消費量測定後に供試個体の全長、体長、湿重量および乾燥重量を測定した。また、供試個体を採取した飼育水槽の生残率を調べた。

[結果] 各魚種の乾燥重量 1mg 当りの酸素消費量と成長との関係で大きく３つの共通した大きな変化が見られた。一つ目は卵から孵化仔魚になるまでの胚発生の過程であった。二つ目は脊索末端上屈開始前後であった。三つ目は浮遊生活から底生生活移行前後であった。また、各魚種の酸素消費量と生残との関係は、仔魚の死亡率が高い時期に酸素消費量が上昇している傾向がみられた。

（6）ワムシ中のタンパク質含量がヒラメ種苗の健苗性に及ぼす影響

松村慶介、小谷知也、伏見　浩

2007 年度日本水産学会春季大会（東京）、講演要旨集、p. 90（2007-3）

＜目的＞ヒラメ *Paralichthys olivaceus* の種苗生産では健苗性の高い生産が求められている。本研究では、異なるタンパク質含量の栄養強化剤を用いて栄養強化したシオミズツボワムシ給餌がヒラメ種苗の健苗性に与える影響について検討した。

＜方法＞孵化から 106 日齢まで飼育した。試験区には、タンハク質含量を変えて

栄養強化剤でワムシを強化する区（タンパク質含量 1 区<2 区<3 区）を設けた。また、ナンノクロロプシスでワムシを強化する区を設置した。各区では 1m3 水槽×3 水槽を用いて飼育した。ワムシは 35 日齢まで給餌した。アルテミアは 15 日齢から給餌し、全ての試験区をマリンオメガ A で栄養強化を行った。5 日齢から 35 日齢まで 5 日毎、46 日齢以降は 10 日毎に全長、体長測定を実施した。
＜結果＞飼育期間中の成長には各試験区間で有意差はなかった。取り上げ時（37～42 日齢）の生残は、各試験区で有意差はなかった。103～106 日齢時の生残では 1 区 25.9%=2 区 35.1%=3 区 18.3%≧ナンノ区 1.9%の傾向があった（$p<0.05$）。白化の出現は 35 日齢では有意差はなかった。106 日齢では 3 区 14%=ナンノ区 10%≧1 区 10%=2 区 10%の傾向があった（$p<0.05$）。黒化は 35 日齢時には出現せず、106 日齢は 3 区 27%>1 区 29%=2 区 48%=ナンノ区 16%であった（$p<0.05$）。骨格異常の出現は 35、106 日齢時では各試験区に有意差はなかった。

（7） ヒラメ種苗の健苗性に及ぼすワムシ培養法の影響

松村慶介、小谷知也、伏見浩

2007 年度日本水産学会秋季大会（函館）、講演要旨集、p. 31（2007-9）

＜目的＞ヒラメ *Paralichthys olivaceus* の種苗生産では健苗性の高い生産が求められている。そのためにワムシ中のビタミン A 含量やリン脂質含量に関する研究が行われてきた。また、栄養強化はワムシの状態に影響されることが明らかにされている。本研究では、連続培養法と植え継ぎ培養法の異なる培養法で培養したワムシを使用してワムシを栄養強化し、それを給餌した場合のヒラメ種苗の健苗性への影響について検討しました。また、ナンノクロロプシス添加効果ににについても同様の餌料条件で調べた。
＜方法＞試験区には、連続培養法（A 区）と植え継ぎ培養法（B 区）で培養したワムシをそれぞれ給餌する 2 試験区、それらの水槽にナンノクロロプシスを添加する 2 試験区（C 区、D 区）、計 4 試験区を設けた。各区では、1 ㎥水槽を 3 水槽ずつ用いて飼育した。ワムシは 35 日齢まで給餌し、全ての試験区を DHA PROTEIN SELCO で栄養強化を行った。全ての試験区、アルテミアは 15 日齢から給餌した。5 日齢から全長が 15 ㎜になるまで 5 日毎に全長、体長の測定と発育ステージの判定を行った。また、生残尾数計数を行い、同時に乾出耐性試験も行った。
＜結果＞成長は 15 日齢時で D 区 7.7 ㎜>A 区 6.9 ㎜=B 区 6.7 ㎜=C 区 7.1 ㎜の傾向があった（$p<0.05$）。25 日齢時と 35 日齢時では有意差はなかった。取り上げ時（37～48 日齢時）の生残は、各試験区間で有意差はなかった。乾出耐性を行った結果 D 区＝C 区＞A 区≧B 区となる傾向があった（$p<0.05$）。

（8） シオミズツボワムシの脂肪酸含量に対する栄養強化法の効果

小谷知也、源河輝久、伏見　浩、林　雅弘

2007 年度日本水産学会秋季大会（函館）、講演要旨集、p. 38（2007-9）

【目的】シオミズツボワムシ（以下ワムシ）への栄養強化は魚類種苗を安定して生産するために必須である。各種栄養強化剤はその用法を指定しているが、用法を変えた場合の効果については知見がない。本研究は栄養強化剤を過剰な条件で使用した時の効果を明らかにすることを目的とした。

【方法】植え継ぎおよび連続培養法でワムシを培養した。植え継ぎ培養からは植え継ぎ後 1、24、48 時間にワムシを収穫し、連続培養からは培養槽および収穫槽からワムシを収穫した。収穫したワムシに市販栄養強化剤を用いて栄養強化を施した。各一次培養から収穫したワムシに対して栄養強化を仕様書通りに行う試験区（通常区: 0.25 g /L, 8 時間）と過剰な条件で行う試験区（過剰区: 0.75 g /L, 24 時間）を設定した。栄養強化を施したワムシの脂肪酸含量をガスクロマトグラフィー法で分析した。

【結果】通常区では一次培養の条件間で 1.5～2 倍の違いがあったが、過剰区では 1～1.5 倍の違いとなった。DHA/EPA 比は、通常区ではどの一次培養条件でも 2:1 となっていた。一方、過剰区では 3.5～4:1 となった。以上から、栄養強化剤を指定された容量より過剰に用いてワムシを強化すると、強化剤量、強化時間の倍率ほど脂肪酸量は増えないが、栄養強化法を変えることにより強化後の脂肪酸組成を変化させることが出来ることが判明した。

（9） 瀬戸内海における紅藻ユナの雌雄配偶体について

山岸幸正、谷昌也、三輪泰彦

日本藻類学会第31回大会（神戸）、講演要旨集、p. 90（2007-3）

紅藻ユナ *Chondria crassicaulis* Harvey は北海道から九州までふつうにみられる海藻であり，四分胞子体および球芽と呼ばれる小枝を形成する藻体が知られているが，配偶体については韓国から報告されている (Lee and Yoon 1996)のみで，日本ではほとんど知見がない。演者らは瀬戸内海の広島県福山市鞆町および尾道市因島において本種配偶体が生育していることを確認し，生態および形態的特徴について調べた。

調査は鞆町海岸において 2006 年 2 月から 2007 年 1 月まで毎月行った。球芽を形成したユナは潮間帯中部ー下部の岩上に一年中みられ，匍匐枝をともなった盤

状付着器を持ち，高さ 20 cm まで，直径 2.6 mm まであり，10 月，11 月には四分胞子嚢を形成した。雌雄配偶体は同じ生育帯に混生する褐藻ヒジキの体上にのみ着生しており，2 月ー7 月および 12 月，1 月にみられた。配偶体は盤状付着器のみで匍匐枝を持たず，そこから多数の直立体が出て房状となっていた。雌性配偶体は高さ 1.0-10 cm，直径 0.5-2.0 mm あり，不規則に互生し，嚢果は球形から卵形で直径 1.0 mm までであった。雄性配偶体は小さく，高さ 0.2-1.7 cm，直径 300-700 μm あり，分枝しないかまたは枝をまばらに生じ，軸や枝の上部に精子嚢板を房状に形成していた。精子嚢板は楕円形で，多細胞列の柄を持ち，1-3 層の周辺不稔細胞がみられた。球芽を形成した藻体と配偶体の *rbc*L 部分塩基配列は同一であった。

(10) Marine Algae from The Central Seto Inland Sea, Japan
Yukimasa Yamagishi and Yasuhiko Miwa
XIXth International Seaweed Symposium (Kobe, Japan) (2007-3)

Marine algae growing at the coasts of In-no-shima and Tomo, Hiroshima Prefecture, located on the central region of Seto Inland Sea, Japan were surveyed. More than 185 species were collected from these coasts. In this presentation, the phenological, geographical and taxonomical knowledgements are shown and discussed. The following species are probably the first record from Seto Inland Sea: *Chaetomorpha linum* (Müller) Kützing, *Callithamnion corymbosum* (Smith) Lyngbye, *Chondria mageshimensis* Tanaka et K. Nozawa. The cystocarpic and spermatangial plants of *Chondria crassicaulis* Harvey, which were not reported from Japan were found in this study. These plants were grown epiphytically only on plants of *Sargassum fusiforme* (Harvey) Setchell in the mid- to lower intertidal zone from February to July 2006, and in the same zone the plants of *C. crassicaulis* forming gemmae and tetrasporangia were grown on rocks.

(12) Microcystis 属シアノバクテリアの株間における窒素欠乏とリン欠乏が切り替わる N/P 比の違い
藤井啓子、北口博隆、満谷　淳
平成 19 年度日本水産学会春季大会(東京)、講演要旨集、p. 233 (2007-3)

Microcystis の細胞内 N/P 比は約 30 であるとの報告から，湖水 N/P 比がそれよりの低い場合に *Microcystis* にとって窒素欠乏と判定する考えがある。しかし,演者らは *M. aeruginosa* NIES0-102 株では，N/P 比約 80 で窒素欠乏とリン欠乏が切り

替わることを明らかにした。本研究では，*Microcystis* 属の他の 2 株において同様の検討を行なった。八田原ダム（広島県）の湖水を 2006 年 7 月から 10 月まで毎月一回採取し，*M. aeruginosa* NIES0-102 株，NIES-104 株，NIES-298 株を用いて湖水が各株にとって窒素欠乏であるのかリン欠乏であるのかを判定した。湖水の N/P 比が 79，および 86 の時には、*M. aeruginosa* NIES-298 株では窒素欠乏，その他 2 株ではリン欠乏と判定された。このことより，*Microcystis* は株によって窒素欠乏からリン欠乏に切り替わる N/P 比が異なり，NIES-298 株では NIES-102 株よりもさらに高い N/P 比で窒素欠乏からリン欠乏に切り替わることが示唆された。

(13) The Commercially Important Seashells in Panay, Philippines
Liberato V. Laureta, Fernand Fagutao, and Hiroshi Fushimi
JSPS Core University Program between the University of Philippines, Visayas, Philippines and Faculty of Fisheries, Kagoshima University, Japan. Seminar on the management of inshore environment and utilization of fisheries resources. (鹿児島) (2007-11)

Many seashells abound in the coastal waters of Panay Island south of the Philippines. Most of these, whether in big quantity or not are sources of livelihood, since they are traded for money for human consumption, as materials for the button and shellcraft industries, as food for aquaculture species, and for keepsake or direct home ornaments.
In 2002-2004 survey of the coastal waters of the four provinces comprising the Panay, 137 shells were found to have commercial values. Of these, 85 were bivalves that belong to 25 Families, and 52 gastropods in 22 Families. There were 75 bivalves used for human consumption, three of these (oysters, *Crassostrea iredalei*, *C. gigas* and mussel *Perna viridis*) are cultured extensively in protected lagoons, while the rest are either gleaned or dived with or without compressor in shallow to deep waters. The angelwing *Pholas orientalis* and *Barnea manilensis*; yellow mangrove shell, *Polymesoda expansa*; tumid venus, *Gafrarium tumidum*;, nylon shells, *Paphia undulata*; meretrix venus, *Meretrix meretrix*; the ark shells, *Anadara inaequivalvis*, *A. granosa* and *A. antiquata*; pen shells, *Pinna bicolor*, and *Atrina vexillum*; scallops like *Amusium peluronectes*, *Chlamys senatoria* and *Annachlamys macassarensis*; spiny oysters like *Spondylus squamosus* and *S. aurantius*; the lucine clams like *Anodontia edentula*, *Eamesiella corrugata* and *Codakia tigerina* and many more are those typically dived or gleaned bivalved shells. For gastropods, 30 species are considered fit for human consumption. Exploited either through handpicking, gleaning or diving, the following species are popularly seen in the

market or peddled in the community; the telescope snail, *Telescopium telescopium*; the abalone, *Haliotis asinina*, the murex shell, *Hexaplex cichoreum*; conchs like the *Strombus canarium*, *S. luhuanus*. *S. labiatus* and *Lambis lambis*, and many more.

The gastropods belonging to the families Cypraeidae, Strombidae, Cerithidae, Conidae, Columbellidae, Fasciolaridae, Neritidae, Naticidae, Turbinidae, Volutidae, Muricidae and Trochidae, are usually used in the shellcraft industries. The shells of the bivalve and gastropod species used as food, are also used for this purpose, like those belonging to the families Veneridae, Cardiidae, Spondylidae, Pectinidae, Arcidae, Glycymeridae, Pteriidae, and Haliotidae. But the bivalve species *Placuna placenta* is the most popular material for the production of shellcraft products like place mats, chandeliers, lampshades, glass coasters, lanterns and others.

For the button industry, shells with nacreous materials like those belonging to the bivalve family Pteriidae (*Pinctada maxima*, *P. margaritifera*, *Pteria penguin*) and gastropods family Trochidae (*Trochis niloticus*, *T. maculatus*) are preferred. Sold for home decors are gastropod species like *Cassis cornuta* and *Melo broderipii*.

Among the four provinces in Panay, Iloilo (120 species: 72 bivalves; 48 gastropods) was found to have the highest number of commercially important shells. Capiz has 66 species (53 bivalves; 13 gastropods); Antique with 64 species (39 bivalves; 25 gastropods); and Aklan has 64 species also (39 bivalves; 25 gastropods).

(14) A case study of present status of coastal resources and fisheries communities in Philippines

Hisashi Kurokura, Jon Parmos Altamirano, Naomi Horita, and Hiroshi Fushimi

JSPS Core University Program between the University of Philippines, Visayas, Philippines and Faculty of Fisheries, Kagoshima University, Japan. Seminar on the management of inshore environment and utilization of fisheries resources. (鹿児島) (2007-11)

Batan Estuary, located in the Northeastern coast of Panay Island in central Philippines is a 2,640 ha semi-enclosed water body and river system supporting some 10,000 fishing households in three municipalities (Altavas, Batan, and New Washington) who are almost solely dependent on estuarine fisheries. Reports show that this was once a very productive fishing ground but at present, people claim of degrading environment and resources. Since there are very few studies and no current data to support this claim, interview surveys and actual field measurements were conducted in order to clarify present status of

the estuary.

Interview surveys were conducted among 105 local fishers to determine their own perception of the conditions of the estuary based on personal experiences. Responses show decrease in daily catch of fish and shrimps from about 24 $kg \cdot d^{-1}$ in 1970, 10 $kg \cdot d^{-1}$ in 1980, and 5 $kg \cdot d^{-1}$ in 2000. Decades ago, the high-priced *Penaues monodon* (tiger shrimp) are abundant in the wild, but the lower-priced *Metapenaeus* ensis (greasyback shrimp) dominated the catch at present with very few instances of *P. monodon*. Ingles et al. in 1991 noted 426+ stationary fishing gears in the Batan Estuary

while in 2000, Babaran et al. mapped 2,097 gears (including inner creeks). In the current study, GPS survey was conducted to map the present distribution of gears in the whole estuary revealing 1,897 gears (excluding inner creeks). In addition, the estuary is degrading, where 96% of mangroves were lost in 50 years; In general, there are three main points of the problem: (1) overcrowded fishing gears caused by low productivity, poverty and possibly population, (2) environmental destruction like mangrove loss and siltation, and (3) lack of efficient government intervention for proper implementation of rules and laws, because the three municipalities covering the estuary have different ordinances and they could not overcome the conflicts among them.

Therefore, the following general recommendations are suggested: (1) decrease and regulate the deployment stationary fishing gears; (2) mangrove rehabilitation especially in abandoned ponds; (3) political will of government to strictly implement laws and rules; and (4) alternative livelihood for the fishing population to decrease fishing pressure.

(15) 培養細胞による魚類の量的遺伝形質解析の意義と問題点
阪本憲司
海洋生物育種工学研究会（福山）(2007-12)

(16) 継代採苗シミュレーションによる遺伝的多様性の長期変化予測
野口大毅、Maria Ortega Villaizan Romo、谷口順彦
海洋生物育種工学研究会（福山）(2007-12)

(17) ヒラメ稚魚期における異物包囲能の発達
倉田　修、畑井喜司雄、多和寛人、小谷知也、 伏見　浩
第５回因島種苗生産技術交流会（福山）、講演要旨集 p. 35、 (2007-8)

(18) シオミズツボワムシ *Brachionus plicatilis* の増殖フェーズにおける個体群の質の評

価
田邊茉莉、木田邦朝、小谷知也、伏見浩
第５回因島種苗生産技術交流会（福山）、講演要旨集 p. 37-38、 (2007-8)

(19) マダイ仔稚魚期の成長に伴う摂餌日周リズム及び排泄速度の変化
須藤健介、上林久記、小谷知也、伏見浩
第５回因島種苗生産技術交流会（福山）、講演要旨集 p. 40-41、 (2007-8)

(20) Supplementation with Zn and Mn through *Artemia* enhanced growth and reduced skeletal deformity of red sea bream larvae
Tien Nguyen Van, Shuichi Satoh, Yuzuru Haga, Hiroshi Fushimi, and Tomonari Kotani
第５回因島種苗生産技術交流会（福山）、講演要旨集 p. 42-43、 (2007-8)

(21) シオミズツボワムシの脂肪酸含量に対する栄養強化法の効果
小谷知也、源河輝久、伏見 浩、林 雅弘
第５回因島種苗生産技術交流会（福山）、講演要旨集 p. 47-48、 (2007-8)

(22) ワムシの培養法がヒラメ種苗生産成績に及ぼす影響
松村慶介、小谷知也、伏見 浩
第５回因島種苗生産技術交流会（福山）、講演要旨集 p. 49-50、 (2007-8)

(23) 魚類仔稚魚期の酸素消費量の変化と成長及び生残との関係
宮嶋 暁、多和寛人、小谷知也、伏見 浩、半田 岳志、難波 憲二
第５回因島種苗生産技術交流会（福山）、講演要旨集 p. 51-52、 (2007-8)

(24) 各種製剤配合飼料の給餌はヒラメの免疫能を向上させるか？
北本恵理、田中麻衣、小谷知也、倉田 修、伏見 浩、畑井喜司雄
第５回因島種苗生産技術交流会（福山）、講演要旨集 p. 53-55、 (2007-8)

(25) 「クロマグロの健苗育成を目指した種苗生産技術開発研究」実施基本計画
伏見 浩
第５回因島種苗生産技術交流会（福山）、講演要旨集 p. 59-60、 (2007-8)

B. 総説

C. 著書

（1） Catabolite control and metabolic networks mediated by the CcpA protein in *Bacillus subtilis*
Yasutaro Fujita, Yasuhiko Miwa, Shegeo Tojo and Kazutake Hirooka
Global Regulatory Networks in *Bacillus subtilis*, edited by Yasutaro Fujita, pp. 91-110, Transworld Research Network, Kerala, India (2007)

In *Bacillus subtilis* and close relatives, global regulation of carbon catabolite control occurs on the binding of the complex of CcpA (catabolite control protein A) and P-Ser-HPr (seryl-phosphorylated form of a phosphocarrier protein of the phosphoenolpyruvate: phosphotransferase system) to the catabolite responsive elements (*cre*) of the target operons, the constituent genes of which are roughly estimated to number three hundred. The complex of CcpA and P-Ser HPr triggers the expression of several genes involved in the formation of acetate and acetoin, which are major extracellular products of *B. subtilis* grown on the glucose. It also triggers the expression of an anabolic operon (ilv-leu) involved in the biosynthesis of branched-chain amino acids which subsequently leads to cell propagation. On the other hand, this complex represses many genes and operons, which include an entrance gene for the TCA cycle (*citZ*), several transporters genes for TCA intermediates, some respiration genes, and many catabolic and anabolic genes involved in carbon, nitrogen, and phosphate matabolism as well as for certain extracellular enzymes and secondary metabolites. Thus, CcpA-mediated metabolic networks play a major role in the coordinate regulation of catabolism and anabolism to ensure the optimum cell propagation in the presence and absence of preferred carbohydrates such as glucose.

D. その他

（1） レポート「世界養殖学会アジア太平洋支部定例大会～アジアの養殖への世界的な注目を実感」
小谷知也
月刊養殖, 44, p. 74-75 (2007-11)

（２） レポート「平成19年度第1回水産増殖懇話会～マツカワの資源回復を目指した種苗生産の現状と展望」
小谷知也
月刊養殖，44，p.36-37（2007-12）

（３） レーヴン/ジョンソン　生物学[下]　原著第7版
P. レーヴン/G. ジョンソン/J. ロソス/S. シンガー共著
山岸　幸正（R/J Biology翻訳委員会、第25章担当）
培風館pp. 509-530（2006-4）

（４） 魚病細菌に対する有機酸の抗菌効果に関する研究報告書
河原栄二郎
福山大学受託研究成果報告書、pp.5（2007-3）

（５） サトウキビ抽出物の免疫増強効果に関する研究報告書
河原栄二郎
福山大学受託研究成果報告書、pp.28（2007-3）

（６） 魚病細菌の感染症予防対策に関する研究報告書
河原栄二郎
福山大学受託研究成果報告書、pp.28（2007-3）

（７） ニシキゴイの生体防御能に及ぼす塩化カルシウム浸漬の影響
河原栄二郎
平成19年度瀬戸内海・四国ブロック魚病検討会、（大阪）（2007-10）

（８） 第4回因島種苗生産技術交流会（水産研究のフロントから）
伏見浩
日本水産学会誌（73）138（2007-1）