

海洋生物科学科 2008 年研究業績

A. 研究発表

1. 論文

- (1) Effects of feeding copepod and *Artemia* on early growth and behaviour of the self-fertilizing fish, *Rivulus marmoratus*, under laboratory conditions
Maria Vivian Camacho Grageda, Tomonari Kotani, Yoshitaka Sakakura, and
Atsushi Hagiwara
Aquaculture, 281, 100–105 (2008)

Growth and survival have often been used as parameters to assess the effects of live feeds on marine finfish, however, behavioural effects, which entail energy cost and may have consequences on fish growth have been given less emphasis. Thus, a 20-day feeding experiment was conducted to determine the effects of copepod *Acartia tsuensis* (104–732 μm), unenriched, and docosahexaenoic acid, DHA-enriched, first instar *Artemia franciscana* nauplii (656–906 μm) on growth and behaviour of the mangrove killifish *Rivulus marmoratus*. Growth was significantly higher in *Acartia*-fed larvae compared with larvae fed *Artemia* (unenriched and DHA-enriched) until day 10. On day 20, *Acartia*-fed larvae had significantly lower growth than fish fed DHA-enriched *Artemia*. Feeding success was highest in larvae fed *Acartia* on day 1. Ingestion rate and satiation time did not differ among fish fed different types of feeds until day 20. Swimming activity before feeding was significantly lower in larvae fed *Acartia* compared with larvae fed *Artemia* (unenriched and DHA-enriched) until day 10. Higher growth in *Acartia*-fed fish on day 10 is probably due to the suitable size and high DHA content of *A. tsuensis*, and lower swimming activity of the larvae. However, on day 20, lower growth observed in *Acartia*-fed fish may be attributed to the shift in the food size preference of the fish. The present study was able to demonstrate the effects of copepods on growth and behavioural development of marine finfish using *R. marmoratus* as a model animal.

- (2) Effect of zinc and manganese supplementation in *Artemia* on growth and vertebral deformity in red sea bream (*Pagrus major*) larvae
Van Tien Nguyen, Shuichi Satoh, Yutaka Haga, Hiroshi Fushimi, and Tomonari Kotani

Feeding trials were carried out to determine the effects of zinc (Zn) and manganese (Mn) supplementation in *Artemia* on growth, survival, body composition and skeletal deformity of red sea bream larvae. Triplicate groups of red sea bream larvae from 15–30 day post-hatching (dph) were fed four types of *Artemia* enriched with Zn (Z), Mn (M), both Zn and Mn (ZM) and without Zn or Mn (control). At 30 dph, significantly higher ($P < 0.05$) growth performance of the fish was recorded in M group (TL = 15.60 ± 0.45 mm) compared to that of the control (TL = 14.90 ± 0.41 mm). Fish fed *Artemia* supplemented with only Zn and with both Zn and Mn showed similar growth performance compared to that of the control. Survival of the fish was not affected either by Zn or Mn supplementation. Increased Mn or both Zn and Mn in *Artemia* nauplii significantly elevated ($P < 0.05$) crude lipid content in 30dph juvenile compared to that in the Z group. At 30 dph, Mn content in juvenile of M and ZM groups was significantly higher ($P < 0.05$) compared to that in the other groups. Similarly, Zn content in the Z group was significantly higher ($P < 0.05$) compared to that in the M and control groups. Skeletal deformities in the experimental fish at 30 dph were highest ($P < 0.05$) in the control group and were significantly improved by supplementation with Zn and Mn. The major skeletal deformities were observed in the vertebral column, neural and hemal spines. In the vertebral column, occurrence of deformities in the neck, hemal and preural were higher than in other regions. The results of the present study demonstrated that maintenance of Mn level in *Artemia* nauplii from 12 to $42.8 \mu\text{g g}^{-1}$ (dry-matter basis) improved growth performance of red sea bream larvae. Zn and Mn supplementation in *Artemia* promoted normal skeletal development of red sea bream larvae.

(3) Improvement of Vaccination Effect on Ocellate Puffer and Japanese Flounder by the Feeding of Artificial Feed with Heat-killed *Enterococcus faecalis* FK-23

Tomonari Kotani, Eri Kitamoto, Osamu Kurata, Norihiro Hirayama, Hiroshi Fushimi, Shuichi Satoh, Kishio Hatai, Aki Miyashima, Takao Nohmi, and Takashi Shimada

Aquaculture Science, 56, 375–382 (2008)

We studied the specific immunostimulant effect of heat-killed *Enterococcus faecalis* FK-23 (FK-23) when the artificial diet including FK-23 was fed on Japanese flounder and

ocellate puffer. The diets with 5 different concentrations of FK-23 was prepared (0-1.25%). Fishes were injected formalin-killed cells of *Vibrio anguillarum* and the injection was performed two times. The second injection was done 3 weeks after the first injection. The agglutination titer of antibody for *V. anguillarum* cells in their serum was evaluated every week after the first injection and the evaluation was performed six times. The agglutination titer in the serum of ocellate puffer fed the diet including highest concentration (1.25%) of FK-23 increased more intensively with the progress of rearing than other concentrations. However those puffers fed the diet with 1.25 % of FK-23 did not grow during the experimental rearing period. Although the agglutination titer in the serum of flounder did not increase, this is possibly because the non-specific immunity worked more intensively than specific one. Consequently FK-23 is possible to increase the vaccination effect of puffer and maintain its effect, and it also make the non-specific immunity of flounder intensify.

- (4) Evaluation of acclimation by measuring the ventilatory frequency in the redfin velvetfish *Paracentropogon rubripinnis* under thermal stimulation
Kenji Sakamoto, Youichi Yokoe, and Iwao Furusawa
Fish Genetics and Breeding Science, 37, 37-41 (2008)

Redfin velvetfish were acclimated at 8-27°C, and then exposed to high temperature in a range of 21-35°C. Ventilatory frequency was measured at 5-300 minutes. The present study showed two patterns for the changes in ventilatory frequency. In the first pattern, once ventilatory frequency exceeded 100 times/min, and then increased or remained at constant frequency. In the second pattern, ventilatory frequency first stabilized at 80 times/min and then gradually decreased. The former pattern shows the inability to acclimate to high water temperature, while the latter shows the ability to acclimate. We termed the water temperature of the upper limit at which a decrease of ventilatory frequency was observed as the "temperature of acclimation turning point"; the relationship between this temperature and the acclimation water temperature is $y=17.865+0.5739x$ ($r=0.997$, $p<0.01$). From these results, ventilatory frequency could be used as an index to clarify the relationship between the rearing water temperature of redfin velvetfish and the temperature of acclimation turning point. To select the fish with resistance to high water temperature, ventilatory frequency may be a useful index.

- (5) Direct evidence of multiple paternities in natural population of viviparous

Japanese surfperches by allelic markers of microsatellite DNA loci
Motohiro Takagi, Kumiko Sakai, and Nobuhiko Taniguchi
Fisheiries Science, 74, 976-982 (2008)

This study was performed to obtain information on the occurrence of multiple paternities in three species of viviparous Japanese surfperches using allelic markers of microsatellite DNA loci. Direct evidence for multiple inseminations was established by reconstructing paternal genotypes of the progeny of pregnant females. Multiple paternities were ascertained in 5 of 10 broods of *Ditrema temmincki* and in 3 of 9 broods of *Neoditrema ransonetti*, but not in *Ditrema viride*. The number of patrilines detected in the progeny of *D. temmincki* and *N. ransonetti* females were 2 or 3 respectively, as determined by the GERUD 2.0 computer program for the reconstruction of parental genotypes from half-sib progeny arrays.

- (6) Inheritance mode of microsatellite DNA markers in maternal and larval kuruma prawns *Marsupenaeus japonicus*
Motohiro Takagi, Takuma Sugaya, Uiko Kubota, Toshiaki Itami, and Nobuhiko Taniguchi
Fish Genetics and Breeding Science, 38, 51-53 (2008)

Based on sequences from *Marsupenaeus japonicus*-positive clones, we identified 4 primer pairs. These loci were highly polymorphic, with the degree of variability differing by locus. The average number of alleles per locus was 22.0 and the average estimated heterozygosity was 0.90. Loci *Mja06*, *Mja14* and *Mja22* are Mendelian markers. However, a null allele was inferred to exist at the locus *Mja27*, based on the assumption that the allele followed the Mendelian inheritance pattern.

- (7) Microsatellite development and survey of genetic variation in skipjack tuna *Katsuwonus pelamis* (L.)
Maria R. MENEZES, Daiki Noguchi, Masamichi Nakajima, and Nobuhiko Taniguchi
Journal of Fish Biology. 73, 463-473 (2008)

A survey of five newly developed microsatellite DNA markers in *Katsuwonus pelamis* revealed high levels of polymorphism in two samples off the west coast of India (Minicoy Island and Kochi coast) and one sample off the Japan coast. Although significant

differentiation ($P < 0.01$) in number of specific alleles was observed between Minicoy and Kochi samples, the F_{ST} values among the samples were very low (average = 0.0014 and not significant ($P = 0.284$)).

- (8) Isolation and characterization of 13 microsatellite markers for the viviparous surfperch *Ditrema temmincki* (Embiotocidae) and cross-species amplification

Motohiro Takagi, Kumiko Sakai, and Nobuhiko Taniguchi

Molecular Ecology Resources, 8, 1030–1033 (2008)

Thirteen microsatellite loci were isolated from a size-selected genomic library of the surfperch (*Ditrema temmincki* Bleeker). All loci displayed a high degree of length polymorphism, as observed in the total number of alleles per locus (2–23) and a high degree of estimated heterozygosity, ranging from 0.080–0.893. The primers developed for *D. temmincki* were also tested for their ability to amplify homologous sequences in *D. viridis* and *Neoditrema ransonetii*. Distinct differences were observed among three species of surfperches, in both genetic variability and the frequency distribution of the alleles.

- (9) The lunar cycle affects at-sea behaviour in a pelagic seabird, the streaked shearwater, *Calonectris leucomelas*

Takashi Yamamoto, Akinori Takahashi, Ken Yoda, Nobuhiro Katsumata, Shinichi Watanabe, Katsufumi Sato, Philip N. Trathan

Animal Behaviour, 76, 1647–1652 (2008)

The lunar cycle has been shown to affect the behaviour of a variety of marine animals. However, changes in the behaviour of seabirds and marine mammals and how they respond to the lunar cycle have been less well documented, principally because of the technical difficulties in long-term recording of their at-sea behaviour. Because seabirds generally rely on visual cues at small spatial scales, ambient light levels by moonlight may have a strong influence on their foraging and predator avoidance behaviours at sea at night. We used global location sensor loggers attached to wintering streaked shearwaters to examine whether the birds' at-sea behaviour varied with the lunar phase. The shearwaters migrated from Japan to the seas off northern New Guinea. Their activities at night changed synchronously with the lunar phase: birds flew for longer periods and

landed on water more frequently on nights with a full moon than when there was a new moon. Our results indicate that at-sea behaviour of pelagic seabirds is closely associated with the lunar cycle.

- (10) シンポジウム記録 水産学と日本水産学会の未来 Part-Ⅲ 一行動する日本水産学会-I (4) 大学の立場から
伏見 浩
日本水産学会誌, 74, 1096-1097 (2008)

与えられたタイトルは極めて大きなもので、私がおのべてを述べられる立場には無い。わが国の大学には国立大学法人、学校法人および地方公共団体が設立しているものがある。それぞれの大学には建学の精神と教育の基本方針がある。大学には、その数だけの個性があり、本シンポジウムの課題についても大学の数だけの考え方があって当然であろう。全ての大学に共通することは唯一つ、大学は高等教育機関であり、学生の教育を行なうところであることであろう。その共通点を有するという一事だけで、著者が話題提供することをお許しいただきたい。

2. 報文

- (1) 瀬戸内海中央部因島・福山の海藻相
山岸幸正、三輪泰彦
福山大学生命工学部研究年報 (7), 21-33 (2008)

瀬戸内海中央部備後灘海域に位置する因島八重子島周辺、福山市鞆町および芦田川河口付近の竹ヶ端において毎月海藻採集を行い、本海域に緑藻 35 種、褐藻 51 種、紅藻 114 種、合計 200 種の海藻が生育することを明らかにした。合計 192 種が得られた因島八重子島周辺は種数も多く褐藻ホンダワラ科数種やクロメなどによる藻場も発達しており、海藻の多様性が高い場所であると考えられる。合計 121 種が得られた鞆町は因島と比べると特に潮下帯の大形海藻の多様性が低かった。竹ヶ端では合計 48 種しか得られず、潮下帯に藻場と呼べるような海藻群落はみられなかった。本研究の結果、瀬戸内海新産種である紅藻オイワケキヌイトグサ *Callithamnion corymbosum* (Smith) Lyngbye、シンカイユナ *Chondria mageshimensis* Tanaka et K. Nozawa が確認された。瀬戸内海特産種の褐藻ナガ

ゲンセンクロガシラ *Sphacelaria nipponica* Kitayama が因島から採集された。これまで日本では報告のなかった紅藻ユナ *Chondria crassicaulis* Harvey の雌雄配偶体が鞆町および因島に生育することを明らかにした。

- (2) A technique to detect synchronous diving behaviour of Antarctic penguins using time-depth recorders

Shinichi Watanabe, Akinori Takahashi, Katsufumi Sato, Phil Trathan, Yutaka Watanuki

Proceedings of the Fifth World Fisheries Congress, 1i_0273 (2008)

Recent studies have shown that several species of penguins synchronize beginning and end of dives with other individuals of the same species, a.k.a synchronous diving. The functions of synchronous diving have been investigated with fortuitous recording of identical diving behaviour of 2-3 individuals obtained by time-depth recorders. To understand fully the functions and mechanisms of synchronous diving, it is necessary to investigate data obtained from almost all members of an aggregation. However, it is a hard task to detect synchronous diving in large amount of data. In the present study, we devised an algorithm to efficiently detect synchronous diving, and applied the algorithm on diving depth records from Adelie penguins near Syowa station, Adelie and chinstrap penguins in Signy Island, Antarctica. Using this algorithm, synchronous diving was efficiently detected in each population of penguins. From the results, we found several differences between synchronous diving of penguins in both species and in both study sites. We believe that this method can be applied on many more populations of penguins efficiently. Furthermore, interspecific, geographical or temporal variations of synchronous diving behaviour in penguins will be discussed.

- (3) Vaccination of Japanese flounder, *Paralichthys olivaceus* against nocardiosis with adjuvanted killed *Nocardia seriolae*

Eriko Nishikawa and Eijiro Kawahara

Proceedings of the WFC 2008, 5th World Fisheries Congress, 2a_0302 (2008-10)

Japanese flounder weighing about 80g were maintained in running sea water. *Nocardia seriolae*, strain number 20 was cultured using a brain heart infusion medium, and was killed with formalin. The fish were immunized with the emulsion by intraperitoneal injection. Control fish were injected with a mixture of the adjuvant and phosphate

buffered saline by the same manner. At three weeks after immunization, serum was separated from peripheral blood of the fish. Serum antibody titer was analyzed with the formalin-killed bacterium. Number of head kidney antibody producing cells was calculated by plaque forming test. Superoxide production and phagocytic activity of head kidney leucocytes were determined using zymosan. And an effectiveness of the vaccine was evaluated by experimental challenge test. The challenge test was performed by intraperitoneal injection of the same strain of *N. seriolae*. In comparison to control group, the immune responses were increased in the vaccinated group was higher than that of control group. These results indicate that oil adjuvanted formalin-killed *N. seriolae* is an effective vaccine for the prevention of nocardiosis in Japanese flounder.

- (4) Immunomodulatory effects of formalin-killed *Nocardia seriolae* on Japanese flounder vaccinated with adjuvanted *Edwardsiella tarda* lipopolysaccharide
Tomoki Hatate and Eijiro Kawahara
Proceedings of the WFC 2008, 5th World Fisheries Congress, 2a_0303 (2008-10)

Japanese flounder, *Paralichthys olivaceus* weighing about 50g were maintained in running sea water. Fish were fed commercial pellets daily. *Edwardsiella tarda* and *Nocardia seriolae* were cultured with brain heart infusion. Crude lipopolysaccharide from *E. tarda* was performed by water-phenol extraction method. *N. seriolae* was inactivated with formalin. Fish were immunized with oil adjuvanted *E. tarda* crude lipopolysaccharide supplemented with or without formalin-killed *N. seriolae* by intraperitoneal injection. At 3 weeks after immunization, serum was separated from peripheral blood of the fish. Serum antibody titer was analyzed against *E. tarda*. Superoxide anion production of head kidney leucocytes was determined with zymosan. And an effectiveness of the immunization was evaluated by experimental challenge test. The challenge test was performed by intraperitoneal injection with *E. tarda*. The immune responses of the fish injected with supplemented immunogen were increased higher than those of the fish injected with non-supplemented immunogen. In the experimental challenge test, survival rate in the fish injected supplemented immunogen was higher than that of the fish injected with non-supplemented immunogen. These results suggest that formalin-killed *N. seriolae* possesses immunomodulatory effects on Japanese flounder for vaccination against edwardsiellosis.

- (5) Effect of nutritional enrichment method on activities and fatty acid contents of euryhaline rotifer *Brachionus plicatilis*
Tomonari Kotani, Teruhisa Genka, Hiroshi Fushimi, and Masahiro Hayashi
Proceedings of the fifth World Fisheries Congress, 2c_0358 (2008)

Nutritional enrichment for rotifer is necessary for the successful production in larviculture. Although manufacturers designated their direction of enrichment diets, it is not known what effect on enrichment the arranged direction shows. We aimed to clarify effects of excessive application of enrichment diet. Two primary cultures were performed with batch and continuous methods. Each population was enriched with commercial enrichment diet. Rotifer populations from two primary cultures were enriched with two methods. One applied the diet with 0.25 g/L for 8 hrs (normal) and another did with 0.75 g/L for 24 hrs (excessive). The fatty acid contents of each population were analyzed with GC after secondary culture. Fatty acid contents of rotifer with the excessive method tended to be higher than normal enrichment. But the magnification was no further than 1.5 – 2 times. In populations enriched normally, fatty acid contents of the population from continuous cultures were twice as high as from batch cultures. However, in populations with the excessive method, fatty acid contents of the population from continuous cultures did not differ from batch cultures. In populations from each primary culture, the DHA/EPA ratio of rotifer enriched with the excessive method was higher than normal direction.

- (6) Immunostimulating effects of a lactic acid bacterium, *Lactobacillus plantarum* on colored carp, *Cyprinus carpio*
Yuko Kondo and Eijiro Kawahara
Proceedings of the WFC 2008, 5th World Fisheries Congress, 2d_0410 (2008-10)

The effects of the dietary administration of a lactic acid bacterium, *Lactobacillus plantarum* on non-specific immune responses of colored carp, *Cyprinus carpio* were investigated. Fish weighing about 50g were fed four different diets: diets supplemented with a liquid media culture of the bacterium; the culture supernatant of the bacterium; formalin-killed cells of the bacterium; or non-supplemented control. After 1 and 2 weeks administration, five fish were sampled each from the four different diet groups. Serum was separated from the peripheral blood of the fish. Superoxide production and

phagocytic activity of head kidney and intestinal leucocytes were evaluated with zymosan. Lysozyme activities of kidney and serum were determined using *Micrococcus lysodeikticus*. Complement activity of serum was analyzed with rabbit red blood cells. The immune response of the group administered with the formalin-killed cells were significantly higher than those of the non-supplemented control group after 1 week administration. After 2 weeks administration, superoxide production of intestinal leucocytes was increased significantly in the group administered with the liquid media culture. These results indicate that the media culture and the killed cells of *L. plantrum* possesses immunostimulating effects to colored carp.

- (7) The Method to Measure Thermal Tolerance Traits Using Caudal Fin Cell of Clonal Fish and its Application to Aquaculture Fish
Kenji Sakamoto, Masamichi Nakajima, and Nobuhiko Taniguchi
Proceedings of the fifth World Fisheries Congress, 2e_0448 (2008)

The method to measure thermal tolerance traits was developed using a clonal fish, silver crucian carp *Carassius langsdorfii*. We used the offspring derived from six different clonal lines, each of which was presumed to be genetically identical.

Juveniles reared at 25°C were exposed to thermal stress at 40°C, and the time to death during the exposure was measured and then compared among the clonal lines. Assay of primary culture cells from each clone was conducted at 43°C using trypan blue assay. We used the same assay at 43°C to determine the thermal tolerance of free cells from caudal fin clips from each clonal line. This method for measuring the thermal tolerance traits were applied to the ayu, *Plecoglossus altivelis*, and Japanese flounder *Paralichthys olivaceus*, and common carp *Cyprinus carpio*. The thermal tolerance values observed in the whole body were significantly correlated with the values obtained from both primary culture cells and free cells. This finding suggests that cell assays with caudal fin cells could be applied to the study for evaluation of the genetic variation and heritability of thermal tolerance traits in fish.

- (8) ペンギン類の同調潜水行動の地域間比較シンクロナイズト・ダイビング：昭和基地と英国基地どちらのペンギンが上手か？
渡辺伸一、高橋晃周、佐藤克文、Phillip Trathan、綿貫 豊
第4回日本バイオロギング研究会シンポジウム論文集, 39-40 (2008)

ペンギン、アザラシ、イルカなど肺呼吸を行なう潜水動物では、複数の個体が潜水と水面への浮上のタイミングを一致させる同調潜水（シンクロナイズト・ダイビング）が知られている。同調潜水に関する研究は、ペンギン類で多く報告されており、深度記録計（TDR）を複数個体に装着し、同時に計測した深度データの比較から、同調潜水を検出し、その機能について考察されている。従来の研究では、同調潜水を行う集団を観察から探し出し、その数個体から得られた短期間の深度データをもとに分析を行っていた。同調潜水のメカニズムを解明するためには、集団中の多くの個体に深度記録計を装着して、その集団内での行動の同調性を詳しく分析する必要がある。しかし、同調潜水を深度データから効率よく検出することが困難だったことから、長期的に得られた多個体の深度データから同調潜水を検出し、その発生頻度や機能について詳しく研究した例はなかった。本研究では、同調潜水を効率よく検出するためのアルゴリズムを考案し、集団のデータから同調潜水を分析することに成功した。分析の結果、同時に取得した TDR のデータから、潜水動物の同調行動を検出することによって、地域間の集団性の違いや採餌環境の相違を知る上で重要な情報を得ることができた。

(9) フィールドワークお助け道具紹介：スネーク・アイ

渡辺伸一

バイオロギング研究会会報 (25), 3-5 (2008)

オオミズナギドリは、ミズナギドリ科の海鳥の一種で、地面に巣穴を掘って、そこで繁殖する。繁殖は曲がりくねったトンネル状の巣穴の先で行われ、トンネル長は数メートルに達するため、外から覗いただけでは、中の様子はまったくわからないことが多い。そこで、著者は巣穴内の様子を詳しく観察するための装置（スネーク・アイ）を開発した。開発した装置は、オオミズナギドリの巣内を観察する上で有効なツールとなった。また、地中だけでなく樹洞に営巣する鳥や、鳥に限らず巣穴を持つ哺乳類の繁殖生態の観察にも有効であることが示唆された。

(10) フィールドワークお助け道具紹介：VHF 電波発信器の再利用法について

渡辺伸一

バイオロギング研究会会報 (31), 4-5 (2008)

動物に装着したデータロガーを回収するために VHF 電波発信器を使用する。本研究では、電波発信器のバッテリーを交換し、再利用する方法を考案した。再生した発信器は、強度や防水性が新品のものと比較して低下することが予想されるが、発信器探索の練習や、捕獲用の罠につけて捕獲探知機として利用するなどの用途

に使用することが可能だった。

3. 学会発表

- (1) Improvement of larval health and quality, with special reference to life stage and morphogenesis of finfish larvae
Hiroshi Fushimi, and Tomonari Kotani
Australian Aquaculture 2008 (Brisbane, Queensland, Australia) Abstracts, (2008-8)

We have a long history of research and development in finfish larviculture technology, targeted to produce high hatchery-raised juveniles for aquaculture and stock enhancement. Deformities such as malpigmentation and vertebral malformation in hatchery-raised juveniles continue to occur to variable degrees. Steady progress of aquaculture and stock enhancement, based on hatchery-raised juveniles, has highlighted the importance of physiological, morphological, and ecological characteristics of the hatchery-raised juveniles in their effectiveness. Larval quality is the key issue in survival in the field after release for stock enhancement, severe mortality in larviculture and low market price of aquaculture products.

While the presence of these problems was reported in the early stages of larviculture in Japan, and characteristics of deformities in adult and larval fish are well documented, the aetiology of skeletal deformities in finfish larvae has not been precisely determined. However, it is widely recognized that several factors have important roles for the occurrence of skeletal deformity of finfish larvae: temperature, salinity, malformation of swim bladders, effect of triploidization; genetics, and nutrition. Dietary components that influence skeletal development in finfish larvae are vitamin C, vitamin A, vitamin D3, vitamin K, tryptophan, peptides, essential fatty acid, highly unsaturated fatty acids (HUFAs), and phospholipids.

We are now investigating the suitable nutritional composition of live food, such as rotifers and *Artemia*, for application in industry to improve finfish larval health and quality. While there are many publications on the roles of nutritional components on the

occurrence of skeletal deformity of finfish larvae, reporting of the effect of stocking density on larval performance is limited. Consequently, we first determined the optimum stocking density of yolk-sac larvae. We examined the allometric growth of finfish larvae from yolk-sac larvae to juvenile. Using the allometric growth coefficient, we defined the stages of the larval period and described the characteristics of each life stage such as allometric growth, body shape, external morphological features and skeletal development.

Second, in order to eliminate the occurrence of skeletal deformity, we tested experimental enrichment diets with different vitamin A concentrations to determine the appropriate vitamin A concentration for rotifers and *Artemia nauplii*. We assessed the occurrence of skeletal deformity at each life stage and the changes in the occurrence rate of skeletal deformity related to vitamin A concentration of live food. Third, we examined the EPA, DHA and DHA/EPA ratio of live food using enrichment diets with fixed EPA concentration, equivalent to *Nannochloropsis* enrichment, and different DHA concentrations. It is important to consider the effect of rotifer condition and cultivation methods which affect the nutritional composition of enriched rotifers. While further studies are necessary to establish practical solutions for improvement of larval health and quality, we summarize our findings to date.

(2) Interspecific and geographical comparisons of synchronous diving behaviour of Antarctic penguins using time-depth recorders

Shinichi Watanabe, Akinori Takahashi, Katsufumi Sato, Phil Trathan, Yutaka Watanuki

3rd International Bio-logging Science Symposium, Monterey, CA, USA.
Abstracts, p. 79 (2008-9)

Recent studies have shown that several species of penguins synchronize beginning and end of dives with other individuals of the same species, a.k.a synchronous diving. The functions of synchronous diving have been investigated with fortuitous recording of identical diving behaviour of 2-3 individuals obtained by time-depth recorders. To understand fully the functions and mechanisms of synchronous diving, it is necessary to investigate data obtained from almost all members of an aggregation. However, it is a hard task to detect synchronous diving in large amount of data. In the preset study, we devised an algorithm to efficiently detect synchronous diving, and applied the algorithm on diving depth records from Adélie penguins near Syowa station, Adélie and chinstrap

penguins at Signy Island in South Orkney Islands, Antarctica. Using this algorithm, synchronous diving was efficiently detected in each population of penguins. We compared synchronous diving behaviour among the populations in terms of frequency of occurrence of synchronous diving, time duration of one synchronous diving period, and spatial difference between diving profiles of synchronously dived birds. Our results showed several differences in synchronous diving characteristics both between species and between study sites. Herein, we discuss interspecific and geographical variations of synchronous diving behaviour in penguins.

- (3) Effect of Zinc and Manganese enrichment in *Artemia* on growth and vertebral deformity in red sea bream (*Pagrus major*) larvae

Tien Van Nguyen, Shuichi Satoh, Yutaka Haga, Hiroshi Fushimi, and Tomonari Kotani

5th World Fisheries Congress (Pacifico Yokohama, Yokohama) Abstracts, p. 162 (2008-10)

Feeding trials were carried out to determine the effects of zinc and manganese supplementation in *Artemia* on growth and skeletal deformity of red sea bream larvae.

Triplicate groups of larvae from 15- 30dph were fed four types of *Artemia* enriched with Zn (Z), with Mn (M), with both Zn and Mn (ZM) and without Zn or Mn (control), respectively.

At 30dph, significant higher growth was recorded in M group than that of the control. Survival was not affected neither by Zn nor Mn supplementation. Moisture, crude protein and ash content in juvenile at 30dph were not different among all groups. However, the Mn content in juvenile at 30dph in M and ZM groups was significantly higher compared to that of the control and Z groups. Similarly, Zn content was significantly higher in the Z group compared to that of the other groups. Skeletal deformity in the 30dph juveniles was highest in the control group and was significantly improved by supplementation with Zn and Mn.

In conclusion, maintenance of Mn in enriched *Artemia* from 12-42.8 $\mu\text{g g}^{-1}$ (dry-basis) improved growth of red sea bream larvae. Supplementation Zn and Mn in *Artemia* promoted normal skeletal development of the red sea bream larvae.

- (4) A technique to detect synchronous diving behaviour of Antarctic penguins using time-depth recorders

Shinichi Watanabe, Akinori Takahashi, Katsufumi Sato, Philip Trathan, and Yutaka Watanuki

5th World Fisheries Congress, Yokohama, Japan. Abstracts, p. 338 (2008-10)

Recent studies have shown that several species of penguins synchronize beginning and end of dives with other individuals of the same species, a.k.a synchronous diving. The functions of synchronous diving have been investigated with fortuitous recording of identical diving behaviour of 2-3 individuals obtained by time-depth recorders. To understand fully the functions and mechanisms of synchronous diving, it is necessary to investigate data obtained from almost all members of an aggregation. However, it is a hard task to detect synchronous diving in large amount of data. In the preset study, we devised an algorithm to efficiently detect synchronous diving, and applied the algorithm on diving depth records from Adelie penguins near Syowa station, Adelie and chinstrap penguins in Signy Island, Antarctica. Using this algorithm, synchronous diving was efficiently detected in each population of penguins. From the results, we found several differences between synchronous diving of penguins in both species and in both study sites. We believe that this method can be applied on many more populations of penguins efficiently. Furthermore, interspecific, geographical or temporal variations of synchronous diving behaviour in penguins will be discussed.

- (5) Effect of nutritional enrichment method on activities and fatty acid contents of euryhaline rotifer *Brachionus plicatilis*

Tomonari Kotani, Teruhisa Genka, Hiroshi Fushimi, and Masahiro Hayashi

5th World Fisheries Congress (Pacifico Yokohama, Yokohama) Abstracts, p. 353 (2008-10)

Nutritional enrichment for rotifer is necessary for the successful production in larviculture. Although manufacturers designated their direction of enrichment diets, it is not known what effect on enrichment the arranged direction shows. We aimed to clarify effects of excessive application of enrichment diet. Two primary cultures were performed with batch and continuous methods. Each population was enriched with commercial enrichment diet. Rotifer populations from two primary cultures were enriched with two

methods. One applied the diet with 0.25 g /L for 8 hrs (normal) and another did with 0.75 g /L for 24 hrs (excessive). The fatty acid contents of each population were analyzed with GC after secondary culture. Fatty acid contents of rotifer with the excessive method tended to be higher than normal enrichment. But the magnification was no further than 1.5 – 2 times. In populations enriched normally, fatty acid contents of the population from continuous cultures were twice as high as from batch cultures. However, in populations with the excessive method, fatty acid contents of the population from continuous cultures did not differ from batch cultures. In populations from each primary culture, the DHA/EPA ratio of rotifer enriched with the excessive method was higher than normal direction.

- (6) Euryhaline cladoceran *Diaphanosoma celebensis* as a live food for marine fish larvae
Atsushi Hagiwara, Yoshitaka Sakakura, Takashi Nakamoto, Yoshikazu Inada, Isao Maruyama, Tomonari Kotani, and Hiroshi Fushimi
5th World Fisheries Congress (Pacifico Yokohama, Yokohama) Abstracts, p. 353 (2008-10)

The size distribution of the euryhaline cladoceran *Diaphanosoma celebensis* ranges between 400 and 1100 µm. This species is easily cultured and HUFA content can be increased by enrichment with DHA containing *Chlorella*. Hence it may be used as a substitute of *Artemia* nauplii. Higher population growth of *D. celebensis* was observed when *N. oculata* (2.5 million cells/mL) and *C. vulgaris* (1 million cells/mL) were co-fed to *D. celebensis* under total darkness. The optimal temperature and salinity were 30-35C and 3-10 pptS. Culture trials in 2L and 80L water volume resulted in highest density at 100 ind./mL and 70 ind./mL, respectively. We conducted rearing trials of mangrove killifish *Kryptolebias marmoratus* larvae from hatching. Ten days rearing trials by feeding *Artemia franciscana* and *D. celebensis* resulted in similar growth, even though feeding success of larvae was 28.5 % with *D. celebensis*, while it was 100 % with *A. franciscana*. We further compared larval growth and survival of Japanese flounder *Paralichthys olivaceus* among feeding regimes using *Artemia* and *D. celebensis*, and there was no difference in survival and growth between treatments.

- (7) The Method to Measure Thermal Tolerance Traits Using Caudal Fin Cell of Clonal Fish and its Application to Aquaculture Fish

Kenji Sakamoto, Masamichi Nakajima, and Nobuhiko Taniguchi
5th World Fisheries Congress (Pacifico Yokohama, Yokohama) Abstracts, p. 364
(2008-10)

The method to measure thermal tolerance traits was developed using a clonal fish, silver crucian carp *Carassius langsdorfii*. We used the offspring derived from six different clonal lines, each of which was presumed to be genetically identical.

Juveniles reared at 25°C were exposed to thermal stress at 40°C, and the time to death during the exposure was measured and then compared among the clonal lines. Assay of primary culture cells from each clone was conducted at 43°C using trypan blue assay. We used the same assay at 43°C to determine the thermal tolerance of free cells from caudal fin clips from each clonal line. This method for measuring the thermal tolerance traits were applied to the ayu, *Plecoglossus altivelis*, and Japanese flounder *Paralichthys olivaceus*, and common carp *Cyprinus carpio*. The thermal tolerance values observed in the whole body were significantly correlated with the values obtained from both primary culture cells and free cells. This finding suggests that cell assays with caudal fin cells could be applied to the study for evaluation of the genetic variation and heritability of thermal tolerance traits in fish.

(8) Genetic change in wild population of red sea bream, *Pagrus major*, for 10 years practice of stock enhancement program

Nobuhiko Taniguchi, Daiki Noguchi, Kenji Sakamoto, Takuma Sugaya, and Masato Aritaki

5th World Fisheries Congress (Pacifico Yokohama, Yokohama) Abstracts, p. 364
(2008-10)

In Japan, the national program of stock enhancement has been done by releasing artificially bred seed of several economically important fish and shellfish species. The loss of genetic variability which may happened in the artificially bred seed population may cause possible risk of reducing the genetic variability of wild population by releasing such hatchery bred fish to the natural water. In this study, we examined the sample lots from the coastal waters of Japan collected in 2007 to detect allelic types of microsatellite DNA markers for evaluation genetic change in wild population of red sea bream, *Pagrus major*. We compared the genetic variability and sub-population structure in 2007 with the parameters from sample lots collected in 10 years before, 1997. Although much amount

of artificially bred seed fish of red sea bream has been released for recent 10 years in the enhancement program, distinct genetic change could not detect in so far the allele richness and heterozygosity of the wild population.

(9) Morphological development of larval pacific bluefin tuna under rearing conditions

Hiroshi Fushimi, Norihisa Kotani, Tomonari Kotani, Yuuji Kamimura, Tomohiko Kawamoto, Makoto Saitou, Takahiro Oosedo, Masaharu Shizuhara, Tsutomu Watanabe, and Takashi Kusano

Current Status and Future Development of Tuna Aquaculture (5th World Fisheries Congress Sattelite symposium) (Pacifico Yokohama, Yokohama) Abstracts (2007-5)

We have long history and experiences on R/D of finfish larviculture, targeted to produce high hatchery-raised juveniles for aquaculture and stock enhancement. In early ages of R/D of finfish larviculture, we usually use a trial and error method to establish the hatchery technologies such as feeding regime, shape of larviculture tank, management of water current of the rearing tank, etc. In modern ages, we are able to develop the ideal larviculture method for new target species using cumulative and established knowledge of finfish hatchery technology.

The larviculture of Pacific Bluefin Tuna has a long history by Kinki University, Amami Yougyo, Amami Station of JASFA and Fisheries Research Agency. However, the state of PBT larviculture is remained a pre-stage of mass production.

In general, the understanding of development and developmental stages of finfish larvae is one of important biological basis of finfish larviculture. The growth of finfish body and body components are not uniform. But, the systems for feeding and swimming must develop simultaneously and preferable in mutual balance (J.W.M. Osse et al. 1997). Therefore, it is very important for establishment of PBT larviculture to clarify the relative growth of body components which is reflecting the development of function such as feeding ability, burst swimming to escape predation.

We measured total length (TL), standard length (SL), head length (HL), trunk length (TrL) tail length (TIL) and body depth (BD) from larvae fixed formalin solution obtained from larval rearing trial of Amami Yougyo Ltd. Co. The relative growth of body components of larval PBT had multiple allometric regression curves. We examined the allometric growth of PBT larvae from yolk-sac larvae to juvenile. Using the allometric

growth coefficient, we defined the thirteen stages of the larval period which has same characteristics of allometric growth of body components and described the characteristics of each life stage such as allometric growth, body shape, and external morphological features.

- (10) Effect of feeding of frozen freshwater cladoceran *Moina macrocopa* on red sea bream larvae

Tomonari Kotani, Hiroyuki Imari, and Hiroshi Fushimi

8th Japan-Korea, Korea-Japan Joint Symposium on Aquaculture (Shirahama, Wakayama) 2008. Abstracts, p. 38 (2008-10)

Artemia nauplii have been utilized as the subsequent live food for fish larvae to euryhaline rotifer *Brachionus*. Because of the shortage of *Artemia* cyst resource and the rise of their cost, any alternative foods for *Artemia* nauplii have been required. This study aimed to investigate the effect of utilization of frozen cladoceran as the alternative for *Artemia* nauplii. In order to compare the effect of feeding, we used the feeding regime of *Artemia* nauplii or zooplankton captured from open seawater. Freshwater cladoceran *Moina macrocopa* were cultivated in 1m³ tank. They were fed freshwater *Chlorella* and the cultivation water was added the extract of chicken manure for the fertilization. After the harvest of cladoceran, they were enriched nutritionally with commercial enrichment diet and then frozen at -30°C. Red sea bream larvae were fed only rotifer until 18 days after hatching (DAH). After 19 DAH, they were fed rotifer and frozen cladoceran, enriched *Artemia* nauplii, or zooplankton captured from open seawater. Duplication was performed in each subsequent feeding condition of rotifer. Total and standard length of 20 larvae of each tank were measured every five days. At 32 DAH, the survival number in each tank was counted and the air dive test was performed in order to evaluate the activity of fishes. Fatty acid contents of each food and fish were analyzed with gas-chromatography. DHA and EPA contents of enriched frozen cladoceran were lower than enriched *Artemia* and zooplankton. Fishes fed cladoceran survived more than ones fed *Artemia* and zooplankton (46.1-47.1%; 31.8-34.7%; 25.9-27.9%, respectively). On the other hand, body lengths of fishes fed cladoceran were smaller and their activity was lower than others. DHA and EPA contents of fishes fed cladoceran were lower than ones fed *Artemia* and zooplankton. It is necessary to improve the nutritional value of frozen cladoceran and the optimum nutritional enrichment diet should be searched. The nutritional improvement will result in higher growth and activity of fishes when we feed

cladoceran.

- (11) Animal-borne cameras show interactions among sea-ice, krill and foraging penguins

Shinichi Watanabe, Akinori Takahashi, Yashuhiko Naito, Nobuyuki Miyazaki, Philip Trathan

31st Symposium on Polar Meteorology and Glaciology Polar Biology, Itabashi, Tokyo, Japan. Abstracts, p. 219 (2008-12)

Pygoscelis penguins feed mainly on Antarctic krill. Distribution of krill varies in relation to changing ocean environment such as sea-ice conditions. In particular, the distribution of krill probably changes vertically in areas where sea-ice condition changes dynamically due to wind or current flow conditions. In such situation, penguins may change their feeding behaviour in relation to changing krill distribution. In this study, we investigated the depth distribution of krill and the diving behaviour of penguins, and examined how prey distribution affects behaviour of predators. Animal-borne camera loggers were used to examine the relationships among sea-ice conditions, the depth distribution of krill, numbers of other birds encountered, and the diving depth of Adélie and chinstrap penguins breeding at Signy Island in South Orkney Islands, Antarctica. From December 2007 to January 2008, we attached the loggers on the backs of 3 Adélie and 9 chinstrap penguins, which recorded an image every 4 seconds and diving depth every second during foraging trips. We obtained a total of 25,474 images for 22.3 h from Adélie penguins and a total of 86,503 images for 72.2 h from chinstrap penguins. During the study period, sea-ice conditions around the island greatly changed probably due to changing sea current and wind direction. As the rate of encounter with pack-ice at the sea surface increases, the depth distribution of krill and the diving depth of penguins tended to be shallower. In addition, the rate of encounter with other birds was relatively low when penguins dived shallower. It was also observed that birds aggregated and fed on krill when penguins dived relatively deep. These results suggest that sea-ice conditions at the foraging habitat of penguins affect the depth distribution of krill, which in turn affect the foraging behaviour of penguins.

- (12) Vaccination of Japanese flounder, *Paralichthys olivaceus* against nocardiosis with adjuvanted killed *Nocardia seriolae*

Eriko Nishikawa and Eijiro Kawahara

5th World Fisheries Congress (Pacifico Yokohama, Yokohama), Abstracts,
p. 345 (2008-10)

- (13) Immunomodulatory effects of formalin-killed *Nocardia seriolae* on Japanese flounder vaccinated with adjuvanted *Edwardsiella tarda* lipopolysaccharide
Tomoki Hatate and Eijiro Kawahara

5th World Fisheries Congress (Pacifico Yokohama, Yokohama), Abstracts,
p. 345 (2008-10)

- (14) Immunostimulating effects of a lactic acid bacterium, *Lactobacillus plantatum* on colored carp, *Cyprinus carpio*

Yuko Kondo and Eijiro Kawahara

5th World Fisheries Congress (Pacifico Yokohama, Yokohama), Abstracts,
p. 358 (2008-10)

- (15) 培養法の異なるシオミズツボウムシを給餌した場合のトラフグ仔魚の飼育成績の比較

小谷知也、田邊茉莉、板倉大樹、伏見 浩、林 雅弘

2008年 日本水産学会 春季大会 (静岡市)、講演要旨集、p. 114 (2008-3)

【目的】ワムシの培養法が栄養強化成績することが明らかにされている。しかし栄養強化成績の差が仔魚に及ぼす影響については明らかではない。そこで、連続培養と植え継ぎ培養で1次培養したワムシの給餌がトラフグ仔魚に及ぼす影響を検討した。

【方法】ポリエチレン黒色円形 1m³ 水槽を用いて、0日齢(孵化日)から20日齢までトラフグ仔魚を飼育した。連続培養法で培養したワムシを給餌する試験区(以下連培区)と植え継ぎ培養で培養したワムシを給餌する試験区(以下バッチ区)の2試験区を設け、それぞれ3反復とした。市販栄養強化剤で栄養強化を行ったワムシを2日齢から20日齢まで給餌した。5日齢から5日毎に全長と体長の測定を行った。また、20日齢に全数計数を行なった。20日齢のトラフグ仔魚および飼育期間中に供したワムシの脂肪酸組成を分析した。

【結果】20日齢の仔魚の平均全長は連培区 10.3±1.0mm、バッチ区 10.2±1.2mmであり、有意差は認められなかった。20日齢までの生残率では、連培区の法が有意に高かった(連培区 66.1±5.9%、バッチ区 47.6±6.5%)。20日齢のトラフグ仔

魚の脂質含量が連培区で有意に高く、EPA と DHA の含量も同様であった。したがって連続培養法によって培養したワムシを給餌することによって飼育成績を改善できると判断された。

- (16) Effect of Zn and Mn enrichment methods for *Artemia* on growth and skeletal development of red sea bream (*Pagrus major*) larvae

Tien Nguyen Van、佐藤秀一、芳賀 譲、伏見 浩、小谷知也

2008 年 日本水産学会 春季大会 (静岡市)、講演要旨集、p. 122 (2008-3)

[Objectives] Enrichment methods for *Artemia* with Zn and Mn were evaluated for growth and skeletal deformity of red sea bream larvae.

[Methods] Red sea bream larvae were fed with 4 types of enriched *Artemia* from 15 to 32 days post-hatching. *Artemia* groups were enriched with marine ω A[®] together with Zn and Mn or without them by regular or novel enrichment method. In novel enrichment method, *Artemia* were enriched with new marine ω A[®] (secondary enrichment) after 24 hours from first enrichment.

[Results] Secondary enrichment with new marine ω A[®] increased protein and lipid contents in *Artemia* ($P < 0.05$). As a results, growth and survival of red sea bream larvae were more superior than the groups fed with *Artemia* enriched by regular method ($P < 0.05$). Zn and Mn supplementation significantly improved growth performance ($P < 0.05$) but did not affect survival rate of red sea bream larvae ($P > 0.05$). Skeletal deformity rate was lower in the red sea bream larvae fed with *Artemia* supplemented with both Zn and Mn compared to non supplemented groups ($P < 0.05$). The present study suggested that supplementation of Zn and Mn improves growth and normal skeletal development of red sea bream larvae. Novel enrichment method is suggested to improve both nutrient and mineral (Zn and Mn) contents in enriched *Artemia*.

- (17) クロマグロの健苗育成技術開発研究-1. 実施基本計画

草野 孝、伏見 浩

2008 年 日本水産学会 春季大会 (静岡市)、講演要旨集、p. 132 (2008-3)

【目的】平成 19 年度から 3 カ年計画で開始した「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」を産学連携のプロジェクト研究の概要を紹介する。

【方法】クロマグロの健苗育成技術を開発し、資源増殖のための栽培漁業の推進と持続的な養殖業の発展に寄与することを目的とする本プロジェクト研究は、研

究総括者を草野孝、共同研究責任者を伏見浩、共同研究者を青木宙（海洋大）、小谷知也（福山大）、川合真一郎（神戸女学院大）、佐藤秀一（海洋大）、萩原篤志（長崎大）、廣野育生（海洋大）および渡辺勤（(有)奄美養魚）として組織された。

【結果】本プロジェクト研究は以下の3課題で構成された：(1)初期飼育技術の向上を図るためにクロマグロ仔稚魚の栄養要求を明らかにし、生物餌料の栄養価を改善する方法の確立し、仔稚魚の形態・消化機能・免疫能の発達と成長・発育に伴う代謝の変化を明らかにする研究、(2)沖出し養成方法を改善するために形態学的、生態学的、遺伝学的健全性の評価に基づく健苗性の向上を図り、行動特性と摂餌特性に基づき種苗性の向上を図るとともに飼育水槽からの取り上げ・輸送方法および飼育用生質網の構造・規模を改善する研究、(3)完全養殖を行なうための人工生産魚を用いた親魚養成と第二世代種苗の生産を行なう研究。

(18) クロマグロの健苗育成技術開発研究-2. 大型水槽による仔稚魚飼育方法の開発-
2007年種苗生産結果

神村祐司、大後戸貴浩、斎藤 誠、川本智彦、鎮原正治、渡辺 勤、小谷知也、
伏見 浩

2008年 日本水産学会 春季大会（静岡市）、講演要旨集、p.132（2008-3）

【目的】本種の種苗生産過程における成長と生残を改善するための基礎資料を収集する。

【方法】養成中の6歳親魚100尾が自然産卵した受精卵を採集し、ふ化管理を行なった後、ふ化仔魚を35および80m³水槽での飼育に供した。L型ワムシ、アルテミア、冷凍コペポダ、ふ化仔魚、キピナゴミンチおよびイカナゴミンチを用い、それらの組み合わせを変えた比較飼育を行なった、飼育水槽の照明条件を変えた飼育を行い、照明条件の検討を行なった。また、飼育水の上下循環の効果の検討を行なった。

【結果】2007年6月中旬から11月初旬まで行なわれた自然産卵から約1.3億粒の受精卵を採集した。産卵時刻は19~20時ごろと推定され、産卵期後半には20~21時となった。飼育試験には823万粒を供した。各種の分析用サンプルの収集と飼育条件の検討を行いながら沖出し種苗の生産を行った。飼育水の上下循環の効果は明らかでなかった。クロマグロ仔魚は全長10mm頃から魚食性が強くなり、小形個体がつつかれ、減耗が生じた。ふ化仔魚を十分に供給できれば、クロマグロ仔魚は10-20日齢に成長停滞を生じなかった。アルテミア、冷凍コペポダの給餌ではクロマグロ仔魚は十分な成長をしなかった。20日齢までの生残は蛍光灯を用いた恒明条件下で高かった。クロマグロ仔魚が異常な遊泳行動をしないよう

に照度を調節しながら 20 日齢までの飼育を行なったところ、日齢の進行とともに夜間の水面照度を下げても良いと思われた。

(19) クロマグロの健苗育成技術開発研究-3. 各増殖フェーズのシオミズツボワムシ個体群の質の評価

田邊茉莉、木田邦朝、小谷知也、伏見 浩

2008 年 日本水産学会 春季大会 (静岡市)、講演要旨集、p. 133 (2008-3)

【目的】シオミズツボワムシ *Brachionus plicatilis* (以下ワムシ) の増殖は 4 つのフェーズを示すことが知られている。本研究では、ワムシの増殖フェーズを経時的に追跡し、ワムシの増殖フェーズと被甲長組成の関係を明らかにすることを目的とし、その後の栄養強化に対する影響についても検討した。

【方法】材料として本研究室で植え継ぎ培養で培養している L 型小浜株を使用した。200L アルテミア孵化槽を使用し、接種密度を約 550 個体/mL として 8 日間培養した。市販の淡水濃縮クロレラを朝と夕の 1 日 2 回給餌した。植え継ぎ培養法と、連培法で培養したワムシについても同様に、朝と夕にワムシの密度計数、水温、塩分濃度、pH、アンモニア態窒素濃度、100 個体の携卵判別及び被甲長のサイズ測定を行った。脂肪酸分析のために 1 日 1 回ワムシを収穫し、1000 個体/mL として栄養強化した後、ガスクロマトグラフィーにより脂肪酸組成の分析を行った。

【結果】8 日間培養では 3 回の培養とも出現時期は異なったが停滞期、対数増殖期、定常期、死滅期を確認できた。被甲長組成は増殖フェーズと共に変化をしたが、共通の傾向は認められず、脂肪酸組成についても同様であった。連培法では被甲長組成の変化は見られなかった。また、脂肪酸組成の変化も少なかった。

(20) クロマグロの健苗育成技術開発研究-4. 培養法の異なるシオミズツボワムシを用いて飼育したマダイ仔稚魚の代謝量の比較

宮嶋 暁、渡辺紀子、小谷知也、伏見 浩、半田岳志、難波憲二

2008 年 日本水産学会 春季大会 (静岡市)、講演要旨集、p. 133 (2008-3)

【目的】シオミズツボワムシは培養法によって栄養強化の成績が異なることが明らかにされている。本研究では連続培養法 (以下連培区) と植え継ぎ培養法 (以下バッチ区) で培養したワムシを用いて飼育したマダイ仔稚魚の酸素消費量を比較することを目的とした。

【方法】実験は卵から 42 日齢まで行った。各試験区は 1 水槽ずつ設けた。飼育

水槽には1m³水槽を用い、初期収容尾数を10,000尾に設定した。酸素消費量の測定には注射筒を用いた密閉式測定方法を用いた。酸素消費量測定後に供試個体の全長、体長、湿重量、乾燥重量を測定して、酸素消費量/尾/分と酸素消費量/mg乾重/分を算出した。全長と酸素消費量との関係から酸素消費量の変化の仕方を判断した。

【結果】酸素消費量/尾/分は両試験区ともに成長に伴い増加した。酸素消費の変化のパターンに差は認められなかった。酸素消費量/mg乾重/分の変化のパターンは同じであった。しかし、開口から脊索末端上屈開始前までの酸素消費量は連培区の方が多かった。しかし、稚魚期になるとバッチ区の方が多くなり、連培区の方が少なくなった。また、連培区では21日齢、バッチ区では23日齢で稚魚になった。そのときの平均体重は連培区の方が大きかった。

(21) クロマグロの健苗育成技術開発研究-5. 各種大型動物プランクトンの仔稚魚に対する餌料価値

小谷知也、今利浩之、伏見 浩

2008年 日本水産学会 春季大会 (静岡市)、講演要旨集、p.133 (2008-3)

【目的】マグロ仔稚魚の初期栄養状態は、その後の成長に重大な影響を及ぼすが、アルテミアの給餌については、その餌料価値が問題視されている。本研究は、アルテミアに代わる生物餌料の探索を目的として、淡水ミジンコ、天然域採取動物プランクトンをマダイ仔稚魚に給餌してその餌料価値の確認を行った。

【方法】福岡県水産試験場が開発したミジンコ培養システムを使用して、タマミジンコの1m³規模の大量培養を行った。このタマミジンコに市販栄養強化剤で栄養強化を施し、その後マダイ仔稚魚に給餌した。比較のため、同じ栄養強化剤で強化したアルテミアと冷凍コペポーダを給餌する区、天然域から採取した動物プランクトンを給餌する区を設けた。マダイ試験魚はワムシ単独給餌終了後(18日齢)、各餌料条件で飼育した(19~32日齢)。試験開始から5日ごとに各試験区20尾の全長および体長を測定した。飼育試験終了時(32日齢)に、空中露出試験を行った。

【結果】マダイ仔稚魚飼育の生残は、ミジンコ給餌区で高くなる傾向が見られた(46.1-47.1%; アルテミア区: 31.8-34.7%; 天然プランクトン区: 25.9-27.9%)。成長はアルテミア(32日齢時全長, 12.6-12.7mm)および天然プランクトン給餌区(12.6-13.3mm; ミジンコ区, 10.0-10.5mm)が優れていた。空中露出後の生残は、アルテミアおよび天然プランクトン給餌区が優れていた。

(22) クロマグロの健苗育成技術開発研究－(6) クロマグロのふ化後の成長に伴う消化酵素活性の変化および消化酵素活性の日内変動

黒川優子、川合真一郎、藤井あや、伏見 浩、小谷知也、神村祐司、大後戸貴浩、斎藤 誠、川本智彦、鎮原正治、渡辺 勤

2008年 日本水産学会 春季大会（静岡市）、講演要旨集、p.133（2008-3）

【目的】クロマグロ (*Thunnus thynnus*) の健苗育成において質的および量的に適正な餌料の選択をする際に、また給餌時間帯や給餌回数などを決める際に、仔稚魚の消化酵素の発達状況や酵素活性の日周リズムを把握しておくことは重要である。本研究ではクロマグロのふ化から22日までの仔魚期において数種の消化酵素活性がどのように変化するか、また、仔魚期における消化酵素活性の日内変動を調べた。

【方法】2007年8月に奄美大島(有)奄美養魚の生贄において受精卵を採取し、ふ化させたクロマグロの仔魚をふ化後22日まで数日間隔でサンプリングした。仔魚はエッペンドルフチューブ内でマイクロペスルを用いて磨砕後、10,000×g、5分間の遠心分離を行い、上清を粗酵素液とした。消化酵素はトリプシン、キモトリプシン、ペプシン、リパーゼおよびアミラーゼの各活性を測定した。また、ふ化後5日と14日において3時間間隔でサンプリングし、消化酵素活性を測定した。

【結果】トリプシンの全活性はふ化直後から認められ、以後、成長に伴って上昇し、特にふ化後20日以後における活性の増加は顕著であった。しかし、体重あたりの活性で示すと、ふ化後18日ごろに最大となった。ふ化後5日におけるトリプシン活性の日内変動を調べた結果、21時ごろに活性のピークが見られたが、ふ化後14日では18時ごろに最大の活性が認められた。飼育の際に、成長に伴い照度の条件を変化させていることが摂餌量に影響し、このことがひいてはトリプシン活性の日内変動に関わっていると思われる。

(23) クロマグロ健苗育成技術開発研究－VIII マグロ仔魚の体成分および餌料の化学組成

佐藤秀一、Tien Nguyen Van、芳賀 穰、神村祐司、大後戸貴浩、斎藤 誠、川本智彦、鎮原正治、渡辺 勤、伏見 浩、小谷知也

2008年 日本水産学会 春季大会（静岡市）、講演要旨集、p.134（2008-3）

【目的】クロマグロ仔魚の栄養要求を検討する目的で、仔魚の体成分および餌料に用いたハマフエフキの化学組成を分析した。

【方法】クロマグロ受精卵、ふ化仔魚、開口仔魚および通常の方法で飼育したふ

化後 4, 5 および 6 日目の仔魚ならびに仔魚後期に餌料として用いたハマフエフキの受精卵, ふ化仔魚, 開口仔魚の化学分析を行った。

【結果】マグロ仔魚では, 摂餌を開始することによって粗タンパク質, カノレシウム, マグネシウム, 鉄およびマンガン含量が, 著しく高くなった。一方, 亜鉛およびドコサヘキサエン酸はふ化後, 漸次減少し, 6 日目には開口時の約 50 および 60%にまで減少した。さらに, メチオニン等の含硫アミノ酸の代謝物質であるタウリンは, ふ化後著しく減少し, 6 日目にはふ化時の 20%近くまで低下した。餌料に用いたハマフエフキには, マグロ仔魚で減少する亜鉛, ドコサヘキサエン酸およびタウリン, 特にタウリンが豊富に含まれていた。これらのことより, クロマグロは仔魚期に亜鉛, ドコサヘキサエン酸, およびタウリンが不足しているのではないかと思われた。以上より, これらの栄養素, 特にタウリンの強化方法を検討する必要があると示唆された。

- (24) 八田原ダムに設置されている躍層低下循環装置のアオコ発生に及ぼす影響
藤井啓子、北口博隆、石田祐三郎、満谷 淳
平成 20 年度日本水産学会大会(静岡市)、講演要旨集、p. 208 (2008-3)

広島県東部にある八田原ダムには, 躍層低下循環装置が設置されており, 水深 20 m 付近より上層の水を気泡により押し上げて循環させている。本研究では, この装置がアオコの発生に及ぼす影響を, 2007 年 5 月から 10 月まで AGP 試験により検討した。その結果, 躍層低下循環装置からおおよそ 300 m 離れた調査定点の表層水では, 多くの月でリンがプライマリーな制限要因となっていたことが示された。調査定点における水温は 8 月から 9 月まで水深 20 m までほぼ一定となっており, 水深 24 ~ 25 m の B-1 m 層ではそれよりも約 4 °C 低かった。また B-1 m 層は貧酸素化しており, リン濃度が表層水の約 15 ~ 30 倍高かった。以上の結果より, 夏季に底層に蓄積しているリンは, 循環装置の働きによって水温が均一化した上層との密度差によって表層への回帰が妨げられていたと考えられた。

- (25) 広島県因島の紅藻ソゾ属およびヤナギノリ属の分類について
山岸幸正、日下あゆみ、太田雄介、三輪泰彦
日本藻類学会第 32 回大会(東京)、講演要旨集、p. 98 (2008-3)

瀬戸内海中央部に位置する広島県尾道市因島において 2005 年から継続的に海藻の採集調査を行っており, 約 190 種の生育が明らかになっている。本研究では, 因島から得られたソゾ属 *Laurencia* およびヤナギノリ属 *Chondria* 海藻の形態観

察および分子系統学的解析を行い、日本の既知種に該当しない2種についての分類学的検討を行った。

ソゾ属の一種の藻体は柔らかく、小盤状の基部から叢生し、扁平で不規則に羽状に分枝し、サクランボ小体を持たない。表皮細胞は縦方向の2次的ピットコネクションを持たず、断面で柵状に配列せず、表面に突出する。四分孢子嚢は母細胞から背軸方向に切り出される。配偶体は得られていない。近年ソゾ属を *Laurencia*, *Chondrophyucus*, *Osmundea* の3属に分けることが提唱されており (Garbary and Harper 1998, Nam 1999), これに従うと因島の本種は *Chondrophyucus* に含まれる。日本から報告されている *Chondrophyucus* の特徴を持つ10種はすべて体が軟骨質で硬く、本種のように柔らかいものはみあたらない。rbcL分子系統樹では、本種は狭義の *Laurencia* クレードの外に位置し、*Chondrophyucus* の種からなる1つのクレードに含まれることが示された。また、因島にヤナギノリ *Chondria dasyphylla* およびそれに形態が類似したヤナギノリ属の未報告種が生育していることを明らかにした。

(26) ダム湖産アユの個体レベルの系統判別—奥津湖におけるアユの陸封化の事例—
谷口順彦、野口大毅、近藤直典、近藤正美

2008年 日本水産学会中国・四国支部会(広島市)、大会講演要旨集、p. (2008-11)

日本列島には、アユ (*Plecoglossus altivelis*) の自然集団として両側回遊型(海産アユ)と陸封型(湖産アユ)の遺伝的2型が存在する。天然遡上のない上流部においては増殖や遊漁を目的とした放流種苗を起源とするダム湖産集団が確認されている。ダム湖および上流域の創出資源として期待されるこのような集団の遺伝的由来を把握することは、今後のダム湖産アユ種苗の効果的な利用および遺伝的管理を行う上で重要と考えられる。

本研究では、最近ダム湖流入河川への遡上および受精卵の産着が確認された岡山県の苫田ダム貯水池・奥津湖のアユ標本を用い、マイクロサテライトマーカーによる系統識別を行った。さらに、ユウ度法を採用することにより、個体レベルの系統判別を試みた。

奥津湖流入河川へ遡上した仔魚(再生産)および奥津湖バックウォーターの産卵場に集まった親魚はいずれも海産アユと判定された。また、種苗放流に用いられた高梁川漁協産人工種苗、揖保川漁協産人工種苗も海産系と判定されたが、鹿児島島の鶴田湖産アユは海産アユと琵琶湖産アユの浸透交雑群と判定された。

(27) ペンギン類の同調潜水行動の地域間比較シンクロナイズト・ダイビング：昭和基

地と英国基地どちらのペンギンが上手か？

渡辺伸一、高橋晃周、佐藤克文、P. Philip Trathan、綿貫 豊

第4回日本バイオロギング研究会シンポジウム（長崎市）、講演要旨集、p. 39-40
(2008-11)

ペンギン、アザラシ、イルカなど肺呼吸を行なう潜水動物では、複数の個体が潜水と水面への浮上のタイミングを一致させる同調潜水（シンクロナイズト・ダイビング）が知られている。同調潜水に関する研究は、ペンギン類で多く報告されており、深度記録計（TDR）を複数個体に装着し、同時に計測した深度データの比較から、同調潜水を検出し、その機能について考察されている。従来の研究では、同調潜水を行う集団を観察から探し出し、その数個体から得られた短期間の深度データをもとに分析を行っていた。同調潜水のメカニズムを解明するためには、集団中の多くの個体に深度記録計を装着して、その集団内での行動の同調性を詳しく分析する必要がある。しかし、同調潜水を深度データから効率よく検出することが困難だったことから、長期的に得られた多個体の深度データから同調潜水を検出し、その発生頻度や機能について詳しく研究した例はなかった。本研究では、同調潜水を効率よく検出するためのアルゴリズムを考案し、集団のデータから同調潜水を分析することに成功した。分析の結果、同時に取得した TDR のデータから、潜水動物の同調行動を検出することによって、地域間の集団性の違いや採餌環境の相違を知る上で重要な情報を得ることができた。

(28) 魚類の初期発育時における赤血球群の動態と甲状腺ホルモン

神田 哲、沖増英治、田川正明、乾 靖夫

第33回日本比較内分泌学会 2008 年度大会及びシンポジウム、講演要旨集、p. 61 (2008-12)

【目的】ヒラメの変態時におこる仔魚型赤血球から成魚型赤血球への移行が、甲状腺ホルモンにより誘起される（乾、1995）ことが知られている。しかし、ヒラメ以外の海産魚の知見はほとんどない。本研究では、異体類以外の初期発育変化と赤血球の動態について、組織化学観察と生化学分析を試みた。

【方法及び結果】変態が生じる異体類のヒラメ、異体類ではないが明瞭な変態が生じるオニオコゼ、卵胎生で変態を伴わないクロソイについて、仔魚期から稚魚期における甲状腺の免疫組織化学像、赤血球群の動態を観察し、甲状腺ホルモンの組織中濃度、血中ヘモグロビン（Hb）濃度を測定して、海産魚の初期発育時におけるホルモン調節を考察した。その結果、いずれの魚においても初期発育時

に赤血球群の移行が生じ、血中Hb量が急激に上昇し、組織内への酸素運搬能の増加が推察された。また、ホルモン投与実験結果より、これらの現象は共通して甲状腺ホルモンにより調節されていることが示唆された。

- (29) ニシキゴイのリンパ球細胞障害性試験法の確立
河原栄二郎、木庭雄二郎
平成20年度日本水産学会中国・四国支部大会(広島)、講演要旨集、p.14 (2008-11)
- (30) ヒラメに対する植物乳酸菌 *Lactobacillus plantarum* の免疫賦活効果
近藤裕子、河原栄二郎
平成20年度日本水産学会中国・四国支部大会(広島)、講演要旨集、p.15 (2008-11)
- (31) ヒラメのエドワジェラ症に対するワクチンと免疫賦活剤の併用効果
旗手友紀、河原栄二郎
平成20年度日本水産学会中国・四国支部大会(広島)、講演要旨集、p.15 (2008-11)

B. 総説

C. 著書

- (1) 水産資源の増殖と保全
北田修一・梶山雅秀・浜崎活幸・谷口順彦 (編著) 234pp, 成山堂, 東京 (2008)

D. その他

- (1) アジュバント添加 *Edwardsiella tarda* LPS で免疫したヒラメに対するホルマリン不活化 *Nocardia seriolae* 菌体の免疫増強効果
旗手友紀、河原栄二郎
第6回因島種苗生産技術交流会(福山市) (2008-8)
- (2) ニシキゴイに対する植物乳酸菌 *Lactobacillus plantarum* の免疫賦活効果

- 近藤裕子、河原栄二郎
第6回因島種苗生産技術交流会（福山市）（2008-8）
- (3) ヒラメのノカルジア症に対するアジュバント添加 *Nocardia seriolae* 不活化菌体によるワクチネーション
西川江理子、河原栄二郎
第6回因島種苗生産技術交流会（福山市）（2008-8）
- (4) 冷凍ミジンコのマダイ仔稚魚に対する餌料価値
今利浩之、小谷知也、伏見浩
第6回因島種苗生産技術交流会（福山市）（2008-8）
- (5) 培養法の異なるシオミズツボワムシを用いて給餌したヒラメ仔稚魚の代謝量の比較
宮嶋 暁、小谷知也、伏見 浩
第6回因島種苗生産技術交流会（福山市）（2008-8）
- (6) シオミズツボワムシ栄養強化剤使用方法の検討
田邊茉莉、板倉大樹、小谷知也、伏見 浩
第6回因島種苗生産技術交流会（福山市）（2008-8）
- (7) 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のか？－製剤混合の効果－
北本恵理、小谷知也、倉田 修、伏見浩、畑井喜司雄、佐藤秀一
第6回因島種苗生産技術交流会（福山市）（2008-8）
- (8) 各増殖フェーズにおけるシオミズツボワムシ個体群の質の評価
田邊茉莉、木田邦朝、小谷知也、伏見 浩
第6回因島種苗生産技術交流会（福山市）（2008-8）
- (9) 培養法の異なるシオミズツボワムシを用いて飼育したマダイ仔稚魚の代謝量の比較
宮嶋 暁、渡辺紀子、小谷知也、伏見浩、半田岳志、難波憲二
第6回因島種苗生産技術交流会（福山市）（2008-8）

- (10) 冷凍ミジンコのマダイ仔稚魚に対する餌料価値
小谷知也、今利浩之、伏見 浩
第 6 回因島種苗生産技術交流会（福山市）（2008-8）
- (11) クロマグロ健苗育成技術開発研究
伏見 浩、草野 孝
第 6 回因島種苗生産技術交流会（福山市）（2008-8）
- (12) 海洋・動物実験「魚類の血液細胞の顕微鏡観察」—私学支部研修会報告、平成 18 年度広島県私学教育研修会生物分科会—
沖増 英治
「広島生物」 広島県高等学校教育研究会理科部会生物部会報（29），32（2008）
- 2006 年 8 月 21～22 日に福山大学グリーンサイエンスセンターで開催された平成 18 年度広島県私学教育研修会生物分科会の実験内容説明として、他の実験とともに「魚類の抹消血液観察」「魚類前腎細胞の核・染色体観察」が具体的に紹介された。
- (13) 甘草抽出物の非特異免疫能に関する研究
河原栄二郎
福山大学受託研究成果報告書、pp. 11（2008-3）
- (14) 魚類の免疫力増強方法
河原栄二郎
特願 2008-164485（2008-6）