

STRATEGY FOR BREEDING STUDY OF FISHERIES ORGANISMS BY FRA

Okamoto, H

Fisheries Research Agency, Japan (2013). Strategy for breeding study of fisheries organisms –A plan of proceedings of fisheries breeding in Japan. 1-20. (in Japanese)

A sea area of around Japan is one of the fertile areas to produce marine natural resources in the world, the product amount of Japanese fisheries and aquaculture is only covered 60 % of the domestic demand itself. Japanese Fisheries Agency made a plan to raise the food self-sufficiency ratio up to 70% for fin-fish, and 73% for seaweeds in 2022. The domestic aquaculture production is mostly consumed in the domestic consumption, and therefore needs to develop itself to achieve the goal of marine food self-sufficient ratio. However, the business condition of Japanese aquaculture is rather serious, because of hardness to keep fish-meal for feeding from overseas, to save the cost for preventing disease, and to compete against cheaper import marine products. To overcome these serious problems of aquaculture farmer in Japan, Fisheries Research Agency (FRA) made strategy for breeding study of fisheries organisms to achieve genetically improvement, for example, about the adaptation in aquaculture environment, or more suitable products for consumer demand. The establishment of breeding in aquaculture is expected to maintain the amount of domestic production, to enhance international competitiveness and to activate related industries, by reduction of production cost and enhancement of domestic consumption. The strategy is comprised of two parts, one is “Present situation of research and development of aquatic breeding study”, the other is “Direction and policy for promotion of aquatic breeding study”.

K. T. Wada (2012). Evaluation of Parental Breeding Values in Aquaculture-Reviews. *Fish Genetics and Breeding Science*, 41, Issue 2, 87-96. (in Japanese)

The strategies in evaluation of parents and in mating design are important in aquaculture breeding programs. This review deals with topics of evaluating the breeding values of parents in fish and shellfish species of aquaculture. (1) In order to evaluate offspring reared at a communal environment, various chemical or physical tags and molecular markers have been developed for assigning the parentage in mating experiments or in selection programs. (2) Accuracy of selection is a main target in genetic evaluation of parental animals and the breeding value has been estimated in many traits of many species. Methodology like BLUP developed in breeding sciences of livestock can be applied to aquatic animals with some modification. (3) Increased cost and inbreeding rate and decreased genetic diversity are

concerns in selective breeding since the number of offspring is much larger in aquaculture breeding program than in those of livestock.

A. Ozaki, W. Kai, J. Aoki, K. Nomura, K. Araki, K. Yoshida, T. Tsuzaki, T. Sugaya, M. Sano, K. Kobayashi-Fuji, T. Sakamoto and N. Okamoto (2012). Application to the genetic breeding using by genomics information in Aquaculture. *Fish Genetics and Breeding Science*, 41, Issue 2, 173-178. (in Japanese)

Ten years ago, genetic breeding study of fisheries organisms was mainly about salmonids because it was the main production of world aquaculture. But now, the aquaculture of marine fish is getting important, the center of breeding study is moving in marine fish. As developing technique of fish seedling, not only the main aquaculture fish species, for example, flounder, puffer fish and yellowtail, but also the hard seedling species like eel and tuna are expected to be applied with breeding study based on genomic information after establishment of complete raising technique in aquaculture farm. We are introducing about studies of Quantitative Trait Loci (QTL) and Marker-Assisted Selection (MAS) in aquaculture fish, and discussing about necessity and possibility of breeding study in aquaculture.

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI (QTL) AND MARKER-ASSISTED SELECTION (MAS) USING BY GENOMICS INFORMATION IN YELLOWTAIL (*SERIOLA QUINQUERADIATA*)

Ozaki, A

Akiyuki Ozaki, Kazunori Yoshida, Kanako Fuji, Satoshi Kubota, Wataru Kai, Jun-ya Aoki, Yumi Kawabata, Junpei Suzuki, Kazuki Akita, Takashi Koyama, Masahiro Nakagawa, Takuro Hotta, Tatsuo Tsuzaki, Nobuaki Okamoto, Kazuo Araki, Takashi Sakamoto. 2013. Quantitative Trait Loci (QTL) Associated with Resistance to a Monogenean Parasite (*Benedenia seriolae*) in Yellowtail (*Seriola quinqueradiata*) through Genome Wide Analysis. PLoS ONE 8(6): e64987. doi:10.1371/journal.pone.0064987

Benedenia infections caused by the monogenean fluke ectoparasite *Benedenia seriolae* seriously impact marine finfish aquaculture. Genetic variation in host has been inferred to play a significant role in determining the susceptibility to this parasitic disease. To evaluate the genetic basis of *Benedenia* disease resistance in yellowtail (*Seriola quinqueradiata*), a genome-wide and chromosome-wide linkage analyses were initiated using F₁ yellowtail families (n = 90 per family) based on a high density linkage map with 860

microsatellite and 142 single nucleotide polymorphism (SNP) markers. Two major quantitative trait loci (QTL) regions on linkage groups Squ2 (*BDR-1*) and Squ20 (*BDR-2*) were identified. These QTL regions explained 32.9–35.5% of the phenotypic variance. On the other hand, the relationship between QTL for susceptibility to *B. seriolae* and QTL for fish body size were investigated. The QTL related to growth was found on another linkage group (Squ7). As a result, the authors present first genetic evidence that contributes to detailing phenotypic resistance to *Benedenia* disease, and the results will help resolve the mechanism of resistance to this important parasitic infection of yellowtail.

Kanako Fuji, Takashi Koyama, Wataru Kai, Satoshi Kubota, Kazunori Yoshida, Akiyuki Ozaki, Jun-ya Aoki, Yumi Kawabata, Kazuo Araki, Tatsuo Tsuzaki, Nobuaki Okamoto, Takashi Sakamoto. 2014. Construction of a high-coverage bacterial artificial chromosome library and comprehensive genetic linkage map of yellowtail *Seriola quinqueradiata*. BMC Research Notes 7:200. <http://www.biomedcentral.com/1756-0500/7/200>

Japanese amberjack/yellowtail (*Seriola quinqueradiata*) is a commonly cultured marine fish in Japan. For cost effective fish production, a breeding program that increases commercially important traits is one of the major solutions. In selective breeding, information of genetic markers is useful and sufficient to identify individuals carrying advantageous traits but if the aim is to determine the genetic basis of the trait, large insert genomic DNA libraries are essential. In this study, toward prospective understanding of genetic basis of several economically important traits, the authors constructed a high-coverage bacterial artificial chromosome (BAC) library, obtained sequences from the BAC-end, and first comprehensive female and male linkage maps of yellowtail using Simple Sequence Repeat (SSR) markers developed from the BAC-end sequences and a yellowtail genomic library. The total insert length of the BAC library the authors constructed here was estimated to be approximately 11 Gb and hence 16-times larger than the yellowtail genome. Sequencing of the BAC-ends showed a low fraction of repetitive sequences comparable to that in Tetraodon and fugu. A total of 837 SSR markers developed here were distributed among 24 linkage groups spanning 1,026.70 and 1,057.83 cM with an average interval of 4.96 and 4.32 cM in female and male map respectively without any segregation distortion. Oxford grids suggested conserved synteny between yellowtail and stickleback. In addition to characteristics of yellowtail genome such as low repetitive sequences and conserved synteny with stickleback, the genomic and genetic resources constructed and revealed here will be powerful tools for the yellowtail breeding program and also for studies regarding the genetic basis of traits.

Jun-ya Aoki, Wataru Kai, Yumi Kawabata, Akiyuki Ozaki, Kazunori Yoshida, Tatsuo Tsuzaki, Kanako Fuji, Takashi Koyama, Takashi Sakamoto, Kazuo Araki. 2014. Construction of a radiation hybrid panel and the first yellowtail (*Seriola quinqueradiata*) radiation hybrid map using a nanofluidic dynamic array. BMC Genomics 15:165.

<http://www.biomedcentral.com/1471-2164/15/165>

Yellowtail (*Seriola quinqueradiata*) are an economically important species in Japan. However, there are currently no methods for captive breeding and early rearing for yellowtail. Thus, the commercial cultivation of this species is reliant upon the capture of wild immature fish. Given this, there is a need to develop captive breeding techniques to reduce pressure on wild stocks and facilitate the sustainable development of yellowtail aquaculture. The authors constructed a whole genome radiation hybrid (RH) panel for yellowtail gene mapping and developed a framework physical map using a nanofluidic dynamic array to use SNPs (single nucleotide polymorphisms) in ESTs (expressed sequence tags) for the DNA-assisted breeding of yellowtail. Clonal RH cell lines were obtained after ionizing radiation; specifically, 78, 64, 129, 55, 42, and 53 clones were isolated after treatment with 3,000, 4,000, 5,000, 6,000, 8,000, or 10,000 rads, respectively. A total of 421 hybrid cell lines were obtained by fusion with mouse B78 cells. Ninety-four microsatellite markers used in the genetic linkage map were genotyped using the 421 hybrid cell lines. Based upon marker retention and genome coverage, the authors selected 93 hybrid cell lines to form an RH panel. Importantly, the authors performed the first genotyping of yellowtail markers in an RH panel using a nanofluidic dynamic array (Fluidigm, CA, USA). Using this, 580 markers containing ESTs and SNPs were mapped in the yellowtail RH map. The authors successfully developed the first yellowtail RH panel to facilitate the localization of markers.. This high-density physical map will serve as a useful tool for the identification of genes related to important breeding traits using genetic structural information, such as conserved synteny. Moreover, in a comparison of 30 sequences in the RH group 1 (SQ1), yellowtail appeared to be evolutionarily closer to medaka and the green-spotted pufferfish than to zebrafish. The authors suggest that synteny analysis may be potentially useful as a tool to investigate chromosomal evolution by comparison with model fish.

ASSESSMENT OF FERTILIZATION ABILITY OF CRYOPRESERVED SPERM IN FISH USING INTERSPECIFIC HYBRIDIZATION

Shimada, Y

M. Suquet, C. Dreanno, C. Fauvel, J. Cosson, and R. Billard (2000). Cryopreservation of

Sperm in Marine Fish. Aquaculture Reserch 31:231-243.

Since 1953, cryopreservation of sperm in fish has been performed on about 30 marine species. The paper reviews the techniques used and the results published in these species. The quality of frozen-thawed semen was evaluated using previously standardized biotests (i.e. fertilization capacity). Of the investigated cryoprotectants, dimethyl sulphoxide (DMSO) generally leads to the best results. Cooling rates range from 8 °C to 99 °C min⁻¹; the thawing rate is generally high. Compared with freshwater species, a high percentage of sperm survives in cryopreservation. Therefore, the cryopreservation of marine fish sperm is suited for application in aquaculture.

T. Morita, N. Kumakura, K. Morishima, T. Mitsuboshi, M. Ishida, T. Hara, S. Kudo, M. Miwa, S. Ihara, K. Higuchi, Y. Takeuchi, and G. Yoshizaki (2012). Production of Donor-Derived Offspring by Allogeneic Transplantation of Spermatogonia in the Yellowtail (*Seriola quinqueradiata*). Biology of Reproduction 86(6):176,1-11.

Authors established a method for the allogeneic transplantation of yellowtail spermatogonia and the production of donor-derived offspring. Donor cells (i.e. type A spermatogonia) were collected from immature (10-month-old) yellowtail males, labeled with PKH26 fluorescent dye, and transferred into the peritoneal cavities of 8-day-old larvae. Fluorescence observation at 28 days post-transplantation revealed that PKH26-labeled cells were incorporated into recipients' gonads. To assess whether donor-derived spermatogonia could differentiate into functional gametes in the allogeneic recipient gonads, gametes collected from nine male and four female adult recipients were fertilized with wild-type eggs and sperm. Analysis of microsatellite DNA markers confirmed that some of the first filial (F₁) offspring were derived from donor fish, with the average contribution of donor-derived F₁ offspring being 66% and the maximum reaching 99%. These findings confirmed that authors' method was effective for transplanting yellowtail spermatogonia into allogeneic larvae to produce donor-derived offspring.

S. Lee, Y. Iwasaki, S. Shikina, and G. Yoshizaki (2013). Generation of Functional Eggs and Sperm from Cryopreserved whole testes. PNAS 110(5):1640-1645.

The conservation of endangered fish is of critical importance. Cryobanking could provide an effective backup measure for use in conjunction with the conservation of natural populations; however, methodology for cryopreservation of fish eggs and embryos has not yet been developed. Authors established a methodology capable of deriving functional eggs and sperm from frozen type A spermatogonia (ASGs). Whole testes taken from rainbow trout

were slowly frozen in a cryomedium, and the viability of ASGs within these testes did not decrease over a 728-d freezing period. Frozen-thawed ASGs that were intraperitoneally transplanted into sterile triploid hatchlings migrated toward, and were incorporated into recipient genital ridges. Transplantability of ASGs did not decrease after as much as 939-d of cryopreservation. Approximately half of triploid recipients produced functional eggs or sperm derived from the frozen ASGs and displayed high fecundity. Fertilization of resultant gametes resulted in the successful production of normal, frozen ASG-derived offspring. Feasibility and simplicity of this methodology will call for an immediate application for real conservation of endangered wild salmonids.

DEMANDS FOR INFERTILITY OF CULTURED MARINE FISHES AND STUDY OF UV IRRADIATION AT DEVELOPMENTAL STAGES IN JAPANESE FLOUNDER

Yamaguchi, T

Toshiya Yamaguchi and Takeshi Kitano 2012. High temperature induces *cyp26b1* mRNA expression and delays meiotic initiation of germ cells by increasing cortisol levels during gonadal sex differentiation in Japanese flounder. *Biochemical and Biophysical Research Communications*, 419(2), 287-292

The Japanese flounder (*Paralichthys olivaceus*) is a teleost fish that has an XX/XY sex determination system. However, the XX flounder can be sex-reversed to phenotypic males by rearing the larvae at high or low water temperatures. Thus, sex in the flounder is determined by genotype plus temperature effects. Therefore, the flounder provides an excellent model to study the molecular mechanisms underlying temperature-dependent sex determination. We previously showed that cortisol, the major glucocorticoid produced by the interrenal cells in teleosts, causes female-to-male sex reversal by directly suppressing mRNA expression of ovary-type aromatase (*cyp19a1*), a steroidogenic enzyme responsible for the conversion of androgens to estrogens in the gonads. In the present study, we found that exposure to high temperature during gonadal sex differentiation upregulates the mRNA expression of retinoid-degrading enzyme (*cyp26b1*) concomitantly with masculinization of XX gonads and delays meiotic initiation of germ cells. We also found that cortisol induces *cyp26b1* mRNA expression and suppresses specific meiotic marker *synaptonemal complex protein 3* (*sycp3*) mRNA expression in gonads during the sexual differentiation. In conclusion, *cyp26b1* expression in gonads is in male-specific manner during gonadal sex differentiation and delays meiosis onset not only in mouse, chick and amphibian, but also in a teleost with TSD.

Therefore, CYP26B1 appears to regulate germ cell fate by controlling retinoic acid signaling in a manner common among vertebrates.

Yamaguchi T., N. Yoshinaga, T. Yazawa, K. Gen, and T. Kitano. 2010. Cortisol is involved in temperature-dependent sex determination in the Japanese flounder. *Endocrinology*, *Endocrinology*, 151(8), 3900–3908

The Japanese flounder (*Paralichthys olivaceus*) is a teleost fish with an XX/XY sex determination system. However, XX flounder can be induced to develop into either phenotypic females or males, by rearing at 18 or 27 °C, respectively, during the sex differentiation period. Cytochrome P450 aromatase, the product of the gene *cyp19*, is expressed in various tissues and plays an important physiological role in the regulation of estrogen biosynthesis. Recently, it has been reported that, in nonmammalian vertebrates, the expression of *cyp19* mRNA in the ovary is very much higher than in the testis during the period of gonadal sex differentiation. In this study, we demonstrated that cortisol causes female-to-male sex reversal by directly suppressing *cyp19a1* mRNA expression via interference with cAMP-mediated activation and that metyrapone (an inhibitor of cortisol synthesis) inhibits 27°C-induced masculinization of XX flounder. Moreover, cortisol concentrations in 27°C-reared juveniles were significantly higher than in 18°C-reared fishes during sexual differentiation. In conclusion, this study has presented the evidence of involvement of cortisol in TSD. Cortisol induced the masculinization of XX fish by directly suppressing *cyp19a1* mRNA expression via interference with the cAMP-mediated activation. These findings provide new insights into the elucidation of molecular mechanisms underlying environmental sex determination, including TSD.

DEVELOPMENT OF INTEGRATED MULTI-TROPHIC AQUACULTURE USING SEA CUCUMBER

Watanabe, S

S. Watanabe, J. G. Sumbing and Ma. J. H. Lebata-Ramos (2014) Growth pattern of tropical sea cucumber, *Holothuria scabra*, under captivity, *Japan Agricultural Research Quarterly*, 48, 457-464

The growth of the juvenile sea cucumber, *Holothuria scabra*, was studied under captivity to elucidate the growth variation pattern and determine the best-fit growth model to estimate age- and size-specific growth rates. Individual growth was extremely variable, with

some individuals below the mean initial weight and some expanding their original body length (L) and weight (W) by up to 6.4 and 156 times, respectively; during 84 days of culture starting at 127 days of age. Some of the smallest individuals showed a higher condition factor than larger individuals in the presence of ample food, indicating that lack of food may not be the only impediment to growth. Among the three growth models compared (von Bertalanffy, Gompertz and logistic), the Gompertz model was considered optimal to express *H. scabra* growth; both in L and W. The age- and size-specific daily growth rate for L and W up to 365 days of age, as estimated by the Gompertz model, had a range of two and nine orders of magnitude in L (0.035 – 0.96 mm/day) and W (3.4×10^{-7} – 3.5 g/day), respectively. Use of the Gompertz model over the linear model, which tends to overestimate growth rates, is encouraged to estimate the growth of *H. scabra* more accurately.

Z. G. A. Orozco, J. G. Sumbing, Ma. J. H. Lebata-Ramos and S. Watanabe (2014) Apparent digestibility coefficient of nutrients from shrimp, mussel, diatom and seaweed by juvenile *Holothuria scabra* Jaeger, *Aquaculture Research*, 45, 1153–1163

The ability of *Holothuria scabra* to digest nutrients, such as organic matter (OM), protein and carbohydrate from animal and plant feed ingredients was investigated. Four test feeds prepared by mixing sand with single ingredients from animal sources (shrimp and mussel) and plant sources (diatom and seaweed) were fed to *H. scabra* to estimate apparent digestibility coefficient (ADC). The total assimilated nutrient (TAN) increased with ADC, whereas ingestion rate (IR) varied slightly among the feeds suggesting that ADC might be a good indicator of nutrient availability to *H. scabra*. The ADC_{OM} of shrimp and mussel was significantly higher than that diatom and seaweed: 86.2%, 77.1%, 55.1% and 32.3% respectively. ADC_{protein} was similar for shrimp (88.7%), mussel (84.8%) and diatom (75.2%), but significantly lower in seaweed (34.4%). ADC_{carbohydrate} was similar in mussel (58.5%) and diatom (58.3%) as well as in seaweed (31.6) and shrimp (28.0%). ADC_{protein} was relatively higher than ADC_{carbohydrate} suggesting that *H. scabra* generally digests more protein than carbohydrate. Furthermore, results indicated that nutrients from animal-based feeds are more efficiently digested by *H. scabra*; thus, animal ingredients rich in easily digestible protein could potentially provide an efficiently balanced diet for *H. scabra* fed with diatom containing high easily digestible carbohydrate.

S. Watanabe, M. Kodama, J. G. Sumbing and Ma. J. H. Lebata-Ramos (2013) Diet-tissue stable isotopic fractionation of tropical sea cucumber, *Holothuria scabra*, *Japan Agricultural Research Quarterly*, 47, 127-134

To provide a basis for a stable carbon and nitrogen isotope ratio ($\delta^{13}\text{C}$ / $\delta^{15}\text{N}$) analysis to determine the assimilated organic matter in sea cucumber, *Holothuria scabra*, diet-tissue fractionations were experimentally determined by mono-feeding rearing with diatom. While $\delta^{15}\text{N}$ fractionation of the whole body wall (2.4‰) was similar to the commonly accepted value (2.6 – 4‰), $\delta^{13}\text{C}$ fractionation of the body wall (4.2‰) showed considerable discrepancy with the commonly accepted value (0 – 1‰) due to the high content (35% dry wt/wt) of calcareous spicules (CaCO_3) in the body wall, which had significantly higher $\delta^{13}\text{C}$ (-8.6‰) than the organic fractions. Computational elimination of spicules based upon spicule content and spicule $\delta^{13}\text{C}$ reduced the $\delta^{13}\text{C}$ fractionation of the body wall to 1.5‰, close to the common value. $\delta^{13}\text{C}$ fractionation after spicule removal by acid decarbonation and subsequent rinsing (3.2‰) did not agree with the common value, and $\delta^{15}\text{N}$ fractionation was significantly elevated by decarbonation. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ fractionations of the intestine (1.5 and 2.2‰, respectively) did not agree with the common values. Since $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the feces did not differ significantly from those of the diet, feces may be used to determine ingested organic matter in the wild.

S. Watanabe, J. M. Zarate, J. G. Sumbing, Ma. J. H. Lebata-Ramos and M. F. Nievaes (2012) Size measurement and nutritional condition evaluation methods in sandfish (*Holothuria scabra* Jaeger), *Aquaculture Research*, 43, 940-948

The aims of this study were to establish an accurate size measurement method and a nutritional condition evaluation method of *Holothuria scabra* (Jaeger). Although 0.5% KCl and 0.05% MgSO_4 did not induce anaesthesia, 2% menthol-ethanol for 20 min was found to be effective and harmless. The anaesthetization significantly reduced the coefficient of variation of the mean body length and weight by 68% and 43% respectively. During starvation, body size and weight decreased concomitantly, resulting in an unchanged condition factor (body weight/volume), suggesting that the condition factor cannot be used as an index of nutritional condition. Protein, cholesterol and carbohydrate concentrations in the body fluid were analysed to study the relationship with starvation. As the protein and cholesterol concentrations initially increased and then decreased during the starvation period, it is difficult to use them as an index of nutritional condition. The carbohydrate concentration showed a gradual one-fold increase during 10 days of starvation, and it may be used as a proxy for nutritional condition; however, further physiological studies are needed. Body fluid density and volume relative to body size gradually increased and decreased, respectively, during starvation. These methods may be used to correctly monitor the conditions of *H. scabra* in studies for aquaculture and stock enhancement techniques.

S. Watanabe, M. Kodama, J. M. Zarate, Ma. J. H. Lebata-Ramos and M. F. J. Nievaes (2012)
Ability of sandfish (*Holothuria scabra*) to utilise organic matter in black tiger shrimp ponds.
ACIAR Proceedings, 136, 113-120

Due to frequent viral disease outbreaks, a large proportion of shrimp aquaculture in South-East Asian countries has switched from black tiger shrimp (*Penaeus monodon*) to *P. vannamei*, an exotic species originally imported from Latin America. One of the causes of disease outbreaks is thought to be poor water and sediment conditions in the shrimp ponds, which may aggravate disease symptoms. To obtain basic information for co-culture methods of black tiger shrimp and sandfish (*Holothuria scabra*) for possible mitigation of shrimp-pond eutrophication and prevention of disease outbreaks, basic laboratory experiments were conducted at the Southeast Asian Fisheries Development Center - Aquaculture Department in Iloilo, the Philippines. A feeding trial of juvenile sandfish showed that they do not grow well with fresh shrimp feed on hard substrate. Another trial indicated that sand substrate enhances the growth of juvenile sandfish fed with shrimp feed. A feeding trial using shrimp tank detritus, shrimp faeces and *Navicula ramosissima* (a benthic diatom) as food sources showed that sandfish grew fastest with the faeces, followed by detritus and *N. ramosissima*. Dissolved oxygen consumption and acid-volatile sulfur levels in the shrimp tank detritus were reduced by sandfish feeding. This suggests that sandfish are capable of growing with organic matter in shrimp ponds, and can bioremediate shrimp-pond sediment.

SUSPENDED CULTURE OF ASARI CLAM, *RUDITAPES PHILIPPINARUM*, AND THEIR ROLES IN THE ECOSYSTEM

Higano, J

Fujiwara M., S. Tsuji, M. Tanaka, Y. Imanishi and M. Nakanishi. 2008. Growth of Manila Clam *Ruditapes philippinarum* On Hanging Culture Using Containers. Bull. Kyoto. Inst. Ocean. Fish. Sci., 30, 49-53 (in Japanese)

In order to examine the growth of the manila clam *Ruditapes philippinarum*, a hanging cultivation experiment using containers was conducted in Kunda and Maizuru Bay. We covered the upper part of the culture containers with a fine mesh net, and put anthracite soil (particle size : 2 - 3 mm) into the containers for bed material. The wild juvenile clams used in this experiment were collected from hanging cockle culture containers in Kunda Bay. We could recognize from the time of discovering juvenile clams whether they were autumn or

summer broods. Autumn broods grew well from spring to summer, but hardly grew in autumn. Summer broods grew well from the first autumn to the summer of the following year, but hardly grew in autumn of the next year. The mean shell length of 1-year-olds was 32 - 42 mm in autumn broods, and 45 mm in summer broods. These results revealed markedly high growth rates not reported to date.

Mizuno T., T. Maruyama and J. Higano. 2009. Transition and Prospect of Asari clam (*Ruditapes philippinarum*) Fisheries in Ise Bay, Mie Prefecture. Bull. Mie Pref. Fish. Res. Inst., 17, 1–21 (in Japanese)

The authors took an overview on the history of the clam fishery in the western-side region of Ise Bay, a large bay (2,342km² with 660 km coastline) in the central part of Japan (as shown in the left figure below). Major commercial clams produced in the bay are Asari clam *Ruditapes philippinarum* living in shallow coastal areas, freshwater clam *Corbicula japonica* and hard clam *Meretrix lusoria* in brackish-water.

The annual yield of these 3 species decreased in response to fishing efforts and environmental changes. Until early 1960s, tidal flats at river mouths covered in excess of 6,000 ha, and eelgrass beds grew there as well as in other parts along the coastline. After the annual catch of Asari clam peaked at ca. 15,000 tons in the late 1960s, it decreased rapidly when coastal developments destroyed tidal flats and eelgrass beds during 1970s (right figure). In this period, the structure of coastal fisheries changed then laver culture and fisheries on clams and pelagic fish were continued. The second peak of Asari yield was seen in 1980s when clams in the subtidal zone were also exploited. Aged fishermen recruited into clam fisheries during 1990s avoiding intensive labors in the laver culture. In 2000s, the 70% of fishermen are working in clam fisheries, and the yield of Asari clam became stagnant in 1,000 to 3,000 tons due to the strong fishing pressure.

In the early 2000s, very few Asari clam larvae, less than 200 individuals/m³ in the main reproductive period (spring and autumn in this region), were observed at the river mouth in the southern part of the bay. This abundance of larvae was contrastingly smaller than that in Mikawa Bay adjacent to Ise Bay where stable annual yields and high abundances of larvae, thousands of individuals in 1 m³, were reported. In the survey point in Ise Bay, it was observed that juveniles immediately after settlement rarely survived after the flood in June through August, while juveniles occurred in autumn survived once in a few years. These survivors were exploited soon after their shell length reached at 25mm, the minimum size allowed to catch, in 2 years.

Catch per unit effort (CPUE) of hard clam and freshwater clam recovered when the

society of local fishermen became aware of the importance of regulating their catch and protecting fishing grounds in the light of field surveys. As contrasted with cases of these 2 species living in or around rivers, the spatial scale necessary for the fishing management of Asari clam should be much larger. In 2009, fishing cooperatives along the bay were organized to conduct resource surveys and activities to protect fishing grounds from undesirable predators. It has been also planned to enhance the quality of some fishing grounds in the bay by creating artificial tidal flats and adjusting the ground height to the known suitable level for the habitat of Asari clam.

Gouletquer, P., J.M. Deslous-Paoli, M. Héral. 1989. Ecophysiologie et Bilan Energétique de la palourde japonaise d'élevage *Ruditapes philippinarum*. Jour. Exp. Mar. Biol. Ecol., 132, 85-108.

Rates of filtration and respiration both follow a nonlinear model based on temperature of the form: $Y = a \times (T - T_0)^b \times e^{-K(T - T_0)}$ with maximal values at 15 and 20°C, respectively. Quantities of seston varying from 0 to 30 mg · l⁻¹ have no effect in reducing the filtration rate. > 8 mg · l⁻¹, ingestion is regulated by the production of pseudofaeces. Maximal assimilation efficiency is ≈ 78%, but this is considerably reduced when the mineral content of the water increases. Assimilation efficiency for the Manila clam is reduced at both high (> 10 mm³ · h⁻¹) or low (< 2 mm³ · h⁻¹) values of ingested ration. The estimated value of growth efficiency (75%) and values of growth efficiency derived from the model $k_1 = 33%$, $K_2 = 51%$ are optimized when ingested volumes are between 1 and 2 mm³. Standard metabolism is estimated as 0.11 ml O₂ · h⁻¹. Zero growth efficiency occurs at a ration level of 2 J · h⁻¹ for an adult. The individual energy budget shows that production is dependent more on temperature than on the energy value of the food. Comparison of calculated and measured production reveals differences resulting from the higher levels of seston found in the field. In particular, during the winter when the mineral content of the seston is high (90 mg · l⁻¹), there is a continuous loss of weight. This results from a lower assimilation efficiency together with production of pseudofaeces. Excretion of organic nitrogen varies throughout the year, ammonia representing no more than a mean of 29.8% of the total nitrogen excretion.

Magni P., S. Montani, C. Takada and Tsutsumi H. 2000 Temporal scaling and relevance of bivalve nutrient excretion on a tidal flat of the Seto Inland Sea, Japan. Mar. Ecol. Prog. Ser.,

Using an indirect and integrated approach, we quantified the magnitude and temporal variability of the contribution of macrozoobenthos to the upward flux of inorganic nitrogen and phosphorus on a sandy tidal flat of the Seto Inland Sea, Japan. From April 1994 to April 1996, we conducted monthly field surveys on the abundance and faunal composition of the macrozoobenthic communities inhabiting the lower part of the intertidal zone. Subsequently, we carried out 24 h day/night laboratory experiments on the nutrient excretion rate by various size-classes of the dominant species. We then obtained the animal nutrient excretion over a 2 yr period, multiplying the species-specific excretion rates by the actual animal biomass found on the tidal flat. Additionally, for all different seasons, we calculated the upward diffusive flux from the sediments from the vertical profiles of nutrient concentrations in the porewater. On the flat, the bivalves *Ruditapes philippinarum* (Veneridae) and *Musculista senhousia* (Mytilidae) were dominant, making up $86 \pm 5.6\%$ when the total biomass exceeded $100 \text{ g DW (dry weight) m}^{-2}$. From our laboratory experiments, the mean (day and night) nutrient excretion rates at 20 to 22°C by the 2 bivalve species were $18.9 \mu\text{mol NH}_4^+\text{-N g}^{-1} \text{ DW h}^{-1}$, $4.8 \mu\text{mol (NO}_3^- + \text{NO}_2^-)\text{-N g}^{-1} \text{ DW h}^{-1}$ and $3.3 \mu\text{mol PO}_4^{3-}\text{-P g}^{-1} \text{ DW h}^{-1}$. In addition, $\text{NH}_4^+\text{-N}$ excretion by *R. philippinarum*, but not that by *M. senhousia*, was significantly higher during the day than during the night. This occurred while the food (*Thalassiosira* sp.) offered in spikes was rapidly taken up irrespective of the concentration (within a field relevant spring-summer range of 10 to $60 \mu\text{g l}^{-1} \text{ chl } a$) and in day/night treatments. The release of nitrogen (N) and the release of phosphorus (P) were highly correlated with each other, for both *R. philippinarum* and *M. senhousia*, with a N/P ratio of 7.8 ± 3.0 and 9.9 ± 3.5 , respectively. In the field, the bivalve excretion rates of nutrients were calculated to be up to $35.2 \text{ mmol NH}_4^+\text{-N m}^{-2} \text{ d}^{-1}$, $8.8 \text{ mmol (NO}_3^- + \text{NO}_2^-)\text{-N m}^{-2} \text{ d}^{-1}$ and $5.8 \text{ mmol PO}_4^{3-}\text{-P m}^{-2} \text{ d}^{-1}$. These values rank in the upper range of nutrient excretion by intertidal macrozoobenthos and are comparable to those found on dense assemblages (800 to $2000 \text{ g ash free DW m}^{-2}$) of the most investigated mussel, *Mytilus edulis*. In addition, nutrient fluxes through bivalve excretion varied strongly within a few months, up to 10-fold (*R. philippinarum*) and 100-fold (*M. senhousia*) between April 1994 and August 1994, as related to the temporal change of bivalve standing stock. The extent of nutrient regeneration through diffusive flux was comparable to that reported for other intertidal zones of Japan and in the eutrophic Seto Inland Sea, within a range of 0.2 to $1.5 \text{ mmol NH}_4^+\text{-N m}^{-2} \text{ d}^{-1}$ and 0.01 to $0.05 \text{ mmol PO}_4^{3-}\text{-P m}^{-2} \text{ d}^{-1}$, thus more than 1 order of magnitude lower than that due to the excretory activity by *R. philippinarum* and *M. senhousia*. Our results indicate that the dominant bivalves, *R. philippinarum* and *M. senhousia*, play a major role in the processes of benthic nutrient

regeneration within the intertidal zone, rapidly and efficiently recycling the inorganic forms of N and P available to primary producers. This study highlighted the importance of considering the temporal scaling of intertidal macrozoobenthos distribution in the quantification of the processes of benthic nutrient regeneration in these highly variable systems.