水研機構研報, 第45号, 125－141, 平成29年
Bull. Jap. Fish. Res. Edu. Agen. No. 45, 125－141, 2017

Abstracts of Poster Presentations

1: Whole genome re-sequencing of fugu populations

Sho HOSOYA*, Satoshi TASUMI*, and Kiyoshi KIKUCHI*

* Fisheries Laboratory, University of Tokyo, Hamamatsu, Bentenjim 2971-4 Japan
E-mail: ahosoya@mail.ecc.u-tokyo.ac.jp

Key words
Fugu, *Takifugu rubripes*, conservation, genetic diversity, single nucleotide polymorphism, *Fst*, linkage disequilibrium, genomic selection

Abstract

Historically, the prevalence of intraspecific diversity, both neutral and adaptive, in many marine species has been unclear. However, the recent advent of new technologies, in particular, high-throughput DNA sequencing is making it feasible to evaluate genetic variability at the whole-genome level. Fugu (tiger pufferfish *Takifugu rubripes)* is one of the important cultured marine fish species in East Asia. The yield of cultured fugu accounted for approximately 5% of total marine fish aquaculture in Japan, and ranked 3rd-4th by value. In addition, this species is becoming an important target for stock enhancement because of severe declines in the wild populations. In recent years, approximately two million hatchery-born seedlings have been released each year. Tagging and releasing experiments have suggested that fugu shows homing behavior to the natal site for spawning following long-range migration, and by extension, that distinct local populations may exist. As expected, recent study using 21 microsatellite loci revealed a shallow divergence between two wild populations from Japan Sea and Pacific Ocean. Nonetheless, the detailed degrees of population and adaptive structuring remain unclear. Moreover there is virtually no information regarding the extent and magnitude of linkage disequilibrium (LD) that play essential roles in choosing marker loci for the management of brood stocks and conservation of wild populations. In this study, we resequenced wild individuals of fugu from Wakasa Bay (Japan Sea) and Mikawa Bay (Pacific Ocean) to compare the genetic diversity within and between populations. Two libraries each containing ten individuals from either of the populations were constructed for paired-end sequencing (2 x 101bp) on the Illumina Hiseq2000. We obtained 43.2M reads per sample yielding coverage of 11.4 per genome, on average. We mapped these reads on the fugu reference genome (Fugu v.5) and called single-nucleotide polymorphisms (SNPs) using BWA, Samtools and GATK software. The number of SNPs detected per individual was about 700 thousand and the SNP frequency was about 480bp per SNP. Missing SNPs because of the shallower depth were estimated as 2 to 2.5 per cent of the total SNPs for each sample. Multidimensional scaling plot clearly separated the two populations, and individuals from Ise Bay were genetically closer than those from Wakasa Bay. However, the global *Fst* (= 0.0057) was small and no outlier locus was detected by BayeScan software. These results suggest that the genetic divergence between the two populations is shallow. Linkage disequilibrium analysis was done using PLINK software. The two populations were similar in the LD state. We detected putative 3,000 LD blocks from each population but 90 per cent of them were smaller than 1kb. The mean r^2 value was 0.48 between two SNPs at 100bp distance whilst that was less than 0.20 at 1kb distance. This rapid LD decay indicates these populations have maintained at relatively healthy states until recently. The expected SNP size for the implementation of genomic selection program for fugu breeding was 300k.

Annotated Bibliography

Katamachi D., Ikeda M., Sato T., Suzuki S., Kikuchi K, and Ojima D. 2014: Development of a multiplex PCR assay for population genetic analysis of the tiger puffer *Takifugu rubripes* using 16 microsatellite DNA loci. *Aquaculture Sci*., **62**, 55−63

The tiger *puffer Takifugu* rubripes is a marine fish species economically important to East Asia, particularly Japan. To evaluate the genetic variability and population structure of the tiger puffer in detail, we generated a multiplex PCR assay of microsatellite DNA loci, a fast and cost-effective technique that allows high-throughput genotyping. In this study, we report the development of four multiplex PCR assays for this species using 16 microsatellite DNA loci located on independent chromosomes. We ensured quality control throughout all steps of the multiplex PCR assay development, i.e., exclusion of loci detected with stuttering, allele dropouts, or null alleles. We evaluated this set of microsatellite DNA loci for polymorphisms using 113 fishes collected from three different locations in the sea around Japan. This combination of loci will prove useful for future investigations of the fine-scale population genetic structure of this species.

2: Incipient transition of a sex-determining gene among closely related species of fugu

Risa IEDA*[1], Sho HOSOYA*[1], Satoshi TASUMI*[1], Shigenori SUZUKI*[2], and Kiyoshi KIKUCHI*[1,3]

*[1,3] Fisheries Laboratory, University of Tokyo, Hamamatsu, Bentenjim 2971-4 Japan
*[2] Minami-Izu Laboratory, National Research Institute of Aquaculture, FRA, Shizuoka, Minami-izu 415-0156 Japan
E-mail: akikuchi@mail.ecc.u-tokyo.ac.jp*[3]

Abstract

Sex determination in teleosts fish is often genetic, where segregation of sex-determining loci assign the phenotypic sex. In these species, sex-linked polymorphic markers can be used for sex identification, potentially providing important information regarding the management of cultured species and conservation of wild species. However, the different master sex-determining (SD) genes in different fish lineages appear to have evolved independently and have been frequently replaced by new genes. Therefore, the sex-linked markers are often specific to a few stock population of the focal species, greatly limiting use of such markers for the management of aquaculture species. To gain more insights into the transition between SD systems in teleosts, we study closely-related species of fugu belonging to genus *Takifugu*. This genus has undergone an adaptive radiation in the last 2-5 million years, resulting in about 20 extant species including fugu (tiger pufferfish). Fugu is one of the most economically important food fish in Japan and also is the first fish with a fully sequenced genome. Previously, we have shown that sex in fugu appears to be determined by a missense single nucleotide polymorphism (SNP) in the *Amhr2* gene. In this study, we have taken advantage of this finding and the rich genomic resources of fugu to explore the genetic basis of sex determination in closely-related species of fugu. We found that while sex in the majority of *Takifugu* species is likely determined by the SNP in the *Amhr2* gene, it is clearly not the case in a few species. To confirm this, we performed genome-wide linkage analysis and identified novel SD loci distinct from the *Amhr2* locus in these species. Interestingly, the transition of the SD system appears to be in progress at least in one species, as a small percentage of males still retains the "sex-determining SNP" on the *Amhr2* gene. These results indicate that fugu and its closely-related species can be an excellent model group for investigating the transitions between alternative master SD genes.

3: Estimation of breeding value in model fish, guppy (*Poecilia reticulata*) and its application for selective breeding in aquaculture

Masamichi NAKAJIMA*, Toyoko NAKAJIMA*, and Syunsuke OBINATA*

* Laboratory of Marin Life Science and Genetics, Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi 981-8555, Japan
E-mail: mnkjm@bios.tohoku.ac.jp

Key words
Guppy (*Poecilia reticulate*), selective breeding, body size, breeding value

Abstract

Selective breeding is one of the most important methods for the genetic improvement in not only livestock animals, but also aquatic organisms. Many varieties and strains were produced in aquatic organisms; however the most succeeded example of selective breeding in aquatic organisms is ornamental fish. In the case of quantitative traits, the example of succeeded selective breeding is scare. In the case of the selective breeding based on phenotypic value, many cases showed inconsistent result. This is due to its inability to remove environmental and dominance effect. Therefore the method to evaluate the accurate abilities of parent is necessary. The breeding value was contrived from such circumstances. Though the breeding value possesses such importance, the applications of the breeding value in the aquaculture are not so much. The reasons why the application in aquaculture is little are 1) the large number of offspring can obtain from small number of parental fish; 2) short life cycle of target species caused difficulties to estimate breeding value in parental fish and their offspring. Although, it is hard to obtain the breeding value, the role of breeding value is very important for the efficient selection. From the above mentioned reason, it is necessary to identify the breeding value for the effective selective breeding in aquaculture. In this study, the breeding values were estimated from selection experiment for body size in the guppy, *Poecilia reticulata*, and examined the efficiency of the selection between used phenotype and breeding value. Comparison of breeding value in parental and offspring indicated significantly positive correlation. Positive correlation also observed between breeding values of parental and body size of their offspring. Selection based on breeding value showed 5% larger in body size compared with selection using phenotype. Selection based on breeding value showed lesser increment in coefficient of inbreeding compared to the selection based on phenotype. These results suggest that the breeding values are effective to the evaluating parental fish and useful for selective breeding. It is expected that the application of breeding value in the industrially important fish, such as Bluefin tuna and Japanese eel.

4: The life table demography and population growth of the rotifer *Brachionus angularis* Gosse, from Kenya; the influence of temperature and food density

Erick Ochieng OGELLO*[1, 2], Hee-Jin KIM*[1], Koushirou SUGA*[1], and Atsushi HAGIWARA*[1]

*[1] Graduate School of Fisheries & Environmental Sciences, Nagasaki University, 1-14 Bunkyo, Nagasaki 852-8521, Japan

*[2] Kenya Marine & Fisheries Research Institute (KMFRI), Kegati Aquaculture Research Station, P.O. Box 3259 - 4200 Kisii, Kenya

E-mail: bb53414801@cc.nagasaki-u.ac.jp

Key words

Rotifera, alga, life table parameters, fecundity, *Brachionus angularis*

Abstract

The nature of reproduction of the Kenyan strain of *Brachionus angularis* was investigated using individual and small batch culture approaches. First, the Kenyan rotifer was identified using morphological and molecular techniques. The life-table demography and the population growth studies were conducted at three temperatures (i.e. 20, 25 and 30°C) using *Chlorella vulgaris* at three densities (i.e. $2.5x10^5$, $2.5x10^6$ and $2.5x10^7$ cells mL^{-1}). The lorica length (85.6 ± 3.1 μm) and width (75.4 ± 3.6 μm) of the Kenyan sample were smaller than those of similar species cited in the literature. The phylogenetic tree grouped the Kenyan sample together with *Brachionus caudatus* and *Brachionus angularis*. However, with additional morphological data e.g. presence of two median occipital spines with either reduced or lacking sub-median spines, *Brachionus angularis* was identified as the most likely match for the Kenyan sample. The rotifers were most fecund (2.11 ± 0.07 offspring $female^{-1}$ day^{-1}) and reproductive (8.43 ± 0.24 offspring $female^{-1}$) at 25°C with 2.5×10^6 cells mL^{-1} of *C. vulgaris*. The highest intrinsic rate of

natural increase (0.74 ± 0.02 d^{-1}), specific population growth rate (0.49 ± 0.01), longest life expectancy at hatching (12.41 ± 0.28 d) and shortest generation time (2.87 ± 0.03 d) were also observed at 25°C with 2.5×10^6 cells mL^{-1} of *C. vulgaris*. However, the duration of hatching to first egg spawning was shortest (2.86 ± 0.21 h) at 30°C with 2.5×10^7 and longest (8.83 ± 0.39 h) at 20°C with 2.5×10^5 cells mL^{-1} of *C. vulgaris*. In the batch cultures, the highest population density (255.7 ± 12.6 ind mL^{-1}) and lowest (122.0 ± 3.6 ind mL^{-1}) were realized at 25°C with 2.5×10^6 and at 20°C with 2.5×10^5 cells mL^{-1} of *C. vulgaris* on day 8 respectively. There was earlier population density peaks at higher food densities (2.5×10^7 cells mL^{-1} of *C. vulgaris*) regardless of temperature. In conclusion, the Kenyan strain of *B. angularis* seems to have favorable morphological and reproductive features making them suitable for aquaculture activities. The life table demography of this strain is optimal at 25°C with 2.5×10^6 cells mL^{-1} of *C. vulgaris*. The results of this study are relevant for improvement of the freshwater aquaculture activities. Further studies on the population growth of the rotifer are recommended using other different food types.

5: Effect of dissolved organic matter on electrochemical removal of ammonia in recirculating aquaculture systems

Satoshi TADA* and Shigenobu TAKEDA*

* Graduate School of Fisheries and Environmental Sciences, Nagasaki University
E-mail: tdsts.goopy@gmail.com

Key words
Recirculating aquaculture system, electrochemical oxidation, ammonia, dissolved organic matter, seawater

Abstract

Improvement of water treatment systems in closed recirculating aquaculture systems (RAS) is necessary for reducing total volume of culture water, which determine the size of the whole system as well as running costs. New RAS using electrochemical oxidation for removal of ammonia in culture seawater are being developed by Nagasaki Prefectural Institute of Fisheries. In this system, hypochlorous acid, which produced by electrolysis of seawater, oxidizes the bromide ions, yielding hypobromous acid. Both hypochlorous and hypobromous acids react with ammonium ion and oxidize it to nitrogen gas. Since these free chlorine and free bromine also react with dissolved organic matter (DOM), efficiency of electrochemical ammonia removal could be affected by quantity and reactivity of DOM in culture seawater. In this study, removal of total ammonia nitrogen (TAN) and DOM by additions of hypochlorous acid was investigated using culture seawater of two RAS for tiger puffer *Takifugu rubripes* (RAS-1) and kelp grouper *Epinephelus bruneus* (RAS-2). The RAS-1 comprised of a culture tank (20 m^3), a solid settler, a circulation pump, a foam fractionator, an electrolysis unit, a reaction tank (for ammonia removal), and activated charcoal tank (for removal of residual chlorine). The RAS-2 has a biofiltration tank in addition to the units provided in the RAS-1. The culture seawater was filtered through Whatman GF/F filter and dispensed into seventeen replicate 250-ml amber glass bottles. Additions of chlorine (Sodium hypochlorite solution) were conducted to achieve 17 steps chlorine doses of 0~16 (or 70) mg/L as Cl_2. After 20 min contact period at 25°C, free available residual chlorine and combined available residual chlorine in the sample were analyzed by the DPD method. TAN was measured using an autoanalyzer. DOM was determined as humic-like and protein-like fluorophores based on the three-dimensional excitation emission matrix spectroscopy. Concentration of TAN in the culture seawater consistently decreased along with the increase in chlorine dose and then disappeared (what is called breakpoint). In the bottles with higher levels of chlorine dose, free available residual chlorine was detected according to the excess amount of chlorine

dose. Concentrations of combined available residual chlorine were very low but small increase was observed around the breakpoint. Humic-like and protein-like fluorophores showed large decrease by the addition of 1 mg/L Cl_2 (the lowest chlorine dose) and then kept relatively constant concentrations until the breakpoint. In the bottled added with excess amount of chlorine (higher than the breakpoint), further decreases in humic-like and protein-like fluorophores were observed. These results suggest that DOM consists of highly reactive fraction and semi-labile fraction, and the former could reduce efficiency of electrochemical ammonia removal in the culture seawater. Compare to the chlorine demand to achieve the breakpoint in DOM-free artificial seawater, the culture seawater from RAS-2 required 17~42% more chlorine to oxidize TAN, but the difference between the artificial seawater and RAS-1 culture seawater was not clear. Skin mucus of kelp grouper seems to be one of the sources of highly reactive DOM in RAS-2.

Annotated Bibliography

Ohwaki H., S. Yamamoto, A. Okamoto, and Y. Kurokawa. 2011. Development of a large-scale closed recirculating aquaculture system for saltwater fish. Report of Industrial Technology Center of Nagasaki, 40, 52-55 (in Japanese)

For the constraction of a large-scale closed recirculating system for aquaculture of saltwater fish on land, we have been developing a new seawater treatment unit using hypochlorous acid produced by electrolysis of seawater with platinum modified titanium electrode, and a closed recirculating aquaculture system with the electrochemical water treatment. Acidic water, which is a byproduct of electrolysis of seawater, can be used for the removal of dissolved CO_2 that accumulated in the culture water. Our computer simulation model could reproduce the flow pattern in the electrolysis unit and the estimated pH value of the out-flow water agreed well with the observation. We found that dissolved CO_2 in the culture water can be easily removed by bubbling of the acidic water.

6: Effect of tetrodotoxin-containing diet on feeding, digestion and growth of tiger puffer *Takifugu ruburipes* juveniles

Kogen OKITA*, Tomohiro TAKATANI*, Osamu ARAKAWA*, Atsushi HAGIWARA*, and Yoshitaka SAKAKURA*

* Graduate School of Fisheries and Environmental Sciences, Nagasaki University
E-mail: jj20140049@cc.nagasaki-u.ac.jp

Key words
Tiger puffer, *Takifugu rubripes*, tetrodotoxin, feeding trial, appetite

Abstract

Marine pufferfish contain tetrodotoxin (TTX), an extremely potent neurotoxin. We recently clarified that a tiger puffer *Takifugu rubripes* juvenile detects TTX by olfactory organ, and actively ingests and accumulates TTX not only in liver and skin but also in central nervous system. We further revealed that gene expression of some appetite peptides in the brain of hatchery-reared non-toxic fish changed by TTX-sensing and TTX-administration. In the present study, feeding trial using non-toxic and toxic diets was conducted with hatchery-reared *T. rubripes* juveniles in order to examine the relation between the appetite and TTX ingestion. A total of 120 non-toxic cultured juveniles (mean body weight of 3.6 g) were randomly divided into two groups where one group was fed with non-toxic commercial diets and the other was fed with TTX-containing diets (5.3 MU/g feed). Fish were maintained in 3 tanks (20 fish/200 l) for each group with flow through system (2 l/min) and vigorous aeration. The fish were fed with non-toxic and toxic diets to apparent satiation three times a day at 08:00, 13:00 and 17:00 hours for 28 days. All fish survived until the last day of feeding trial. There were no significant differences in the growth performance between fish fed non-toxic diets and toxic diets: total length (85.8±1.2 vs 85.1±2.7 mm), standard length (81.1±1.0 vs 80.1±1.5 mm), degree of loss of caudal fin (77.7±0.6 vs 75.5±5.5%), body weight (18.4±1.7 vs 17.6±1.1 g), feed

intake (11.7±0.6 vs 11.3±0.8 g), assimilation rate (91.1±1.6 vs 86.3±3.0%), weight gain (14.9±1.6 vs 14.0±1.1 g), feed efficiency (127.3±7.1 vs 123.7±5.3%). Total amount of administered and accumulated TTX in the fish fed toxic diets are 59.9±4.1 MU/fish and 33.6±2.0 MU/fish (56.4±7.1% of administered), respectively. These results indicate that TTX at this high dose may not have function as feeding stimulant for *T. rubripes* juveniles, but that feeding activity and growth of the juveniles are not inhibited by potent neurotoxin. We will further perform quantitative analysis and investigate the immunohistochemical localization of appetite peptides in the brain of TTX-sensed and TTX-administered hatchery-reared *T. rubripes* juveniles.

7: Temperature tolerance in two clonal strains of mangrove killifish, *Kryptolebias marmoratus*

Marina YAMADA* and Yoshitaka SAKAKURA*

* Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Japan.
E-mail: bb53115032@cc.nagasaki-u.ac.jp

Key words
Mangrove killifish, *Kryptolebias marmoratus*, thermal tolerance, hybrid

Abstract

Mangrove killifish *Kryptolebias marmoratus* is the only known self-fertilizing vertebrate. This unique species broadly distributes in coastal mangrove habitats from southern Brazil through the Caribbean Islands and Central America to North Florida. They are capable of synchronous self-fertilization, producing homozygous clones as a consequence. Our laboratory has two clonal strains, PAN-RS and DAN, which were originally collected from near Bocas del Toro, Republic of Panama and Dangriga, Belize, respectively. PDHy strain which is the hybrid of PAN-RS and DAN was produced by artificial insemination (Nakamura et al. 2008). The descendants of PDHy are divided into 4 strains, PDHyI, PDHyII, PDHyIII and PDHyIV according to the growth rate. Since Panama and Belize show different climate, we hypothesized that PAN-RS, PDHy and DAN show different temperature tolerance.

We used two clonal strains (PAN-RS and DAN) and hybrid strains (PDHyI, II, III, and IV). Fish were kept in plastic containers filled with 60 mL of 17 ppt artificial brackish water under 25 ℃ and photoperiod of 14L:10D. "Upper and lower thermal acclimation limits" were quantified for each strain using chronic thermal tolerance methodology (Fangue et al. 2006). All fish were kept under 25 ℃ for one week, and 20 fish from each strain were subjected to either increasing or decreasing water temperatures of 0.5 ℃ per day. This experiment was continued until all fish died. The respective chronic thermal maximum or minimum value was taken as the high or low temperature at which 50% morbidity was observed. The respective chronic thermal maximum was significantly higher in DAN (36.6 ℃) than PAN-RS (35.7 ℃), (Log-rank test, $p<0.01$). The respective chronic thermal maximum was 30.7 ℃, 33.6 ℃, 33.7 ℃ and 32.5 ℃ for PDHyI, PDHyII, PDHyIII and PDHyIV, respectively. PDHyI showed the lowest respective chronic thermal maximum among all strains. Respective chronic thermal maximum of each PDHy strain was lower than their parents. The respective chronic thermal minimum was 9.4 ℃ for PAN-RS and 9.6 ℃ for DAN, with no significant difference (Log-rank test, $p=0.59$).

8: Effects of coexistence of protozoa *Euplotes* sp. coexistence on the population growth of minute monogonont rotifer *Proales similis*

Naoshi WAKIMURA*, Yoshitaka SAKAKURA*, and Atsushi HAGIWARA*

* Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Japan.
E-mail: bb53115036@cc.nagasaki-u.ac.jp

Key words
Proales similis, Euplotes sp., monoculture, mixed-culture

Abstract

The rotifer *Brachionus rotundiformis* (SS-type) is commonly used as starter food for rearing small-mouthed marine fish larvae. However, several tropical marine fishes have much smaller mouth gap, hence cannot feed on the *B. rotundiformis*. To solve this problem, the minute monogonont rotifer *Proales similis* is preferred due to its smaller size than *B. rotundiformis*. However, the culture of *P. similis* is easy to be collapsed due to inability to withstand the handling stresses and environmental changes in the culture medium. In this study, we investigated the interaction between *P. similis* and the protozoa *Euplotes* sp. in the rotifer culture water. We independently cultured *P. similis, Euplotes* sp. and a combination of *P. similis* and *Euplotes* sp. in 144 ml glass jar containing 50 ml of seawater (22 ppt) at an initial density of 1 ind./ml at 28°C in darkness for 14 days. All the treatments were triplicated and daily fed with *Nannochloropsis oculata* at 8.0 x 10^5 cells/ml without water exchange. A similar experiment was conducted separately with Chlorella vulgaris as food at 2.9 x 10^5 cells/ml daily without water exchange. When fed with *N. oculata*, population density increased in *Euplotes* sp. in the monoculture and mixed-cultures. However, the population growth of *P. similis* in the mixed-culture decreased after 8 days and *P. similis* disappeared after 14 days of culture. There was a significant difference in the population density of either *P. similis* or *Euplotes* sp. between the monoculture and mixed-culture on day 14. When cultured with *C. vulgaris*, population density of *P. similis* decreased from day 6, while that of *Euplotes* sp. reached its highest peak on the same day. *P. similis* dissapeared completely on day 14. These results suggest that the presence of *Euplotes* sp. in the *P. similis* cultures significantly suppressed the population growth of *P. similis* presumably due to stressful interactions. Furthermore, the competition between *P. similis* and *Euplotes* sp. for bacteria may also have existed. Even though we did not observe a behavioral interaction of *Euplotes* sp. and *P. similis*, the swimming speed of the *Euplotes* sp. was higher than the *P. similis*.

9: Distribution of larval and juvenile greater amberjack (*Seriola dumerili*) around the Penghu Islands, Taiwan

Takamasa HASEGAWA*1, Hsin-Ming YEH*2, June-Ru CHEN*3, Ryo KAWABE*4, and Yoshitaka SAKAKURA*1

*1 Graduate School of Fisheries and Environmental Science, Nagasaki University, Japan
*2 Coastal and Offshore Resources Research Center, Fisheries Research Institute, Council of Agriculture, Taiwan
*3 Fishery Research Institute, Council of Agriculture, Taiwan
*4 Institute for East China Sea Research, Nagasaki University, Japan
E-mail: bb53512002@cc.nagasaki-u.ac.jp

Key wards
Greater amberjack, *Seriola dumerili*, early life, spawning ground, otolith

Abstract

The greater amberjack *Seriola dumerili* (family Carangidae) widely distributes around the world. Because of its commercial importance, rapid growth and good adaptation to captivity, *S. dumerili* is a very important species for aquaculture in Japan. Juveniles of *S. dumerili* associate with floating objects such as drifting seaweeds. However, there is very limited knowledge about larval and early-juvenile stages of this species in the wild. In order to investigate the early life history of *S. dumerili*, firstly we validated the otolith daily increments using artificially-raised fish (11-51 days after hatching). Then, field surveys were made by R/V Hai-an, Fishery Research Institute, Council of Agriculture, Taiwan around the Penghu Islands, Taiwan, from May to August 2015. Frontal zone and drifting seaweeds were visually observed during survey, and drifting seaweeds were scooped together with associated fishes by a hand net (Φ45 cm, 3 mm mesh). Surface tows of plankton net (Φ1.3 m, 0.33 mm mesh) were conducted for 10 minutes with towing speed of 2 knots in frontal zones and other areas. At each sampling station, vertical

profile of water temperature and salinity were measured by a CTD (SBE-19 plus, Sea-Bird Electronics Inc.). Fish species were identified and zooplankton abundance (mg DW·m^{-3}) and species composition (%) were calculated. We also measured four *S. dumerili* samples caught in 2014 deposited at Penghu Marin Biology Research Center, Fishery Research Institute, Council of Agriculture, Taiwan. Increments of sagittal otolith of reared fish showed the same number as their age in days after hatching (ANCOVA, df=1, p=0.32). Relationships between otolith diameter (y_{dia}, μm) and age (x_{day}, days), and between otolith diameter (y_{dia}, μm) and TL (x_{TL}, mm), were described as following equations: $y_{dia}=0.0003 \cdot x_{1.93}^{day}$ (r=0.991), and $y_{dia}=0.1937 \cdot x_{TL}-0.1018$ (r=0.981), respectively. We caught a total of four larval and juvenile *S. dumerili* by surface towing, but not from drifting seaweeds. Total length of *S. dumerili* ranged from 7.4 to 42.5 mm, and age ranged from 18 to 56 days after hatching. All *S. dumerili* were caught in open water and in frontal zone, however we could not detect significant differences in zooplankton abundance between frontal zone and other station (t-test, p=0.87). Thecostraca was significantly abundant in the stations away from the frontal zones (t-test, p<0.01). In 2014, larval and juvenile *S. dumerili* were caught in January and May, and body length ranged from 5.8 mm in NL to 54.2 mm in TL. Our study indicates that *S. dumerili* spawns from April to June in 2015, and frontal zone in open water is nursery ground for *S. dumerili* around the Penghu Islands, Taiwan.

10: Effect of starvation on mixis induction in offspring and genetic mechanism of the monogonont rotifer *Brachionus manjavacas*

Shohei KAMIZONO*, Koushirou SUGA*, Yoshitaka SAKAKURA*, and Atsushi HAGIWARA*

* Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Bunkyo 1-14, Nagasaki 852-8521, Japan.
E-mail: bb53415001@cc.nagasaki-u.ac.jp

Abstract

Short time starvation of neonates hatched from rotifer resting eggs induces active mixis up to the 10th generation. However, this phenomenon remains unexplained whether it can last for further generations. Also, the mode of heredity and the acquired parental characteristics inherited by the subsequent generations are not clear. In this study, we used the rotifer *Brachionus manjavacas* and *Brachionus plicatilis* to investigate the heredity of mixis induction in rotifers up to the 80th generation and the inheritance of the acquired traits by methylation of DNA. The maternal rotifers hatched from resting eggs were starved for 12 hours before determination of the acquired characteristics. The control group was not starved. A set of 8 individual rotifers were randomly selected from starved and control group. Rotifers were individually cultured in 0.2 ml of sea water (22 ppt.) in 8 well microplate at 1 individual per well untill the 80th generation. The rotifers were daily provided with *Nannochloropsis oculata* suspension at 6.0 × 10^{6} cells/ml. The mixis induction in each generation was determined. We determine the genetic factors that changed the mixis induction rate of the parent and future generations. First, we designed the primer using partial base sequence of the methyltransferase, which was the DNA methylase which EST analysis of *B. plicatilis* performed PCR as template cDNA of *B. manjavacas*. The mixis induction of the offspring from starved parents increased until 38th generation. In addition, mixis induction during accumulated generations peaked at the 17th generation. We observed repetitions of increase and decrease of beyond the 17th generation. The amplification of the DNA fragment of the predicted size was confirmed through PCR analysis using the primer designed from the DNA methyltranferase (DNMT) gene of *B. plicatilis*. This phenomenon could be explained by the epigenetic inheritance involving methylation of the DNA. It was estimated that the DNMT gene fragment by BLAST analysis. We have been investigating this by comparing the *DNMT* gene expression level among generations.

11: Body size, culture and fish larval ingestion on a minute rotifer *Colurella* cf. *adriatica*

Stenly WULLUR*1, Petrus LETSOIN*2, Rina P. ASTUTI*3, and Atsushi HAGIWARA*4

*1 Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado - Indonesia
*2 State Polytechnic of Fisheries. Tual, Southeast Maluku – Indonesia
*3 Gondol Research Institue for Mariculture, Bali – Indonesia
*4 Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, Japan
E-mail: miracle_given@yahoo.com

Key words
Minute rotifer, *Colurella* cf. *adriatica*, body size, population growth, fish larvae, ingestion

Abstract

Current procedure of rearing small mouthed marine fish larvae is using the Super Small (SS) type of rotifer *Brachionus rotundiformis* as starter food during first days of larval first feeding. The *B. rotundiformis*, however, is ineffective or even unsuitable for larvae of several marine tropical fish with even smaller mouth size including, Napoleon fish (*Cheilinus undulatus*), groupers (genus *Epinephelus*), angelfishes (family Pomachantidae). In present study, we examined the feasibility of a minute rotifer *C.* cf. *adriatica* as live food by measuring its body size, analyzing population growth and fish larval ingestion on the rotifer.

Rotifer *C.* cf. *adriaticawas* isolated using a plankton net (45 μm mesh size) from an estuary in Mangket, North Minahasa, North Sulawesi, Indonesia. Water temperature and salinity at the time of sampling were 28±1 °C and 30±1 ppt, respectively. Sixty adults of the rotifer were measured for its body length and width. As comparison, body length and width of a local strain *B. rotundiformis* were also measured. Population growth of *Colurella* cf. *adriatica* was assessed by culturing the rotifer under four densities (3, 6, 9, 12 x 10^6 cells/ml) of *Nanochloropsis oculata* as food source. The rotifer was cultured at salinity of 20 ppt and placed in a controlled room temperature at 25±1℃. Water volume of the culture was 4 ml (using 3x4, multiwell plate) and the initial density of the rotifer was 1 ind./ml. Observation was made daily by counting the numbers of rotifer in each well until the density decline. Larval ingestion on the rotifer was investigated in Gondol Research Institute for Mariculture, Bali-Indonesia. Approximately 10 ind./l eggs of humpback grouper (*Cromileptes altivelis*) were transferred to four 200-l larval rearing tank. The first two tanks were fed with 10 ind./ml of rotifer *C.* cf. *adriatica* from day 2 till day 5 after hatching, while the other two tanks were left without any addition of food. All surviving larvae were harvested on day 5 and the numbers of remaining larvae were counted. Gut content of the surviving larvae was analyzed to see the presence of rotifer. Body length and width of *C.* cf. *adriatica* were distributed from 82.8-103.2 and 46.8-61.7 μm, respectively. The mean body length (95.9±3.8 μm; mean± standard deviation) and width (46.8-61.7 μm) of *C.* cf. *adriatica* were significantly smaller/narrower than *B. rotundiformis* (175.2±9.2 and 123.5±7.7 μm, respectively) (*t-test*, $p<0.05$). Rotifer *C.* cf. *adriatica* grew well in all *N. oculata* treatments. The rotifer attained highest population densities on day 16 (774±167 ind./ml) and 18 (656±139 ind./ml) at *N. oculata* densities of 6 and 9x10^6 cells/ml, while it was reached on day 26 (646±85 ind./ml) and 34 (560±58 ind./ml) at *N. oculata* densities of 3 and 12x106 cells/ml, respectively. Humpback grouper larvae show higher survival (1.5%) on *C.* cf. *adriatica* treatment than control (0.3%) (*t-test*, $p<0.05$). By analyzing gut content of the remaining larvae, it was found that individual of rotifer *C.* cf. *adriatica* presence in gut of the larvae indicating that larvae of humpback grouper ingested the rotifer.

12: Production of myostatin gene-knockout Japanese anchovies (*Engraulis japonicus*) using TALEN-based genome editing

Keishi SAKAGUCHI*1, Kanako NAKASHIMA*2, Hajime KITANO*1, Naoki NAGANO*1, and Michiya MATSUYAMA*2

*1 Fisheries Research Institute of Karatsu, Department of Joint Research, Faculty of Agriculture, Kyushu University
*2 Laboratory of Marine Biology, Faculty of Agriculture, Kyushu University
E-mail: keishi_s@agr.kyushu-u.ac.jp

Key words
Japanese anchovy, *Engraulis japonicus*, myostatin, knockout, TALEN, genome editing

Abstract

Genome editing techniques such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR) have attracted considerable attention in recent years since these technologies can mediate targeted and efficient genetic modifications (knockout, knock-in, and gene modification) in various organisms. Thus, targeted genome editing, which enables researchers to tailor genomic loci of interest, is one of the most promising approaches for plant, animal, and fish breeding. Nevertheless, the application of the techniques to teleost fishes have been limited almost exclusively to popular experimental fish models, such as zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*). There are no literature reports on the application of these techniques to marine fish, which includes many important species for the fisheries industry. In this context, we regard the Japanese anchovy (*Engraulis japonicus*) as a most suitable candidate for genome-editing experiments in marine fish, because they have the following advantages: 1) easy rearing and breeding in a small-scale fish tank. 2) year-round and multi-year spawning under photoperiod and temperature control. 3) quick growth into mature individuals, which produce another generation of eggs, about three months. Myostatin (MSTN), previously referred to as growth differentiation factor 8 (GDF8), is a negative regulator of skeletal muscle growth. In mammals, MSTN-deficient animals resulted in an increase of skeletal muscle mass with both hyperplasia and hypertrophy. Likewise, recent studies revealed that the MSTN gene inhibits skeletal muscle growth even in fish. Thus, to produce a fish breeding model generated by genome editing, we performed targeted gene disruption of the MSTN gene in Japanese anchovies using TALEN technology. We constructed three TALEN pairs targeting the first intron of the MSTN gene and the in vitro transcribed RNAs of the pairs were injected into the yolk of embryos at the one-cell stage. As a result, mutant F0 embryos were obtained with a very high insertion and/or deletion (indel) mutation rate (~96.9%), and thus the F0 founders were mated with each other to produce MSTN-knockout anchovies at the F1 generation. To our knowledge, this is the most advanced study for genome editing in marine fishes. The rearing of F1-individuals and their genotyping is now in progress.

13: Isolation and screening of novel probiotic lactic acid bacteria for aquaculture

Nguyen Thi Hue LINH*1, Masato OTANI*2, and Yousuke TAOKA*3

*1 Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki
*2 Graduate School of Agriculture, University of Miyazaki
*3 Department of Marine Biology and Environmental Sciences, Faculty of Agriculture, University of Miyazaki
E-mail: nb14005@student.miyazaki-u.ac.jp

Key words
Probiotics, lactic acid bacteria, *Lactobacillus* sp., pathogen, antagonistic activity, tolerance in gastrointestinal juice

Abstract

Most of probiotics used in aquaculture are considered as alternative therapies for the use of antibiotics to prevent diseases in aquatic animal. Among of probiotic bacteria, lactic acid bacteria are one of potential candidate due to several strains have been isolated from fish gut as beneficial microflora and acted antagonistic against Gram-negative fish

pathogens. Therefore, in this study we conducted to isolate lactic acid bacteria from fermented food as candidate strains which may be used to reduce the antibiotics using for sustainable aquaculture. The probiotic properties of isolates were surveyed. Lactic acid bacteria were isolated from samples such as fermented foods by an agar plating method using GYP and MRS media. The antagonistic activity test against fish pathogens *Lactococcus, Streptococcus* and *Edwardsiella* was carried out according to the method of the double layer agar method. The tolerant ability of isolates on NaCl (0%, 3%, 5%, 10%), pH (from 2 to 9), artificial gastric juice (at pH range 2-4) with pepsin and intestine juice (at pH 8) with gall powder were evaluated. Isolates were identified based on the sequences of 16 S rRNA gene (~700bp). Totally 55 strains of lactic acid bacteria were isolated from rice bran and several kinds of fermented vegetables. In antagonistic test, three isolates showed positive results against three strains of *Edwardsiella tarda*, three strains of *Streptococcus disgnactie*, three strains of *S. iniae* and three strains of *Lactococcus garvie*. These three strains of GYP 31, GYP 69 and GYP 4-20 were identified as Lactobacillus sp.. The relative growth of GYP 31 strain at pH range 5-8 was from 100% to 80%, at pH 2-4 and pH 9 were below 20%; while GYP 69 strain and GYP 4-20 strain only grew well at pH 5, pH 6 with 100% of the relative growth. Three strains grew well at NaCl concentration from 0-5%, 3-5% and 0-3% with the relative growth from 80% to 100%, respectively. In tolerance test on acid and artificial gastrointestinal juices, GYP 31 strain expressed the sustained ability and survival itself better than GYP 69 strain and GYP 4-20 strain. GYP 31 strain showed the highest viable count as $1x10^8$, $1.4x10^8$, $5.6x10^7$cfu/ml in acid solution (at pH 3,5-4), artificial gastric juice at (pH 4) and intestine juice (at pH 8), respectively, although the viable count was lower than those in the control group ($2.2x10^9$cfu/ml at pH 7). From these results, *Lactobacillus* sp.. GYP 31 strain is considered as a potential probiotic candidate in aquaculture due to its ability competition with pathogens and high tolerance in the gastrointestinal tract of fish.

14: Effect of protease addition to EP diet on the growth of amberjack, *Seriola dumerili*

Yousuke TAOKA*1, Takayuki MINAMI*2, Nguyen Thi Hue LINH*3, and Terumi KOGA*4

*1 Department of Marine Biology and Environmental Sciences, Faculty of Agriculture, University of Miyazaki
*2 Miyazaki Prefectural Fisheries Research Institute
*3 Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki
*4 TOA Pharmaceutical CO., Ltd.
E-mail: yousuketao@cc.miyazaki-u.ac.jp

Key words
Seriola dumerili, protease, digestibility, growth rate, feed, low temperature, aquaculture

Abstract

In aquaculture, the late growth of the cultured fish in winter season is serious problem for economical aquaculture. The extruder pellet (EP) has been used as an composed feed for the culture fish because EP can be produced efficiently. However, the EP is very hard to decompose it by digestive enzymes as compared with raw diet. The activities of digestive enzymes in the gastrointestinal tracts of the cultured fish significantly decrease due to the low temperature during winter season. Therefore, it is very difficult that the digestive enzymes with low activities decompose the EP diet efficiently. To dissolve this problem, in this study, the application of proteolytic enzymes from microorganisms was examined to accelerate the digestion of the EP diet for the cultured fish. Acid and alkaline proteases from microorganisms was used. The effect of pH and temperature on enzyme reaction was also investigated. Alkaline and acid protease were added to the EP diets in a 0.1M Tris-HCl buffer and 0.1M glycine-HCl buffer, respectively. The reaction mixtures were incubated at 15, 20, 25, 30 and 35°C for 180 min. The degradation of the EP diet was evaluated by weighing the solid bodies. The nitrogen concentration of the centrifugal supernatant in the reaction mixtures were determined according to the

Kjeldahl method. The alkaline proteases showed stable activities at 20-35°C, and the activity at 15°C significantly decreased by 44%. The decomposition of the EP diet was enhanced by addition of both proteases at 20°C. These results indicated that the addition of proteases from microorganisms is effective to enhance the decomposition of the EP diet at low temperature. Amberjack, *S. dumerli* was fed with EP diet with or without alikaline protease (control group) for 60 days. After 60 days of rearing, the average fish body weight was higher in the group with alikaline protease as compared with that in the control group. These results showed that it is possible that the addition of protease enhance the growth rate of *S. dumerili* in winter season.

Annotated Bibliography

Satoh K. 2005. Study on improvement of composed diet for yellowtail culture. Bulletin of Oita Institute of Marine and *Fisheries Science*, **6**, 19-77 (in Japansese)

To date, Japanese aquaculture, mainly for yellowtail Seriola quinqueradiata, has been developed using raw-fish as their feedstuff, such as sardine and mackerel obtained from adjacent sea, that used to be abound and available with low cost. The reduction of sardine resources definitely caused the nessesity of composed diet in yellowtail culture. Therefore, the studies examined to improve and to improve accommodate the composed diet for yellowtails as to feedstuff, protein digestibility, feed additives, and feeding regime.

15: Amino acid profile of thraustochytrids cells and potential of application to aquafeed

Kenya HORII*[1], Masahiro HAYASHI*[1], and Yousuke TAOKA*[2]

*[1,2] Department of Marine Biology and Environment Sciences, Faculty of Agriculture, University of Miyazaki
E-mail: gd12020@student.miyazaki-u.ac.jp※[1], yousuketao@cc.miyazaki-u.ac.jp※[2]

Key words

Aquaculture, feeds, fishmeal, protein source, thraustochytrid, amino acid composition, byproducts

Abstract

Fishmeal is used as protein sources for aquaculture feeds. However, the price is drastically increased to around 170,000/ ton in 2013 during recent 10 years because of the decrease of fish resources, anchovy. This is serious problem for sustainable aquaculture. Therefore replacement of fishmeal to another resource is urgently needed. Thraustochytrids are marine protists and classified to one of Stramenopiles. They are widely distributed in marine environment and accumulate large number of lipids in cell bodies. Therefore, thraustochytrids have attracted strong interests for production of valuable lipids as single cell oils (SCOs) such as biodiesel and omega-3 fatty acids. In the process of lipid extraction from the cultured cells, some solids (extract residue) are produced as byproducts. It is considered that this extract residue except for lipids is mainly consists of protein. From the viewpoints of industrial application of thrautochytrid cells as protein sources, we have planned to use the byproducts as resources instead of fishmeal for aquafeeds. In this study, we evaluated the recovery rate of protein in a thrausotchytrid, *Aurantiochytrium limacinum* strain mh0186 known as a docosahexaenoic acid (DHA) producer under the lipid extraction process. For selection of adequate strains for protein production, thraustochytrids were isolated from marine environment. *A. limacinum* strain mh0186 was cultured in a GY broth. The cultured cells were collected by centrifugation and lyophilized for proximate analysis. The content of protein, lipid and ash in the lyophilized cells was determined according to Kjeldahl method, Folch method and heat-ashing method, respectively. The composition of amino acid and fatty acid were analyzed by liquid chromatography-mas spectrophy (UF-Aminostation, Shimazu Co. Ltd., Japan) and gas chromatography (GC-2014, Shimazu Co. Ltd., Japan), respectively. In the process of lipid extraction, the extract residue was collected and re-lyophilized. The protein content and amino acid composition were analyzed by same method described as above. Seawater, sands, leaves, seaweed were collected for

the isolation of thraustochytrids from coastal area in Miyazaki, Kumamoto and Oita, Kyushu, Japan. Thraustochytrids were isolated on a B12 Culture Agar "*Nissui*" plate medium by pine pollen-baiting method. The isolates were cultured in a GY broth, and the cultured cells were collected by centrifugation for analysis of the composition of amino acid and fatty acid. Isolates were identified at genus level based on the 18S rRNA sequence analysis. The content of crude protein lipid and ash per g of the cultured cell of mh0186 strain were 333 mg, 440 mg and 42 mg. On the one hand, the protein content of the extract residue obtained from 1g of the cultured cells was 226 mg (recover rate, 68%). In both samples of the cultured cells and the extra residue, glycine, leucine, isoleucine, glutamate and arginine were mainly detected. One hundred twenty thraustochytrids strains were isolated. In some isolates including *A. limacinum* strains SR21 and mh0186, *T.aureum* ATCC34304, *Schizochytrium aggregatum* ATCC28209, glycine, leucine glutamate, arginine and were detected as major amino acids. Strain Tak2 specifically accumulate 25% cystathionine to total amino acids.

16: Diurnal changes in frequency of the burst swimming behavior of adult Pacific bluefin tuna (*Thunnus orientalis*) in a land-based tank

Akiko TSUJITA*, Toshinori TAKASHI*, Kentaro HIGUCHI*, Tatsuru KADOTA*, Koichiro GEN*, Ayako SUZUKI*, Junpei KONISHI*, Keiichi MUSHIAKE*, and Masakazu OKA*

* Research Center for Tuna Aquaculture, Seikai National Fisheries Research Institute, FRA
E-mail: tsujiaki@affrc.go.jp

Key words
Pacific bluefin tuna, collision death, burst swimming behavior, diurnal change, broodstock tank

Abstract

In aquaculture of Pacific bluefin tuna, *Thunnus orientalis* (PBT), development of a stable system of artificially-reared fingerlings is needed to ensure the sustainability of aquaculture through a reduction on the reliance on wild captured juveniles. Therefore, we constructed two large indoor land-based PBT broodstock tanks at Seikai National Fisheries Research Institute, FRA, Japan, and are examining the environmental cues such as water temperature and photoperiod which are essential for successful and stable spawning of PBT. However, heavy mortality of adult PBTs occur in the tank due to collisions with the tank wall. It is considered that the collision deaths are associated with the burst swimming behavior. In this study, to clarify the process of the collision death, we examined diurnal changes in frequency of the burst swimming behavior of three-year-old PBTs in the land-based tanks. Nineteen three-year-old PBTs were reared in the land-based tanks (20 m in diameter, 6 m in depth) and their swimming behaviors were recorded using a video camera for six days. A day was compartmentalized into six periods defined as dawn (7:00 to 9:00), daytime-I (11:00 to 13:00), daytime-II (15:00 to 16:00), dusk (16:00 to 18:00), night-I (20:00 to 22:00) and night II (0:00 to 2:00) according to changes in illumination. We counted the frequency of the burst swimming behavior at each period from the recorded video imagery. As a result, the frequency of the burst swimming behavior at dawn was significantly higher than that in other time periods. Notably, the burst swimming behaviors were frequently observed in 30 min just after illumination (from 32 to 85 lux) during dawn. Additionally, the frequency of the burst swimming behavior of multiple PBTs in the tank like "a panic" were often observed during dawn, whereas solitary burst swimming behaviors were observed at the other time periods. These results suggest that collision deaths of PBT in the tank were caused by burst swimming behavior associated with sudden increases of light intensity during dawn.

17: Interval and spawning frequency of Pacific bluefin tuna *Thunnus orientalis* in a land-based tank

Ayako SUZUKI*[1], Koichiro GEN*[1], Tatsuru

KADOTA*1, Kenji SAITOH*2, Takuma SUGAYA*3, Masakazu OKA*1, and Keiichi MUSHIAKE*1

*1 Research Center for Tuna Aquaculture, Seikai National Fisheries Research Institute, FRA
*2 Research Center for Aquatic Genomics, National Research Institute of Fisheries Science, FRA
*3 Research Center for Marine Invertebrates, National Research Institute of Fisheries and Environment of Inland Sea, FRA
E-mail: ayakos@affrc.go.jp

Key words
Pacific bluefin tuna (*Thunnus orientalis*), spawning frequency, mtDNA *D-loop* region, haplotype

Abstract

Recently, the exploitation of natural stocks of Pacific bluefin tuna *Thunnus orientalis* (PBT) has increased to dangerous levels though increased fishing pressure. The scarcity of this species and its high commercial value, together with its very high growth rates, makes it a potential candidate for marine aquaculture. Therefore, the artificial propagation and seed production techniques for PBT represent objective for sustainable utilization of this resource. Fundamental information on the spawning ecology of PBT is essential for the development of production techniques. However, this information is still largely lacking. In this study, we investigated spawning frequency of PBT by comparing mitochondrial DNA *D-loop* region haplotypes of broodstock fish, with those of fertilized eggs and hatched larvae. Broodstock PBT, fertilized egg and hatched larval samples were obtained from a land-based tank at Research Center for Tuna Aquaculture, Seikai National Fisheries Research Institute in Nagasaki, Japan. Spawning activity in a land-based tank was observed over a 98 day period during May 16 to August 28 in 2014. The sampling of fertilized eggs and hatched larvae was conducted for a total of 15 days within the 98 days. A total of 15 samples were collected for an initial consecutive 3 day period and then at approximately 1 week intervals from May 16 to August 28 in 2014. The 36 broodstock individuals and 659 eggs and hatched larvae were observed to have 3 haplotypes. These haplotypes were named A, B and C, respectively. Among the broodstock fish, haplotype B was detected at high frequencies, secondly haplotype C, and thirdly haplotype A. The number of each broodstock individuals was 32, 3, and 1, respectively. Each haplotype of eggs and hatched larvae was detected on 14 days, 1 day and 8 days, respectively, during the 15 sampling days. Haplotype B occurred in the 3 days of consecutive spawning. This is consistent with genetic or histological observations that other wild *Thunnus* species spawn multiple times and on consecutive days. These results demonstrate that PBT in a land-based tank may have the potential to spawn consecutively and multiple times.

18: Effect of timing of restricted feeding on sexual maturation in the yellowtail, *Seriola quinqueradiata*

Kentaro HIGUCHI*1,2, Kazunori YOSHIDA*1, Koichiro GEN*1, Toshinori TAKASHI*1, Kiyoshi SOYAMA*2, and Keiichi MUSHIAKE*1

*1 Seikai National Fisheries Research Institute, FRA, 1551-8 Taira-machi, Nagasaki, Nagasaki 851-2213, Japan
*2 Institute for East China Sea Research, Nagasaki University, 1551-7 Taira-machi, Nagasaki, Nagasaki 851-2213, Japan
E-mail: higuken@affrc.go.jp

Key words
Yellowtail (*Seriola quinqueradiata*), sexual maturation, restricted feeding, broodstock management

Abstract

In aquaculture of large sized marine fish such as bluefin tuna and yellowtail, extensive amounts of fish feed are needed for the broodstock management because of their large body size. In order to save on the feeding cost for their broodstock management, development of a restricted feeding technique without affecting reproductive performance is required. However, available information about the

effect of food supply on reproduction is limited in fish. In this study, we examined the effect of restricted feeding during the gonad immature and vitellogenic phases on sexual maturation in females of the yellowtail, *Seriola quinqueradiata*. Two-year-old cultured yellowtail females, which the average body weight was 5.0 kg, were divided randomly into three sea cages on November 2012 and reared until the next spawning period (April 2013) under natural conditions at the Goto station, Seikai National Fisheries Research Institute, FRA, Japan. The feeding regimes in each cage were defined as follows: control group fed to satiation three times a week throughout the experimental period, two restricted groups fed 30% of the amount of feed given to the control group (100%) during the immature (from November to January) or vitellogenic phase (from February to April), respectively. At the end of the experimental period, the average body weights were 6.6 kg in the control group, 5.8 kg in the restricted group during the immature phase and 5.9 kg in the restricted group during the vitellogenic phase, which shows that the restricted feeding reduced approximately 50% of somatic growth throughout the experimental period. Interestingly, the gonad weights in the restricted feeding group during the vitellogenic phase were low as compared with the control group and the restricted feeding group during the immature phase. Histological observations revealed that females in all groups had oocytes that completed the accumulation of yolk globules at the spawning period. However, the mean diameter of most advanced ovarian follicles in the restricted feeding group during the vitellogenic phase was significantly smaller as compared with the other groups. Furthermore, plasma estradiol-17β levels in the restricted feeding group during the vitellogenic phase were significantly lower as compared with the other groups at the spawning period. These results indicate that the restricted feeding during the vitellogenic phase alters the gonadal development in relationship with the plasma estradiol-17β levels in the yellowtail females.

19: Evaluation of nitrogen excretion in young, immature and adult Pacific bluefin tuna (*Thunnus orientalis*) measured in the land-based tank

Toshinori TAKASHI*1, Takeshi EBA*2, Tadashi IMAI*3, Tetsuo MORITA*3, Yoshihisa YAMAMOTO*3, Hiroyuki MATSUNARI*4, Junpei KONISHI*1, Kazuki KUMON*2 , Koichiro GEN*1, and Masakazu OKA*1

*1 Seikai National Fisheries Research Institute, FRA, 8-1551 Taira, Nagasaki, Nagasaki 851-2213, Japan
*2 Amami Laboratory, Seikai National Fisheries Research Institute, FRA, 9555 Hyousakiyamahara, Oshima Setouchi, Kagoshima 894-2414, Japan
*3 Yashima Laboratory, National Research Institute of Fisheries and Environment of Inland Sea, FRA, 234 Yashima-higashi-machi, Takamatsu, Kagawa 761-0111, Japan
*4 National Research Institute of Aquaculture, FRA, 422-1 Nakatsuhamaura, Minamiise, Mie, 516-0193, Japan
E-mail: ttakasi@fra.affrc.go.jp

Key words
Pacific bluefin tuna (*Thunnus orientalis*), nitrogen, ammonia excretion rate, ration size

Abstract

Ammonia excretion in marine teleosts accounts for 70 to 90% of their total nitrogen excretion. Ammonia is toxic to fish and is a major factor limiting fish biomass and stocking density in intensive culture systems and aquariums. Quantification of ammonium nitrogen is important for estimating stocking biomass/density, water flow and size of the biological filter in the culture system. Several studies have suggested that ammonia excretion is affected by several factors such as species, body weight, water temperature and ration size. Although several tuna species such as Pacific bluefin tuna (PBT) and yellowfin tuna have been reared in aquariums and research facilities including Seikai National Fisheries Research Institute, there is a lack of studies about ammonia excretion in tunas. In this study, we investigated the effects of body weight and ration level on nitrogen excretion for PBT in captivity.

Young (0.67 ± 0.14 kg, n = 50), immature (14.4 ± 1.88 kg, n = 2) and adult (42.9 ± 6.5 kg, n = 2) PBTs were introduced to experimental land-based tanks from net cage or rearing tank, and thereafter they were acclimated to running sea water conditions. Experimental tank size varied according to fish size (20 kl for young fish; 65 kl for immature fish and 150 kl for adult fish). The PBTs were fed raw fish or artificial feed until the experiments. Before the fasting and postprandial experiments, the fish were deprived of food for 48 hours. Rearing water was sampled every 2 h for the first 12 h, and at 4 h intervals from 12 to 24 h. In the postprandial experiment, the experimental fish were fed bait fish (chub mackerel, *Scomber japonicas*, or sandlance, *Ammodytes personatus*) or artificial feed. Fish feces were collected from the tank bottom after 24 h. Determination of fecal nitrogen and concentration of ammonium, nitrite and nitrate nitrogen was carried out on each fecal and water samples. Weight-specific ammonia excretion rates of fasted fishes showed an inverse relationship with body weight (W). The relationship for total ammonia nitrogen (TAN) was: TAN (mg N W^{-1} d^{-1}) = 297.4·$W^{-0.36}$ (r^2=0.99). The ammonia excretion rate at 10 kg in PBT was twice as much as it is for red seabream (unpublished data). Although postprandial ammonium excretion rate was in relation to the ration size (R, mg feed-N W^{-1} d^{-1}), linear regression analysis indicated that TAN excretion rates increased with ration size: TAN (mg N W^{-1} d^{-1}) = 332.4·R−179.6 (r^2=0.87). There was no significant difference in the rates between PBT fed the bait fish and the artificial feed. Postprandial fecal nitrogen was positively correlated with ration size. Fecal nitrogen was excreted at a level of 0.9-1.9% for the baitfish and 0.3-1.3% for the artificial feed. Comparing the results of PBT with other fish, these values were lower than that of red seabream (12.8%) and pufferfish (16.1%). This study highlights the need for effective evaluation of nitrogen loading and water quality management in PBT rearing facilities and aquaculture grounds.

20: A high density genetic linkage map for yellowtail (*Seriola quinqueradiata*) containing 6,275 EST-based SNPs

Akiyuki OZAKI*1, Jun-ya AOKI*1, Kazuo ARAKI*1, Hironori USUKI*1, Kazunori YOSHIDA*2, Tsutomu NODA*2, Takuro HOTTA*2, Hirotaka MIZUOCHI*2, Taro CHUJO*2, and Yasuhiro SHIMA*1,2

*1 National Research Institute of Aquaculture, FRA, 422-1, Nakatsuhamaura, Minamiise-cho, Watarai-gun, Mie, 516-0193, Japan
*2 Seikai National Fisheries Research Institute, FRA, 122-7, Nunoura, Tamanoura-machi, Goto-shi, Nagasaki, 853-0508, Japan
E-mail: aozaki@affrc.go.jp

Keywords
Yellowtail (*Seriola quinqueradiata*), EST-based SNPs, genetic linkage map, quantitative trait loci (QTL), Affymetrix

Abstract

The marine products industry has developed as majority of the fishery, which are captured and directly using of aquatic resources. Only recently the breeding are considered as important research because available of aquatic resources are restricted gradually. The expectation of aquaculture research is getting higher in response to the prediction of aquatic resources depletion. Also the genetic improvement of economic traits are needed, it hopes apply to superior fish breeding, because artificial juvenile have a possibility to improve the phenotype for suited to aquaculture condition in every generation. We are researching practical application about selection of economic important traits from natural genetic resources using yellow tail (*Seriola quinqueradiata*) as target species. High density SNP arrays have become the tool of choice for QTL mapping, Genome-wide association studies, marker-assisted selection (MAS) and genomic selection (GS). More recently, high-density linkage maps generated by SNP array data have proven to be crucial for the accurate assembly of scaffolds and contigs in whole-genome sequencing efforts. Earlier mapping studies

have identified QTL for important commercial traits including parasite disease resistance, and combining the resources of a high density genetic map with genome sequence data will facilitate the fine mapping of these loci and the identification of candidate genes. In this study, Affymetrix SNP array was used to genotype 460 samples collected across five families from wild population in coastal waters of Goto Fukue-island. To establish EST (expressed sequence tag) -based SNP array, a cDNA library was generated from pooled RNA samples extracted from 11 tissues from a single individual. Sequencing on Roche/454 GS FLX platform generated 1,353,405reads. The sequencing of SNP identification produced 570,846 raw reads derived from the full-length library and 456,482 raw reads derived from the 3'-anchored library derived from 5 hundred juveniles. Quality – based variant calling using CLC Genomics Workbench detected 9,356 biallelic putative SNPs in 6,025 contigs, with a minor allele frequency (MAF) >25%. A Linkage analysis was performed using application package of LINKMFEX version 2.3. This application can separate originated alleles from male or female. In order to avoid the error of genotyping, the accuracy of genotypes in their progenies was checked from parental male and female alleles. Genotype data were converted to a backcross format as though the grandparent genotype was unknown. Pairwise analysis was performed, and markers were sorted in linkage group at a minimum LOD threshold of 5.0. Linkage phases were determined retrospectively by examining the assortment of alleles among linked markers. A total of 6,275 EST-based SNPs were mapped to 24 linkage groups. The total distance covered by the male and female maps were 1,230cM and 1,031cM. This map is currently being used to map QTL for a number of commercially important traits, and will be used to improve the assembly of the yellowtail genome. It is possible to rapidly develop domesticated strains having commercially important traits in yellowtail aquaculture.