Proceedings of the session on

FISH GENETICS - FUNDAMENTALS AND IMPLICATIONS TO FISH MANAGEMENT

(And other supporting materials to provide an information package for the design of a stock concept symposium/workshop)

Supported by the
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Great Lakes Fishery Commission

1451 Green Road
Ann Arbor, Michigan 48105
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FOREWORD

The 1976 Report of the Lake Ontario Committee (LOC) to the Great Lakes Fishery Commission (GLFC) included the following recommendation:

Genetic Integrity of Fish Stocks:

A. There is concern that current stocking practices in Lake Ontario will lead to genetic dilution and impairment of established self-reproducing stocks.

It is recommended that a policy statement based on the "Stock Concept" be developed by the GLFC and that the Scientific Advisory Committee be instructed to examine the problem, specify guidelines and references for the selection of naturally-reproducing stocks, and where necessary, recommend appropriate research studies. It is suggested this subject could well warrant a GLFC-sponsored symposium-workshop.

B.* The LOC requests the Commission contact each agency that has stocked fish in the Great Lakes and request they provide all available information on the origin and known characteristics of those stocked fish. The compiled information should be in report form for the LOC 1977 annual meeting.

In responding for the Commission at the Annual Meeting, June 1976, Chairman Loftus noted that similar recommendations had been submitted by the Upper Great Lakes Committees, acknowledged that the Commission is in full accord with the expressed concern, and stated that the Commission is considering initiation of a large-scale binational workshop or symposium to address the whole question of the stock concept and needs for research in fish genetics

*Part B of this recommendation is similar to a request from the Lake Michigan Lake Trout Technical Committee (LMLTTC) forwarded to the GLFC through the Lake Michigan Committee. Cataloging such information for lake trout has been an ongoing activity of the Commission for many years, but the requests of the LMLTTC and the LOC resulted in renewed efforts to keep the information timely and in more usable form.

and selection as applied to rehabilitation of Great Lakes fish stocks. The Commission then charged the Scientific Advisory Committee (SAC) to investigate the feasibility of such a workshop.

As of November 1977 the genetic origins of lake trout stocked in Lake Michigan have been summarized and mapped in draft form for use by the LMLTTC, similar summarization has been initiated for Lake Superior lake trout, and information gaps in our records for splake and lake trout plants in lakes Huron, Erie, and Ontario are being filled. Plans are to summarize this information for all Great Lakes lake trout and splake plants. Similar efforts will be expended for other salmonids as appropriate.

Reporting at the Commission's Interim Meeting, December 1976, the SAC proposed an exploratory session of invited papers supported by the GLFC and co-sponsored with the American Fisheries Society at the Annual Meeting of the International Association for Great Lakes Research, May 1977, with Dr. H. T. Booke, U.S. Fish and Wildlife Cooperative Fishery Research Unit, University of Wisconsin--Stevens Point, as Convenor. The Commission endorsed this proposal and appointed an ad hoc committee of Commissioner K. H. Loftus (Ontario MNR) and Alternate Commissioner J. Hemphill (USFWS) to work with Dr. Booke to organize a session on the fundamentals of genetics as applied to fish and their interaction with their environment.

This session was to be a prelude to the proposed binational workshop. The Commission agreed to support publication of the papers in the Journal of Great Lakes Research if the participants desired, but the main thrust of the effort was to supply background information and a resource base from which to consider further the design of the stock concept workshop.

The participants decided against formal publication, and agreed that the most practical way of dissemination of the materials was through informal distribution by the Commission. The invited presentations should not be judged harshly from an editorial viewpoint. They are author-edited talks for information purposes. Considering the purpose of this document--provision of a resource base--two other pieces have been added: excerpts from Kenneth H. Loftus' paper, "Science for Canada's Fisheries Rehabilitation Needs" (J. Fish. Res. Board Canada 33:1822-1857); and "Species Management" by Dwight A. Webster and William A. Flick, originally published by Trout Unlimited in their 1975 publication, "Proceedings of the Wild Trout Management Symposium". The excerpts from the Loftus paper are placed early in the document because they reflect the concern of the Commission in general terms.

The SAC will report their progress to the Commission at the Interim Meeting, December 1 and 2, 1977. At this time the SAC has asked A. H. Berst, Research Scientist, Fisheries Section, Fish and Wildlife Branch, Ontario Ministry of Natural Resources, to organize a meeting to discuss the proposed stock concept workshop and set in motion the preparatory stages. His correspondence soliciting ideas and suggestions follows.

The Commission is particularly grateful to authors Dr. Henry E. Booke, University of Wisconsin at Stevens Point; Dr. Peter Ihssen, Ontario Ministry of Natural Resources; Dr. Edward Massaro, University of New York at Buffalo; Dr. Fred Allendorf, University of Montana; and Dr. Raymond Simon, U.S. Fish and Wildlife Service for preparing their presentations on short notice; to Henry Booke for his efforts to organize the session; to the International Association for Great Lakes Research and their Program Chairman, Stan Bolzenga, for accommodating us; to authors Ken Loftus, Dwight Webster, and Bill Flick, JFRB Canada Editor J. C. Stevenson and Trout Unlimited for permission to include the reprinted material; to Jane Herbert for recording and transcribing the session; and to Trudy Woods, Becky Andress, and Pat Lindvay for secretarial services in putting the document together.

Carlos M. Fetterolf, Jr.

Executive Secretary
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Our file number .
Your file number .

1977 10 07

Mr. Carlos Fetterolf,
Executive Secretary
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Dear Carlos:

Please find enclosed, a copy of a self explanatory memorandum to scientists in the Fisheries Section of the Fish and Wildlife Research Branch. I would appreciate if you would forward copies of the memorandum to U.S. individuals and/or agencies whom you feel might contribute useful ideas and suggestions.

By copy of this letter, I am informing Dwight Webster and trust that he may see fit to send copies independently to U.S. people.

Thank you.

Yours very truly

A.H. Berst, Research Scientist
Fisheries Section
Fish and Wildlife Research Branch

AHB/mh
c.c. K.H. Loftus
A.H. Lawrie
J.D. Roseborough
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Ontario

Ministry of
Natural
Resources

1977 10 07

Our file number .

Your file number .

MEMORANDUM TO:

All Research Scientists
Fisheries Section
Fish and Wildlife Research Branch

SUBJECT: Proposed Conference on Stock Concept.

I have been asked by the Scientific Advisory Committee of the Great Lakes Fishery Commission to organize a meeting in the near future on the above subject. The purpose of the meeting will be to set in motion the preparatory stages for a conference to be held some time within the next couple of years.

I believe that the paper "Science for Canada's fisheries rehabilitation needs" by Ken Loftus (J. Fish. Res. Bd. Canada 33: 1822-1857) provides a basis of logic in support of such a conference. The stock concept is obviously a component of the current management strategy for west coast salmon fisheries. Many of us feel that it is also relevant to the management of freshwater fisheries.

Perhaps the objectives of such a conference would include (i) a review of evidence in support of the stock concept and its impact on marine fisheries, (ii) a review of documented evidence and presentation of new evidence of the stock concept in freshwater fisheries including salmonids, percids, coregonids,and centrarchids and (iii) a forecast of how the application of the stock concept in the strategy for management of freshwater fisheries would influence research and management programs.

I would greatly appreciate your views on the above subject, and would especially appreciate knowing of any significant references (other than those cited in Loftus' paper) and/or the names of any scientists either in research or management, who are directly or indirectly involved in this field.

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Since there is a commitment to hold the initial meeting and report to the Scientific Advisory Committee by 1977 12 01, I would appreciate your reply as soon as possible.

A.H. Berst, Research Scientist
Fisheries Section
Fish and Wildlife Research Branch

c.c. K.H. Loftus
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THE STOCK CONCEPT

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The following is excerpted from "Science for Canada's fisheries rehabilitation needs," K. H. Loftus, 1976, Journal of the Fisheries Research Board of Canada 33:1822-1857 and reproduced with the permission of the author and JFRB Canada Editor J. C. Stevenson.

The Stock Concept

The reality of discrete spawning populations has been recognized for several years as an important consideration in the management of west coast salmon. There has been an explicit attempt to harvest each recognized stock at the different levels appropriate to their continued productivity. In practice, however, this laudable and necessary objective has been difficult to achieve with more than general precision because of the mixing of stocks at sea, and because most of the harvesting gear does not discriminate between stocks. Only when the stocks are close inshore and near their "home" stream do they become adequately separated to allow an opportunity for fully selective harvesting. By the time this sort of separation occurs there is little, if any, opportunity for harvest by the traditional gear, and furthermore, the quality of the fish may have seriously deteriorated.

In spite of the difficulties, the objective has been partially achieved and, in the process, much additional information on the movements and distribution of stocks in the ocean has been generated.

Ricker (1972) presented a comprehensive review of the knowledge of discrete stocks of west coast salmon and trout. Impressive indeed is the listing and description of discrete stocks within species, and particularly within single river systems. The question whether genetic and/or environmental influences are involved in the emergence of discrete stocks is vital to management and to rehabilitation. The epilogue of Ricker's exhaustive work is, therefore, quoted here.

> "In almost all cases where both genetic and environmental influences affecting natural stock differences among Pacific salmon and steelheads have been searched for adequately, both have been found; though sometimes one, sometimes the other, is relatively weak, or is infrequently expressed.
> "Since season of return to the river is strongly under genetic control, and ability to 'home' is apparently under genetic control, a corollary is that most or all of the identified stocks of these fish differ genetically to some extent.
> "Such conclusions sometimes encounter criticism on the grounds that they are too complicated. Nature (the argument runs) should not be made more complex than it really is; species can exist in one and the same river system, a dozen or so even within a single lake — particularly when it is known that there is some interchange between them.
> "Certainly when two or more hypotheses are equally possible, it is customary to prefer the simplest one. But how are we to know which one is simplest? What is the test of simplicity? With respect to any recurring difference between two salmon stocks, we have three alternatives:
> "1. The difference is completely environmentally determined.
> "2. The difference is completely hereditarily determined.
> "3. The difference is determined partly by heredity, partly by environment.
> "Superficially at least, hypothesis (1) and hypothesis (2) both seem simpler than hypothesis (3); perhaps they really are so.
> "If we were to reject hypothesis (3) because it is too complicated, then which of (1) and (2) is the simpler? Some writers have seemed to believe that (1) is simpler than (2), or at least 'more conservative' than (2), apparently because it implies a more homogeneous genetic constitution among the various populations of a species. For example, if it has been demonstrated that the number of scales on fish of a certain species is affected by environmental temperature during development, they will then decide that 'there is no need to postulate' any genetic basis for an observed difference in scale number between two local stocks of that species.
> "But what if we were to apply the same procedure in reverse? Suppose we have demonstrated a hereditarily-determined difference in scale count between two stocks, as Dr. Neave did for *Salmo gairdneri*; should we then decide that there is no need to postulate any effect of the environment on scale number in that species? That obviously isn't logical; but then the converse proposition cannot be logical either.

> "My strong opinion is that we should avoid any appeal to simplicity or conservatism in such questions. Time and again it has been discovered that nature is more complex than anyone dreamed possible. Hence we should stick to whatever evidence is available, however sketchy it may be.
> "In the matter at hand, the evidence available is now quite considerable. It indicates that most of the studied differences between local stocks can and usually do have both a genetic and an environmental basis. Be it simple or complex, this should now be our normal expectation in respect to any as-yet-unstudied difference between stocks of salmon or trout. Only direct investigation can show which type of influence predominates in a particular situation."

Larkin (1972) provides a view of the management implications of the stock concept. The difficulties in achieving selective harvest of discrete stocks are emphasized.

Available evidence strongly suggests that full utilization of complex river systems by salmon, giving maximum production of young salmon from the system, and maximum returns to it, is achieved only when a complex of stocks is present. Salmon rehabilitation projects face the task of optimizing reproduction on a stock-by-stock basis and of learning how to recognize or to create stocks comparable to those which have already been lost. Care may be necessary to avoid overloading parts of the river system to the detriment of other stocks and projects will require careful balance between stocks and careful evaluation.

The significance of discrete spawning stocks is recognized now as well in Atlantic salmon (Saunders 1967; Moller 1970; A. W. H. Needler, personal communication). Probably because of the relatively small size of the total resource and because it is comprised of small runs scattered over so much coastline, less detail is available to describe these stocks. Less is known too of the movements of separate stocks at sea, witness the recent surprise off Greenland which precipitated harvest restrictions. It seems probable that many stocks have already been lost and that rehabilitation will be the more difficult for that reason.

In the freshwater area little attention has been given to the significance of discrete spawning stocks. I am now of the opinion that our inattention in this respect may have rendered some of our management ineffective, and in some cases where routine plantings have been involved, even counterproductive. A few examples may serve to establish the possibility of parallels in some freshwater species to the stock concept described for Pacific salmon.

Martin (1957; 1960) described very precise homing behavior in lake trout at spawning time in 1480-acre Louisa Lake where the spawning grounds were separated by only a few yards. His confirming evidence of discrete spawning times and within-lake locations for lake trout in a number of Algonquin Park lakes (personal communication) is extensive. Loftus (1958) described populations of river-spawning lake trout in Lake Superior. The distribution of these trout stocks overlapped in the open lake but they separated year after year and moved to the "home" (presumably natal) rivers. He recorded some additional spawning times and places for Lake Superior lake trout as reported by fishermen and hatchery personnel. Eschmeyer (1955) in discussing lake trout reproduction in southern Lake Superior suggested that lake trout returned to the same spawning shoals year after year. J. B. Smith (1968) was able to map more than 150 separate former lake trout spawning grounds on the basis of recollections by commercial fishermen. Lawrie and Rahrer (1972) and Regier and Loftus (1972) considered that the system of accumulating catch statistics in Lake Superior and in other lakes, a system which did not reflect stock-by-stock distribution, served to mask the reality of the fishing-up process to the extent that some stocks may have been decimated long before total harvest figures showed a decline. In many instances, the trout rehabilitation program in the Great Lakes implicitly, but not explicitly, recognizes the stock concept. Successful rehabilitation may be delayed by the lack of appropriate brood stocks. Olver and Lewis (1971) were able to find only a few examples of planted lake trout which successfully reproduce themselves.

Whitefish in Lake Huron are comprised of apparently discrete stocks. Two are recognized by J. J. Collins (personal communication) in the North Channel; two in South Bay (Budd 1957); two in Georgian Bay (Cucin and Regier 1965), and two in Lake Huron proper (Budd and Cucin 1962; Spangler 1974). These stocks may be the few remaining from a larger original number of stocks. Christie (1972) recognized two stocks of whitefish in Lake Ontario, one of which now appears to be extinct.

Walleye literature also suggests strong parallels to the stock concept. Lake Erie contains two apparently discrete stocks, one which reproduced in the Western Basin and which has suffered a severe decline in recent years; another which reproduces in the eastern basin and which has remained relatively stable. Ferguson and Derksen (1971) described mixing of a Lake St. Clair walleye population with those of southern Lake Huron and of western Lake Erie. In the Bay of Quinte, Lake Ontario, Payne (1963) studied a population, then flourishing, now virtually extinct. Within the same bay certain river spawning populations persist at low levels (W. J. Christie, personal communication). In Lake Huron a

number of discrete river spawning walleye stocks are recognized in the Moon, Shawanaga, French, Pickerel, Mississauga, and Echo rivers. Others no doubt occur. The same story is probably verifiable for stocks in Lake Superior's Goulais, Batchawana, and Michipicoten rivers, and Ryder (1968) recorded the demise of one such population near Red Rock in Nipigon Bay. The work of Uthe and Ryder (1970) presents further evidence that the stock concept may be valid for walleyes.

Rainbow trout, a species introduced to the Great Lakes several decades ago have now become naturalized and appear to exhibit "stock" characteristics similar to those described for steelhead on the west coast.

To date the significance of discrete spawning stocks, the stock concept of west coast salmon, appears to have failed to attract the explicit attention it merits for management and rehabilitation throughout the freshwater area.

It is recognized that many stocks are seriously depressed and that some have already been lost. There are a number of causes for these losses which need to be more widely recognized, better understood, and guarded against. They include (1) direct excessive exploitation, (2) incidental catch by gear directed at adjacent stocks or species which are in good supply, (3) genetic drift imposed by the selectivity of gear fishing for a particular size. This may tend to remove early maturing and/or fast growing individuals from the stock, (4) hatchery plantings from unselected stock origins may survive to maturity and mix with native stock during spawning thereby diluting the gene pool of a well-adapted stock, (5) environmental change may force desegregation at spawning of formerly discrete but closely related stocks causing interbreeding. No doubt a number of other mechanisms could contribute to loss of stocks essential to the full use of an ecosystem.

Over several thousands of generations, most species appear to have evolved discrete stocks adapted to similar yet discrete and specific habitats. Fisheries science is not yet able to recognize either the habitat differences or the specific characteristics which differentiate stocks. Man's attempts to date to move stocks from one place to another have been mostly unsuccessful at least in terms of achieving natural reproduction by the transplanted stock. Given that a naturally evolved complex of stocks is apparently essential to full use of the productive capacity of a river system, and perhaps of lakes, it follows that it is important to learn to recognize and to preserve representative stocks. It is also important to learn how to reestablish stocks which have been decimated or lost, and to achieve a natural balance among stocks. There is a strong implication that rehabilitation requires the culture and planting of numerous small lots of the appropriate stocks until natural reproduction is reestablished. Such planting requirements suggest a need for small, versatile, and mobile hatchery facilities rather than the major "efficient" fish factory type of facility currently in favor.

It may be necessary to establish a national gene pool which would provide for the preservation of representative, particularly valued or endangered stocks.

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FISH GENETICS AND SELECTIVE BREEDING

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The following is excerpted from "Science for Canada's fisheries rehabilitation needs," K. H. Loftus, 1976, Journal of the Fisheries Research Board of Canada 33: 1822-1857 and reproduced with the permission of the author and JFRB Canada Editor, J. C. Stevenson.

Fish genetics and selective breeding — A number of fish stocks on the west coast, east coast, and in the freshwater area are depressed to critical levels or are extinct. In the Great Lakes where multiple stresses have been evident, the discrete stocks lost may number in the dozens and involve species such as lake trout, whitefish and other coregonids, and walleye. In all areas, it seems prudent to accept as fact the real possibility that we have lost many more stocks than can readily be identified now. Most of these stocks have been lost as a result of man's intervention, direct and/or indirect: fishing, industrializing, dam and/or canal building, trying to reduce the nuisance from black flies, mosquitoes, or budworms, etc. We would be naive to assume that, having recognized the importance of discrete stocks, we will now be able to organize the activities of competitive water users in such a way as to avoid the loss of additional stocks.

Former high abundance levels were supported by communities of species, and by within-species stock complexes. Rehabilitation programs, therefore, are directed toward the rebuilding of these species and stock complexes to levels at which they are again able to reproduce effectively. It is intended that management will be such as to allow them to maintain themselves through natural reproduction. The implication here is that rehabilitation of the Fraser or Saint John River ecosystem, and of lakes Superior or Ontario requires the reestablishment of naturally spawning populations of each of the species and stocks which formerly made up the resource base in those waters. It is recognized that habitat degradation by virtue of new species invasions, and by water quality changes may have eliminated some of the precise habitats formerly occupied by some species or stocks, and that, therefore, we may be unable to fully regain the former levels of abundance. Aggressive habitat management programs such as those undertaken by the Great Lakes Fishery Commission and the International Joint Commission in the Great Lakes are needed to make some of those habitats available again, while others must be considered lost. Having accepted that we are unlikely to fully regain former abundance levels in our rehabilitation programs, there are still major improvements attainable.

In many instances where stocks persist, though at precariously low levels and where their habitats remain healthy, the science necessary for their rehabilitation appears to be available. In cases where stocks are at extremely low levels, it may be advisable, or necessary, to use sex products from males only for cloning with females of a closely related stock, as suggested by Calaprice (1969) and by Ihssen (1976) to obtain adequate numbers of fish containing at least some of the genes necessary to effective rehabilitation. Further careful testing of this technique would be useful.

In many cases where stocks have become extinct, and where they may go to extinction despite our best efforts, and/or in those cases where the habitats have been modified in some irreversible way, the science required for rehabilitation is not yet available. A major new initiative of basic research in fish genetics and selective breeding is necessary.

The necessary new research initiative may not be difficult to mount because scattered existing initiatives have already shown promise of success. In Ontario, for example, beginning in the late 1950s under the guidance of F. E. J. Fry, an attempt has been made to create a new trout stock by hybridization and selective breeding, capable of occupying the vacant lake trout niche in Lake Huron. Hybrids were made between lake trout and brook trout, and the selection techniques to concentrate the deep-swimming lake trout character and the early maturing character of brook trout (Tait 1970; Straight 1969) were developed and applied through four or five generations. The project has been successful in the laboratory and large-scale field testing of the selected hybrid is currently under way in Lake Huron. In this research an attempt was made to build a new trout to occupy the former lake trout habitat which has been modified by the sea lamprey invasion and the several fish community consequences of that event. It is suggested that similar research projects may be necessary for other trout, salmon, whitefish, and walleye populations where original stocks have vanished and where water management cannot be expected to

restore habitats of the quality required by the original stocks.

More recently, work in selective breeding and hybridization has been initiated with salmon at St. Andrews on the east coast, Nanaimo on the west coast, and with rainbow trout at Winnipeg. Some of these studies reflect the anticipated need for selection of special characters in aquaculture programs rather than rehabilitation programs.

Whatever the specific objectives of the several beginnings that have been made, all have been contributing to a better definition of the basic questions that must be addressed by research. Some of these follow:

1. What are the mechanisms or patterns of inheritance for discrete characteristics? It will be necessary to select for certain characters (e.g. deep-swimming ability, or tolerance for low oxygen levels) and at the same time avoid the incidental selection of an unwanted character (e.g. stream spawning).
2. What are the specific parameters in a changed environment which have become critical to survival of a stock?
3. Having identified the parameters in (2), what are the selection techniques appropriate to adapting the new stock to the changed environment?
4. How are closely related stocks recognized? By time of spawning, spawning behavior, direction of fry movement after emergence, etc? It was mentioned earlier that some stocks are thought to have been depressed through genetic dilution from indiscriminant plantings of other stocks of the same species. Still the prospect of being able to select from elsewhere a stock appropriate to the vacated niche of an extinct stock is intriguing. Further, if sex products from "closely-related" stocks can be helpful in rehabilitating a critically low, or lost stock, how can the appropriate stock be identified?
5. Is the approach suggested by Calaprice (1969) and by Ihssen (1976), that of maximizing heterozygosity, the best direction to take in rehabilitating extinct stocks? How much variety of parental material is required?

The above are some of the questions for which answers will be needed in rehabilitation programs. It is recommended that a major research initiative in basic fish genetics and selective breeding be undertaken. Genetics, physiology, and behavior are three of the disciplines which need to be part of such an initiative.

While applauding the forward-looking research in genetics and selective breeding that has already been initiated in at least four locations in Canada, it appears now to be appropriate to examine these in detail to determine whether some consolidation or further coordination might be appropriate to more rapid progress on the basic questions before us.

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HISTORY OF FISH GENETICS AND CYTOGENETICS: ALPHA, BETA, GAMMA, AND RESOLUTION

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I wish to thank the Great Lakes Fishery Commission and the American Fishery Society for sponsorship. I'm Henry Booke, Assistant Unit Leader, Wisconsin Cooperative Fishery Research Unit. I am supposed to give a talk on the history of fish genetics and fish cytogenetics. Why did I divide the topic? Primarily because of prejudice. I have an interest in cytogenetics. As I did more thinking about the subject, I realized that there's more to it than genetics and cytogenetics. There are many other topics that should be covered. In a sense my title is a bit of a misnomer. So I will be covering fish culture, selective breeding, physiological genetics, cytogenetics, population genetics.

What is genetics? Essentially the science of heredity and variation. Cytogenetics can be defined as the study of behavior of chromosomes during mitosis and meiosis. A person working in that area is interested in the morphology of the chromosomes and the resulting descriptions of the chromosomes and this is known as the karyotype. This person is working to define the specific morphology of each chromosome and then obtain the diploid number. And then he follows his interest by seeing what happens to the chromosomes during mitotic and meiotic processes.

What I am going to do now is simply treat each area with a bit of history with the idea of getting some thought of where we stand now, and perhaps pose a few questions in terms of where we should go in the future. Then each of our speakers will treat things a bit more in detail, and then we hope that you will question us and stimulate good discussion.

1. History of Fish Culture

If anyone at all is worried about how animals change from generation to generation or how we can maintain animals, we then can start talking about fish genetics developing 2500 years ago in terms of a fish cultural activity. The earliest recordings of this activity appear to be around 475 BC (The Classic of Fish Culture by Fan Lai). This was in China. What they did was use wooden sticks, or faggots, and simply place these in ponds. The spawn would be collected around the sticks and was transferred to various other ponds and maintained. Carp, goldfish, and medaka were so maintained. I found records of these activities from 475 BC and 1243 AD (Kwei Sin

Chak Shik by Chow Mit). Later on, in 1639, there was a book published called, "The Complete Book of Agriculture," again Chinese by Heu. Most fish cultural activities that were developed by the Chinese were done because they were worrying about whether they were going to have an adequate food supply on a steady basis. It appears that independently the Romans also developed a series of fish cultural activities. Primarily cutting canals to the sea and then fencing off the canals after the fish came in to hold them in these areas as a resource or as a food reserve.

There seems to be no great fish culture activity for almost 500 years. From the fifth century through the 5th, 6th, 7th centuries you see some activity, and then, around the 13th century, in France, you have a record of a monk, Dom Pinchon, involved in fish culture. Apparently there was quite a bit of fish culture activity going on in the European monasteries. Pinchon mixed ova and sperm. He was able to artificially impregnate and maintain eggs in wooden boxes and move them about according to his needs. Further fish culture activity in Europe beyond monastery walls was not done until 1763. At that time in Germany, Stephan Ludwig Jacobi rediscovered the activities that went on during Pinchon's lifetime. In France, 100 years later (1863), J. Remy and A. Gehin started to get into fish culture activities. All these activities seemed to be motivated by food needs.

Now I'd like to move on to North America and there you see notes in the literature with the indication of the terrible condition of the rivers. This is in the late 17th century. In the 19th century, the first fish hatchery in North America was established in Mumford, New York and the name Seth Green appears in the literature as the manager of this fish hatchery. Hatcheries were thought to be needed To restore our fish depleted waters. By 1857 there is a manuscript by Thaddeus Garlick on artificial propagation of fish. In 1868 Thaddeus Morris published the book, "American Fish Culture." By 1898, the U.S. Bureau of Fish and its first manual on fish culture. What's interesting about this manual? On page 181 of this manual it tells of the mixing of whitefish eggs (Coregonus clupeaformis) times lake herring (C. artedii) sperm. If Great Lakes hatchery workers ran out of sperm for the whitefish, they used lake herring sperm. And you wonder why you may have problems today in the Great Lakes.

The next accomplishment, in terms of a fish culture manual, comes during the 1950's. H. S. Davis's book on the culture and diseases of game fishes. So briefly, this is our history of interest in fish culture. What, as far as the 20th century goes, do we have in North America for fish culture activities today, between Canada and the United States? There are over 100 federal hatcheries and over 500 state and provencial hatcheries. What is my sublime deduction? We know how to grow fish. No question about that. We are going to leave fish culture and go into another branch of genetics, selective breeding.

II. Selective Breeding

I'll define selective breeding in the qualitative sense first and later in the quantitative sense. In other words, we look at particular fishes and we decide on an empirical basis we like the color or we like this particular size or we like this growth rate and we select for them on an empirical basis. There is no statistical basis to it. We first see this qualitative approach in 1919 in Hackettstown, New Jersey. Charles Hayford was responsible and he published his first paper on the subject in 1930. He worked with the first North American pioneer in this field, Dr. George Embody of Cornell University. They were able to breed and select brook trout for bacterial disease resistance, increased growth, size of fingerling, and increased number of eggs. Later in the 1930's, Dr. Loren Donaldson started his work. For the first time that I've ever seen noted, George Embody was given credit for influencing Donaldson. This was done in a recent book some of you may want to look at and is called "Fish of Rare Breeding" by Neil Hines (Smithsonian Inst. Press). This book is a description of Donaldson's fish selective breeding work for the better part of his lifetime. In 1931, H. S. Davis was breeding brook trout for a specific spawning regularity or spawning date. Louis Wolf, in New York during the 1930's was detecting strain differences in brook trout in terms of specific disease resistance. This seems to be the basic highlight history of North American selective breeding programs.

The quantitative approach, or the statistical approach, to measure the effects of genetic and environmental contributions to the development of a particular trait is called, in genetics, simply heritability. Where is this being done to any extent? I would say in the last 5-7 years there has been a definite sign of activity in the United States, in Washington, Wyoming (U.S.F.W.S. Fish Genetics Laboratory), California, Canada, perhaps at St. Andrews (for Atlantic salmon), Israel (carp and Tilapia), Norway (for some of the salmonids), and England (flatfish). More on the subject of selective breeding will be dealt with in terms of a quantitative approach by Ray Simon.

Now, I want to slip back a little bit in time to talk about a book that was published in 1926. This book was called "The Biology of Fishes" and was written by H. Kyle. It is one of those traditional books that shows up perhaps every generation and it sort of summarizes the field. In fact it is still available and TFH publishes it. In 1926, what sort of appreciation did Kyle have of genetics as applied to fish? You might think that his impressions could be the general beliefs held by fishery workers at that time. He said what was done in genetics in 1926 did not fit Mendelian Laws of Heredity. In other words, the expected ratios that you would expect to appear as far as experimental results are concerned did not. But this man did not have appreciation of what was going on. All of you are probably aware of the name, Johannes Schmidt. This was the man that worked with the eel; discovered their spawning

grounds in the Sargasso Sea. Schmidt, in his earlier years, worked on fish genetics and specifically with the guppy which at that time was called Lebistes reticulatus. He showed that dorsal fin spot color patterns had a father-to-son inheritance and these were male sex-linked characters and also limited as far as sexual expression to the males. This was done in 1919. Kyle apparently didn't appreciate this work. He felt that there was nothing in the field of genetics to apply to fish work. Winge, another worker, confirmed the color pattern of inheritance that Schmidt had worked out for the guppy. If you look at the Japanese literature, there are the names Ishikawa (1913), Toyama (1916), and Ishihari (1917). All of them did work that definitely showed a Mendelian pattern of inheritance for color in the medaka. In Germany, at K. Kosswig's laboratory, there was work on means of determining sex in fishes. In the United States, here on this campus, in the late 1930's, some work was done on parthenogenic fishes or fishes that reproduce in that manner and at that time the genus name was Molliensa, now it is Poecilia. This is the famous molly. This work has been continued by R. Miller, here on this campus, and by R. Jack Schultz at the University of Connecticut, Storrs. In Sweden, a highlight publication on chromosomes studies on salmonids was published in 1945. This was G. Svardson's work. It would appear that Kyle was too hasty in his review work and opinion.

III. Physiological Genetics

Last night the group that will be appearing today on this panel had a little bit of an argument and they said that essentially what I will deal with in physiological genetics is not what they want to call physiological genetics. They said I'm talking about morphological genetics. Well, in terms of the development of this field and the type of information known with its specific level of resolution or the amount of detail we were aware of in the 20's and 30's, fish physiological genetics dealt with the study of genes in the development of the organism. We didn't have Watson-Crick models and weren't dealing with specific molecules. We were dealing with characters on a much higher level.

The early workers were worried about things like sex determination and what sort of mechanisms existed for it in fish. Well, fish have done everything. There are XX-XY, and WY-YY mechanisms. In other words, where the female is the XX or WY form. In fact, in one fish species there is the reverse. An XX, where the female is XX, and also where the female is WY. Why do workers use these different letters? It's by tradition. If the female is the homozygote, it's always XX. If she is a heterozygote, workers use a WY, in terms of the sex chromosomes. Not all fish have, or at least as far as we know, have a distinct sex chromosome. We can't seem to detect it morphologically as a sex chromosome. There is some indication that in many fishes there is some influence in sex determination by autosomal chromosomes.

In the field of physiological genetics, it was demonstrated in the 1920's, 1930's, 1940's, that specific genes influenced the development of certain embryological stages. Pigmentation, for example, was demonstrated to be controlled by gene action. Who did much of this work? Mainly Myron Gordon, at the New York Zoological Society Aquarium, that is now in Brooklyn, N.Y. where K. Kallman carries on this work.

Meristic traits have been demonstrated to be under influence of genetics or gene action. It has been shown that not all hybrids were necessarily intermediate in meristic characters. In other words, if you cross species A times species B you were supposed to expect that all hybrids were to be intermediate in character. The character intermediacy idea was strongly influenced by Carl Hubbs in a 1955 publication. As far as species isolating mechanisms involving reproductive behavior are concerned, this type of study is starting to come into the literature primarily with work on cyprinodontid fishes. In 1957, the state of art in this field of fish physiological genetics could be summed up in a statement by Gordon. Gordon indicated that, "more highly inbred lines of fish should be used for experimental purposes." Why? Because you could get more valid and verifiable results because you would decrease genetic variability which is probably being expressed in your experimental results.

IV. Cytogenetics

In the field of fish cytogenetics we are at a very beginning level or descriptive morphology stage for chromosomes. The first activity that I can find recorded was in 1916. The diploid numbers of the cyprinodontid fishes that were recorded were incorrect, and they were primarily incorrect for many other fish until the mid-1940's. Where do we get the prowess to say that they are incorrect? Fish chromosomes are very small, very hard to work with. Even with the best of methods, we are dealing with materials that are probably less than 10 microns in size. It wasn't until methods, spin-off methods, that were developed in the human cytogenetics field, were available that accuracy in fish chromosome studies increased. These investigators developed methods for blocking the mitotic process in cultured cells with a chemical called colchicine. Chromosome counts of blocked metaphase chromosomes could then be made. Once the methods came on the scene, they permitted all sorts of studies, not only in fish (genetics), but other mammalian and vertebrate forms were able to be studied. However, you have got to have a laboratory set up to culture these cells in order to do this particular work. From 1945 on, I would say we were getting good valid results because, even though these chromosomes are small, you could still get them at a stage where you could define their specific morphology, and then count them. Now what can you do with this sort of information?

Initially, there was a great state of activity in terms of species-specificity. You would think that each specific animal would

have a specific chromosome number. Well for a while that was so. But it happens to be that fish tended to be a bit more conservative than we thought and I'm basing this conclusion on approximately 500 species of fish that have been examined for chromosome number. Most fish tend to have diploid chromosome numbers around 48-50. However, in the salmonids, they are not conservative or boring. They vary from around 50 to 130. When you examine the cypriniform fishes, you find a variation too. A similar diploid number variation as in salmonids. From around 20 up to 104 (for carp), 100 for goldfish depending on whose results you are interpreting. Most of the perciform fishes have a tendency to center their diploid number around 50. However, in certain cases you find that you can get highly reasonable karyotype data which can be used with other characters to determine species-specificity.

We have a case for using the karyotype or the formal number type of chromosomes for a species as a systematic character. There is some indication, however, that you may find chromosomal polymorphism. What is that? In terms of one species, there might be a varibility in chromosome number; perhaps by river system. There is some evidence for this in Atlantic salmon. You might find a chimaeric condition. Meaning, in the same species you can find a mixture of chromosome numbers depending on what organ is examined. This has been demonstrated in the rainbow trout on the West coast.

Where have we gone with fish chromosome work? We are at the very beginning level. What I will call alpha level or the descriptive level. We are just looking at fish and describing the chromosomes, and we have found that, in general, the most primitive fishes, the earliest fishes, tend to have a high chromosome number. And in the more recent fishes, you have a reduction in chromosome number. The chromosome number, in time, is reduced by a process called fusion or where two chromosomes become joined. The reverse of this can happen but it hasn't been shown to any great extent in fish. This latter process is called fission or the splitting apart of chromosomes. In the Great Lakes, what is the status of karyotype information? All the salmonids, all the esocids, lampreys, and a moderate number of the minnows, suckers, catfishes, have been karyotyped. So we have a good level of information about chromosome morphology and diploid number.

V. Population Genetics

About 1938, the biochemical method called moving-boundary electrophoresis was developed in Europe by Arne Tselius. This electrophoresis method allows you to separate particular forms of molecules depending on their electric charge. In whatever form of electrophoresis used, whether you're using starch, paper, or cellulose acetate as a medium for carrying proteins or other molecules with a charge on them and then applying an electric field to the medium, we have found that the most information, in terms of recognition of specific forms of fishes, recognition of races, recognition of populations, has come from the use of this particular tool. The

situation that held up its application in fish work was that we did not make the association between a protein molecule with a charge and the fact that this protein molecule was inherited.

In some genetic work with pigeons in 1941, it was demonstrated that certain serum proteins showed up generation after generation. But it wasn't really appreciated that this association between a specific molecule, a protein, and its inheritance existed. Remember this was before Watson & Crick. There was a great amount of literature, perhaps a couple of hundred papers, published from about 1945 to about 1961 where investigators were studying different molecules using electrophoresis and looking specifically for species-specificity in these molecules. Then in the early 1960's, there was some work started in Clement Markert's lab, at John Hopkins University. Markert was interested in serum proteins and how they acted in terms of the embryological development of an organism. And here we have the beginning of the story of lactate dehydrogenase (LDH) and its many isozyme forms.

Markert's group could show, and other labs also demonstrated, that there was a certain specificity of this protein (LDH) as far as a species was concerned. But it wasn't until around 1966 in this country and independently in England at Henry Harris' Laboratory, that Lewontin and Hubby discovered that there was a considerable amount of polymorphism at each gene locus for LDH and other enzyme molecules. What do I mean by gene polymorphism? It is simply where a gene that exists on a chromosome at a specific site, a locus, is expressed differently because that gene has been altered by mutation in a fish species. These genes are called alleles (genes exist as pairs or alleles on sister chromosomes), and their expressed products, allelic forms. If allelic forms are expressed, and we could demonstrate it by electrophoresis, we have a natural means of marking a fish population. From 1966 on, this field has developed in terms of application and papers published. The most immediate application is the ability to not only identify a species, but to identify a form at the population level, subpopulation level, race level, or however you want to treat a particular fish variant.

I'd like to pose a few questions in terms of my quick review of fish genetics history. Alpha is a standard systematic, or systematist's term for the descriptive stage. But whatever walk of life you are dealing with, whenever you are learning something new, there is always a beginning descriptive stage and thus I've adopted the term alpha from the systematist. The question being posed is "Will I be here tomorrow"? For 2500 years man has been capable of culturing fish and having at least from an empirical approach some understanding of how to do it. I would say now with the great number of hatchery production units in this country, on this continent, yes, we can successfully culture fish. But the question should be, "Why should we just judge that a fish culturist should be given his full credit for doing a wonderful job because he produces so many pounds of flesh per unit food fed?" The fish culturist is judged on the amount of product he ships out of the hatchery and not on the

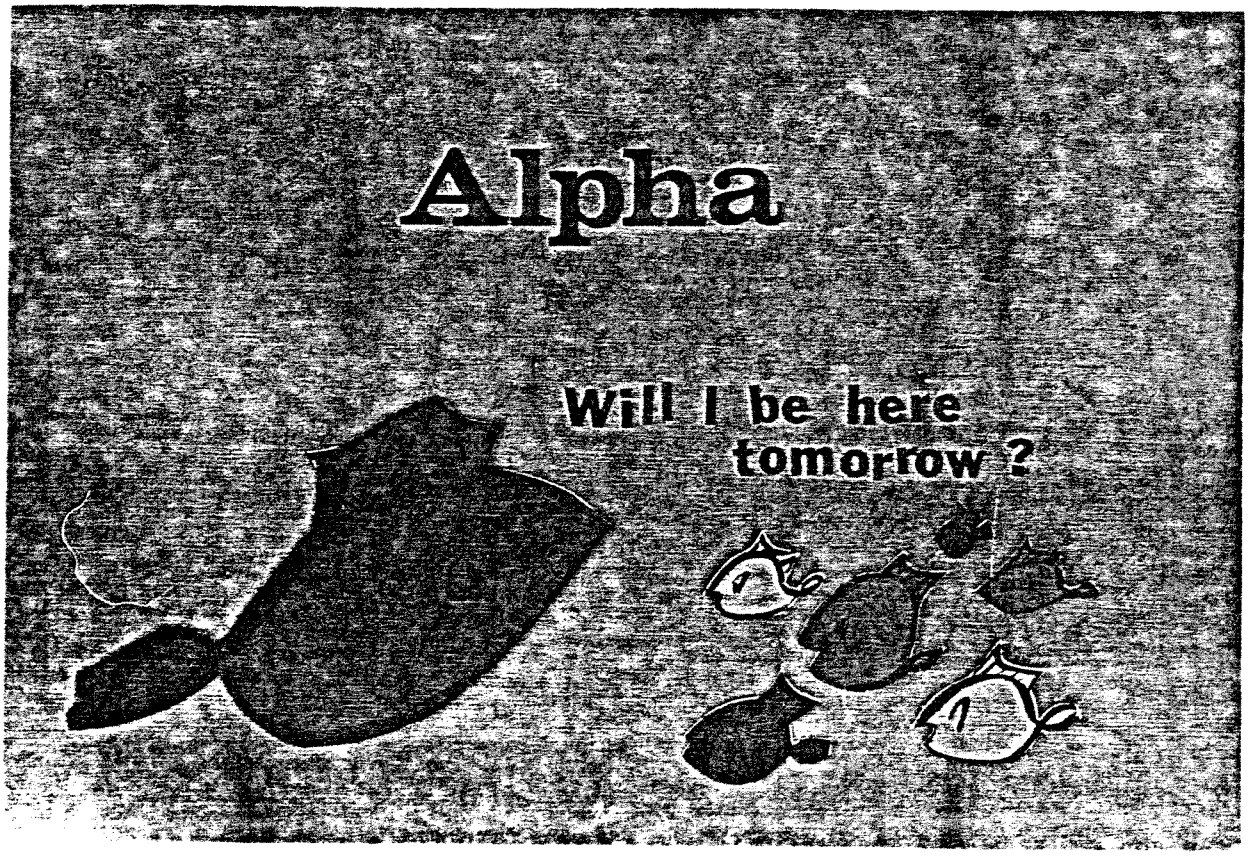

Figure 1

functional relationship of that fish to being able to sustain itself in an environment. We should be asking questions about whether there is another way we should be judging our products as they go out of our shops, in other words, our hatcheries. If you want to be a bit more mundane about it, I would like to ask what industry on the face of this earth can produce a product, throw it away, and not judge it? This industry is not going to be in business very long. But our government agencies do it every day in terms of hatchery products. If you disagree, please question me about it when we get into our discussion.

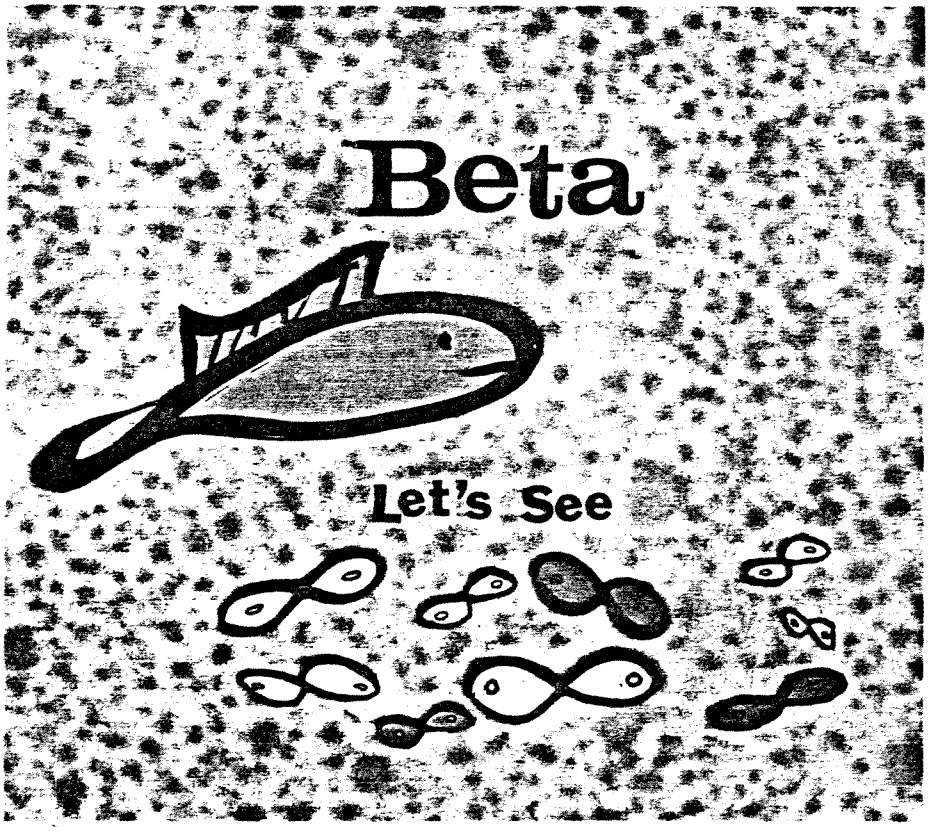

Figure 2

The next level a systematist would study would be the arrangement or beta level. Have we approached this level at all in fish genetic studies? In terms of selective breeding we are getting to that point and Dr. Simon will talk about this later. I would say, in terms of use of internal markers, in other words, isozyme systems or proteins in general, we are getting to the point where we can understand something about specific populations and variation in those populations.

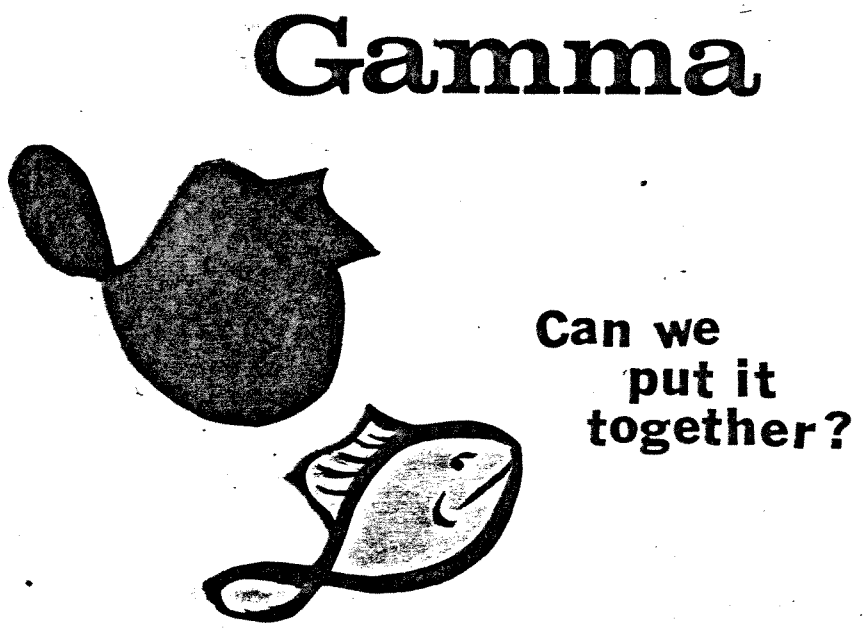

Figure 3

The next level of study for the systematist would be the synthesis level or gamma. We are far from that in terms of the kinds of products we are putting out in our environment and in our knowledge of their ability to inherit particular characteristics.

Figure 4

Resolution: What can we resolve in terms of fish genetics knowledge today? Definitely, we have the ability to define a population in terms of certain genes, not every gene in the fish body, but genes that are representative of that particular animal. As far as selective breeding is concerned, we are just starting at the point where we are having an understanding of what trouble our house is in. Again, I am going to leave that discussion to Dr. Simon and at this point I am going to stop and permit Dr. Peter Ihssen to speak about fish physiological genetics.

Physiological and Behavioral Genetics and the Stock Concept for Fisheries Management

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I believe that it is now well accepted that fish species are generally subdivided into more or less genetically isolated populations or stocks. The stock has been defined as a population of organisms that share a common gene pool and a common environment. Adaptive as well as non-adaptive genetic changes within species occur at the stock level. Adaptation to the common environment occurs at the stock level as well as non-adaptive genetic changes due to sampling variation of the alleles for small populations, called genetic drift. These processes, coupled with some genetic isolating mechanisms, bring about the genetic differentiation among stocks. This leads to geographic clines or sharp distinctions among stocks, for example, due to discontinuities in environmental conditions. For some salmonids, their strong homing behaviour provides the mechanism for genetic isolation. Migration counteracts these processes of differentiation among stocks and leads to greater genetic uniformity.

That a species can be composed of genetically distinct sympatric stocks has been recognized for a long time. The German ichthyologist Heincke has been credited with the pioneering work in this area. He published a paper in 1898, in which he suggests that the Atlantic herring of the North Sea is represented by 30 small groups, which he called races, and defined as groups of individuals which live in the same area, share the same habitat, and have a close blood relationship. The distinctive features, in this case, were morphological features. This definition is very similar to the one I gave except Heincke used the term "close blood relationship", where we now say "share a common gene pool". Of course his work preceded modern genetic theory and the recognition of Mendel's work. The stock concept has received renewed interest in the last two decades due to the discovery of very considerable intraspecific genetic variability for structural genes by electrophoretic techniques. I will be discussing genetic intraspecific physiological, and behavioral variability. Before I do, however, I would like to say a few words about technique. Intraspecific variability has been studied traditionally by looking for differences for morphological characters. Body measurements, meristic counts, and morphological features such as color variants have been observed for specimens collected from the field. As soon as fish were reared in controlled environments (and even now of course this is very difficult for marine species) it was found that morphological characters are very much influenced by environmental events, particularly during early

embryonic development. Consequently, at least part of the variability observed is not due to genetic differences, but environmental effects. Hence the differences observed among stocks may only partially, if at all, reflect the genetic relationship among stocks. Morphological characters, however, have received renewed interest with the availability of high speed computers that can simultaneously analyze a large number of characters using statistical techniques such as discriminant function analysis; but again, the differences that are found do not necessarily reflect genetic differences. For example, we looked at a number of lake trout populations from Ontario lakes and hatcheries for characters such as number of anal and dorsal rays, vertebrae, gill rakers, and pyloric caeca. The difference in number of gill rakers, anal and dorsal rays, and pyloric caeca that could be induced simply by rearing from fertilization in a different environment (hatchery) were of the same magnitude as the differences observed among these populations. Hence one cannot draw conclusions from such phenotypic characters about the genetic variability among stocks.

Behavioral and physiological characters have not been studied to the same extent for stock differences. However, usually in these studies more care was taken in controlling the environment, because it is very obvious that specimens collected from the field are not suitable for comparative studies due to their different environmental histories. The great advantage of the electrophoretic technique is that the phenotypes observed are very representative of the genotypes and little influenced by the environmental effects. For behavioral and physiological characteristics of course this is not the case since many processes intervene between the genotype and the observed phenotype and usually a number of genes control each character. However, many links between the electrophoretic genetic variability and character of adaptative significance have not been convincingly made to date.

Intraspecific variation for characters associated with migration have been studied for a few species of fish. Little is known about the genetics of these characters, but it can be postulated from work on the behavior of other animals that these characters are polygenetically controlled as has been found in Drosophila for example. Differences in spawning behavior have been observed for different lake trout stocks of the Great Lakes. For example, Loftus has reported on river spawning of Lake Superior lake trout and Royce reported on differences in spawning time within season for New York state lake trout stocks. To what extent these differences reflect genetic differences is not known since no controlled experiments have been performed to my knowledge. Brannon showed that sockeye fry reared from inlet and outlet stream populations of the Fraser River system exhibited behavioral patterns under controlled laboratory conditions corresponding to the up-stream and down-stream migration behavior in the wild with hybrids intermediate in behavior. Johnson and Groot studied the orientation behavior of smolts from different Babine Lake populations and discovered differences in orientation in the laboratory corresponding to the orientation required in the lake to find the outlet stream. In these experiments, gametes were

collected from the wild and reared in the hatchery without any of the environmental cues that were thought necessary for this behavior.

Another technique to demonstrate genetic differences are transplantation experiments. This work has shown, for example, that differences in growth and size among stocks due to feeding behavior was environmentally induced, rather than genetically, at least in some instances. Recently a very interesting transplantation experiment was reported on by Bams on homing of pink salmon. This work is particularly convincing since the maternal effects not ruled out in the studies I mentioned so far were ruled out by crossing local and donor males to identical donor females. Eggs were collected from a stream, which is called the donor stream, and crossed with donor and local males. "Local" in this context means the environment in which these crosses were tested. For a number of females, the eggs were divided in half, and fertilized by males from the two different areas. Hence, differences found could not be due to maternal effects associated with the eggs. However, maternal effects were not ruled out in the other studies because eggs from different populations were used. Bams found that the local x donor strain returned about 10 times as frequently to the local stream as the pure donor strain. It appears that some local genes are required to bring these fish back to the home stream. Also, it was found that the migration behavior in the stream (of returning precisely to the planting site) was much more precise for the local x donor strain that had the local genes. I think this is a very convincing piece of work indicating inheritance of a behavioral character.

In our lab we have done some work on the inheritance of seasonal spawning time and found that there is a strong correlation among sisters for this character. Also, the mean spawning date of sisters is correlated with their mothers' spawning date and hence their birth date. The spawning time within season for individual females is very precisely replicated in other seasons. Hence a strong maternal influence is indicated.

With regard to physiological characters for a number of brook and lake trout populations of Ontario, no differences for high temperature tolerance were found. These were carefully controlled replicated experiments for different acclimation temperatures. Similar comparisons were made for different naturalized Ontario rainbow trout populations and again no population differences for upper temperature tolerance were found. Also in our laboratory we have been comparing two rainbow trout stocks for a number of physiological characters. One of these stocks is a hatchery strain commonly used by the Ontario Ministry of Natural Resources for plantings in lakes and streams. The other stock is a self-sustaining naturalized stock from the Nottawasaga River which flows into Lake Huron. Males and females from these two stocks were crossed in a diallel experimental design (eggs from each female are split into two lots and each lot fertilized by a male from each strain and the same males are then used in the same way on a female from the other stock producing four crosses) which was repeated twelve times with about 20 fish per family. This experimental design permits one to

separate male and female effects. It was found that most of the variability in growth was accounted for by female effects with the families derived from the hatchery stock growing faster than the families derived from the naturalized strain. The rate of maturation of males on the other hand was almost entirely determined by the males with the families derived from hatchery males having a higher percentage males in their second year. It is too early to assess maturation of females since only a small percentage have matured to date.

Hemoglobin concentrations for these fish were compared and it was found that fish derived from the hatchery stock had highly significant lower total amounts of hemoglobin in whole blood than the fish derived from the naturalized stock. Also the amount of hemoglobin per red blood cell volume was lower for the hatchery stock. Also it was found that the amount of hemoglobin in whole blood is positively correlated with the length of the fish within these strains.

In conclusion I want to make the point that usually man's activities have the greatest impact at the stock rather than the species level. And consequently it has been suggested by a number of fishery scientists that without a clear idea of the specific features of the biology of these stocks, a sound approach to fisheries management is not possible. I think it is fair to say that until recently this concept has not been given the emphasis it deserves. The consequences have been not only the loss of some irreplaceable stocks of fish but also most probably the failure of some management programs to re-establish self-sustaining stocks.

The LDH Isozyme System of Salmonids

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When Henry Booke asked me to do this talk several months ago he said that my specific task would be to try to explain to the audience of what value electrophoresis techniques might be in the identification of stocks and environmental influences and things like that. I have a question, therefore to ask the audience: Will I be wasting your time while I explain to you what electrophoresis is and how electrophoretic techniques are utilized in the identification of fish species?

LDH isozyme patterns of the rainbow trout. Now, to some of you this just might be a lot of jibberish. However, what we have done here is to separate the multiple molecular forms (isozymes) of the enzyme lactate dehydrogenase (LDH) from skeletal muscle, heart, intestine, and the eye by electrophoresis in a stabilizing medium. Now, how do we go about accomplishing this?

First of all, let me say something about lactate dehydrogenase. Lactate dehydrogenase is an enzyme, therefore it is a protein, therefore the information for its biosynthesis is encoded in the genome of the organism.

Lactate dehydrogenase is a soluble enzyme and can be extracted from tissues simply by homogenizing a sample of tissue in distilled water or in some type of buffer solution (at high protein concentrations, a buffer solution is not necessary). Usually, the homogenate is clarified by centrifugation and the supernatant solution, the extract, is applied to a stabilizing medium in preparation for electrophoresis. In our case, the stabilizing medium was starch gel.

The technique of electrophoresis (free boundary electrophoresis) was developed by Tiselius. Tiselius used a U-tube into which he placed a solution containing protein molecules, keep in mind that these molecules are charged. Into one arm of the U-tube he placed a negative electrode and into the other, a positive electrode. By passing a D.C. electric current through the solution Tiselius discovered, using a UV optical system, that he could separate the proteins according to charge differences. By having this apparatus constructed of movable sections, he was able to separate out certain proteins. This was a remarkable technique. However, it presented problems because the protein molecules were free in solution, and many influences can affect the resolution of the protein molecules in solution. For example, small changes in temperature can accelerate the rate of diffusion and, therefore, the resolution of the molecules. Thus, the apparatus has to be maintained at a constant

temperature. This is difficult and costly to achieve.

In more recent times, it was discovered that a more stable system could be achieved employing a strip of filter paper which is nothing more than a cellulose matting as a stabilizing medium. The paper is dipped into a buffer solution, the excess is blotted off and a sample of a soluble protein mixture is spotted on the strip. The strip is subjected to electrophoresis; that is, a voltage gradient is established across the paper and the component proteins of the mixture are resolved into individual bands. Now, this is a very simple technique, basically requiring nothing more than a power supply, a strip of filter paper and a tissue sample. Very convenient. A few years after this technique was developed, somebody came up with a substance called cellulose acetate. Electrophoresis on cellulose acetate was somewhat of an improvement over paper electrophoresis because of the properties of the material. Filter paper has been greatly improved over the last fifty years, but you still can't get the type of resolution on filter paper that you can on cellulose acetate. Unfortunately, both of these methods suffer from several inadequacies. One of these, which is, perhaps, not obvious, is that their capacity, the amount of material which can be applied to paper or cellulose acetate, is extremely limited. In some cases this might not be a problem, in other cases it is an extreme problem. A second problem, very similar to the problem encountered by Tiselius is that their resolving power is limited; fundamentally because what we are doing is separating the protein molecules strictly according to charge and diffusional movement of the proteins is not restricted.

Protein molecules are charged molecules. The charge can be negative or positive. and one protein molecule can either be more charged or less charged, negatively or positively, in relation to some other protein molecule. Another characteristic of protein molecules is that of size. Size is a characteristic of a given type of protein molecule, as is charge. Some types of protein molecules are smaller than other types. Thus, we have two characteristics, charge and size, that we can exploit to separate protein molecules. Why not exploit both characteristics?

A while back someone came up with the brilliant idea to convert starch into starch gel and use the gel as a medium for separating proteins. A starch granule is nothing more than a gigantic curled-up sugar polymer. Starch granules moistened with a buffer solution and packed into a trough can be used as an electrophoretic stabilizing medium. But, unfortunately, the interstices between packed starch granules are so large that resolution is reduced by diffusion. Fortunately, someone discovered that the starch granule can be unfolded and the linear starch polymers formed can be packed into a dense matting of controllable pore size. The pore size of the gels formed is such that diffusional movement of protein molecules is greatly inhibited which greatly improves resolution. All one has to do is suspend the starch in a buffer solution, boil it carefully (to unfold the starch granules), pour the hot suspension into a mold and allow it to cool. On cooling, a dense meshwork of starch polymers

is formed. By controlling the starch concentration (i.e. the linear polymer concentration), the pore size of the meshwork can be controlled. By controlling the pore size, the rate of migration of proteins (which differ both in size and charge) can be manipulated which is of great importance in the study of the composition of complex mixtures.

I would like to pass this technique on to you because it has great potential in your field. Most certainly, it will find wide applicability in fisheries management in that it provides a simple means for sampling the genetic composition of fish stocks. It requires only simple equipment. All you need is a hot plate, stirring motor and stirrer, starch, buffer solution, molds, and a D.C.

To prepare the starch gels I use in my research, the starch is boiled in a buffer solution, poured into a mold and allowed to cool. Sample wells into which the tissue preparations are placed are preformed in the gel in the mold. For those of you who have never seen a starch gel, it looks like a stiff block of gelatin. I prepare my tissue samples, such as skeletal muscle (red or white muscle or a mixture of both red and white muscle), heart, a piece of intestine, eye, etc. by homogenizing minced tissue in buffer solution (1 to 20 ml buffer per gm tissue). The homogenates are centrifuged and the clear supernatant solution, containing the soluble proteins, is placed into the sample wells of the gel. A voltage gradient is generated across the gel and the proteins are separated according to size and charge intensity. As is well known the enzyme lactate dehydrogenase can be separated into a number of bands of activity. These multiple molecular bands are called isozymes or isoenzymes. Now what does this all mean? Well, proteins like the enzyme lactate dehydrogenase are encoded in the genome. Therefore, they are subject to alteration via mutation. Perhaps they are also subjected to change or variation in expressibility due to environmental factors. These alterations, variations, etc. are detectable by starch gel electrophoresis.

The technique can be used for the identification of the multiple molecular forms of enzymes, or other types of proteins as a purely descriptive tool. However, it can also be used as an analytical tool. Lactate dehydrogenase is composed of four sub-units. These four sub-units form the functional molecule. So far as we know, the individual sub-units, by and of themselves, are not active. The sub-units have to be put together in a tetrameric form before they will function, before the enzyme is active, so far as we know. Using starch gel electrophoresis, or other types of biochemical techniques, like chromophotography, it is possible to isolate individual isozymes. There are five major bands of lactate dehydrogenase in the cow, the horse, etc. If you isolate the slowest moving band, which is called LDH-5, and the fastest moving band, which is called LDH-1, and combine them in a phosphate buffer solution in the presence of a small amount of sodium chloride and freeze and thaw this solution, five isozymes are formed. LDH-1 and LDH-5 remain; but LDH-2, -3, and -4, migrating between LDH-1 and LDH-5, are generated. What has happened is that, on freezing in this solution, the tetramer dissociates

and on thawing, the individual sub-units reassociate at random into every possible combination of four. Thus, if there are two different types of sub-units, as there are in mammalian LDH, five isozymes will be formed which can be separated by gel electrophoresis. Now, employing the freeze-thaw molecular hybridization technique in conjunction with gel electrophoresis we have an analytical tool that can get some information on the actual molecular composition of the groups of LDH isozymes of the rainbow trout. For example if we attempt to hybridize different groups of rainbow trout isozymes with equine or bovine LDH-5, we can get some interaction (certainly not a binomial recombination by any means) between LDH-5 of the horse or cow and certain groups of trout isozymes. By using this technique, this intraspecific hybridization, we can obtain some information about the structure of LDH molecules without resorting to techniques like amino acid and peptide analysis, or other types of analyses. Using the hybridization technique, it is possible, for example, to show that during the evolution of the salmonids (and other species of fish) that, by the process of gene duplication, as in the case of the hemoglobins, new genes were established in the genome. These genes are still undergoing variation and can be used for the identification of specific strains and specific stocks of fishes.

Genetic Variation in Populations of Fish

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Introduction

The era of modern genetics began at the beginning of the 20th century with the rediscovery of Mendel's principles. The field of population genetics quickly emerged in the following 30 years. By 1930, a large proportion of our present total body of population genetic theory had been described by the three early giants of this field--R. A. Fisher, J. B. S. Haldane, and S. Wright. The theoretical foundation of population genetics, therefore, was established very early.

Unfortunately, however, the practice of population genetics did not develop as rapidly. It is very difficult to examine genetic variation in natural populations. Thus, although armed with this large body of theory, geneticists could not go out and look at genetic variation in natural populations. They went out and looked at morphological differences; they looked at physiological differences; they looked at behavioral differences. There was no way, however, they could interpret these differences in terms of Mendelian genetics (i.e., allelic variation at single genetic loci).

The overwhelming majority of the experimental work performed was with the fruit fly (Drosophila spp). This early work was almost exclusively accomplished with genetic markers associated with visible phenotypic effects (e.g., white eyes or wingless). These low frequency genetic variants with a large, usually harmful, effect do not represent the type of variation in natural populations we are most interested in. We would like to be able to examine the genetic variation which is present at high frequencies in natural populations and can be used to characterize genetic differences between different populations within a species. This, however, was impossible for a long period of time following the origins of population genetics in 1930.

In 1966, two independent publications described a technique which for the first time allowed the detailed examination of genetic variation in natural populations (Harris, Proc. Roy. Soc. Ser. B 164: 298; Lewontin and Hubby, Genetics 54: 595). These two papers described the use of starch gel electrophoresis to examine genetic variation in populations of humans and Drosophila. One of the beauties of this technique is that it can be applied equally effectively to almost any organisms desired. Within the last year in my lab alone, the same basic techniques have been used to examine genetic variation in tent caterpillars, fish, frogs, mice, bears, and pine trees.

We now have a tool which allows us to examine in detail genetic variation in fish populations. How do we use it?

Interpretation of Electrophoretic Data

Dr. Massaro has spent considerable time and effort outlining the biochemical basis of electrophoresis. I would like to spend some time outlining the genetic interpretation of these results.

Figure 1 shows the same enzyme (lactate dehydrogense - LDH) that Dr. Massaro spoke about. The differences seen in this figure represent genetic differences between individual rainbow trout (Salmo gairdneri). Each column (1-12) is a liver sample from individual fish. The observed differences represent two different genetic forms (A and A') of this enzyme in rainbow trout. These two genetic types (i.e., alleles) are detected by their different rates of migration when placed in a starch gel matrix which has an electric current passing through it.

Three different genetic types, or genotypes, can be seen in this figure--AA, AA', and A'A'. Each individual carries two copies of this gene, one received from the mother and one from the father. Individuals can therefore possess two copies of the fast migrating allele (genotype AA), two copies of the slow migrating allele (A'A'), or one copy of each (genotype AA'). The five electrophoretic bands (or isozymes) seen in the AA' type results from the biochemical structure of LDH. As discussed by Dr. Massaro, four protein sub-units join together to form an active LDH molecule. If two genetically different sub-unit types (A and A') join together four at a time, the result is the five different isozymes seen in the AA' phenotypes.

Now, just so you don't go away with the impression that LDH is the only enzyme biochemical geneticists work with, I should discuss other enzymes which are also used in this work. There are presently some 40 enzymes being used in my lab to detect genetic variation in populations of salmonids (Allendorf, Mitchell, Ryman, and Stahl, Hereditas 86: (in press)). The basic biochemical and genetic principles involved using these other enzymes are analogous to those presented for LDH here today. The more enzymes, and therefore genetic loci, we can examine the more detailed a picture we can develop of the genetic structure of populations.

A valid question at this point is--How do we know these differences I have presented represent simple genetic differences? In this regard, I am very fortunate to be working with salmonids. It is relatively easy to perform experimental matings under controlled conditions with fish of known genetic types to test the inheritance of these observed electrophoretic differences. These matings have been performed and confirm the genetic basis of this variation (Allendorf, Ph.D. thesis, Univ. of Washington, 1975).

Now that we have established the experimental basis of this technique, how do we use electrophoresis to characterize genetic

variation in fish populations? Let's return to Figure 1 showing different genetic types of LDH in the rainbow trout. If we assume these twelve individuals represent a sample from a population of rainbow trout, we can characterize that population for this genetic locus by calculating the frequencies of these two allelic types (A and A') in this sample. A total of 24 genes are represented in this figure (two times the number of individuals). By examining the numbers of different genetic types in our sample we can estimate allele frequencies for the population from which this sample was taken. Each AA genotype individual has two copies of the A allele; similarly, each AA' individual has one copy of the A allele. Therefore, the frequency of the A allele in this sample is two times the number of AA individuals plus the number of AA' individuals, divided by the total number of genes examined. Thus,

$$\text{freq}(A) = \frac{((2)(5)) + (6)}{24} = \frac{16}{24} = 0.67$$

Likewise,

$$\text{freq}(A') = \frac{((2)(1)) + (6)}{24} = 0.33.$$

We have now estimated the allele frequencies in the total population through this sample of twelve individuals. The more individuals we examine, of course, the more accurate our allele frequency estimates are. Using these techniques, we characterize different populations by the frequencies of such genetic types for as many different genetic systems (i.e., loci) as possible. If we carry out this procedure on the 70 some loci we can now examine in salmonids, we can derive a fairly detailed analysis of the genetic structure of these populations.

Applications

How can we apply this technique to the problems involved in managing populations of fish? The first question I would like to approach is--How much genetic variation is there within individual populations? This is obviously a very important question. If we are concerned with evolution, whether via natural selection or via artificial selection in a hatchery, the amount of genetic variation present is the key factor. If there is no genetic variation between individuals, then there will not be any evolutionary change in that population. The rate of evolutionary change is directly proportional to the amount of genetic variation present. Therefore, if we wish to select a fish population for some particular characteristic, it is necessary that there be genetic variation present for us to succeed. In addition, the loss of genetic variation following inbreeding, which often occurs in hatchery stocks, has been demonstrated to have harmful effects (Kincaid, J. Fish. Res. Bd. Canada 33:2420). It is,

therefore, desirable to be able to measure the amount of genetic variation within individual populations of fish.

Electrophoresis provides a simple objective technique for measuring the amount of genetic variation within a population. We can analyze a sample of fish from the population in question and directly quantify the amount of variation present.

How is this information directly important to managing fish populations? There has been much speculation that hatchery practices have reduced the amount of genetic variation in many of our hatchery stocks of fish. These concerns were previously limited to speculation. Now, however, we have a method for directly estimating how much genetic variation is actually present in these stocks. A comparison of stocks within a particular species will identify which stocks do possess significantly lower amounts of genetic variation. We thus have a method for objectively estimating the loss of genetic variation in hatchery stocks.

An extension of this technique is the creation of stocks with exceptional amounts of genetic variation. When selecting new stocks to be cultured, there are advantages for selecting stocks with as much genetic variation as possible. Such stocks can be created using these techniques by incorporating individuals from stocks which are initially extremely genetically different, as measured with electrophoresis. Thus, we can both monitor the loss of any genetic variation and also artificially increase the amount of variation in a particular stock.

The next level of genetic variation of interest is the amount of genetic variation between different populations. Through the examination of allele frequencies at many loci we can estimate the amount of genetic divergence between populations. This gets us into the problem of the stock concept. Having separate genetic populations within a species is a critical factor in the management of such a species. A difficult problem has long been the identification of such stocks. Electrophoresis allows us to collect samples from a number of natural populations within a species, and objectively define the population structure of that species. We can both outline the major population groups and also estimate how genetically different local populations are. We can, thus, for the first time describe the patterns of genetic diversity within a particular species.

This genetic structure has very important implications for the management of a species. A good example of this on the local population level is a study I am involved in dealing with brown trout (S. trutta) populations present in Swedish Lakes (Allendorf, Ryman, Stahl, and Stennek, Hereditas 82, 19). We have found two completely genetically isolated populations are equally frequent in this lake. However, they have different growth rates. We are currently attempting to detail other environmental or physiological differences between individuals in these two stocks.

The presence of these two separate stocks is an important factor in managing this lake. A harvesting system based on simple quotas and size restrictions could have devasting effects. Proper management in this situation must treat these stocks separately. For all practical purposes these two stocks represent separate species and must be managed as such.

On a larger scale, the genetic structure within a species can be used as natural genetic tags. This is of critical importance when dealing with populations of salmonids which often travel long distances from their waters of origin.

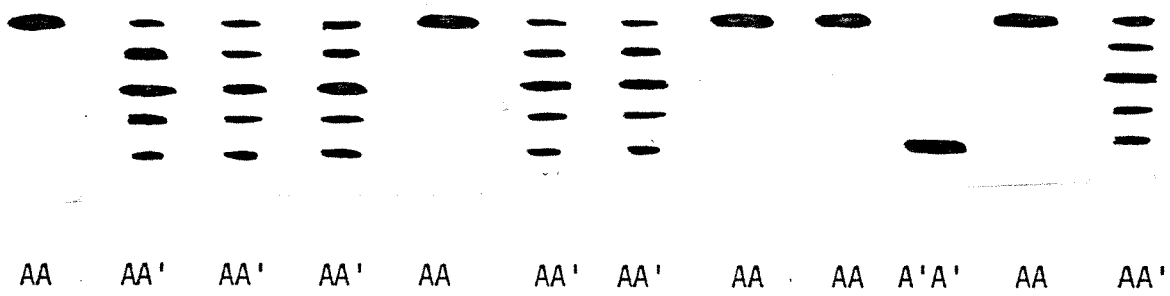
Populations of anadromous salmonids on the Pacific Coast are generally harvested before returning to their freshwater streams for reproduction. Harvesting quotas must take into account the stream of origin of returning fish so that sufficient numbers are allowed return to each local population in order to maintain that population. Genetic differences among local stocks, as measured by the techniques described here today, are currently being used to approach this problem with populations of both Oncorhynchus and Salmo species along the Pacific Coast.

We can go at least one step further in this use of genetic differences as natural tags in the management of fish populations. This technique is dependent on finding genetic differences among stocks. We can, however, create stocks which are genetically distinct via these techniques through systems of artificial selection.

An exciting example of this application is being applied to the management of steelhead (S. gairdneri) in the State of Washington. Genetically marked hatchery fish are being planted in previously unplanted steelhead streams to monitor the effect of hatchery planting on the native stocks. The biological nature of genetic tags allow us not only to identify the planted hatchery fish but also their progeny. Thus, we can examine both the spawning success of hatchery fish and the interaction between the progeny of the hatchery and native fish.

Well, I'm already ten minutes over my alloted time so I had better stop right here. Thank you for your attention.

Figure 1. Diagram of electrophoretic patterns reflecting genetic variation for LDH in the rainbow trout.



Genotypes

Selective Breeding: The "Specialist" Approach versus Crossbreeding: The "Generalist" Approach

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Trout hatchery personnel have repeatedly observed differences in growth and performance of different trout strains. It is in fact axiomatic that if two or more strains of trout are retained separately, some differences, either great or small, are always expected. Strain differences are typically found in bioassay tests, nutritional studies and response to disease challenges. For example, some strains of trout are known to be highly tolerant of furunculosis and remain asymptomatic, while other strains are severely decimated within the same hatchery environment. Furthermore, the difficulty in repeatability of bioassay results is understood to be due partly to use of different strains at different localities.

Given that strain differences in trout are the rule rather than the exception, the idea of matching strains to particular management objectives or particular environments is clearly one way of deriving benefit from these differences. Just such an activity was charged as a major objective of the Fish Genetics Laboratory in 1973. The project approaching this issue was titled Genetics of Wild and Hatchery Trout Strains and has served to catalog a variety of strain attributes with a final aim of being able to specify which attributes were correlated with superior performance. This activity then is one of defining a SPECIALIST which is predicted to match certain environments or management circumstances. Numerous differences in strains have been identified by FGL studies. Several of these differences are of obvious economic value, such as hatchery survival, food conversion efficiency and survival after release.

A stringent limitation on the specialist approach, however, is the difficulty of testing performance under any appreciable fraction of the hundreds of different environments that exist. Difficulties associated with this limitation are several, if survival and performance (return to the fisherman) are adopted as major criteria to measure success.

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For example, we can define several environmental factors which would be expected to discriminate the superiority of certain strains among two or more strains being tested simultaneously:

1. abundance of food
2. type of food
3. pH
4. water hardness
5. temperature
6. pollution level
7. type of pollutant if present
8. oxygen level
9. physical factors (deep lake, farm pond, sluggish stream, etc.)
10. competition and predation levels

Furthermore, seasonal fluctuations affect many of these factors, thus they are variables more often than they are constant. Given then that large numbers of different environments exist and that they are subject to change, the search for ideal strains is apt to become very complicated, and then perhaps for limited usefulness. The natural idea springs forth that the strain could use some help in the form of selective breeding. Thus the few individuals which show the most dramatic expression of what is desired are chosen out as parents for the next generation and the specialist idea is advanced to yet further degree. The procedure is repeated for several generations with the hope of attaining maximum levels of change. These kinds of selection have been performed in hatcheries rather often (few managers would use poor-looking fish for breeders unless they had no choice). Generally speaking, these activities present a problem because they are uncontrolled experiments in the sense that unselected controls are rarely retained for purposes of measuring the changes anticipated. A second problem may be more important, namely, that practitioners of artificial selection must assume they know what is best. Almost inescapably, this leads to an attitude of paternity concerning the experiment. In other words, having once decided what is best, it is increasingly more difficult to believe that other alternatives might be better. There is yet a third problem, caused by the fact that selective breeding, by definition, reduces the numbers of breeding individuals to be used as parents. The price paid for gains which may have been made can be measured from the effects of inbreeding, which inevitably has been accumulated from the reduced numbers of parents. Some costs of inbreeding are shown in the following two tables. These data, where each number is based on several hundred measurements, are thought to approximate the inbreeding levels of some (possibly a majority) of trout hatcheries. Eggs of each female were divided into two groups thus I x I (inbred matings) and I x 0 matings from the same female are comparisons of half-sibs. The adverse result of inbreeding is clearly appreciable.

The alternative approach is to use crossbred individuals derived from crosses between different strains for production purposes. These crosses have often, but not always, been shown to perform better than either of the parent strains. The main problem in this case is the

large number of different crosses that are possible. Twenty strains, for example, would yield 380 possible unique crosses. Results to date indicate that some strains do not do well in crossings while others are impressive. There are two clear advantages to the use of strain crosses. First, the idea of "paternity" is minimized because of the need to measure what is superior, rather than prognosticating it. Secondly, the inbreeding which has accumulated can be put to advantage because of the vigor displayed in many strain crosses.

In closing, one point requires heavy emphasis. Because environments are changing, what does well today cannot be guaranteed to do similarly in the future. For this reason, it is essential to retain broodstocks in unmixed state to permit construction of the full possibility of crosses for the future.

These remarks have been made in reference to fish which are released from a hatchery with subsequent requirement for fairly extended, or greatly extended survival time to permit contribution to a fishery. Traditionally used hatchery stocks may not be quite so severely compromised in uses where the fish are under total captive control. Even here however, the advantages to be gained from crossbreeding deserve evaluation.

Body Weight (grams)

Summary of Means

	Inbred Male	Outbred Male
Inbred Female	I x I 2.25	I x O 3.40
Outbred Female	O x I 3.47	O x O 4.09

Mortality (Hatching to 147 days)

Summary of Means

	Inbred Male	Outbred Male
Inbred Female	I x I 34.9	I x O 13.6
Outbred Female	O x I 10.1	O x O 12.0

Roundtable Discussion

Mark Noveck: Dr. Massaro, would you like to delineate some of the staining methods you used for enzyme identification:

Massaro: A number of different types of enzymes can be detected by starch-gel electrophoresis. However, I'll just confine my remarks to the dehydrogenases because I don't wish to go into a lot of explanation concerning substrates, etc., and I can use lactate dehydrogenase as an example. The same basic system is used for the detection of dehydrogenases. The reaction, very simply, is one of a reducing series in which lactate is converted to pyruvate. In effect, two hydrogens are removed from lactate. What do you do with these two hydrogens? This reaction, the conversion of lactate to pyruvic acid, requires a substance called a cofactor which accepts hydrogen atoms. This cofactor is called nicotanamide adenine dinucleotide (NAD). All you have to keep in mind is that NAD is reduced in the conversion of lactate to pyruvic acid. In effect, the two hydrogens are removed from the lactate molecule by the cofactor. Different dehydrogenases use different types of cofactors, but the same type of a reaction occurs. Years ago it was discovered that this dehydrogenation reaction could be made visible by using a tetrazolium dye (nitro blue tetrazolium) commonly used in histochemistry. If, in a buffered solution, the substrate, our enzyme, lactate dehydrogenase, plus its substrate, lactate, plus the cofactor, NAD, plus the tetrazolium are mixed together, a reaction will take place in which the hydrogen is transferred from lactate to the cofactor and then to the tetrozolium. When the tetrazolium is reduced, it precipitates forming a blue or blue-purple precipitate. If a tissue homogenate is prepared, for example, from skeletal muscle, a whole host of soluble enzymes are going to be present in the supernatant of this homogenate. How do we stain specifically for lactate dehydrogenase? Lactate is used as the substrate. Enzymes are highly specific and they will do their job, usually only on a particular type or a particular group of substrates, in this case lactate. Following electrophoresis, the starch gel is placed into a buffer solution containing lactate, the cofactor, and another substance which facilitates the reaction called PMS (phenazine methosulfate). The hydrogens are removed from the lactate by the cofactor. PMS removes the hydrogens from the cofactor and the tetrazolium removes the hydrogens from PMS. Reduced tetrazolium is precipitated within the pores of the gel in the vicinity of the individual isozymes of LDH. The reduced tetrazolium, which is called formazan, is precipitated in the pores of the gel and is very difficult to leach from the gel so that, if treated correctly, a rather permanent record is obtained. The gel can be preserved and you can keep it for a long period of time. Lactate, which contains the hydrogens, is converted to pyruvate and the cofactor is repeatedly reduced and reoxidized. Thus, the cofactor is recycled. If one uses a large quantity of lactate one can, in effect, recycle this reaction for a long period of time. In simple terms, this means that you can start off with a very small amount of lactate dehydrogenase and by recycling, for a period of time, the very small amount of lactate dehydrogenase can be detected because formazan is continuously being precipitated in the vicinity of the

isozyme. A similar reaction, cofactor, PMS, nitro-blue tetrozolium, is used to detect all types of dehydrogenases. Even coupled reactions can be used in which the enzyme of initial interest is not a dehydrogenase.

Dan Cobal: There are millions of lake trout stocked in the Great Lakes. It is my understanding that there seems to be minimal natural reproduction and I would like to ask the panel what they would recommend be done to rectify that situation.

Ray Simon: One of the things about biological problems is that they are technically unique and to attempt to categorize an answer is probably stupid. Presumably it would clarify the situation if the historical background of that situation were known. That is, is the failure due to intervention through the adjunct of the hatchery? Habitat conditions during the year between the good reproduction and poor--how have physical conditions of the habitat changed? It's unanswerable unless you begin to glue together the description of that particular circumstance which I'm not able to do here because I don't know it. I presume you resolve it by digging further into the question which puts it back on you. That's a poor answer.

Stanford Smith: The primary problem, Ray, is that in the areas where you don't have natural reproduction you have lost your native strains. Where you do have natural reproduction you still have them. Like in Lake Michigan, Lake Huron we lost all of the lake trout, now we are coming back with other strains and they aren't reproducing.

Ray Simon: It sounds like we'd better get Fred Allendorf to build one that resembles the ones that do succeed in their natural habitat. I'm not being facetious, I don't know how to solve the problem.

John Driver: You are mentioning the historical significance of the lake trout and I'm in charge of the Marquette Lake Trout Hatchery. I've gone back to the records as far as I can, 1946, 1943, when they were first starting and they are very scanty and as you have indicated there has been much inbreeding within those stocks. But on Lake Michigan, three years ago at Charlevoix, eggs were taken from wild fish (planted fish which were mature), and the viability of those eggs was very good. So I wouldn't say that you are not getting reproduction in Lake Michigan. You are getting reproduction but not necessarily natural from the standpoint of them reproducing on the reef. They have the capability of reproducing because those wild fish eggs, a couple million of them, have been taken and have done very well in a hatchery situation. So it lends me to believe maybe that they haven't been planted correctly in the past, to some extent. I don't know. Maybe the habitat has been destroyed, but they still have the capability of reproducing well. At least in certain areas--off of the research facility at Charlevoix, for one.

Henry Booke: One of the questions I was going to pose before this audience in terms of stimulating questions like we are getting right now is how do we match some of these markers that we have discovered for these particular fishes against successful forms? Successful forms from particular hatcheries that we wish to maintain as a natural resource. We have to wait awhile before we can do that but at least attempts are being made. I think the best approach is to look at what we have now and try to characterize what we have and are defining as successful. Then see if there is some genetic basis for this success such that we can select for it. Maybe we are dealing with the same fish that are being affected by different environmental conditions. Is there a genetic basis for differences found among representatives of one species? I can recall a paper published in the early 60's, probably around 1964, by Eschmeyer and Phillips where they described crossings of lake trout, fat and lean forms of Lake Superior lake trout, and obtained intermediate forms. There was a strong implication that there was a genetic basis for the difference between the two forms. This work hasn't been appreciated as far as using it in the hatchery systems around the Great Lakes.

Peter Ihssen: I have a comment to that same question. Indications are that the lake trout of a lake like Lake Superior was constituted of a number of genetically isolated stocks. For example, there used to be river spawning, deep shoal spawning and shallow shoal spawning lake trout. Now we are trying to rehabilitate the lake with essentially one or two strains which may not have the proper potential for the spawning behavior that is required in the lake under present environmental conditions. For example, it may be that because of the change in species composition in the lake that certain spawning areas are more suitable than others as for example deep shoals compared to shallow shoals or river spawning areas. It may take some time for the stocks used in present plantings to select for individuals who have adapted to this new set of conditions. I think such a process of adaptation is sometimes indicated. For example, for the initial rainbow trout plantings in the Great Lakes, at first only little reproduction was noticed and then slowly there was a buildup of the population. A similar effect is being noticed for pink salmon which have a shorter life cycle and consequently the process of adaptation is more noticeable. For lake trout, on the other hand, because of their long life cycle, it may take a long time before we notice an appreciable buildup of the stock due to natural reproduction.

Ed Brown: Did you indicate earlier that, from the standpoint of rainbow trout that very little of the natural, or genetic, variation had been lost?

Fred Allendorf: Yes. In the great majority of populations that I have examined this is true.

Ed Brown: I guess, along this same line, we are interested in the lake trout situation and such questions are layman-type questions. Just how fast is natural selection in the hatchery in terms of a salmonid population.

Fred Allendorf: The loss of genetic variation that I was referring to is the kind that is associated with having small numbers of individuals reproducing the population. But in regard to your question, yes, that's a real problem because you are not only losing important genetic variation but also you are selecting for characteristics which are good in the hatchery. And if you want to use your product out in the wild, then you are going against your ultimate goal. I think the important thing is to define what you want to do with your hatchery product.

Mark Noveck: In the lakes, my particular area of interest is the effects of certain chlorinated hydrocarbons on lake trout development and my research has shown that early developmental stages of the lake trout fry are probably more susceptible to toxicity of these compounds than later stages. This could possibly play a part in the difficulty in natural reproduction of the species. So I think something like that should also be considered in their natural habitat. Especially because of the large lipid area of the yolk sac, for instance, which acts like a sink for compounds like PCB's.

Neil Foster: I just wonder if our preoccupation with hatcheries might be missing the point, to some extent, when in fact one of the things we should be examining very carefully is the adaptive physiology of the lake trout egg on the actual spawning reefs--if we are trying to establish self-sustaining populations in lake trout, shouldn't we know more about the genetic variability in egg physiology, for example?

Henry Booke: Well I think one of the implications, at least I have pointed it out and I think others on the panel have enforced this idea either directly or indirectly, is that you first of all must be able to characterize what you have there and then if that is the form that you are saying is the successful form, then work from that point. But if you're working from the point of view where you are building a husbandry and superimposing those fishes on the natural population, I think that is the long route. Unless of course you are dealing with a put-and-take fishery, then you don't care what you've got as long as the fisherman gets the hooked product, but this is also what I was aluding to at the very beginning. I am worried about the attitude that hatchery people base the rates of success on how many pounds produced--and there is nothing wrong with that. But in the final analysis we are really interested in what is the functional status of that individual fish when it gets into the environment. How does it sustain itself? If it doesn't, then we are blaming it on the environment and not the hatchery. Maybe we're wrong and maybe we're right. But right now we don't have much of a basis or much of an approach, except by these methods which are beginning to be appreciated.

Russ Daly: I think historically we have had year classes, perhaps something like 14 to 16 out there at one time. We only started stocking lake trout in 1965, in realistic numbers anyway. Basically, I think it just takes a lot of lake trout to make a lot of lake trout. And I don't believe that on some of the historic reefs we can do that yet. When we begin to analyze and think about it we took off 6 million pounds, harvested over 17 inches, during the hay-day of Lake Michigan and I don't think we can approach that right now. So I think, as the gentleman pointed out, we are a little premature in expecting something to happen. Even with the original native stocks on Gull Island Shoals, in Lake Superior, it took quite a few years once that population was brought down to its knees to have it restore itself naturally. And then to have it not only restore itself, but to have it virtually wiped out two years ago by an intensive fishery. So we are dealing, I think, a numbers game too, and, getting back to the hatchery product, not withstanding PCB's and the DDT found in sac fry, some of our Lake Michigan progeny now are quite healthy to the fingerling stage. I think we're playing a numbers game.

Andy Lawrie: I would like to make a comment and I'd like to perhaps ask the panel for their judgement on something. Apropos of the rehabilitation of lake trout, at least in Lake Superior, I think something that is very often forgotten is that what we know about this process is the result of a sampling program which is carried out in the largest fresh water body in the world. And it is dependent, at least on the Canadian side, to a very considerable degree, on the patterns of fishing that are carried out by commercial fishermen. This tends to define the places from which the samples come and the time of year in which they are taken. And it thereby determines the information which you get and which you use to judge the success of your program. Now I am not sure that this is equally true on the U.S. side but it is my understanding that the U.S. government utilizes their commercial fishermen who were selected for their faithful reporting in the days when that fishery was free and that they asked these people to continue fishing in the same way and in the same places that they had traditionally done. So, to some extent, I would expect that there is a sampling problem on that side of the line as well. A more explicit comment, that I find interesting and which I must confess I have sat on for a long time, largely because I haven't managed to look at the data from that particular point of view until recently, has to do with the widespread belief and practice that planting large yearling lake trout is the route to success in this game. It has something to do with Dr. Simon's reference to the fact that larger fish at planting yield larger returns to the fisherman--and this is certainly true. I think in terms of everybody operating on Lake Superior, at least. On the other hand, I have recently put together the data from the first four year classes which Ontario planted into Lake Superior--at the two ends of it. By chance those that went in at the east end were small, about 1/5 the size of those that went in at the west end. They didn't come from exactly the same stocks, but they came from stocks that originally were derived from Lake Superior waters and had been held for the most part in inland

lakes in Ontario. Generally speaking, those four year classes survived in very small numbers to reach, despite lamprey predation, ages 11, 12 and 13. First of all, each cohort in the lake very clearly shows increasing survival at a very rapid rate as the result of lamprey control in 1961. That is, the later year classes have been suffering less predation and have done better, and quite substantially better. The data also show a strong bias in favor of the larger fish at the west end, if you look at the total production through their entire period in the lake. But it shows a bias of almost 10:1 in favor of the small fish at the east end of the lake if you look at the numbers that have been present after they have reached the age of maturity; at about 8 years. Being rapid growing and reaching a large size early, in a fishery which is prosecuted by a size selective gear and in the face of a predator which is also size selective for larger fish, is clearly not a very advantageous thing to do if what you are looking for is numbers of mature fish to deposit eggs for you eight years after you have planted them. Now whether or not the analysis will hold true with later year classes, I don't know. But I think that we have been party to an activity which has pleased us very much at the time because we've had visible evidence of success early in the game and which has defeated, or partially defeated, our purpose in the long run. The question that I would like to pose to the panel is this: As a person with responsibility, or some responsibility, for management of fisheries in a province that has 800,000 lakes, I get a little bit up tight at the thought of trying to apply the traditional population dynamics solutions to management problems over bodies of water of that number; not to mention for the infinitude of stocks which you are now presenting me with to occupy those waters. And one thought which has occurred to us, and on which we had been operating and which I think is shared by our colleagues in other jurisdictions, is that we may be able to reduce the magnitude of our problem by looking for so called type-sets of lakes. Lakes which have sufficient in common in terms of the community that they support and the limnology which goes toward determining that community and the climate in which they happen to reside. And if you know one you can say at least with some degree of resolution that you know them all and that you therefore have some kind of handle on how to deal with the populations and communities that reside in these lakes. So the question for you--Dr. Simon's made the comment I think at one point in his reply to an earlier question, and I appreciate why he said it, that the biological problems unfortunately always have a high degree of specificity attached to them. How are we going to cope with this problem of multiple stocks with their different characteristics and their potential adaptation to rather specific parts of the major environment that we are in, if we cannot find some mechanism for recognizing and delineating generalities amongst these?

Henry Booke: I posed that same question before a Sea Grant group about a week ago and we didn't arrive at an answer, even after we were able to describe what we think are two populations of the lake whitefish in the boundary waters of Wisconsin and Michigan. Ultimately when we resolve

fish groups where we can almost pinpoint individuals, certainly populations or subpopulations of fish, our problems will increase. How would you manage fish stocks especially when you have commercial fishing mortality as opposed to all other types of fish mortality? I can't answer that question now but it is something I would like to think about for a long while because I think many of our problems, evolving either from our successes or our failures, are based on the fact that we haven't recognized that we do have genetic variation in fish and it has to be maintained. How do we maintain it under disturbances created by man and perhaps exotics? I consider the lamprey an exotic in the Great Lakes. I wouldn't even attempt to try and answer the question, but I think we should be aware of genetic variation in view of the approaches that we have to study it. We have the stocks. We might just leave them alone and we would always have them. But that won't be the case. That's not realistic. Perhaps someone else on the panel would like to also answer Mr. Lawrie's question?

Peter Ihssen: We now have talked at some length about these genetically differentiated stocks for these 800,000 lakes. Very little is known about the genetic variability among these stocks and I think one of the first things we have to do is get a better inventory of what is out there. We spend a lot of effort looking at the characteristics of these lakes, but we have not spent much effort in looking at the characteristics of the fish in these lakes and I think we have to get some feeling for the genetic resource we have and so I suggest that greater emphasis be placed on collecting this information.

Edward Massaro: I'd like to back up what Peter has said. We have a lot of information about the physical-chemical characteristics of lakes, but in comparison to the gross effort and quantity of money spent in this direction very little has been spent on understanding what the genetics ultimately mean, i.e., what are the physiological and biochemical characteristics of these individual strains, species, or whatever they might be. We don't know at this time. The only way we will find out is by more study.

Fred Allendorf: In terms of 800,000 lakes I'd like to further state what Peter said. I think an important point is that sitting here, we can't tell you how much you can generalize and how much you can't. But there are certain basic questions, which if you had some baseline data, you could answer. You want to know: How different are the different stocks in these 800,000 lakes? You can look at populations from different lakes and you can estimate how genetically similar they are. Maybe you don't have discrete stocks; maybe they are all very similar. The next level is genetic sub-division within lakes. These general principles have to be found; but, they only can be found by going out and looking at the stocks which are present and trying to come up with some handles.

Andy Lawrie: I think a cofactor has been identified here as money. The discussion might be furthered little by another kind of cofactor at a later time and I would challenge you to start breeding biochemists who live to be 99. We are currently in the process of trying to carry out an inventory on these same lakes in Ontario at an apparently substantial rate per annum. Something like 15 or 20 lakes in each of 40 some odd districts every year and our present reckoning is that by spending one day on these bloody lakes collecting the kind of information you can get from a single overnight gill net set, plus the limnological data that you can acquire in 99 years, we may have just begun to get an idea of the problem that we are talking about. So its a problem of very substantial magnitude and of course I agree with all of you. We support genetics activity in our agency because what you say is obviously correct--we don't have a handle on it and we have to have.

Carlos Fetterolf: The Great Lakes Fishery Commission hopes to have a binational workshop on the stock concept at some time in the future. Through the Commission and a recommendation of the Scientific Advisory Committee, Henry Booke put this special session together to serve as a prelude to the stock concept workshop and to also provide us with a resource document which the workshop participants may read and be brought up to some sort of speed before entering the workshop. On behalf of the Commission I would like to thank Henry and his participants for their fine presentations.

Species Management

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The topic "Species Management" may be developed in several ways, but because of the special interest of this group, it appears appropriate to focus on intraspecific diversification as it relates to management or potential management. The special case of wild *vs.* domestic strains will be noted in somewhat greater detail as a practical example of the benefits of how certain stocking requirements can benefit from the attributes of wild trout.

Historically, we have tended to regard a species of salmonid as a homogenous organism, regardless of origin. True, certain life history differences were strikingly apparent within anadromous species, and these were more or less taken into account when fish culture was involved in management. But with more readily domesticated trout, it has been easier, indeed customary, to develop brood stocks as a convenient source of eggs. Thus with the commonly cultured species. . brook, brown, rainbow and cutthroat trouts. . there has been the opportunity to evolve a different philosophy and management procedure based on the fact that the entire operation could be conducted independently of the realities of natural environments. Not so with the cultural programs for salmon and such species as steelhead and lake trout, where either availability, economics or logistical constraints have worked against widespread establishment of brood stocks. The genetic con-

sequences of these two divergent procedures are potentially considerable. The management consequences are no less so, but do seem to be well recognized or considered. Generally, culturists of anadromous species and lake trout lack the brood stock option and are forced to more or less maintain the integrity of the gene pool as it relates to performance in the natural environment. Alternations in genetic make-up inevitably occur, though perhaps inadvertently, within the cultural phases of the life cycle. It does not necessarily follow that these changes are favorable when judged by the ultimate objectives of the program or by standards set by native stocks in natural habitat.

Examples of species or intraspecific diversity are numerous, and only a sampling can be included in this review. We note in passing the substantial evidence for racial or stock differences in certain anadromous species and concentrate on sometimes more subtle variations that may be of equal management importance to inland fisheries. One sport fishing example in the anadromous group, however, that demands attention, is the summer steelhead program developed in Washington (Millenbach 1972). Summer steelhead *Salmo gairdneri* are more desirable from an angling standpoint than winter steelhead, since they enter freshwater from May to late summer in prime condition, with the spawning season upwards of a year away. Through selection of the earliest spawners of the summer run, by advancing the spawning time another two months by light control and by the development of improved fish cultural techniques, it has been possible to produce the smolt-sized yearlings in time to coincide with the natural spring outmigration. The spectacular success of this program is well known.

Production of one-year hatchery smolts has long been the goal in cultural programs for Atlantic salmon *Salmo salar*. But production of smolt-sized salmon does not necessarily mean production of physiological smolts, as a number of agencies have found out. A series of experiences currently underway in Iceland may be cited to illustrate the need to meld manipulation of growth with physiological requirements. This country is especially favored to accelerate salmon growth in hatcheries as abundant geothermal water greatly facilitates raising rearing water temperatures. But returns from releases of smolt-sized yearling salmon, reared inside at elevated temperatures, were much lower than 2-year smolts reared under an ambient temperature regime (Isaakson 1973). When the former group was exposed to the normal natural light cycle and several weeks of winter temperatures, survival response, measured by return to the stream, was immediate. Returns from the cohort of the 1972 year class reared under these conditional manipulations, released in spring 1973 and returning as grilse in 1974, was the highest among several groups, including those of the traditional two-year smolts (Isaakson, personal communication).

In Norway, Professor Skjaerwold is currently conducting an elaborate experiment to test for differences in growth of juveniles among a number of stocks of Atlantic salmon. Norwegian colleagues inform us that already there are indications of substantial strain differences in this parameter that could have application in management (Kjell Jensen, personal communication). Earlier, Carlin (1969) showed striking differences occurred in survival, ranging from one to seventeen percent, between different families of salmon reared under comparable conditions.

A New York example illustrates different results from stocking two forms of indigenous lake trout *Salvelinus namaycush*, one from the Adirondack uplands, another from the Lake Ontario – Finger Lakes basin. These forms represent two different stocks that invaded the area following the retreat of the Laurentian ice sheet. A stock from an eastern refugium gained access to the mountain areas via high level glacial lakes that predated the opening of waterways to the west, when lake trout from a Mississippi refugium invaded the Great Lakes basin. Both Adirondack and Finger Lakes stocks of trout were used for stocking purposes, but since eggs were much more readily obtainable from the latter source, use of the Finger Lakes strain in the Adirondacks was inevitable. Marked plantings over the past 25 years have shown virtually no survival of the Finger Lakes strain when planted in Adirondack waters, although companion releases of native strains did show reasonable recoveries. Reasons for this difference have not been inevstigated, but the management implications are obvious.

Some of us have given thought to the need for chemical lamprey *Petromyzon marinus* control in Lake Ontario. Lamprey were there for some time before the lake trout declined, and lamprey and substantial salmonid populations coexist in two of the Finger Lakes tributary to Lake Ontario. Was there some significance to the high recovery of a token planting of Finger Lakes strain lake trout made in Lake Ontario in the 1950's, recoveries that were made in commercial nets that completely decimated the hatchery stock after two or three years? A group of scientists discussing for two days the apparent anomaly of lamprey-trout relation in central New York lakes, *vis-a-vis* the upper Great Lakes, came up with the euphemism of "accommodation". In our simplistic approach to solving an immediate problem have we overlooked something in species management that may have been already worked out by nature?

Acute environmental degradation through acid precipitation is developing regionally in parts of eastern Canada and the United States and the southern tip of Scandinavia, where increased acidity of precipitation originating in the Ruhr Valley of Germany and from Britain has rendered many lakes and streams fishless (Oden and Ohl, 1970). In the past two decades, many lakes in the southwest corner of New York's Adirondack Mountains have experienced 10 to 100 fold increases in acidity, and, as in Scandinavia, entire fish populations of all native species have been decimated in some of these waters. Solutions to the problem are long term, and, while local alkalization may be practical on a limited basis, what about the existence of strains of fish more tolerant of low pH? Recent findings in Pennsylvania, where the problem is acid mine drainage, have indicated a wide range in acid tolerance of brook trout *Salvelinus fontinalis*, both by individuals in the same group as well as between groups (Dunson and Martin 1973). One of the several strains involved in these tests was a domesticated New York strain that proved the least resistant to this kind of environmental stress. A remarkable body of water exists in New York, however, Honnedaga Lake, where brook trout live at pH values fluctuating about 5.0 and with the additional burden of concentrations of zinc at 0.02 to 0.15 ppm. Nonacclimatized test fish live only 1 to 2 days under most conditions (Schofield 1965). We do not know if there is any genetic basis for this adaptation.

Thermal degradation has claimed many former trout waters. There is a marginal range of habitat where a more thermally resistant strain of trout might be useful in management. Unfortunately, the choice of "ideal" water for hatchery purposes, i.e., spring water with minimal seasonal variations in temperature, probably mitigates against retention of genes controlling extremes of temperature tolerance when brood stocks are maintained as closed systems. Studies on the hybrid splake *fontinalis x namaycush* where temperature preference and lethal temperatures differ widely in the parental stocks, demonstrated thermal inheritance of resistance to high temperatures in one of the backcross hybrids involving the brook trout maternal parent (Ihssen 1973). Geographical intraspecific variations in lethal temperatures have been demonstrated for two subspecies of Arctic charr *Salvelinus alpinus* (McCauley 1958).

Splake are a new breed of salmonid filling a useful ecological role in management. Experience in Ontario (Martin and Baldwin 1960), New York and elsewhere indicated that the hybrid had satisfactory survival and excellent growth in lakes containing non-trout species such as suckers, minnows and sunfish. Like lake trout, splake turn readily to a piscivorous diet, but seem more readily available seasonally for shallow water angling.

Ontario has also conducted a forthright selection program for splake retaining the deep swimming habit of lake trout, but earlier age at maturity of brook trout (Berst and Spangler 1970; Tait 1970; Ihssen and Tait 1974). The objective was to develop a fish occupying the niche vacated by lake trout with the advent of lamprey, but mature at a size below that most subject to lamprey attack. The breeding program was successful in retaining these characters by the F_6 or F_7 generation, although it is not yet clear if management objectives have been achieved.

Food habits of salmonids, either intra- or interspecifically, provide some interesting biological differences and probably more management options than we now take advantage of. Scandinavians, especially in Sweden, have long been preoccupied with interactions of populations of charr *Salvelinus alpinus* and/or brown trout *Salmo trutta* because of the intense program of river impoundment and lake level regulation. Nilssen and Pejler (1973) and Aass (1973), among others, have shown that when charr or brown trout occur allopatrically, food habits are quite different than when they occur sympatrically. In the former case, both tend to feed on benthic organisms, while in the latter, charr become plankivorous, leaving the benthic littoral fauna to trout. In North American lakes it is common to manage for more than one species, frequently with rainbow trout *Salmo gairdneri* as one of the combination. Since this species tends to utilize plankton more efficiently than either brook or brown trout, for example, differences in the feeding habits and other requirements provide for variations in season and methods of capture, a good example of management at the full species level.

Sympatric populations of Arctic charr showing great divergencies in growth rates are most intriguing and common throughout the range of this species. A stunted form, rarely exceeding 9 to 10 inches in length, is largely a plankton feeder, while a larger or "normal" form has benthic or even piscivorous food habits. Both forms also occur allopatrically. Recent studies making use of electrophoretic techniques have confirmed that observed ecological and morphometric characters (size and number of gill rakers, position of mouth) are indicative of real population differences (Lenart 1972). This differentiation is believed to have taken place prior to the last deglaciation (Behnke 1972 and others). What use can be made of this kind of ecological diversity?

In some of the European lakes containing both dwarf charr and brown trout, trout may reach a large size feeding on charr. Evidence is accumulating that fish eating proclivities are, at least to some degree, under genetic control. Aass (1973, and personal communication) describes instances

of where strains of fast growing, charr-eating brown trout retained this habit when introduced into other high-mountain Norwegian stunted charr lakes, but were out-performed by the native strain of brown trout when transferred to lowland lakes. Aass points out that while the fish eating habit may have evolved over thousands of years in some populations, there are examples of its development in reservoirs during the past 40 years, and that transfers from these populations also retained the fish eating habit.

In North America we have similar examples of predator-prey relationships that may be more than casual or opportunistic: the giant Lahontan cutthroat of Pyramid Lake *Salmo clarki henshawi*, the pure form of which may be extinct (Behnke, MS 1971); the Gerrard strain of Kamloops rainbow trout from Kootenay Lake (Hartman 1969), and Kamloops trout in Pend d'Oreille, both populations utilizing kokanee salmon as forage; and the large brook trout fomerly inhabitating the Rangeley Lakes of Maine that fed on the now extinct population of stunted charr or blueback trout *Salvelinus alpinus oquassa* (Kendall 1918).

There is much evidence bearing on differences in vulnerability to angling, a reflection of varying food habits and/or behavioral characteristics. Many of these involve domesticated stocks and are not considered here. Two strains of cutthroat living in the same body of water in Colorado showed up quite differently in angling catches over a two year period (Trojnar and Behnke 1974). One, the Snake River cutthroat, appeared three to four times as often as expected on the basis of the proportion present in the lake. This same phenomenon is commonly evident in Adirondack test waters stocked with more than one strain of brook trout. One experiment involved a native Adirondack strain (Horn Lake), a Canadian strain (Assinica Lake) and domestic fish, and the angling catch expressed as a percentage of the stock on hand at the beginning of the season was: Assinica 70, Domestic 50, Horn Lake 20. Trout caught while surface feeding during the heat of mid-day were invariably of the Assinica strain.

We have reviewed a sampling of options that have been employed in species management, others that might be explored to a greater extent. In either case, the innovation in some way usually exploits diversification combined with an awareness of physiological or ecological needs. These examples refute the notion of a homogeneous equally useful strain for multipurpose management.

The examples have focused on natural populations or those resulting from plantings of fish from non-domestic stocks. Of particular relevance and interest in this Symposium is the relative performance of wild and domesticated strains of trout. A substantial body of information of wide-spread origin has accumulated on this subject, but at the risk of being provincial, we have to review the essence of this phase of species management on the basis of our New York experiments and experiences (Vincent 1960; Flick and Webster 1962, 1964; Flick 1971; and unpublished data). Current awareness and contributions in this area in California, however, should be noted in passing (for example, Cordona and Nicola 1970, and W. D. Weidlein, personal communication).

New York fish managers, as well as others, have noted that generally low angling or test netting recoveries followed plantings of domesticated strains of brook trout in the Adirondack Mountains, New York (Zilliox and Pfeiffer 1960). These observations included the mitigated environment of reclaimed waters devoid of competitive fish, where few survivors were found after age two. The longevity of natural populations of wild brook trout, in contrast commonly extended to three or four years, and age five fish were not rare (loc cit and personal observations of authors). An early experiment by Greene (1952) suggested that wild hatchery reared trout offered management potential. This and other considerations prompted a program initiated in 1958 to quantify more precisely relative performance between domestic and F_1 wild[1] hatchery reared strains. The investigation was funded from private sources and conducted on private lands, thus greatly enhancing flexibility of operations and control over angling.

Data on survival within the first year of life was obtained from a small (0.5 acre) drainable pond fed by a small cold brook. Spring fingerlings, released in spring, were inventoried in autumn, and sometimes returned to the pond and inventoried a final time the following spring. Data on survival through the life span of any given cohort was obtained by planting spring or fall fingerlings in natural ponds containing no fish or only brook trout, and estimating standing crop at semiannual intervals. All of the waters used (Long, Bear and Bay Ponds) were located within distances of five miles of one another in the headwaters of the St. Regis River in the northern Adirondack Mountains.

Six wild strains of trout were involved in these studies, four native to the Adirondacks and two recently naturalized in New York waters from the James Bay area of Quebec; performance of only four will be cited in this paper. The New York locations were a mountain-top pond containing only native trout (Horn Lake), a large, deep, acidotropic body of water (Honnedaga Lake), and a small upland brook (Long Pond Outlet). Longevity in all populations was a minimum of five years, but the stream population was stunted, averaging 6 to 7 inches in length in trap and angling

samples, and natural mortality was excessive after age two. Canadian strains of trout were from the Assinica or Temiscamie area southeast of James Bay, where they attained a size of over five pounds and longevity extended to nine years (confirmed by known age naturalized trout).

Domestic strains of trout were from two sources, a so-called "Berlin" strain cultured at the National Fish Hatchery at Cortland, New York, and a "New York" strain, generally used for stocking in that State. Both had been propagated from brood stocks with a long history of a closed gene pool. Under cultural conditions, both showed rapid growth, attaining lengths of 4 to 6 inches as fall fingerlings and an early age at maturity (0+ in larger males and I+ for females). Compared with wild hatchery reared groups, they were robust in appearance and exhibited a wide range of behavioral differences (Vincent, loc cit).

Results of a series of experiments involving five year classes comparing two to seven different New York brook trout strains in each experiment were consistent in showing substantially higher survival of wild groups between spring and fall of the first year of life (Flick and Webster 1964 and unpublished). Among the twelve paired comparisons, survival in wild strains averaged 25 percent higher, with a range of 12 to 43 percent. Two experiments evaluated the effect of parental history on survival, but it made no difference whether all groups were reared to maturity in a hatchery environment on a standard hatchery diet or in a natural environment on natural food: survival of wild strains was always higher. One experiment included two interstrain hybrids between wild and domestic stocks, and both hybrids also proved superior in survival to that exhibited by the domestic parent.

Survival and production data over the life span of the several strain cohorts obtained from semi-annual estimates of population size and growth in natural ponds were no less convincing on the positive attributes of wild strains in a pond management program for brook trout. All five experiments with two or more strain-cohorts led to essentially the same conclusions. The 1960 year class in Long Pond provides an example of the results obtained and data on planting is given in Table 1. The larger size of domestic fingerlings reflects the adaptation of this strain to cultural practices and would be regarded of positive survival value. To eliminate possible effects of fin clipping, one of the ventral fins was used to identify the domestic group and one of the wild strains.

Population size of the three cohorts in the 1960 year class is shown in Figure 1. Domestic strain fish were essentially extinct at age three, while both wild strains existed in substantial numbers. Wild fish at all ages dominated the population. Since angling took place from age two onwards, the curves reflect losses due to this source as well as natural causes. Domestic strain fish were initially larger in weight, but all groups reached a climax size of about 0.8 pounds (Figure 2). This size is not a definitive parameter, since it merely relfects response to conditions of stock density during the course of the experiment.

The combined effects of growth and survival are depicted by the biomass (population size x mean weight) present through the life span (Figure 3). Also shown are the pounds of fish in each strain removed by angling. After age one, it is clear that a substantially high biomass is on hand in the two wild strains, also reflected in angling catch. Here a total of 40, 73 and 68 pounds was harvested from Domestic, Long Pond Outlet and Honnedaga strains, respectively. Reduced catches during ages 4 and 5 were due to decreases in fishing effort.

Comparison of the three groups is facilitated by computation of gross production (Table 2); this eliminates the effects of angling and includes an estimate of all biomass elaborated, including losses to natural causes during each semi-annual interval (Ricker 1958). Gross production is readily transformed (after subtracting the weight at stocking of the strain-cohort) to a ratio showing the number of pounds produced in the pond per pound of trout stocked. For the Domestic strain, this amounted to 7 pounds, for the two wild strains, 80 and 51 pounds or a proportional rating of 1:12:7. A similar, but independent rating, can be calculated from biomass harvested by angling divided by biomass stocked, or 1:13:8. These relative ratings were calculated for five experiments, and without exception, wild strains gave substantially higher recoveries when judged by these parameters (Table 3).

Performance of the interstrain hybrid of Assinica x Domestic in Bay Pond was especially notable. This cohort exhibited faster initial growth and higher survival than either of the pure parental strains, resulting in lifetime gross production estimates of about 1,600 pounds for the hybrids, compared with 460 and 265 pounds of the wild and domestic parental stocks. In five angling seasons, 432 pounds of hybrids were removed, averaging 1.4 pounds. In several other waters, these interstrain hybrids have consistently outgrown domestic strains stocked at the same time, suggesting hybrid vigor in this character.

Aside from intrinsic values in data showing wide disparity of performance under natural field conditions, the ratios in Table 3 have direct bearing on benefit-cost judgments when hatchery reared

fish are used in the kind of management programs under consideration. They form a viable alternative for judging the effectiveness of a hatchery product compared with traditional methods based on hatchery performance alone. The several experiments exhibited a range of values in favor of wild strains, but an average of about five times higher weight recovery can be taken as the basis for a working estimate (Table 3). Thus, for any given unit cost per pound of fish in the hatchery, wild strains produced five times as much poundage in nature as domestic strains. This assumes no differential in rearing costs between the two groups, but there is considerable cushion to absorb any likely additional cost associated with raising wild strains. Furthermore, most hatchery techniques and diets have been developed with domesticated strains in mind so that changes favoring wild strains could modify potential added production costs.

The use of wild strains of trout for stocking appropriate waters in rehabilitation or maintenance programs may constitute only a small part of the total propagation program of governmental agencies. But economic payoffs seem assured, and the development of high quality angling experiences through appropriate regulations and use of special strains has a definite spot in the repertoire of fishery managers.

Finally,we would like to echo the plea, so earnestly expressed on numerous occasions by Dr. Robert Behnke of Colorado State University, that we preserve the genetic integrity of such of our heritage of salmonid fishes as we will have left, and that as managers, we recognize the genetic diversity and plasticity of salmonid fishes in the context of species management and use it when appropriate for innovative improvements in the more traditional approach to management (Behnke 1971).

[1]The term F_1 wild is used here to clarify the semantics connected with the term wild as loosely applied in the literature, and signifies the first generation of hatchery reared stock from original native populations unaltered (presumably) by previous cultured introductions. This convention was suggest by Moyle (1966 MS).

Table 1. Stocking data on the 1960 Year Class released in Long Pond. October 1960.

Strain	Number	Average Length (inches)	Total Weight (pounds)	Fin Clip
Domestic	350	4.9	17.5	RV
Long Pond Outlet	350	2.7	2.4	Ad
Honnedaga	350	2.9	3.8	LV

Table 2. Computation of relation of the biomass of gross production to biomass of trout stocked. 1960 year class - Long Pond.

Strain	Gross Production (pounds)	Biomass Stocked (pounds)	Gross Prod. / Biomass Stocked	Rating
Domestic	116	17.5	7	1
Long Pond Outlet	193	2.4	80	12
Honnedaga	194	2.9	66	10

Table 3. Gross production and yield to angling of brook trout in relation to biomass stocked in five experiments. (Angling ratio in parentheses.)

Strain	Bear '59s	Long '60f	Long '61s	Bear '61s	Bay '68f
Domestic	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
Long Pond Outlet	3 (4)	12 (13)			
Honnedaga	4 (5)	10 (10)			
Horn			7 (6)	4 (3)	
Assinica					3 (3)
Assin x Dom					7 (12)
Temiscamie					2 (4)

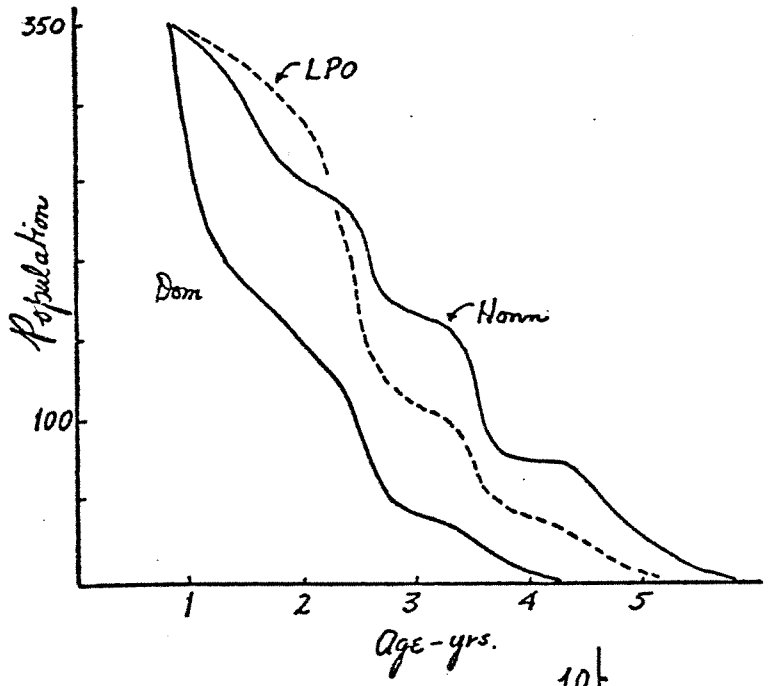

Figure 1. Population size of three strains of brook trout planted as fall fingerlings and estimated at semiannual intervals. 1960 year class, Long Pond.

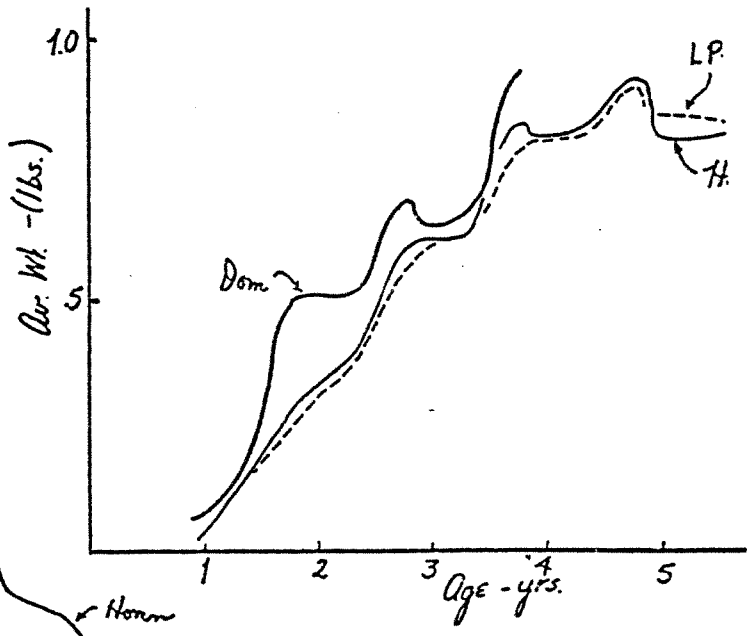

Figure 2. Growth in weight of three strains of brook trout. 1960 year class, Long Pond.

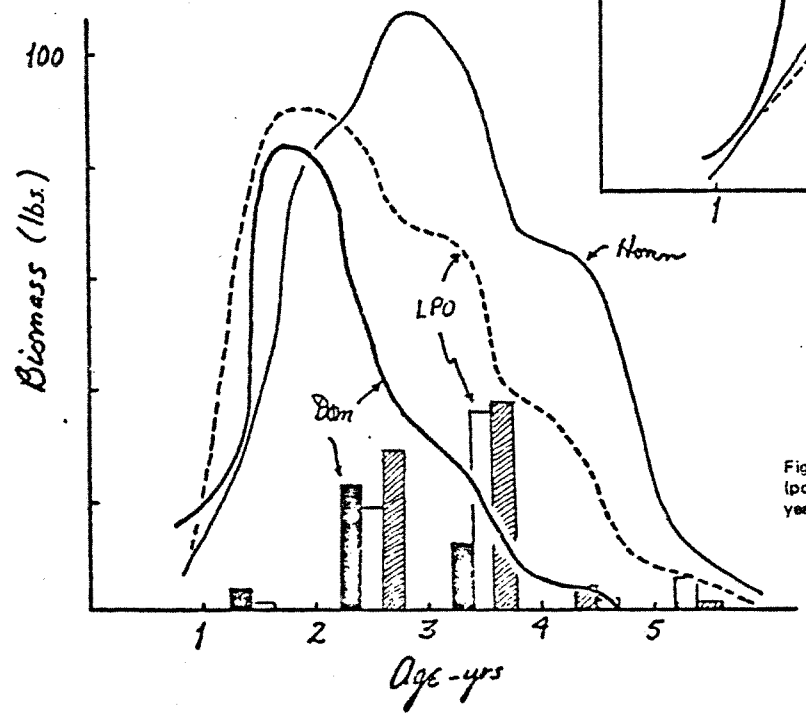

Figure 3. Estimated biomass and angling catch (pounds) in three strains of brook trout. 1960 year class, Long Pond.

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