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Thyroid and LDH Activity in Coho Salmon from West Coast and Lake Michigan Regions

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THYROID AND LDH ACTIVITY IN COHO SALMON FROM WEST COAST AND LAKE MICHIGAN REGIONS

by

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INTRODUCTION

The Pacific salmon have been of physiological interest for some time because they undergo great transitions in their environment (fresh water to salt water and back) during their life span, as well as being subject to the degenerative changes of "aging" over a very short period of time. They have been studied with respect to osmotic and migratory phenomena and have been proposed as "model" organisms for the pathology of aging.

The translocation and successful establishment of a breeding population of coho salmon Oncorhynchus kisutch in Lake Michigan from eggs collected in Washington and Oregon presented several unique opportunities for study. The salmon of Lake Michigan undergo physiological and morphological changes similar to other coho salmon but they are not migrating to, nor maturing in, salt water. Still their survival rates and growth characteristics are substantially better (based on records of the Michigan DNR) than the Oregon populations from which the eggs were originally obtained. The West Coast adult salmon average seven pounds as compared to twelve pounds for Lake Michigan adults.

In addition to the many obvious differences between the Pacific Ocean and Lake Michigan, there are several more subtle ones which are of real importance as they influence fish physiology. These are: 1) the substitution of iodine deficient environment for one of iodine abundance, 2) dependency upon forage species with low caratenoid content in Lake Michigan as opposed to the rich caratene content of

plankton and shellfish of salt and brackish water, 3) confinement to an area of high pesticide residue during final maturation and migration, and 4) substitution of no osmotic gradient for the radical one from salt to fresh water.

Due to heavy industrial demands, many of the major rivers of the Washington and Oregon areas have been altered by hydroelectric dams making them unsuitable for natural coho reproduction. As a result, West Coast populations of the Columbia River Basin are maintained through hatchery systems just as in Michigan.

The one area in which Michigan coho appear to be comparatively substandard is in reproductive fecundity and fertility. Reduced fertility may be due to either low viability of the eggs or very early embryonic mortality. The most commonly proposed cause is the impact of pesticide residues. However, in view of all the other environmental changes, there is the very real possibility that this is not the answer or at least not the whole answer. The solution of this problem becomes of practical importance when establishing "no effect" levels for pesticide contaminants as proposed by the Environmental Protection Agency. In establishing the point at which a substance is no longer limiting, it must first be established that it alone is limiting, or the relationships among all the limiting factors must be understood. In light of past experimentation, thyroid gland activity (as reflected in hormone assays and tissue examination) and the LDH content of blood was determined to be the most useful of the known physiological parameters.

This study approached the problem by determining plasma

thyroxine levels, plasma triiodothyronine saturation, plasma LDH activity, and histological examination of the thyroid gland at different life stages of two coho salmon populations. Comparisons within and among populations gave some insight into the probability of thyroid and liver malfunction being major causes of observed reproductive problems and the degeneration that is unique to these species of fish.

It was hypothesized that significant physiological differences exist between the two salmon populations of coho relative to thyroid gland function and that differences resulting from variable iodine content of the respective environments and possible synergistic effects with pollutants in Great Lake waters could explain reproductive problems associated with Lake Michigan salmon. LDH titers were expected to be higher in both adult and subadult (age one year less than adults, hereafter referred to as a jack) populations compared to the smolt salmon since the adult and jack salmon apparently undergo tissue necrosis during their spawning run and subsequently die after spawning. Tissue necrosis was found to be accompanied by elevated LDH levels in blood by Wroblewski and Gregory (1961).

Similarity content between adult and jack salmon would indicate that t sponse is reproductive in nature and not associated with an age factor which would be indicated by significant differences in LDH content between adult and jack salmon. By comparing the amount of LDH present in the three life stages (adult, jack and smolt) of salmon which occur approximately one year apart, information on the effects of migration and osmotic stresses at varying periods of maturation can be evaluated. West Coast adults

and jacks undergo radical osmotic transitions from salt to fresh water while the West Coast smolt experiences opposite gradient changes going from fresh to salt water. The Lake Michigan salmon do not undergo radical osmotic changes while migrating to or from spawning streams, and the distances traveled are considerably less than the characteristic lengthy migrations associated with West Coast salmon.

Steelhead trout, Salmo gairdneri were introduced in the 1870's from California to Lake Michigan. Since they undergo nearly identical environmental conditions as those experienced by the salmon but do not succumb after one spawning, comparisons between steelhead trout and coho salmon give insight into the impact of the environment on these fish and their ability to cope with stresses associated with reproduction. The role of thyroid and liver malfunctions as factors involved with reproductive and degenerative problems occuring in coho salmon at the time of spawning were examined in this study.

Hoar and Randall (1969) reported that low iodine waters promote thyroid hyperplasia in trout and this condition may be prevented by the addition of exogenous iodine to the water. This is supported by the work of Hunn and Reineke (1963) who experimented with steelhead trout and reported that iodine is readily obtainable from food sources via gut adsorption or from water via the gills and further that the level of iodine stored is closely related to that present in the food and/or water. In a study comparing the state of activity of the thyroid gland between spawning Lake Michigan and Pacific Ocean rainbow trout, a marked hyperplasia was found in the Great Lakes fish compared to the normal condition of the thyroid observed in the Pacific

Ocean steelhead (Robertson and Chaney 1953). The evidence indicated that this hyperplasia of the thyroid gland present in the steelhead from Lake Michigan represented a hypofunctioning thyroid due to an iodine deficiency in the water.

The thyroid gland apparently functions both in migration and also salinity preference in coho salmon. In yearling coho, thyroid gland activity increases (expressed by thyroid clearance) before the young salmon show a change of preference from fresh water to salt water (Orr 1970; Eales 1963; Baggerman 1960a; Baggerman 1963). Baggerman (1960b) indicates that in juvenile coho salmon, the length of the daily photoperiod controls the time at which salinity preference takes place and likely the induction of migration-disposition.

Six environmental factors are considered to contribute to the eventual death of Pacific salmon after their return migration to the spawning grounds. These are as follows: 1) the change involved in moving from a fresh water to salt water environment, 2) the lengthy fresh water migration, 3) bodily infections, 4) increased water temperature, 5) lack of food, and 6) body depletion resulting from sexual maturation (Orr 1970).

Several authors have shown a relationship between increased levels of lactic acid and death of salmon. Black (1957) in a study of sockeye salmon showed that blood lactic acid increased seven to ten times the normal values and recovery after severe muscular exer-- cise was not as rapid in fresh water compared to salt water. Black (1958) and Huntsman (1938) concluded that while it may not be possible to prove that endogenous production of lactic acid as

a result of severe muscular exercise is the primary cause of death in salmon, it would appear safe to conclude that muscular activity and lactic acid are significant correlates of a condition resulting in death.

Excess accumulation of lactic acid in a fish which had not recently undergone severe muscular activity may be indicative of hepatic degeneration. Robertson and Wexler (1960) showed that pronounced degeneration occurred in liver and other tissues of spawning Pacific salmon. The leakage of LDH into tissue fluids and resulting abnormally high blood titers would indicate such cellular breakdown (Wroblewski and Gregory 1961; Hsieh and Blumenthal 1956). Therefore, information on liver necrosis (suggested by macroscopic examination of the tissue) as an associated factor in the death of coho salmon would be indicated by elevated LDH levels in the blood.

MATERIALS AND METHODS

Adult and jack coho salmon were sampled (blood and thyroid tissue) in the Fall of 1971 at the Bonneville Dam on the Columbia River in Oregon and at the Platte River Salmon Hatchery at Honor, Michigan. Three steelhead trout were also collected with the salmon at the Platte River Hatchery. Smolt salmon were sampled in the Spring of 1972 at the Wolverine State Fish Hatchery in Wolverine, Michigan, and collected at the Fall Creek Hatchery on the Alsea River in Oregon and sampled at the Western Fish Toxicology Laboratory in Corvalis, Oregon, which draws its water from the Willamette River. A second sampling of smolts one half maintained in salt water and one half maintained in fresh water for a period of two weeks was also acquired from the Western Fish Toxicology Laboratory to check on whether the high LDH values obtained from the initial sampling of smolts from the West Coast were an abnormal condition. It was possible that retaining the smolts in fresh water constituted a stressful condition in salmon preparing to enter salt water. The collection of steelhead trout was made to compare the current conditions with those reported in previous literature based upon histological condition of the thyroid gland in the steelhead trout. Information on the LDH content of the blood was also obtained from these steelhead.

Seventeen adult, 17 jack, and 20 smolt salmon were sampled on the West Coast; while 25 adult, 16 jack, 30 smolt salmon and three steelhead trout were sampled from the Lake Michigan region. Male fish were used exclusively throughout the study except for smolt salmon whose sex was undetermined.

All adult and jack fish were stunned by a sharp blow on the head prior to sampling blood and thyroid tissue; in addition the fish collected at the Platte River Hatchery were electrically shocked. Approximately ten cc of blood were collected from each West Coast fall fish, with 0.5 cc of ten percent sodium citrate per syringe used as an anticoagulant. In contrast, five cc of blood were collected from each Platte River salmon with 0.2 cc of heparin per syringe used as an anticoagulant. Only one cc of blood was obtained per smolt salmon at both locations, with 0.1 cc of the respective anticoagulants used per syringe. Dilution factors negate themselves in comparing adults to jacks, and smolts to smolts, but do not when comparing smolts to either adult or jack salmon. Since all smolt salmon values were twice the concentration of adult and jack salmon, the smolt sample values were divided by two making them comparable. In addition, a small number of serum samples were obtained from the coho salmon sampled at the Platte River station. Serum was collected in order to evaluate its use for future studies since serum samples are more convenient to obtain than plasma samples.

Blood from all the fall collected samples was obtained by inserting the needle via the oral cavity into the dorsal aorta, while the method used for spring smolts involved cutting off the tail and drawing blood from the caudal artery. The blood samples were centrifuged as soon as possible after collection at 3,000 r.p.m. for five minutes. The supernantant plasma was then removed, frozen in dry ice, and sent in sterile vials to the laboratory. All plasma and serum were stored at minus 18 F^{o} until analyzed.

Thyroid tissue from all the fish was obtained by removing the isthmus in the area of the second gill arch and immediately placing the tissue in Bouin's picric acid solution. The tissue was decalcified in a solution consisting of equal portions of 50% sodium citrate and 50% formic acid for one week. The tissue was prepared for histological analysis by routine methods (Humason 1967). Tissue was serially sectioned at the area of the second gill arch ten to twenty microns in thickness and stained with haematoxylin and eosin. Photomicrographs of the thyroid tissue were obtained with a Nikon M-35 Interference Phase Microscope.

LDH assays of the plasma and serum samples was obtained by using Sigma Chemical kits for LDH determination and following the procedures described in Sigma Technical Bulletin #500 (Berger and Broida 1971). LDH is an enzyme which catalyses the conversion of pyruvic acid plus B-DPNH to lactic acid plus B-DPN. Pyruvic acid reacts with 2,4-Dinitrophenylhydrazine to form a colored "Hydrazone" which has a high O.D. over the wavelength of 400 to 500 millimicrons. Lactic acid, B-DPN, and B-DPNH do not contribute a significant O.D. at this wavelength. Therefore, by starting with Sigma Standardized Pyruvate Substrate which yields a constant hydrazone O.D., the varying O.D.'s resulting from the conversion of part of the pyruvic acid to lactic acid due to LDH activity may be measured. Analysis was accomplished by employing a Hitachi Parkin-Elmer model 139 UV-VIS Spectrophotometer, the wavelength used throughout the study was 460 millimicrons.

Plasma thyroxine assays were obtained using kits and procedures developed by Abbott Laboratories. The Tetrasorb-125 T-4 diagnostic

kit yeilds data expressed in micrograms of serum thyroxine/100 ml of serum. Serum samples are dissolved in thyroxine binding globulin I 125 test solution. Thyroxine in the serum competes for TBG-binding sites and is quantitatively determined by the amount of ^{125}I-thyroxine it releases from binding sites in the ^{125}I-TBG solution. An anionic resin-sponge is used to seperate unbound ^{125}I-thyroxine from that which is bound to TBG. The Trisorb-125 T-3 diagnostic kit data was expressed in differences in percent uptake of I^{125} by a resin-sponge. Circulating thyroxine is bound mainly to thyroxine-binding globulin. In hyperthyroidism, the primary thyroxine-binding sites are nearly saturated, an added liothyronine (triiodothyronine) I^{125} is taken up by secondary binding sites provided by a resin-sponge. In hypothyroidism, it is the primary binding sites which are relatively unsaturated which takes up the liothyronine I^{125}, and thus uptake by the resin-sponge is decreased. Counting was achieved by employing a Packard Tri-Carb Liquid Scintillation Counter. Approximately a six percent error (determined by Abbott Laboratory) was experienced during the analysis of the T-4 assay due to an inadvertent 20 minute delay in counting, which was accounted for in the affected samples.

The level of significance accepted throughout the study was the 95% confidence interval (P>.05). An analysis of variance for independent samples was computed for the T-4, T-3, and LDH data. A t test for independent measures was used to test differences among the T-4, T-3, and LDH population means as indicated by the analysis of variance computation. Bartletts test statistic was also computed to test the equality of the population variances.

RESULTS

The results of the thyroxine (T-4) assays expressed in micrograms of T-4 per 100 milliliters of serum are presented in Table 1. The hypothesis that differences between the West Coast and Lake Michigan salmon populations exist in relation to thyroid gland activity was reflected in the results of the T-4 analysis. In general, significant differences (P>.05) existed between Lake Michigan and West Coast adult and jack salmon groups, and also between Lake Michigan and West Coast smolt salmon groups. In addition, the smolt salmon groups from both regions differed significantly (P>.05) from the adult and jack salmon groups from both locations (Table 1).

The results of the triiodothyronine (T-3) assays expressed in relative percent uptake of T-3 by a secondary binding site are presented in Table 2. The T-3 assays was a reflection of the degree of saturation of thyroxine binding globulin in the serum samples. The T-3 analysis therefore should be a reflection of the T-4 analysis. In general, the West Coast and Lake Michigan adults and jacks were not significantly different (P>.05) from each other. The Lake Michigan smolts were significantly different (P>.05) from the adults and jacks of the Lake Michigan region but not from the adults and jacks of the West Coast region (Table 2).

The hypothesis of differences in thyroid gland activity between the West Coast and Lake Michigan salmon populations was supported by the histological examination of the thyroid tissue from the two populations of fish. The contrast between the West Coast coho salmon and

Table 1. Plasma Thyroxine Assays From West Coast And Lake Michigan Salmon

Salmon Group	n	Mean Values mcg T_4/100 ml Serum	Standard Deviation
Group a) West Coast adults	7	3.70	.920
Group b) West Coast jacks	6	3.72	.872
Group c) Lake Michigan adults	6	1.82	.254
Group d) Lake Michigan jacks	6	1.74	.506
Group e) West Coast smolts	4*	1.29	.099
Group f) Lake Michigan smolts	5*	0.70	.120

The mean values of the following salmon groups were found to be significantly different ($P>.05$): a-c, a-d, a-e, a-f, b-c, b-d, b-e, b-f, c-e, c-f, d-f, e-f.

* Two fish pooled per sample.

Table 2. Plasma Triiodothyronine Assays From West Coast And Lake Michigan Salmon

Salmon Group	n	Mean Values Percent Uptake	Standard Deviation
Group a) West Coast adults	8	39.8%	.054
Group b) West Coast jacks	8	38.1%	.041
Group c) Lake Michigan adults	5*	42.4%	.032
Group d) Lake Michigan jacks	6**	47.8%	.085
Group e) Lake Michigan smolts	5***	34.2%	.058

The mean values of the following salmon groups were found to be significantly different ($P > .05$): a-d, b-d, c-e, d-e.

* Two fish pooled per 4 samples.

** Two fish pooled per 2 samples.

***Six fish pooled per 5 samples.

both the Lake Michigan coho salmon and the Lake Michigan steelhead was striking. The results of the histological examination of the West Coast adult and jack salmon are shown in Figure 1 and 2 respectively. The follicles contain an abundance of dense colloid and the follicular cells are low cuboidal type in appearance. These two conditions are characteristic of a normal appearing thyroid gland (Turner, 1966). This condition contrasts sharply with the results obtained from the Lake Michigan adults and jacks shown in Figure 3 and 4 respectively. The follicles contain little or no colloid and the follicular cells are high columnar type. Marked hyperplasia and hypertrophy is evident in this tissue. The Lake Michigan steelhead trout thyroid gland is shown in Figure 5. The follicles contain little colloid and the follicular cells are high columnar type. The marked tissue hypertrophy in coho salmon from Lake Michigan is not evident in this tissue, although a hyperplastic condition is present.

The hypothesis that differences would exist in LDH content of the blood between smolt salmon as compared to adult and jack salmon appears to be supported by the results obtained in this analysis. The adult and jack salmon from the Lake Michigan region were all electrically shocked, the adult and jack salmon from the West Coast were dumped on a pier while sampling occurred, and the smolt salmon had their tails removed and blood drawn from the surrounding area which would likely yield some tissue fluid. All these processes might be expected to increase variability in the amount of LDH found. Bartletts test statistic, which compares the equality of population variances, is presented in Appendix A and Appendix B, and shows that

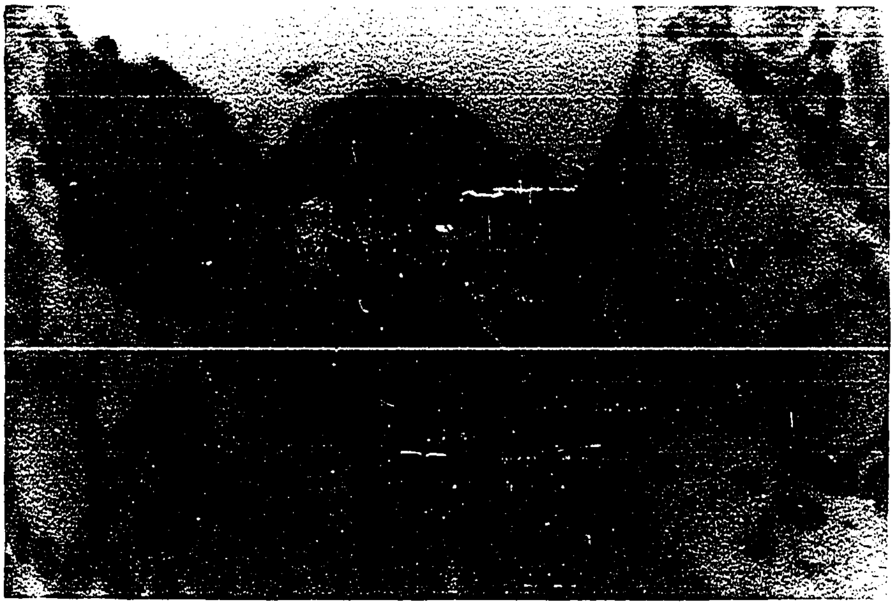

Figure 1. West Coast adult salmon. The follicular cells are low cuboidal type, while the follicles are filled with a dense colloid. x 400.

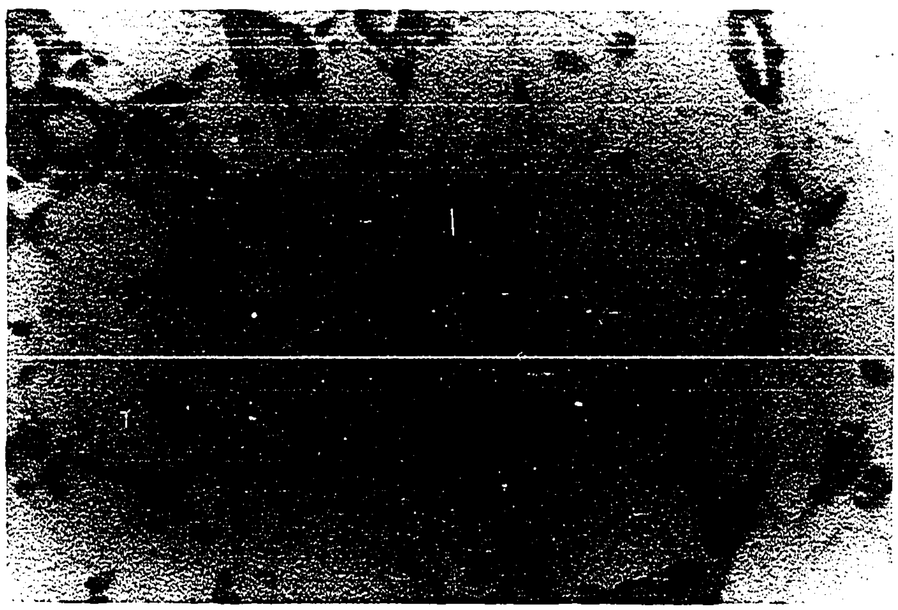

Figure 2. West Coast jack salmon. The follicular cells are low cuboidal type, while the follicles are filled with a dense colloid. x 200.

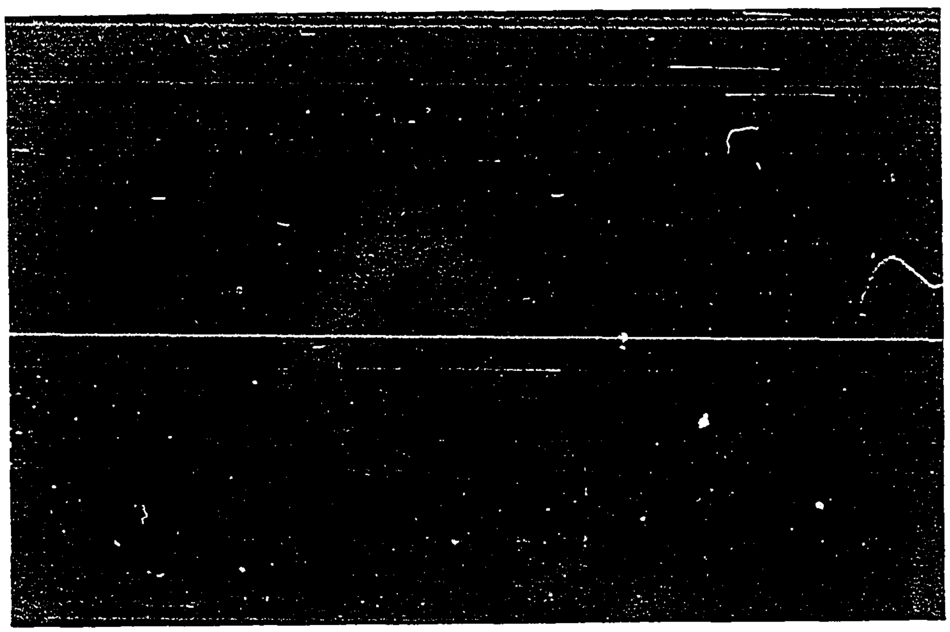

Figure 3. Lake Michigan adult salmon. The follicular cells are high columnar type, while the follicles are devoid of colloid. Marked hyperplasia and hypertrophy is evident in this tissue. x 400.

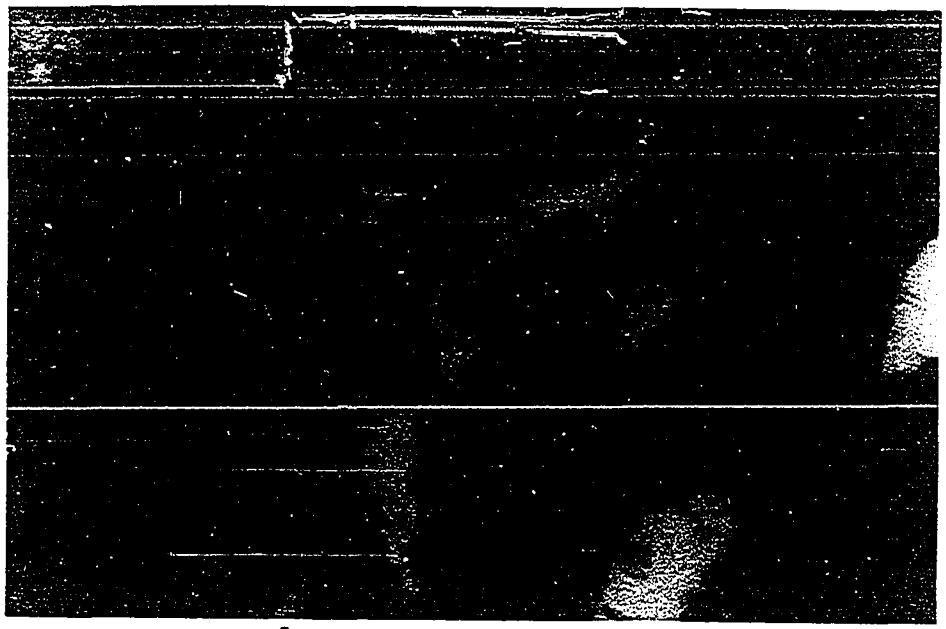

Figure 4. Lake Michigan jack salmon. The follicular cells are high columnar type, while the follicles are devoid of colloid. Marked hyperplasia and hypertrophy is evident in this tissue. x 400.

Figure 5. Lake Michigan steelhead trout. The follicular cells are high columnar type, while the follicles are devoid of colloid. Marked hyperplasia is evident in this tissue. x 400.

the variances of the groups in question were not statistically different (P>.05) indicating that the observed differences in LDH analysis were not apparently due to handling procedure differences. Plasma and serum samples obtained from the Lake Michigan adult and jack salmon did not differ significantly in LDH content, and were therefore combined in the LDH analysis.

In general, the Lake Michigan and West Coast adult and jack salmon both between and within groups were not significantly different (P>.05). The Lake Michigan smolts were statistically significant (P>.05) from all other salmon groups. The West Coast smolts were not significantly different (P>.05) from Lake Michigan and West Coast adult and jack salmon (Table 3). It was believed that the West Coast smolt LDH values were unusually high (the cause of these high LDH values was undetermined) and therefore additional West Coast smolt samples were obtained to check on the initial values. The second sampling of West Coast smolts both from fresh water and salt water was significantly different (P>.05) from the initial West Coast smolt samples, the Lake Michigan and West Coast adult and jack salmon groups, and they also were different from each other (Table 3).

Table 3. Blood LDH Assays From West Coast Salmon, Lake Michigan Salmon, And Lake Michigan Steelhead.

Salmon Group	n	Mean Values B-B Units *	Standard Deviation
Group a) West Coast adults	17	870	479
Group b) West Coast jacks	17	1044	407
Group c) Lake Michigan adults	25	1009	430
Group d) Lake Michigan jacks	16	1193	398
Group e) West Coast smolts	5	809	82
Group f) Lake Michigan smolts	10	34	49
Group g) Michigan Steelhead	3	1127	545
Group h) West Coast Fresh Water smolt	5	599	105
Group i) West Coast Salt Water smolt	5	417	69

The mean values of the following fish groups were found to be significantly different ($P>.05$): a-d, a-f, a-i, b-f, b-h, b-i, c-f, c-h, c-i, d-e, d-h, d-i, e-f, e-h, e-i, f-g, f-h, f-i, g-i, h-i.

* One B-B Unit reduces 4.8×10^4 umoles of pyruvate per minute at 25°C.

DISCUSSION

It was apparent from the results obtained in this study that significant physiological differences in thyroid gland activity existed between the populations of coho salmon from Lake Michigan and the West Coast. These differences were demonstrated in the values obtained in the T-4 analysis, the T-3 analysis, and the histological examination of the thyroid tissue from these salmon.

The magnitude of the difference between West Coast and Lake Michigan adult and jack salmon T-4 levels was a factor of about three. The adult and jack salmon from the iodine rich environment of the Pacific Ocean resulted in values of 3.70 to 3.72 ug of T-4/100 ml of serum respectively compared to the 1.82 to 1.74 ug of T-4/100 ml of serum in adult and jack salmon from the iodine poor environment of Lake Michigan (Table 1). Robertson and Chaney (1953) noted significant differences in protein bound iodine and total serum iodine between the rainbow trout from the Lake Michigan and West Coast regions, inferring from their data that the resulting thyroid hyperplasia in the Lake Michigan trout represented a "hypofunctioning due to iodine deficiency..." in the Lake Michigan waters. The thyroxine values obtained from the coho salmon suggests that available iodine may in fact be a significant factor involving the lowered T-4 values in the Lake Michigan salmon. The T-4 analysis indicates a hypothyroid condition exists in the Lake Michigan coho salmon compared to the West Coast coho.

It has been shown by Murray and McGirr (1954) that iodine deficiency in the environment favors T-3 formation in the thyroid gland of vertebrates present in such an environment. The Lake Michigan smolts have a T-3 value of 34% resin-sponge uptake compared to the West Coast adults and jacks with T-3 values of 39% and 38% respectively. These values were not significantly different at the level accepted in this study although they were borderline in this respect (Table 2). A T-3 analysis on the West Coast smolts was not performed due to lack of sufficient quantities of serum.

The Lake Michigan adult and jack salmon T-3 values were 42% and 47% resin-sponge uptake which were significantly different from the Lake Michigan smolts T-3 value of 34% resin-sponge uptake indicating a hyperthyroid condition relative to the smolts (Table 2). The West Coast jack and adult salmon also differed significantly from the Lake Michigan jacks in T-3 analysis (Table 2).

Factors reported to increase resin-sponge uptake are hyperthyroidism, thyrotoxicosis, and liver diseases (Hamolsky, Stein, and Freedberg 1957; Hamolsky and Freedberg 1960). The Lake Michigan smolts, the West Coast smolts, the West Coast adults, and the West Coast jacks were comparatively free from the gross level liver necrosis present in the Lake Michigan adult and jack populations. This liver necrosis was characterized by a yellowish-greenish coloration in the necrotic portion of the tissue, and also by the increase in size (two to three times larger than a normal liver) in the diseased livers. Liver disease could account for the reason that the T-3 values were higher in the Lake Michigan adult and jack populations

compared with the West Coast adults and jacks. Liver disease was also reported to decrease resin-sponge uptake in T-3 analysis so that the actual effects of liver disease is uncertain (Friis 1960; Robbins 1959). Certainly the visible liver necrosis evident in the Lake Michigan adult and jack salmon populations could have been significant in increasing resin-sponge uptake indicating an incorrect diagnosis of a hyperthyroid condition rather than the expected hypothyroid condition indicated by the T-4 analysis. If a true hyperthyroid condition existed in the Lake Michigan adult and jack salmon, then T-4 values at least equal to West Coast salmon would be expected in the Lake Michigan salmon. Since this is not the case, the liver necrosis (Table 3) is suspect in raising the T-3 values in the Lake Michigan adult and jack salmon (Table 2).

The histological examination of the thyroid tissue in the West Coast adult and jack salmon showed that a normal appearing condition existed in this tissue, quite similar to West Coast steelhead from California examined by Robertson and Chaney (1953). The characteristic features of a normal thyroid gland (Turner 1966) are low cuboidal epithelium in follicular cells which surround a dense colloid filled follicle. This is essentially the condition present in the thyroid glands of the West Coast salmon (Figure 1 and 2). In contrast, the Lake Michigan adult and jack salmon have a marked hyperplasiac and hypertrophic condition present in the thyroid tissue. The follicular epithelium is high columnar type and the follicles are devoid of colloid (Figure 3 and 4). This condition is similar to the spawning Lake Michigan rainbow trout examined by Robertson and Chaney (1953).

Their conclusions on the trout from this region were that a hypothyroid condition existed due to an environmental deficiency in iodine.

The steelhead trout collected in this study were apparently not different histologically from those steelhead examined by Robertson and Chaney (1953) from the Lake Michigan area. The follicles are generally devoid of colloid and the follicular cells are high columnar type (Figure 5). A marked hyperplasia exists in this tissue and these steelhead are not histologically unlike the adult and jack coho salmon examined from Lake Michigan.

It is now appropriate to address the question whether a hypothyroid condition exists in the coho and steelhead populations in Lake Michigan and is it a limiting factor as indicated in this study and suggested by Robertson and Chaney (1953). The available evidence collected in this study indicates that a hypothyroid condition did exist in the Lake Michigan population of coho salmon and probably existed in the steelhead population. If this lack of iodine is a limiting factor in the reproductive capacity of these fish, then the steelhead trout are a key element in analysing the problem since they are "native" to the Great Lakes in the sense that their introduction from California was in the 1870's while the salmon have only been recently introduced.

The steelhead trout seem not to have adjusted to a normal physiological state in relation to thyroid gland activity in the Lake Michigan environment since a hyperplastic thyroid condition was still present in these fish. With the increasing numbers of steelhead presently in Lake Michigan, the obvious conclusion is that the iodine content of

the Great Lakes is not a limiting factor, at least for steelhead trout and presumably coho salmon. Contradicting this conclusion is the fact that approximately thirty generations have elapsed since their introduction to the Great Lakes region, a very short period of time genetically. Presumably a limited number of individuals with a subsequent limited gene pool started the strain in Lake Michigan. Following the decrease in the steelhead population due to sea lamprey predation, artificial propagation was used to increase the steelhead population. Again the probable result of this artificial propagation was a limited gene pool with little natural selection occurring except for the percentage of naturally reproducing steelhead which was presumed small compared with the artificial stocking, since artificial means were deemed necessary to maintain and increase the population. Therefore, one could argue that given ample time the trout will adapt to the environment, reproduce naturally, and maintain a substantial population without assistance from artificial stocking procedures. With the large increase in trout populations due to artificial propagation programs it is difficult to argue that a future reduction in reproductive capacity will occur, but the contention of iodine being a limiting factor which may express itself in the future is still valid. The increase in the steelhead populations was probably due to two factors: 1) the reduction in lamprey followed by restocking programs producing great increases in fish living to maturity, 2) the introduction of great numbers of salmon which brought a new host for the existing lamprey to infest, thus diluting the impact on steelhead populations.

The hypothyroid condition present in the Lake Michigan salmon is important in aspects other than possibly limiting the reproductive capacity of these fish. Increasing evidence by the Environmental Protection Agency and other investigators have shown that fish accumulate pesticides, herbicides, and other toxic elements (Rudd 1964; Woodwell, Craig, and Johnson 1971; Conservation Foundation 1969). Cope (1964) and Woodwell, Craig, and Johnson (1971) have shown that pesticides are concentrated from the environment by fish and these pesticide residues tend to accumulate in the lipids of the affected animals. Holden (1965) discussed the effects of pesticide contaminants on salmon and points out that at the time of spawning lipid reserves are depleted and the pesticide content of the remaining lipids will be further increased to a level which may become toxic for some target organ.

Pesticide accumulation does occur in coho salmon as amply demonstrated by Aulfrich et al (1971) when they correlated pesticide residues in coho salmon with reproductive decline and kit mortality in mink fed on a diet of Lake Michigan coho salmon. West Coast coho salmon did not produce any impaired reproductive performance and in many respects was superior to that of mink fed control rations. Salmon are presently banned from commercial sale in Michigan, due to pesticide residues above the level determined to be safe for human consumption by the Food and Drug Administration. A plausible explanation for this problem lies in the hypothyroid state of the Lake Michigan coho. Metabolic disturbances associated with hypothyroidism include a consistent rise in serum lipids due to a greater retardation

of lipid degradation. A reduced metabolic rate occurs resulting in a reduction of tissue fat catabolism and utilization, so that body fat increases (Macgregor 1954). The result is an obese fish with increased fat deposition which allows for increased accumulation of pesticide in the fatty tissue of these salmon. The consequences of this probable sequence of events as they influence the future of the coho salmon cannot be underestimated.

From the results obtained in this study, it is suggested that the steelhead population will increase until a plateau is reached, then the population will eventually start to decline due to the impact of the environmental factors of low iodine and added deleterious effects due to increasing influx of pollutants into Great Lakes waters. It is conceivable that the increasing numbers of steelhead trout in the Great Lakes at the present time are occurring in spite of decreasing reproductive capacity of the population as a whole. The decrease in lamprey and the restocking program allows for a greater number of trout to reach maturity, so that a decrease in numbers of fry fish produced per spawning could occur and not be reflected in the numbers in the general population. This condition would become evident in time with a leveling off of the general population followed by a gradual decline in numbers of fish in the population, or increases in the number of fish spawned artificially needed to maintain the population at a given level.

The hypothesis that liver malfunction may contribute to the degeneration associated with the salmon at the time of spawning appears to be supported by the LDH analysis. The Lake Michigan

smolt salmon were significantly different (P>.05) from the Lake Michigan adults, the Lake Michigan jacks, the West Coast adults, and the West Coast jack salmon (Table 3). The West Coast smolts appeared to be high in LDH activity. Therefore, a second sampling of smolts, some retained in salt and some in fresh water, was tested for LDH to compare with the initial sampling of West Coast smolts. Both the smolt groups, fresh water having a mean value of 599 B-B units and salt water having a mean value of 417 B-B units differed significantly (P>.05) from the initial sampling of West Coast smolts which had a mean value of 809 B-B units (Table 3). The West Coast smolts sampled in salt water had significantly lowered LDH activity than either group of West Coast smolts sampled in fresh water. Some investigators believe that at the time of migration young coho salmon are under osmotic "stress" because these fish are no longer fully adapted to a fresh water environment. Black (1957) and Bates and Vinsonhaler (1957) in separate studies reported the apparent benefit of sea water on the recovery of salmon exhausted through exercise. Salt water was an apparent aid to young salmon recovering from severe exercise since fish similarly exercised in fresh water died. The data collected from the West Coast smolts in this study indicates that the smolts retained in sea water were apparently more efficient in coping with environmental conditions than their counterparts retained in fresh water. The smolts sampled in salt water from the West Coast were significantly different (P>.05) in LDH content from the West Coast jacks, the Lake Michigan adults, the Lake Michigan jacks, and the Lake Michigan smolts (Table 3). The second

sampling of West Coast smolts in fresh water differed significantly (P>.05) from the West Coast adults, the West Coast jacks, the Lake Michigan adults, the Lake Michigan jacks, and the Lake Michigan smolts (Table 3). At least three factors could account for the increased LDH activity and degeneration experienced by the adult and jack salmon: 1) osmotic changes associated with migration, 2) progressive response due to the age of salmon, and 3) changes associated with achieving reproductive maturity.

Osmotic changes associated with migration can be discounted as causing the high LDH activity and degeneration as observed in the adult and jack salmon. The Lake Michigan adult and jack salmon do not undergo the radical osmotic changes experienced by the West Coast salmon, but still exhibit the increased LDH and degeneration at spawning found in the West Coast samples. The Lake Michigan smolts undergo no radical osmotic changes and do not migrate to salt water. They are retained throughout their life cycle in fresh water as are the adult and jack salmon from Lake Michigan. However, they are significantly different in LDH content from the adult and jack salmon in Lake Michigan. Therefore, if osmotic gradient changes are an influence in the increased LDH values experienced by the West Coast adult and jack salmon, then significantly lower LDH values in Lake Michigan adult and jack salmon would be expected. The smolt salmon from the West Coast retained in salt water have undergone osmotic changes opposite but equal to those experienced by the West Coast adult and jack salmon, but are significantly lower in LDH content from the West Coast jacks, the Lake Michigan adult and the Lake

Michigan jack salmon. Lack of osmotic change appears to have had a stressful effect on the salmon as indicated by the LDH content of the West Coast smolt salmon retained in fresh water compared with those sampled in salt water, but osmotic changes alone cannot account for the degeneration observed in the salmon.

If the increased LDH values and degeneration present in the adult and jack populations were a progressive response due to age, then the jack salmon would be expected to have LDH values intermediate between the adult and smolt salmon. This would be expected since the jack salmon are approximately one year older than the smolt salmon and approximately one year younger than the adult salmon. There is no significant difference in LDH content between the jack and adult salmon from either the West Coast or Lake Michigan populations. Also, the jack salmon would not be expected to degenerate and die after spawning if age were a critical factor in initiating this process. Age per se, therefore, has no apparent influence on the LDH content of the coho salmon and does not account for the increased LDH activity nor the hepatic degeneration observed in these salmon.

The one factor which apparently accounts for the observed differences in LDH content and the associated degeneration process was changes associated with achieving reproductive maturity. The adult and jack salmon from the West Coast and Lake Michigan locations were reproductively mature, experienced high LDH titers, and underwent tissue degeneration at the time of spawning. The smolt salmon are not reproductively mature and have significantly lowered LDH values in both West Coast and Lake Michigan regions. The physiological

changes associated with achieving reproductive maturity apparently alter the fish in some manner so as to preclude their recovery from the rigors of spawning. Osmotic gradient changes and age apparently do not have a major effect in causing the degenerative processes associated with spawning in these salmon.

It is necessary to discuss the variables which could have affected the LDH values obtained in this study. The Lake Michigan adult and jack salmon were all electrically shocked prior to sampling, the West Coast adult and jack salmon were dumped on a pier prior to sampling, and the smolt salmon were sampled by cutting off the tail and drawing blood from the caudal artery. In all these instances the LDH content in the blood of these salmon could have been altered producing results which were not of biological interest. Bartletts test statistic was therefore used to test the equality of the population variances (Appendix A) and to determine the equality of variances by testing meaningful combinations of salmon groups used in the LDH analysis (Appendix B). The results of Bartletts test showed that the variances of the salmon groups in question were not significantly different ($P>.05$) and therefore the results obtained are not attributable to handling procedures alone. LDH content of the blood is probably not the method of choice to study degeneration associated with spawning in these fish, but the results warrant further investigation into this area of fish physiology.

Investigations of salmon in September of 1972 prior to their entry into the spawning streams indicated that the gross level liver necrosis was absent at this point in their migration. Apparently

the marked liver necrosis evident in the spawning or spent salmon does not manifest itself until the salmon are well up the streams in the Michigan area.

SUMMARY AND CONCLUSIONS

Blood and thyroid tissue samples were obtained from adult and jack coho salmon from the West Coast and Lake Michigan areas at the time of spawning in the Fall of 1971, and from smolt salmon in the Spring of 1972. Blood samples from the salmon were analyzed for thyroxine, triiodothyronine, and LDH activity while the thyroid tissue was examined by histological methods. Significant differences in thyroxine, triiodothyronine, and histological examinations both between and among populations indicated that a hypothyroid condition existed in the Lake Michigan salmon compared with West Coast salmon. This condition was probably caused by a lack of iodine present in the Lake Michigan environment. Possible effects upon the reproductive capacity of salmon in the Lake Michigan environment were discussed.

Adult, jack, and smolt salmon were analyzed between and among populations for LDH activity. Significant differences in LDH content between adult and jack salmon compared with smolt salmon indicated that degeneration associated with salmon at the time of spawning was not attributable to osmotic changes or aging, but was apparently linked with physiological changes associated with reproductive maturation.

Appendix A. Bartletts Test And Analysis Of Variance For T-4, T-3 And LDH Analysis

Analysis	Bartletts Test		Analysis Of Variance	
	Chi Square	D.F.	F Statistic	D.F.
T-4	24.2*	5	24.3*	33
T-3	4.7	4	4.56*	31
LDH	52.9*	8	11.9*	102

* (P>.05)

Appendix B. Bartletts Test Between Salmon Groups In LDH Analysis

Salmon Group	Bartletts Test		
	Chi Square	D.F.	Significance
L.M. adult - L.M. jack *	1.17	1	N.S.***
W.C. adult - W.C. jack **	0.40	1	N.S.
L.M. smolt - W.C. smolt	1.46	1	N.S.
W.C. adult, jack - L.M. adult, jack	1.93	1	N.S.
W.C. Salt Water smolt - W.C. Fresh Water smolt	0.61	1	N.S.

* L.M. - Lake Michigan

** W.C. - West Coast

***N.S. - Not Significant (P>.05)

LITERATURE CITED

Aulfrich, R.J., R.K. Ringer, H.L. Seagran, and W.G. Youatt. 1971. Effect of feeding coho salmon and other Great Lakes fish on mink reproduction. Can. J. Zool., 49:611-616.

Baggerman, Bertha. 1960a. Salinity preference, thyroid activity and the seaward migration of four species of Pacific salmon (Oncorhynchus). J. Fish. Res. Bd. Can., 17(3):295-322.

Baggerman, Bertha. 1960b. Factors in the diadromous migrations of fish. Sym. Zool. Soc. Lond., 1:3-60.

Baggerman, Bertha. 1963. The effect of TSH and antithyroid substances on salinity preference and thyroid activity in juvenile Pacific salmon. Can. J. Zool., 41:307-319.

Bates, D.W., and R. Vinsonhaler. 1957. The use of louvers as a means of guiding fish at the Tracy, California pumping plant. Trans. Am. Fish. Soc. for 1956, 86:38-57.

Berger, L., and D. Broida. 1971. The colorimetric determination of lactic dehydrogenase in serum, urine, or other fluids. Sigma Technical Bulletin #500. Sigma Chemical Company. St. Louis,Mo.

Black, E.C., 1957. Alterations in the blood level of lactic acid in certain salmonid fishes following muscular activity. J. Fish. Res. Bd. Can.,14(6):807-814.

Black, E.C., 1958. Hyperactivity as a lethal factor in fish. J. Fish. Res. Bd. Can., 15(4):573-586.

Conservation Foundation. 1969. "Pollution by Pesticides" Washington, D.C.

Cope, O.B., 1966. Contamination of the fresh water ecosystem by pesticides. J. Applied Ecology., 3:33-44.

Eales, J.G., 1963. A comparative study of thyroid function in migrant juvenile salmon. Can. J. Zool., 41:811-823.

Friis, T., 1960. In Vitro uptake of ^{131}I labelled L-thyronine by human erythrocytes. Acta Endocrinol., 33:117.

Hamolsky, M.W., and A.S. Freedberg. 1960. The Thyroid Gland. New England J. Med., 262:129.

Hamolsky, M.W., M. Stein, and A.S. Freedberg. 1957. The thyroid hormone-plasma protein complex in man. II A new in vitro method for study of "uptake" of labelled hormonal components by human erythrocutes. J. Clin. Endocrinol., 17:33.

Hoar, W.S., and D.J. Randall. 1969. Thyroid function and its control in fishes. "Fish Physiology". Acad. Press, N.Y. and London. pp. 241-274.

Holden, A.V., 1966. Organochlorine insecticide residues in salmonid fish. J. Applied Ecology., 3:45-53.

Hsieh, K.M., and H.T. Blumenthal. 1956. Serum lactic dehydrogenase levels in various disease states. Proc. Soc. Exptl. Biol. Med., 91:626.

Humason, L., 1967. Animal Tissue Techniques. W.H. Freeman and Co.

Hunn, J.B., and E.P. Reineke. 1963. Influence of iodine intake on iodine distribution in trout. Proc. Soc. Exptl Biol Med., 115(1):91-93.

Huntsman, A.G., 1938. Overexertion as cause of death of captured fish. Science., 87:577-578.

Macgregor, A.G., 1954. Hypothyroidism. "The Thyroid Gland". R. Pitt-Rivers and W.R. Trotter. Butterworths, London. Vol. I. pp. 112-150.

Murray, I.P.C., and E.M. McGirr. 1954. Iodine metabolism in thyroid dysfunction. "The Thyroid Gland". R. Pitt-Rivers and W.R. Trotter. Butterworths, London. Vol. I. pp. 39-70.

Orr, R.T., 1970. Animals in Migration. The MacMillan Company, Collier-MacMillan LTD., London.

Robbins, L.R., 1959. Experience with the in vitro erythrocyte uptake of ^{131}I-labelled l-triiodothyronine in a rountine clinical laboratory. J. Clin. Endocrinol., 19:1292.

Robertson, O.H., and A.L. Chaney. 1953. Thyroid hyperplasia and tissue iodine content in spawning rainbow trout: a comparative study of Lake Michigan and California sea-run trout. Physiol. Zool., 26(4):328-340.

Robertson, O.H. and B.C. Wexler. 1960. Histological changes in the organs and tissues of migrating and spawning Pacific salmon (Genus Oncorhynchus). Endocrinology., 66:222-239.

Rudd, R.L., 1964. Pesticides and the Living Landscape. University of Wisconsin Press, Madison.

Turner, D.C., 1966. General Endocrinology. W.B. Saunders Company. Philadelphia-London.

Woodwell, G.M., P. Craig, and H.A. Johnson. 1971. DDT in the biosphere: where does it go? Science., 174:1101-1107.

Wroblewski, F., and K. Gregory. 1961. Lactic dehydrogenase isoenzymes and their distribution in normal tissue and plasma in disease states. _Ann_ _N_. _Y_. _Acad_. _Sci_. _94_:912.