**[Symposium on the Point of Contact between Zoological Science and Fisheries Science: From Basic to Application, and vice rersa]**
**Organized by Katsutoshi Arai**

# Primordial Germ Cells in Fish: From Basic to Application

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If *in vitro*-cultured cells could be converted into individual fish, they would have numerous applications in the field of genome biology and biotechnology. In spite of the benefits, however, no such system is presently available in fish. Because primordial germ cells (PGCs) have the potential to be converted into individual fish via maturation and fertilization processes, we chose them to be the starting material for our system. As the first step, we visualized live PGCs in rainbow trout (*Oncorhynchus mykiss*). Because the *vasa* transcript is restricted to the germ cell lineage, its regulatory regions are activated only in PGCs. Therefore, we produced transgenic strains carrying the green fluorescent protein (GFP) gene driven by the *vasa* gene regulatory regions. The resulting transgenic embryos showed green fluorescence specifically in PGCs. As the second step, the GFP-labeled PGCs were purified by flow cytometry. The genital ridges isolated from the transgenic embryos were dissociated by trypsin and sorted into GFP-positive and GFP-negative cells. The GFP-positive cells possessed morphological characteristics typical of PGCs. In addition, the *vasa* gene was expressed only in the GFP-positive cells, confirming that they were PGCs. To obtain functional gametes derived from isolated PGCs, a technique for converting PGCs into eggs and sperm is necessary. For this purpose, we developed a method for transplanting PGCs into developing embryos in order to incorporate them into the germ cell lineage of the recipient embryos. Approximately 10 PGCs isolated from newly hatched embryos were transplanted into the peritoneal cavity of recipient hatchlings. The transplanted PGCs actively migrated towards, and were finally incorporated into, the genital ridge. The PGCs that settled in the genital ridges of the recipient embryos proliferated, started meiosis, and differentiated into eggs and sperm in synchrony with the germ cells of allogenic recipient embryos. Further, the donor-derived gametes produced normal progenies through fertilization. These techniques, in combination with *in vitro* culture, genetic modification, and cryopreservation of PGCs, are expected to have numerous applications in the field of fish bioengineering.

# Sex-Specific Differences of Recombination and QTL Analysis in Fish

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The majority of species and strains reared globally for aquaculture are relatively unimproved for commercially important traits. The potential for genetic improvement in fish species compared with domestic livestock, is very high. Therefore, we are integrating molecular genetic technologies into aquaculture to help solve some of the major genetic problems. Our long-term goal is to use genetic markers to increase the efficiency of artificial selection in fish stock improvement. To do this, marker-assisted selection (MAS) has been proposed. MAS can be carried out with an understanding of the linkage relationships between quantitative trait loci (QTL) and markers. To identify QTL controlling traits of economic importance, a genetic linkage map is required, with variable markers distributed throughout the genome.

Recombination is a gene-shuffling process between two homologous chromosomes during meiosis. Recombination rates were used to estimate genetic distances for construction of the genetic maps. Sex-specific differences in recombination rates have been observed in some fish species. To analyze sex-specific differences of recombination rates on chromosome regions in Japanese Flounder *Paralichthys olivaceus*, we have constructed a genetic linkage map using microsatellite markers, and identified centromeric regions with a half-tetrad analysis using gynogenetic diploids.

The linkage map consists of 24 linkage groups. 23 centromeric regions have been identified. We found sex-specific differences in recombination rates on chromosomal regions in Japanese Flounder.
These results have practical applications for the strategy of breeding programs, experimental designs of QTL analysis, and MAS. As a first step of MAS, QTL associated with Lymphocystis disease resistance in Japanese Flounder have been identified using this linkage map. The genetic linkage map based on microsatellites could be useful for QTL analysis and MAS in aquaculture.

# Territoriality and Stocking Effectiveness in Ayu

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Tomo-duri, which depends upon the territorial defense of ayu, *Plecogolossus altivelis*, is popular among leisure anglers. The upstream migration of the species is frequently cut off by artificial constructions settled along a river line. For the shake of stock enhancement, releasing seedlings has been undertaken. The effectiveness of stocking is evaluated by recapture rate. In the early days of fish release, seedlings would go downstream without remaining in the fishery ground. Jumping behavior in ayu was found to be a predictive index for the stocking effectiveness. Fish in a school without defending territory are never angled using the tomo-duri, even though they stay in the fishery ground. Testosterone was proved to be a hormone that promotes aggressiveness in immature ayu. Stock release is of an economic act, which requires profits. Based on the cost and benefit, optimal territory size was examined corresponding to the amount of fish released. These behavioral and ecological studies contribute stock enhancement in ayu.

# From the Viewpoint of Systematics and Phylogenetics: Efficiency of the Molecular Tools

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Molecular techniques recently bring new frame of reference to fields of the systematics and phylogenetics. Here I will talk about the present condition with two examples. One of them is a study on the species identification and taxonomic reconsideration of marine lobsters in southern Japan. A simple PCR-RFLP procedure produced a convenience way to identify phyllosomae of *Panulirus* species, which have a long planktonic period in the early life history. This technique is very useful especially for the ecological study of the Japanese spiny lobster (*P. japonicus*: ise-ebi), a very important resource in marine fishery. I also refer to efficiency of the molecular tool to reconsider some taxonomic problems of this genus. The second example is about a new powerful phylogenetic tool called the "mitogenomics." Although molecular phylogenetic studies are now vigorously carried out for various organisms, most of them were based on highly restricted information and were not able to arrive a fruitful conclusion. In the mitogenomic conception, Drs. Miya and Nishida, who thought out and grew this methodology, recommend to use the whole mitochondrial DNA to sequence analysis, which can be conveniently collected with a series of PCR-based techniques. The study group of them has approached to serious phylogenetic problems among vertebrates and invertebrates (especially ichthyological lineages), and made way to resolute deeper (older) and more complex diversifications.