SOME IMPRESSIONS REGARDING GENETICS AND THE FISHERIES OF JAPAN

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INTRODUCTION

This report is based on a tour of Japanese laboratories concerned with aquaculture or subjects pertinent to it, 8-13 October 1972; and on attendance at the Second International Ocean Development Conference, Tokyo, Japan, 5-7 October 1972.

Tour included: Oyster Research Institute at Kesen-numa on Mohne Bay; Pearl Research Laboratory of the Fisheries Agency at Kashikojima, Mie-ken (Director, T. Hayashi); Ocean Research Institute of the University of Tokyo; Nikko Branch of Freshwater Fisheries Research Laboratory at Nikko on Lake Chuzenji (Director, K. Onodera); Tohoku University School of Fisheries; Tohoku Regional Fisheries Research Laboratory.

BACKGROUND INFORMATION ON GENETICS IN JAPANESE FISHERIES

There has been, in the past, no overall, concerted effort at applying genetics to the aquaculture endeavors of Japan which are without dispute the most extensive in the world. Now, however, in conjunction with a national research program on aquaculture it appears that some special committee on the application of genetics to aquaculture is being organized.

Since the advent of genetics at the turn of the century, Japan has had a number of highly trained geneticists, many of world renown, invariably prominent at International Congresses of Genetics. Breeding sciences and genetics are regarded important enough for school children to memorize the names of famous Japanese (Kihara) and American (Sears) wheat geneticists. Genetic applications to breeding have, however, been with few exceptions in agriculture not in fisheries, the same situation as in the United States.

Japan has had nothing at least in recent years equivalent to the symposium the Russians held in 1968, "Genetics, selection, and hybridization of fish," published in 1969 (Cherfas, 1969), and translated in 1972 through the auspices of the National Oceanic and Atmospheric Administration. (Nor has the United States.) It is relatively in recent years that there has been a reinstatement of Mendelian, "Western" genetics in Russia after years of propaganda—serving Lysenkoism (which proposed that most of "heredity" was due to environment). Perhaps the Russians, with all vested interests in traditional fields of genetics disbursed, are now more free to address the reestablished discipline of genetics to the new research challenges of the time of which aquaculture is certainly one.

In any comparison of the application of genetics to agriculture and to fisheries, irrespective of what nation's research programs are being discussed, it should be noted that even prehistoric man was working on the cultivation of land crops and domesticating animals. Modern man, on the other hand, is still for the most part content, correctly or incorrectly, with leaving marine organisms wild. University schools of fisheries are dedicated to the advancement of the science and art of fishing wild fish in the wild. No doubt, this is partly the result of man's lack of day-to-day familiarity with aquatic organisms, one measure of his failure to control the seas as he does land. Also, the advance of civilization necessitated the promotion and ultimate success of agriculture as wild crops and animals disappeared in its wake. In the same way pollution of the seas may assure the promotion of aquaculture over the span of the next few decades.

Whatever the background reasons only presently is genetics beginning to be applied to the fisheries in Japan, which rank among the most important industries of this island nation. By contrast triploid seedless watermelons are bred in Japan from sterile crosses made between special lines, and giant,

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highly polyploid apples are sold at railroad station food stands.

Until now there has been no pressing economic necessity in Japan to employ genetics to produce an organism better able to perform in an intensive aquaculture system. Aquaculture could be inefficient, depend on capture of young wild animals or gravid females for a kind of semifarming, be labor intensive, and still be worthwhile as a means of producing food and obtaining an income. This is no longer the case, and the change may be attributable, in part, to rising labor costs and to increasing demands for high quality seafood by a population with a higher living standard.

EXPANSION OF INTENSIVE AQUACULTURE, A PREREQUISITE FOR GENETIC APPLICATION

Certainly, the genetic study of wild fish populations has some profound implications for fishery management. Genetic knowledge of wild populations, in addition, makes for more judicious selection from such wild populations of the founding populations used to breed hatchery stocks with particular characteristics. The hatchery breeding of special, though still undomesticated, stocks for release to the wild represents still another area of genetics little considered in the fisheries. Such methods have been employed in forestry and grassland management, at least, in the United States. However, the deliberate severe alteration of the genotype of the wild organism, which aquaculturists need to transform the wild fish or invertebrate into a domesticated or cultivated form, requires, to begin with, intensive and fairly successful aquaculture of the organism. For that reason, it is important to note here the current status of aquaculture programs in Japan.

At the opening of the Oceanology Conference in Tokyo (Second International Ocean Development Conference, 1972), it was indicated in a special talk that one of five major projects recommended by a special Council for Ocean Development created in July 1969, as an advisory organ to the Prime Minister, was the development of fish culture with undersea experimental farms. This is, it was explained, because Japan's coastal fishing has declined owing to pollution and overfishing, because of the exclusion of Japan from some fisheries in their part of the world by neighboring nations, and because of an intensification of the world "fishing race." New fishing grounds will be developed and underutilized species exploited. Still of great importance, the Council believes, will be the switchover in the fish industry to farming-type endeavors. The necessity of breed improvement for maximum success in such intensive culture under artificial conditions seems to be well understood.

At the same conference Akio Honma from the Fishery Agency, Ministry of Agriculture and Forestry, gave a presentation on "Fish farming in Japan." Honma said that in the fishing field, farming, along with concomitant management and breeding, has been applied in the past only to a few freshwater species and to a few species of seaweeds and crustaceans. Over the years 1962-1966 the government's Fishery Agency established five Fish Farming Centers to encourage coastal fishing in the Seto Inland Sea. The entire Seto Inland Sea is being considered as one huge pond. Several important marine species are to be preserved and multiplied. Fry are bred at the centers and released to the prefectures for raising and releasing. In this program for the Seto Inland Sea a certain period of growth will be left to nature eliminating the necessity of using cheaper fish for the farming of more expensive fish.

Catching and releasing fry, as a source of seed, proved entirely inadequate. Seed breeding from artificially spawned animals is now the work of the project. It was found that, on the whole, breeding techniques were inadequate for the scope of the undertakings. (Here, genetic improvement in reproductive performance under artificial conditions could lead to better breeding techniques in the hatcheries as an intermediate step in the total program. Nothing was said of this.) The main work of the center is now accordingly the development of mass production schemes. As the need arises, fishing experiment station and university personnel work together at the center.

It has become the policy of the government to subsidize research work for seed production to some nongovernment public corporation where they believe management is better. Seed production is the area to which most money is now being directed.

Efforts are now being made to rear artificially produced fish to maturity in larger tanks so that they can subsequently be used to breed another generation. When this is accomplished, of course, genetically controlled breeding will be possible.

Systematic work in the release and control of seed has been limited since so much of the project had to be aimed at developing seed and better seed-producing techniques. It was found that, if young

prawns are protected for a certain period and released after adapting them to natural water, 5 to 10% of the released numbers can be harvested.

As a result of this program, prawn has been produced on a commercial scale. Also red sea bream, octopuses, globefish, scorpionfish, sea-eel, blue crab, and abalone. The prawn project is emerging from its experimental to a semibusiness stage.

The prawn commonly cultured in Japan is *Penaeus japonicus*. It is one of the most expensive of seafoods in Japan. Artificial culture techniques for this species have been developed over the last 10 yr by fisheries biologists and professional aquaculturists. Despite a number of technical and socioeconomic difficulties in the commercial production of prawn in Japan, it is evident to the Japanese that an increasing demand for prawn and shrimp will stimulate the development of its culture as an industry wherever in the world it is at all feasible. Culture should be particularly promoted in the untouched, widely ranging swamplands in the tropical zones. However, presently in Japan, poor culture techniques, defects in the present system of intensive culture, laborious rearing operations, rising land costs, and unfavorable rearing conditions owing to pollution make for unstable and costly production on prawn farms. A decrease in artificial production is anticipated. Culturists wonder whether prawn farms can even be maintained in Japan in competition with more productive enterprises. Yet, an overwhelming demand continues to exceed production even though at the Tokyo Central Fish Market a kilogram of live prawn has been sold for as much as 8 to 30 U.S. dollars (Second International Ocean Development Conference, 1972).

The prawn fishery, just as the shrimp fishery in the United States, might well be the fishery that would benefit monetarily most quickly from a well-integrated, sensibly supported genetics program.

Experimental studies on the hybridization of the freshwater shrimp are being carried on at the Tokyo Fisheries University (Uno and Fujita, 1972). Crosses of *Macrobrachium nipponense* ♀ × *M. formosense* ♂ and reciprocal crosses were reared from the larval stage to sexual maturity. Morphological comparisons of hybrids to each parent were made. These interspecies crosses were achieved by exposing the excised spermatophores to 50% seawater for 15 to 30 min, and then attaching them to the ventral thoracic part of the female of the other species just before she spawned. Such hybrids could have advantages over nonhybrids for intensive aquaculture under artificial conditions, or for "colonization" of a new area in the wild.

POLLUTION AND INTENSIVE AQUACULTURE WITH "SEMIDOMESTICATED" STRAINS

Possibly, even before breed improvement, with concomitant genetic adaptation to the cultivated state, aquaculture of some "semidomesticated" forms will become a success with the disappearance of their wild progenitors from Japan's coastal waters, or when these wild forms accumulate enough contaminants to become unfit for human consumption. Oyster seed production has been eliminated from around the City of Hiroshima with implications for the entire fishery. Yet, while pollution of wild stocks and their demise by marine contaminants may make some aspects of commercial hatchery production more attractive, too widespread pollution would render even artificial production in natural seawater virtually impractical. The Japanese regard the future success of artificial culture to be intimately tied to a resolution of pollution problems.

APPLIED AND BASIC GENETIC RESEARCH TO SUPPORT FISH BREEDING

For many years Japan has emphasized applied research in the fishing fields. A system of extension services for the fisheries is well established. In the United States the Sea Grant Program stresses applied research and extension services, but Japan is looking in the opposite direction. Unlike the Americans, the Japanese have made near maximum and efficient use of applied research. Now, Japanese scientists actually carrying out the work and their research administrators alike are of the opinion that, to increase further the productivity of their fisheries, more basic research is required. In the past, such research has been largely confined to universities where it has not been well funded and to special national institutes. Basic research, they believe, is necessary to develop reliable means of carrying regularly large numbers of fish from egg through sexual maturity, and this in the face of serious pollution. Such work should be deliberately associated with breed improvement programs, and the Japanese recognize this. Even if they did not, some useful genetic selection would occur inadvertently.

Food and disease appear to be presently regarded as more important constraints on aquaculture generally than lack of special breeds. However, the

Japanese regard water quality—pollution—as the current most serious point to consider in establishing aqua-farms. In considering this ordering of pollution, nutrition, then disease, then genetics, in terms of priority, it should be noted that:

1) Genetics will not succeed in accomplishing much of anything if the animal, to begin with, is not reasonably easy to culture;

2) but that genetics can in an early stage of aquaculture contribute to the development of types better able to utilize artificial foods, resist disease, be more vigorous, and develop good gametes;

3) so nutrition, disease, and genetics should not be regarded, either in the United States or Japan, as three separate research entities—they overlap.

STORAGE OF STOCKS AND COLLECTIONS FOR BREEDING PURPOSES

A directory prepared for distribution at the Twelfth International Congress of Genetics, Tokyo, 1968, lists important genetic stocks of plants and animals and microorganisms being maintained in national universities (Oshima, 1968). Tohoku University, Faculty of Agriculture, is named as holding oyster stocks, but no other fish stocks are listed. There are plans for establishing future "storage" centers to maintain genetic stocks. Fish are not mentioned in these proposals for storage of genetic stocks prepared by classic geneticists. "Stocks would be managed by an expert geneticist so that uncontrolled, random breeding" of the closed stocks would not result in genetic changes in the stocks making them unrepresentative of the genotypes of the wild populations or cultured lines from which they were sampled for preservation.

JAPANESE GENETICISTS

In this same directory, names of 944 Japanese geneticists are registered. Only four are cited as involved in research on fish: one was listed at the Nippon Institute of Scientific Research on Pearls, two as working on the genetics of sexuality in fish, and one on the genetics of invertebrate sex-determination. An exhibit, "Genetics in Asian countries," at this Twelfth International Congress of Genetics," featured research on the origin, differentiation, distribution, and breeding of a number of plants and animals especially associated with the life of Asian peoples; nothing was included on fish.

Japan was represented by one of six members of the FAO ad hoc Working Party on Genetic Selection and the Conservation of Genetic Resources of Fish, which met in Rome in 1971 (Food and Agriculture Organization, 1972). Their representative was K. Suzuki, Chief Fish Culture Section, Ueda Branch of Freshwater Fisheries Research Laboratory, Nagano Prefecture. The report published by this panel is an excellent statement. It is mostly, however, oriented towards freshwater fish. In this report the case for widespread utilization of genetics in the fisheries is put more strongly than presented here where emphasis is on genetics in the total perspective.

JAPAN'S NATIONAL INSTITUTE OF GENETICS

Japan has an excellent National Genetics Institute, which consists of 10 departments and was established in 1949 as the governmental institute for fundamental studies of genetics. One of its first directors was the now retired H. Kihara, world renowned wheat geneticist. In the November 1972 edition of Nature devoted to "Science in Japan" there is an article by Kihara, "Activities of the National Institute of Genetics."

One of the most active groups is the Department of Developmental Population Genetics. The work of this group is currently all theoretical and not at all concerned with fish. Training of this staff though could be well utilized in fishery research.

The silkworm is a much used organism for genetic research at this Institute. Genetic mutations interfering with the feeding patterns of the silkworm larvae are being studied. Such mutations no doubt also present themselves in larvae of marine invertebrates, as the oyster, which have delicate larval stages often difficult to culture. Studies on radiation and chemical mutagenesis are being actively pursued using the silkworm. Emphasis is on dose-rate effects. (This is a very important area. There is a necessity internationally to clarify effects or lack of effects at very low doses of radiation, something most difficult to carry out experimentally.) With embryonic death as a criterion, radio-sensitive and radio-resistant strains of silkworms have been isolated, differences being 6- to 9-fold. Using *Drosophila,* induced mutations affecting viability rather than simply inducing lethality have been found to occur 40 times as often as lethal mutations. This work in the field of mutagenesis might be directed toward establishing control standards for

marine contaminants as they affect the lethal gene load and reproductive cells of breeding fish populations. Recently, workers at this National Institute of Genetics have developed a very sensitive test system for the detection of chemical mutagens in polluted environments.

The Department of Cytogenetics is studying chromosome polymorphism in populations of the black rat around the world. With emphasis on the Japanese population, the aetiology of Down's syndrome in humans is being studied. Expertise is certainly available then for breeding-related cytogenetic work.

Microbial genetics is also being pursued. Training in such a field could be the basis for studying disease organisms of aquatic plants and animals.

In the Department of Applied Genetics, rice is most extensively investigated - aquatic, marine organisms not at all. A worldwide collection of rice species is maintained; there is a complete collection of wheat and its relative species.

OYSTERS OF JAPAN, SPECIFIC USE OF HYBRIDS AND HYBRID VIGOR

There is a very real separation of *Crassostrea gigas,* commonly called the Japanese oyster, into several distinct races (Imai and Sakai, 1961).

There may even be a variation in the chromosome number in some Japanese populations of *C. gigas*. This is at variance with all the recent reports of American workers (review, Longwell and Stiles, in press). However, all the *C. gigas* sampled in the United States for chromosome analysis derive from seed imported from Japan to the U.S. West Coast, and most of the spat exported from Japan has been taken from one particular region.

C. gigas is collected widely in Japan in the shallow waters on the coast from Hokkaido to Kyushu. Seed were, until recently, collected most actively in Hiroshima, where oyster culture is said to have begun 400 yr ago. The Miyagi district is next in production to Hiroshima. Main seed-producing beds in Japan are Hiroshima, Miyagi, Mie, and Kumamoto Prefectures. The Miyagi Prefecture is famous for their export of oyster seed. This is most likely where most seed imported to the U.S. West Coast has been originating. Hiroshima culturists seldom bought oyster seed and seldom exported their seed to other prefectures or countries.

There are 20 different species of oysters in Japan, all edible. Only three of these are of direct economic importance, *C. gigas, C. rivularis,* and *Ostrea nipponá*.

It is believed that *C. laperousi* and *C. ariakensis,* native Japanese oysters, reported some years ago by Kobayashi (1954) to have four more chromosomes than *C. gigas,* are really *C. rivularis*. The species *C. rivularis* is not cross-compatible with *C. gigas* (Imai and Sakai, 1961). Confusing here is the fact that American malacologists regard *C. laperousi* and *C. ariakensis* as merely forms of the variable *C. gigas*.

C. rivularis, imported with seed of *C. gigas,* has been planted in Puget Sound and, according to Galtsoff (1964), has established itself there.

Some of this taxonomic confusion in classifying oysters may stem from the existence already of several hybrid-type populations in the wild. Nothing was learned in Japan regarding the opinion of some U.S. workers that *C. angulata,* the Portuguese oyster, and *C. gigas* are the same species.

Lest an understanding of the nature of these species be regarded as esoteric, it should be pointed out that such much publicized benefits of genetics, as miracle rice, resulted from a combination of two species or varieties. The characteristics of each of these types of rice were clearly known and understood by the breeders who test-hybridized them initially along with many others which were not "miracles" at all.

Part of the vigor of *C. gigas* relative to the more sensitive commercial East Coast American oyster, *C. virginica,* may derive from a slow though steady rate of introgressive hybridization. That is, by the addition of other species genes to *C. gigas* on a small scale over a long period. It could also derive from larger scale hybridization in various oyster populations in the more distant past. Both of these probabilities can be checked experimentally. This is basic research, of course, but it would provide some information on how important hybridization programs are in developing vigorous hatchery stocks. Such would remove some of the present unavoidable guesswork as to the usefulness of wide species crosses.

Spat of *C. gigas* are now being shipped to Frânce from Japan for planting on oyster beds once populated by *C. angulata*. There is a chance of natural hybridization on the wild beds in France between imported *C. gigas* and the remains of the once plentiful *C. angulata* populations. These two species readily hybridize in the laboratory, the hybrids survive and are fertile (Imai and Sakai, 1961).

It would be hard to overemphasize the importance hybrid vigor will have in developing hatchery lines of oysters, lobsters, scallops, fish and other marine food animals, and algae. Hybrid vigor is so important because, unless artificial seawater is used, water pollution will always be a threat to the commercial success of a hatchery. Because a hatchery must carry its product through its most sensitive larval stages, in smaller numbers than in the wild, and in one spot as opposed to many in the wild, water pollution can have a more disastrous effect in a hatchery than in the field.

Widespread use of artificial seawater would necessitate the breeding of lines commercially productive in such a media. Drastic changes in the genotype would most probably be necessary. This is not a genetic improbability by any means, but aquaculturists do not give the idea much thought.

LABORATORY VISITS

Even though there has been no general, broad program of fish or aquacultural genetics in Japan, some fine genetic research has gone on in various fishery laboratories. This work has not yet had a great deal of impact on the general fisheries. It is related here along with comments and discussions regarding aquaculture-related genetics. The research cited is a sampling of the sort of work that will most likely be conducted in the future on a larger scale.

Oyster Research Institute at Kesen-numa on Mohne Bay—Oyster, Scallop, and Abalone

The Oyster Research Institute at Kesen-numa (chief researchers now H. Kan-no and T. Seki) developed, under the leadership of T. Imai, methods for the artificial rearing of the Japanese oyster, *C. gigas*. This Japanese work paralleled the prior work of Loosanoff and Davis (1963) of the Milford Biological Laboratory, now part of the National Marine Fisheries Service. From the Kesen-numa Laboratory also came a breeding study on *C. gigas*, which included the effects of inbreeding, hybridization between members of different geographic races, and interspecies crosses using the Japanese oyster as one of the species parents (Imai and Sakai, 1961). Additional aquaculture and breeding information is contained in a recent book edited by Imai et al., 1971, "Through culture in shallow seas (progress in shallow seas culture)." This book is now being translated from the Japanese through the auspices of the National Oceanic and Atmospheric Administration. At least for some considerable period of time adult, fertile hybrids of the cross *C. gigas* × *C. angulata* were maintained at the Kesen-numa Laboratory.

This Institute is now rearing the European oyster, *Ostrea edulis*, which does well in Japan as a hatchery species. These oysters are sold as spat to growers and marketed. *C. angulata* is also being reared. No genetic studies are now being conducted on the oyster.

The sea scallop, *Patinopecten*, is also being raised. The scallop fishery still depends on the capture of juveniles, not on hatchery rearing. However, at least in Mutsu Bay annual catches of *P. yessoensis* fluctuate widely. This is attributed to a natural instability of the reproduction of this species. Natural reproductive instability would make this scallop an excellent candidate for reproduction in the hatchery. Some of the scallops are known to be functional hermaphrodites. Use could so be made, for both experimental and commercial breeding work, of their rapid inbreeding potential by self-crossing.

Research at the Kesen-numa Laboratory is now concentrating on the abalone. The market for this marine gastropod is excellent, and there is an urgent need to mass produce the young. Unlike the situation for the oyster and scallop, mass collection of wild abalone is fairly difficult. Young abalone escape from the collectors, and in their natural habitat, they shelter themselves under boulders or rocks. To increase production it is, therefore, essential to produce the young artificially.

In Japan 10 species of abalone are found. Only four of these species constitute the staple food products—*Haliotis haliotis discus hannoi*, *H. discus*, *H. sieboldii*, and *H. gigantea*. Of the total catch *H. discus hannoi* supplies 58%.

At present, no genetic studies on abalone are being conducted at Kesen-numa. However, there is interest in possible genetic causes of the less than desired percent survival of the young larvae, in the chromosomes of the abalone, and in genetic resistance to disease. Disease is anticipated as a potential serious problem in the very intensive system under which the abalone will be reared. The intensive system includes a period of growth in the heated waters of a nearby power plant at Shiogama. An aboveground running water tank system contains the abalone at this time, and a hatchery with experimental facilities has been built at the power plant near the tank farm.

At the Iwate Prefectural Roes and Fries Distribution Center the gametogenesis of adult abalone was observed, as well as the normal development from fertilized egg to creeping stage larvae (Shibui, 1972).

Once the artificial production of abalone in Japan progressed to the commercial scale, interest developed in the potential of interspecies crosses. At Chikura Branch of Chiba Prefectural Fisheries such hybrids were made and studied. These were between *H. discus* and *H. gigantea;* between *H. discus* and *H. sieboldii;* and between *H. gigantea* and *H. sieboldii* (Oba, Toyama, and Kaneko, 1972). All were reared through metamorphosis in the laboratory. Hatching, fertilization abnormalities, and shell form and structure were compared to the parental characteristics. Such hybrids could have advantages over nonhybrids for intensive aquaculture under artificial conditions, or for colonization of a new area in nature.

Some hybridization work was also conducted at the Kesen-numa Institute (J. W. McBeth).[2]

Pearl Research Laboratory of the Fisheries Agency at Kashiko-jima, Mie-ken

Research is confined exclusively to study of those mollusks used to culture pearls. Work is ultimately directed at improving the quality of the cultured pearl. All the research appears to be basic. It seems that, because the science and art of pearl culture are well worked out, these researchers have more freedom to pursue fundamental work. There is a persistent near 40% mortality of the pearl oyster, *Pinctada fucata,* in the trays and racks used to hold them in the pearl farming. Mortality appears to be accepted as part of the business. The demand for pearls has dropped and there is now a government restriction on the amount of pearl farming to be conducted. There are no projects aimed at breeding disease resistant organisms, as those for the commercial *Crassostrea virginica* in the U.S. mid-Atlantic states.

Research is being carried out on the artificial culture of this pearl oyster's mantle (A. Machii, pers. comm.). Mitosis and cell proliferation have been obtained in vitro. American, European, and Japanese workers can all generally attest to the fact that success in invertebrate tissue culture has been most elusive. A persistent measure of culture success would find application in numerous fields of fishery research. It would apply to work on this epidemic MSX disease of the American oyster, which just about devastated the oyster industry of the U.S. mid-Atlantic states a few years ago (Sindermann and Rosenfield, 1967).

Some inbreeding and some hybridization studies are being conducted on the pearl oyster (K. Wada).[3] Also, some studies have been conducted on its chromosomes in spite of technical problems caused by the presence of so much yolky material in the eggs (K. Wada, see footnote 3). This latter work will probably be published soon. It seems that some research effort will be directed in the future towards study of biochemical polymorphisms in the wild stock.

Ocean Research Institute, University of Tokyo—Genetic Polymorphisms of Wild Populations

In the division, Biology of Fisheries Resources, studies are being conducted on enzyme variation in fish populations (Numachi, 1972a).

Shortage of genetic markers has been a handicap in the study of fish genetics. Now variation in different enzymes can be used as genetic markers by employing gel electrophoresis followed by histochemical staining methods. This method makes possible an examination of the structure of a population of fish in genetic terms. Identification of self-sustaining subpopulations of fish is a most basic problem in fisheries management.

Enzyme polymorphisms reflect a mutation in the structural gene of the enzyme concerned. A few years ago variants of enzymes were regarded as rare events. It is now known that such variants are very common in most enzymes in various organisms. An increasing number of electrophoretically distinguishable variants has been discovered in fish, as well as in other organisms. Marine mammals, such as whales, dolphins, and seals, all contain five lactate dehydrogenase forms. Their enzyme "patterns" are similar to those of terrestrial mammals, including man.

Numachi (1971, 1972b, c) has specifically reported in the literature on interspecific and intraspecific variation of enzymes in fish species and on analysis of the genetic control of such enzymes in fish—on

[2] Communication through Japanese staff member acting as Director at the time.

[3] Personal communication from a fellow staff member. K. Wada was away at a fisheries meeting at the time the laboratory was visited, so more precise information on this work was not obtained.

electrophoretic variants of catalase in black rockfish, *Sebastes inermis;* genetic polymorphisms of tetrazolium oxidase in black rockfish; genetic control and subunit composition of lactate dehydrogenase in *Pseudorasbora parva* (a cyprinoid fish); duplicate genetic loci and variant forms of malate dehydrogenase in chum salmon, *Oncorhynchus keta,* and rainbow trout, *Salmo gairdneri.*

Evidence has importantly been provided for the tetraploid condition of salmon. This means that salmon, like many higher plants, basically have four of each chromosome instead of the usual two each found in normal diploid organisms. Diploidy is the common state in the animal kingdom. Further examination of the evolutionary process of salmonids remains to be done. Already though recognition of the tetraploidy of salmonids should be of intrinsic importance in genetic studies. Because of the tetraploid nature of salmon, big salmon obtained by selection are not a good general example of what might be expected by similar programs of breeding using truly diploid species. Tetraploidy lends itself particularly well to gigantism.

Study of enzyme variation in species transplanted and farmed in the not-so-distant future will be an important means of stock identification, and of determining whether desired or unwanted hybridization has occurred in the wild between the local and transplant type. This will be no small job since the present most effective utilization of different genetic characters of various populations is still by transplantation of populations and species suitable for the environmental conditions of the areas intended for farming (Numachi, 1972a).

Another application of enzyme variants to genet improvement of aquatic organisms may be as mark ers linked with economic characeters. Economic characters are usually polygenic and so are impossible to study by simple Mendelian genetics as are distinct, qualitative, morphological characters.

Nikko Branch of Freshwater Fisheries Research Laboratory on Lake Chūzenji

Breed improvement is now regarued as one of the main problems in the freshwater fish culture in Japan. Some selective breeding is underway at the Nikko Branch of Freshwater Fisheries Research Laboratory (for preliminary work on *Salmo gairdneri* see Kato and Sakamoto, 1969; Kato, 1970). Extensive hybridization studies have been carried out and reported in the literature (Suzuki and Kato, 1966; Kato, 1967; Suzuki and Fukada, 1971, 1972). Most likely, such programs will be stepped up as breed improvement is stressed in the future.

It is believed that no fish populated Lake Chūzenji until 1873 when some local people volunteered to stock the lake with such fishes as the char and carp. Since that time, many species of salmonid fishes have been stocked frequently in the lake. The eggs of *Salvelinus fontinalis,* the brook trout, were imported in 1901 from the United States and fry released in the lake.

It is well known that distinct species and genera of fishes hybridize frequently in nature. A considerable number of natural hybrids has been reported, especially in the freshwater families—Cyprinidae and Castostomidae; also, in Percidae, Centrarchidae, Poeciliidae, etc; and importantly in Salmonidae. Natural hybrids seem to occur much less frequently in marine fishes. Information about natural crosses is most useful in determining the relationship of groups of fishes.

Natural hybrids between *S. pluvius,* the Japanese char, and *S. fontinalis* were collected from a brook flowing into Lake Chūzenji. They are estimated to constitute about 12% of the population at the area of the headspring.

Growth and survival rates of artificially produced hybrids between *S. pluvius* and *S. fontinalis* were studied from 6 mo after fertilization until they were 2 yr old and compared to the nonhybrid parents. This was to determine their relative suitabilities for pond culture. Best growth was in *S. fontinalis* followed by the hybrid, then by *S. pluvius.* Survival rate was highest in the hybrids. All the hybrids and *S. fontinalis* came to maturity during this time, but a third of *S. pluvius* was still immature. Hybrids were fertile and, in fact, produced a significantly larger number of eggs than did the nonhybrid parents.

With the hope of directly utilizing the information obtained to increase annual trout harvests, hybridization experiments with 62 combinations of Salmonidae have been going on since 1965. The purpose of this work was to find F_1 hybrids suitable for pond culture and for stocking lakes or rivers. Until a few years ago the freshwater fishes of Japan were abundant in nature. They were used mostly by rural people as a main protein source. Due to a reduction in their natural habitats, such fish are now less plentiful. At the same time the demand for them has gone up, making them a luxury food. Presumably it was believed that genetic and breeding work would have some bearing on this situation. It was further hoped

that some of these hybrid crosses would be sterile. Sterile hybrids might, because of their sterility, have a faster rate of growth or greater ultimate size than fertile nonhybrids.

Hybrid development did occur in all combinations. There were, however, wide differences in survival rates. Hybrids between 32 combinations survived until they reached the free-swimming stage. Nine of these showed survival rates similar or better than those of parental species. Those combinations that did survive are currently being studied at later growth stages.

Among hybrid characteristics already ascertained is a heterosis against disease. This is an important feature since prevention of epidemic disease is a serious problem in trout culture.

Even some intergeneric hybridization was achieved. These hybrids though presented special difficulties in culturing and rearing.

Tohoku University School of Fisheries and Tohoku Regional Fisheries Research Laboratory—Radiation Effects on Oyster; Seaweeds

The effects of ionizing radiation on *Crassostrea gigas* are being studied at Tohoku University (L. Maeda, pers. comm.). This work may be similar in nature to some done on *C. virginica* (Longwell and Stiles, 1972).

Recent advances in cultural techniques for the seaweeds, like *Porphyra* and *Undaria*, have brought substantial benefit to a considerable portion of fishermen engaged in seaweed production in Japan. (At the same time once important nori grounds about Tokyo have been irretrievably lost to pollution, and nori products have come to contain mercury and cadmium.) Little attention though has been given to the possibility of artificially crossing seaweed types to give a more productive, profitable strain. Since 1958, S. Suto has been making interspecies hybrids always using a local commercial species as one parent. This work has been done with a view of obtaining a new seaweed with good characteristics for culture (Suto, 1963; also see Suto, 1972). Fortunately, self-fertilization rates are low, facilitating artificial fertilization. However, much preliminary work had to be done before artificial fertilization could be achieved with certainty. Also, the relationship between *Porphyra* species had to be clarified.

Artificial crosses were attempted between five species of the *Porphyra* genus, including four local forms. Crosses occurred easily between any two species tested. Descendants from crosses of two dioecious parents and from two monoecious parents grew normally. Those from dioecious-monoecious combinations died in mass at a young stage, but left a few survivors. The three dioecious species studied appear to be very closely related. At least in hybrids of the dioecious species, there is normal reproduction with typical genetic segregation.

Recently, some lines started from an interspecies hybrid have yielded two and three succeeding generations. Some of the lines grow more vigorously than did their native parents.

This work demonstrates that new lavers, more suitable for commercial production than the present wild lavers, can be bred. Probably the F_1 interspecies hybrids will first have to be subjected to a program of artificial selection in the laboratory, or one of natural selection in the wild before being used commercially.

Introduction to Japan of foreign species of *Porphyra*, which may be disease resistant, for field trials and for use in hybrids with Japanese species, is regarded important. High quality species already being utilized are, unfortunately, not the most productive, and disease is a factor in lowering productivity.

There is a likelihood of some natural hybridization occurring on the wild beds between closely related species. The genetic and other implications of this are recognized.

Some cytogenetic studies on *P. yezoensis* have been done elsewhere at Nagasaki University Faculty of Fisheries (Migita, 1967). There are three chromosomes in vegetative and spermatogonial cells in the leaf thalli, and six chromosomes in fertilized carpogonia. The haploid chromosome number of this alga is then three. Meiosis takes place in the cell division of the conchospore at the time of spore formation. Such information is pertinent to the making of artificial crosses. Also for the artificial somatic replication of a single plant for multiplication prior to larger scale commercial production.

Aside from the great commercial value of seaweeds in Japan (their most important fishery), the Japanese probably have here one of the most interesting areas of the future field of marine genetics with most promise of great commercial application. This is because plants, in general, lend themselves so well to genetic study with subsequent genetic manipulation and because so many genetic and breeding techniques are so well worked out for plants.

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