NATIONAL MARINE FISHERIES SERVICE
COASTAL ZONE & ESTUARINE STUDIES
NORTHWEST FISHERIES CENTER
2725 MONTLAKE BLVD. E.
SEATTLE, WASHINGTON 98112

INPFC ANNUAL REPORT 1971 (1973)

possible taxonomic group. Analysis of such samples at the plankton sorting center established at the NMFS Northwest Fisheries Center will provide an additional parameter to assess the distribution and relative abundance of important fishes as well as of forage fishes and plankton which constitute the food chain.

Endorsement of and participation in the ichthyoplankton phase of the MARMAP Program by national sections of the INPFC through the aegis of the Oceanography Subcommittee of the Committee on Biology and Research would greatly accelerate the acquisition of our knowledge not only of the salmon environment but that of other species as well.

Literature Cited

Favorite, F., and W. J. Ingraham, Jr. MS 1971. Influence of Bowers Ridge on circulation in Bering Sea and influence of Amchitka Branch, Alaskan Stream, on migration paths of sockeye salmon. *In* Biological oceanography of the northern North Pacific Ocean. Motoda Commemoration vol., Fac. Fish., Hokkaido Univ. (In press.)

Favorite, F., W. J. Ingraham, Jr., and D. M. Fisk. 1972. Oceanography. INPFC, Anna. Rep. 1970: 90–98.

National Marine Fisheries Service. 1970–71. Fishing information. Natl. Oceanic Atmos. Admin., Natl. Mar. Fish. Serv., Fish.-Oceanogr. Center, La Jolla, Calif. Issues of December 1970, March and June 1971. Various pagination.

INHERITANCE OF BIOCHEMICAL VARIANTS IN THREE SPECIES OF PACIFIC SALMON AND RAINBOW TROUT

by Fred M. Utter, James L. Mighell, and Harold O. Hodgins*

In previous INPFC Annual Reports we have described several biochemical variants in fishes that, based on several lines of indirect evidence, we have assumed to be genetic (Utter and Hodgins, 1971; Utter, Hodgins, and Johnson, 1972). If biochemical variants are truly genetic in nature, they can be useful in identifying species and races of fish and in determining relative influences of genetic and/or environmental factors on ocean distribution, areas of origin, and on stock capabilities and characteristics.

The strongest tests for determining genetic or nongenetic mechanisms that control biochemical variants are tests with data from breeding experiments. In interpreting the results of breeding experiments we look for evidence of multiple loci or a single gene locus, for evidence of different genes (alleles) at a locus, and for numerical ratios of progeny that permit interpretation of dominant, codominant, or recessive patterns of expression of genes. If numbers of progeny of various biochemical types fit closely enough a pattern consistent with that predicted from parental types, strong evidence will then have been obtained that the biochemical variants directly reflect genes and that the inheritance is Mendelian.

Allelism (genes at a single locus) of numerous biochemical variants described by this laboratory has been inferred by lines of evidence including: (1) starch gel electrophoretic patterns for particular proteins that are consistent with allelic variants of the same proteins in other species, (2) repeatability of expression from duplicate samplings of a given individual, (3) stability of expression over long developmental periods, and (4) conformance of frequencies of phenotypes to a Hardy-Weinberg statistical distribution. (See Utter et al., 1972, for a review of the biochemical systems through June 1970 and Hodgins, 1972, for a more detailed statement of our criteria for allelism.) Although, as stated above, we have assumed that biochemical variants that meet these criteria are indeed allelic, we have not previously demonstrated such a relation through data from breeding experiments.

This paper presents biochemical genetic data for progeny of parents of known phenotypes for the following species and biochemical systems: sockeye salmon—phosphoglucomutase (PGM); chinook salmon—tetrazolium oxidase (TO); coho salmon—transferrin (Tfn); rainbow trout—alpha glycerophosphate dehydrogenase (AGPD), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), TO, and Tfn. These data substantiate hypotheses of Mendelian inheritance for the systems investigated and support such hypotheses for the same systems in other salmonid species where family data are not, as yet, available.

Experimental Procedures

Parents of progeny used in this study were obtained as follows: coho salmon—Skykomish Hatchery (Washington State Department of Fisheries); chinook and sockeye salmon—adult fish returning to this facility (the National Marine Fisheries Service laboratory in Seattle): rainbow trout—adult fish reared at this facility; and male anadromous rainbow trout (steelhead) from the Chambers Creek Hatchery of the Washington State Department of Game. All progeny were hatched and reared under similar conditions at this facility. Chinook and sockeye salmon progeny were tested between 2 and 4 months after hatching. Coho salmon and rainbow trout were

* National Marine Fisheries Service, Northwest Fisheries Center, Seattle.

tested between 6 and 9 months after hatching; somewhat larger fish were required from these species because a blood sample—difficult to obtain from small fish—was needed to test for Tfn.

All biochemical systems but Tfn were found in skeletal muscle extracted as described by Utter and Hodgins (1970). Blood plasma for Tfn typing was obtained by withdrawing blood from the pericardial cavity of freshly killed fish with a capillary pipette. Approximately one drop of whole blood was expressed into two drops of Alsever's solution (a citrate-dextrose-saline anticoagulant) in 10×75 mm culture tubes. Each sample was centrifuged at 1000×g for 3 minutes before testing.

Details of electrophoresis were described by Utter and Hodgins (1969). Buffer systems for the respective biochemical variants were as described by Utter and Hodgins (1972). Specific staining methods for enzymes followed those described by Shaw and Prasad (1970). Tfn was detected by a nonspecific protein staining method using a 0.1% nigrosin-buffalo black solution dissolved in a 5:4:1 water-methanol-acetic acid mixture. Destaining was carried out with the water-methanol-acetic acid solution.

Descriptions of each of the biochemical systems studied in this report have been given elsewhere (Utter and Hodgins, 1970; Utter, Ames, and Hodgins, 1970; Utter, 1971; Utter and Hodgins,

TABLE 16. Observed and (in parentheses) expected PGM phenotypes of parents and progeny in sockeye salmon matings assuming Mendelian inheritance.

Lot	Progeny phenotypes			Phenotypes of parents	
	AA	AB	BB	Male	Female
1	0 (0)	90 (99.5)	109 (99.5)	AB	BB
2	0 (0)	90 (98.5)	107 (98.5)	BB	AB
3	0 (0)	78 (84)	90 (84)	BB	AB
Control	0	0	100	BB	BB

TABLE 17. Observed and (in parentheses) expected TO phenotypes of parents and progeny in chinook salmon matings assuming Mendelian inheritance.

Lot	Progeny phenotypes			Phenotypes of parents	
	AA	AB	BB	Male	Female
2–10	12 (10.5)	9 (10.5)	0 (0)	AB	AA
2–11	12 (10)	21 (20)	7 (10)	AB	AB
2–13	10 (10)	20 (20)	10 (10)	AB	AB
4–14	8 (11)	14 (11)	0 (0)	AA	AB
5–10	0 (0)	29 (30)	1 (0)	AA	BB
5–12	0 (0)	40 (40)	0 (0)	BB	AA
7–13	0 (0)	14 (14.5)	15 (14.5)	BB	AB
Control	100	0	0	AA	AA

TABLE 18. Observed and (in parentheses) expected Tfn phenotypes of parents and progeny in coho salmon matings assuming Mendelian inheritance.

Lot	Progeny phenotypes						Phenotypes of parents	
	AA	AC	CC	AB	BB	BC	Male	Female
23	0 (0)	17 (17.5)	18 (17.5)	18 (17.5)	0 (0)	17 (17.5)	BC	BC
51	0 (0)	0 (0)	27 (30)	0 (0)	0 (0)	33 (30)	BC	CC
53	0 (0)	31 (25)	19 (25)	0 (0)	0 (0)	0 (0)	AC	CC
Control	0	0	20	0	0	0	CC	CC

TABLE 19. Observed and (in parentheses) expected TO phenotypes of parents and progeny in rainbow trout matings assuming Mendelian inheritance.

Lot	Progeny phenotypes						Phenotypes of parents	
	AA	AB	BB	AC	BC	CC	Male	Female
1313×1301	0 (0)	19 (20)	21 (20)	0 (0)	0 (0)	0 (0)	BB	AB
1343×1347	0 (0)	0 (0)	18 (15)	0 (0)	26 (30)	16 (15)	BC	BC
SH2	0 (0)	20 (21)	22 (21)	0 (0)	0 (0)	0 (0)	AB	BB
Control	0	0	45	0	0	0	BB	BB

TABLE 20. Observed and (in parentheses) expected Tfn phenotypes of parents and progeny in rainbow trout matings assuming Mendelian inheritance.

Lot	Progeny phenotypes			Phenotypes of parents	
	AA	AB	BB	Male	Female
1313 × 1301	7 (8.3)	22 (16.4)	4 (8.3)	AB	AB
1356 × 1341	32 (30)	28 (30)	0 (0)	AB	AA
Control	50	0	0	AA	AA

TABLE 21. Observed and (in parentheses) expected AGPD phenotypes of parents and progeny in rainbow trout matings assuming Mendelian inheritance.

Lot	Progeny phenotypes			Phenotypes of parents	
	AA	AB	BB	Male	Female
SH2	0 (0)	20 (21)	20 (21)	AB	BB
Control	0	0	140	BB	BB

TABLE 22. Observed and (in parentheses) expected LDH phenotypes of parents and progeny in rainbow trout matings assuming Mendelian inheritance.

Lot	Progeny phenotypes			Phenotypes of parents	
	$B^{2'}B^{2'}$	$B^{2'}B^{2''}$	$B^{2''}B^{2''}$	Male	Female
SH8	0 (0)	45 (45)	0 (0)	$B^{2''}B^{2''}$	$B^{2'}B^{2'}$
SH14	0 (0)	11 (11)	0 (0)	$B^{2''}B^{2''}$	$B^{2'}B^{2'}$
Control	200	0	0	$B^{2'}B^{2'}$	$B^{2'}B^{2'}$

1972). Phenotypic designations given in these papers are retained here.

The phenotypes of most of the systems studied here are codominantly expressed by starch gel electrophoresis (i.e., all alleles of a genetic system are fully expressed in heterozygous individuals). Thus the presumed genotype can be interpreted directly from a given phenotype. The data for the fully codominant systems are given in Tables 16 through 22. In these systems, the observed phenotypes of the parents and progeny are presented and the expected numbers of progeny—assuming simple Mendelian inheritance—are shown in parentheses. In systems involving two alleles, all three possible phenotypes (the two homozygous phenotypes and the heterozygote) are listed, although some of these phenotypes may not be expected to occur from these matings. Similarly, all six possible phenotypes are listed in systems involving three alleles. Controls are data from one or more matings where both parents have the same homozygous phenotype.

VARIANTS IN PROGENY AND THEIR PARENTS

Pacific Salmon

Each of the sockeye salmon PGM crosses (Table 16) involved a heterozygous individual with a common (BB) homozygote. Although none of the crosses deviated significantly from expected ratios, an excess of homozygous progeny was found in all three matings. These deviations become significant when the data are pooled (Chi-square=4.04, degrees of freedom=1, .05>P for chi-square test >.01), suggesting that some selective factor favored the BB phenotype.

The ratios of TO phenotypes in chinook salmon progeny (Table 17) approximate expected values based on parental phenotypes. The only qualitative exception to Mendelian inheritance in all of these matings is seen in lot 5–10. In the AA×BB cross, only heterozygous progeny are expected; however, one BB individual was found in this lot. It seems most likely that this individual is from another lot and had been placed in the wrong holding tank because the remaining individuals are all of the expected phenotype.

With the possible exception of lot 53, all of the ratios of Tfn phenotypes of coho salmon progeny (Table 18) conform closely to expected values. This support of the assumption of Mendelian inheritance of coho salmon transferrins clears the way for more extensive use of this system as a population marker in this species. The marked differences in distribution of transferrins between Puget Sound and Columbia River coho (Utter et al., 1970) indicate that this system should be useful for comparisons of populations separated by relatively small distances.

Rainbow Trout (Steelhead)

The numbers of progeny of the different TO phenotypes (Table 19) all approach expected values based on the phenotypes of the parents. We were fortunate in finding two BC phenotypes (a reasonably rare phenotype in our stocks) among our parent fish.

Although numbers of progeny of both Tfn crosses (Table 20) fell within acceptable limits, the double heterozygous cross (1313×1301) approached significance (i.e., chance might not account for differences between observed and expected—.2>P for chi-square test >.05). Should this trend persist upon further sampling, Tfn may prove useful in demonstrating effects of environmental variables as it has in voles (Tamarin and Krebs, 1969) and as has been suggested for skipjack tuna (Fujino and Kang, 1968).

Only one of our spawning rainbow trout had a heterozygous AGPD phenotype. The ratio of phenotypes among its progeny (Table 21) clearly in-

dicates Mendelian inheritance.

Both matings involving LDH variants (Table 22) were between homozygous $B^{2''}B^{2''}$ males and $B^{2'}B^{2''}$ females. All progeny tested, as expected, had heterozygous ($B^{2'}B^{2''}$) phenotypes.

A possible exception to the rule of codominance occurs in MDH variants of rainbow trout. Bailey et al. (1970) observed that two loci of rainbow trout appear to code for MDH subunits (B′) giving rise to active enzymes having the same electrophoretic mobility. One of these loci also coded for an allelic subunit (B). Because the B′ subunit was synthesized in all individuals, it was impossible to qualitatively differentiate between BB′ heterozygotes and BB homozygotes. (Using densitrometric methods, Bailey et al., 1970, were able to quantitatively differentiate these two genotypes on the basis of different intensities of staining of bands having the same mobilities; our MDH phenotypes do not appear to reflect these differences as clearly.) In spite of this limitation, our MDH data (Table 23) support the hypothesis of Mendelian inheritance. The heterozygous parent was taken from a population where the frequency of the MDH variant was reasonably low (proportion of variant MDH phenotypes=0.17); it is, therefore, most likely that its genotype was BB′ (rather than BB). The progeny data indicating approximately equal numbers of homozygous and heterozygous individuals are consistent with this assumption.

TABLE 23. Observed and (in parentheses) expected MDH phenotypes of parents and progeny in rainbow trout mat- assuming Mendelian inheritance.

	Progeny phenotypes		Phenotypes of parents	
Lot	B′B′	BB′	Male	Female
SH8	20 (22.5)	25 (22.5)	BB′	B′B′
Control	68	0	B′B′	B′B′

INFERENCES FROM VARIANT DATA

Data from each of the crosses described above are consistent with an assumption of Mendelian inheritance for the genes controlling the observed biochemical variants. This finding was expected and virtually eliminated the possibility that these variants could be artifacts reflecting different environmental stresses (although they may indirectly reflect environmental differences through processes of natural selection). The data also suggest that these variants do not vary in chinook salmon, coho salmon, sockeye salmon, nor in rainbow trout between the juvenile life history stage and time of spawning.

The data give strong support for hypotheses of Mendelian inheritance for similar (probably homologous) variants of other salmonid species where family data have not yet been obtained. Such variants we have studied include: LDH of sockeye salmon (Hodgins, Ames, and Utter, 1969), PGM of rainbow trout (Roberts, Wohnus, and Ohno, 1969; Utter and Hodgins, 1972), LDH variants of the B^2 locus in chum salmon and coho salmon (Utter et al., 1972), and MDH variants in pink salmon (Utter et al., 1972).

It is now possible to extend use of these variants with increased confidence in their genetic nature. Their use in racial studies has been well documented (Utter et al., 1972) and will continue. Studies of possible genetic linkages in rainbow trout are in progress within our stocks. The variants also are potentially important in studying possible selective factors that influence their presence in different environments. An understanding of these factors may lead to a much clearer understanding of the significance of these biochemical genetic variants in populations.

LITERATURE CITED

BAILEY, G. S., A. C. WILSON, J. E. HALVER, and C. L. JOHNSON. 1970. Multiple forms of supernatant malate dehydrogenase in salmonid fishes. J. Biol. Chem. 245(22): 5927–5940.

FUJINO, K., and T. KANO. 1968. Transferrin groups of tunas. Genetics 59: 79–91.

HODGINS, H. 1972. Serological and biochemical studies in racial identification of fishes. *In* Raymond C. Simon and Peter A. Larkin (editors), The stock concept in Pacific salmon, p. 199–208. H. R. MacMillan Lectures in Fisheries, Univ. Brit. Columbia, Vancouver, B.C.

ROBERTS, F. L., J. F. WOHNUS, and S. OHNO. 1969. Phosphoglucomutase polymorphism in the rainbow trout, *Salmo gairdneri*. Experientia 25: 1109–1110.

SHAW, C. R., and R. PRASAD. 1970. Starch gel electrophoresis of enzymes: A compilation of recipes. Biochem. Genet. 4: 297–320.

TAMARIN, R. H., and C. J. KREBS. 1969. *Microtus* population biology. II. Genetic changes at the transferrin locus in fluctuating populations of two vole species. Evolution 23: 183–211.

UTTER, F. M. 1971. Tetrazolium oxidase phenotypes of rainbow trout (*Salmo gairdneri*) and Pacific salmon (*Oncorhynchus* spp.). Comp. Biochem. Physiol. 39B: 891–895.

UTTER, F. M., W. E. AMES, and H. O. HODGINS. 1970. Transferrin polymorphism in coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 27: 2371–2373.

UTTER, F. M., and H. O. HODGINS. 1969. Lactate dehydrogenase isozymes of Pacific hake (*Merluccius productus*). J. Exp. Zool. 172: 59–67.

———. 1970. Phosphoglucomutase polymorphism in

sockeye salmon. Comp. Biochem. Physiol. 36(1): 195–199.
———. 1971. Biochemical variants in the Pacific hake. INPFC, Annu. Rep. 1969: 112–113.
———. 1972. Biochemical genetic variation at six loci in four stocks of rainbow trout. Trans. Am. Fish. Soc. 101(3): 494–502.
UTTER, F. M., H. O. HODGINS, and A. G. JOHNSON. 1972. Biochemical studies of genetic differences among species and stocks of fish. INPFC, Annu. Rep. 1970: 98–101.

PITUITARY GONADOTROPIC ACTIVITY AND OVARIAN ANTIGENS FOR PREDICTING AGE AT MATURITY OF HIGH SEAS SOCKEYE SALMON

by William D. Gronlund, Harold O. Hodgins, and Ethel A. Blood*

Previous studies at the National Marine Fisheries Service (NMFS) laboratory in Seattle of a maturity-related lipoprotein (SM antigen) in female sockeye salmon blood (Ridgway, 1961; Hodgins and Utter, 1969) and of gonadotropic hormone (GH) activity in pituitary glands of sockeye and chum salmon (Gronlund, 1969; Gronlund and Hodgins, 1970) have demonstrated that both provide valid criteria for the identification of maturing fish taken at sea several months before spawning. For accurate forecasting of inshore runs from high seas sampling, data on age at maturity of sockeye salmon taken at sea are essential. The possibility of detecting the onset of sexual maturity in sockeye during the year before spawning led us to investigate pituitary GH further and to investigate the relations between antigens in ovaries and the maturation process.

Blood SM antigen can be induced with estrogens (Ames, 1967) in immature male and female sockeye salmon; blood SM antigen can also be induced in immature female rainbow trout but not in immature female coho salmon with pituitary extracts (Gronlund, 1969). The antigen is detected in both the blood and ovaries of maturing female salmon, and its presence in blood of high seas sockeye salmon is consistent with known age-maturity data (Hodgins and Utter, 1969). The GH is detected in extracts of maturing sockeye salmon pituitary glands, and levels of activity—using gonad weight augmentation bioassays—are consistent with other maturity criteria (Gronlund and Hodgins, 1970).

Because of lack of sensitivity, the bioassay by gonad weight augmentation used for GH was not applicable to individual pituitary glands from high seas sockeye, and a more sensitive test was needed if earlier maturity predictions were to be possible using GH as an indicator.

Whereas SM antigen is detectable in the ovaries of all high seas sockeye salmon with SM antigen in blood (SM+), some fish without detectable SM antigen in blood (SM−) did have detectable SM antigen in ovaries. To assess ovarian SM antigen as a maturity factor, we examined the relation between blood and gonad SM antigen.

The purposes of the present studies were to: (1) examine ovarian and serum proteins from maturing sockeye salmon to determine how many ovarian constituents were detected by anti-SM sera and to make preliminary investigations of their physico-chemical properties for use in identification of their roles in the maturation process and (2) investigate a new GH assay procedure based on radioactive phosphorous (^{32}P) uptake by immature salmonid testes. Using this method, we examined the GH activity of individual pituitary glands of high seas sockeye salmon. We also partially characterized the hormone as a first step in the development of immunochemical assay procedures, which are extremely sensitive and can be used to quantitate GH activity in blood as well as in pituitaries.

EXPERIMENTAL PROCEDURES

Ovarian SM Antigen Tests

Salmon captured at sea on 1967–71 cruises of the RV *George B. Kelez* were frozen whole and transported to the NMFS laboratory in Seattle. The fish were partially thawed and ovaries removed. Ovaries were either homogenized with phosphate buffered physiological saline in a glass hand homogenizer, in a mechanical homogenizer, or were cut in small pieces and crushed. All methods gave essentially the same results. The saline extracts were then tested for SM antigen using the immunodiffusion test that was used with blood (Ridgway, Klontz, and Matsumoto, 1962).

Ovarian SM Antigen Purification

Ovaries pooled from female sockeye salmon captured on the high seas and kept in frozen storage were processed by a modification of the method described by Wallace, Jared, and Eisen (1966) for separation and purification of phosvitin—a protein containing a high percentage of phosphorous—from eggs of several vertebrate species including teleosts. As part of this process, a water-insoluble material which they called the "phosvitin-lipovitellin complex" is separated. We previously found that protein material from mature salmon ovaries, serologically cross-reactive with serum SM antigen, is water insoluble. This in-

* National Marine Fisheries Service, Northwest Fisheries Center, Seattle.