Quantitative Interlake Comparison of Thyroid Pathology in Great Lakes Coho (Oncorhynchus kisutch) and Chinook (Oncorhynchus tshawytscha) Salmon

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ABSTRACT

Coho salmon (Oncorhynchus kisutch) and chinook salmon (Oncorhynchus tshawytscha) from Lakes Ontario, Michigan, Erie, or Huron were found to suffer epizootics of thyroid hyperplasia and goiters which appeared to have an environmental etiology. There were 10-fold differences in goiter prevalence within the Great Lakes, and the differences in goiter frequency were correlated with the degree of thyroid hyperplasia. A means of assessing the degree of thyroid hyperplasia (thyroid index) is described, and the derived index was used to facilitate statistical interlake and interspecies comparisons. Despite the hyperplastic (or goitered) condition in all prespawning or spawning Great Lakes salmon, serum thyroid hormone levels were generally higher than in prespawning coho salmon from the Fraser River, British Columbia, indicating that the Great Lakes fish were not necessarily hypothyroid. The hyperplastic lesions appear to undergo progressive changes: (a) large follicles, partly colloid depleted, surrounded by cuboidal epithelial cells; (b) small follicles, largely colloid depleted, surrounded by columnar epithelial cells (in this form, the follicles commonly assume a trabeculate arrangement); (c) "microfollicles" with greatly enlarged columnar epithelial cells encompassing very small follicles; (d) apparently afollicular lesions with little or no colloid in evidence. There was some evidence of benign invasiveness, although the lesions generally resembled simple hyperplastic parenchymatous goiters seen in humans.

INTRODUCTION

Thyroid hyperplasia or goiter in Great Lakes fishes was reported in the early 1900’s (18, 36, 37). It has only been in more recent years, however, that epizootic outbreaks of goiter in feral fish species of the Great Lakes have been recognized (4, 44, 53), and there appears to have been a significant increase in goiter frequency in Great Lakes coho salmon populations over recent years (44). The evidence previously reported also suggests that extrinsic environmental factors working in concert with low iodine levels in the lakes are involved in the etiology of the disease (44, 53).

In order to elucidate the etiology and pathogenesis of goiter in these fish, an interlake and interspecies comparison of thyroid activity in Great Lakes coho and chinook salmon was undertaken to quantitatively and qualitatively assess thyroid status. This paper describes epizootiological findings of a study of thyroid pathology in coho and chinook salmon in the Great Lakes and presents a histopathological assessment of thyroid condition from an interspecies and interlake perspective. This information should be useful as base-line indicators with which to compare future generations of these species to determine the extent of annual variations in the incidence and severity of thyroid disease in Great Lakes salmonids.

The results also add further substance to our earlier claims (33, 44, 53) that the etiology of goiters in these fish has an environmental basis other than, or in addition to, iodide deficiency.

MATERIALS AND METHODS

Coho (Oncorhynchus kisutch) and chinook (Oncorhynchus tshawytscha) salmon were captured by netting, shocking, and herding in several locations on the Great Lakes (Table 1). The gill nets used were 3.5- or 4-inch multifilament, or 5-inch monofilament stretch mesh. The nets were monitored at 2- to 4-hr intervals, or after 9-hr overnight sets, to minimize post-mortem effects. Electroshocking techniques were variable depending on stream access and water conditions. In all cases, an effort was made to minimize handling and trauma from the various capture techniques used.

All fish caught were from feral populations which had originated from hatchery-reared stock in either Canadian or United States facilities. Wherever possible, the source of individual fish was identified from fin clips. Except for the spring 1977 sample, all collections were of sexually mature fish in either prespawning or spawning condition.

To serve as a control, 12 three-year-old spawning coho salmon from Big Qualicum Hatchery, British Columbia, were sacrificed, and blood samples and preserved lower jaws were shipped to Guelph for examination.

Blood samples were collected, by caudal severance, into a plastic tissue culture tube and kept on ice overnight. Serum was separated by centrifugation and stored at -30°C in plastic serum storage vials until analyzed for thyroid hormones. Serum triiodothyronine and thyroxine analyses were performed using the Ames assays (Miles Laboratory, Mississauga, Ontario, Canada) as described previously (53).

The presence of overt goiter was determined by retracting the operculum and examining the base of the gill arches for swellings or nodules. Fish with one or more distinct nodule(s) greater than 1 cm in diameter were recorded as having overt goiters.

Thyroid tissue was excised from the base of the third gill arch, fixed in Bouin’s solution for a minimum of 48 hr, decalciﬁed in 5% formic acid solutions (27) for 7 days, embedded in...
Paraplast, and sectioned at 6 to 7 μm. Sections were stained with hematoxylin and eosin according to routine procedures (27).

In order to facilitate quantitative interlake comparisons of thyroid histology, a "thyroid index" was derived using several histological criteria. Use of any single criterion could result in a misleading index; thus, a number of criteria believed to be indicative of thyroid activity or status were examined. It was also desirable to derive an index which would give reference to the relative degree of pathology of the gland. The following criteria were considered to be useful parameters with which to assess thyroid function: follicular diameter; follicular shape; colloid content; epithelial cell height; nuclear diameter; afollicular morphology; and hyperplastic response.

Follicular diameter was measured with an ocular micrometer on a Zeiss microscope (in μm), and it was assumed that a decreasing follicle diameter indicated an increasing activity (40). The progression of a normal follicle to a microfollicular morphology (i.e., the formation of clusters of very small follicles with little luminal area) has often been associated with an active or pathological gland and was therefore considered as a partial diagnostic criterion of alteration in thyroid function.

In a goitered condition, follicles may lose their sphericity and hence make measurement of follicle diameter inappropriate. Nonspherical follicles were measured along both the long and short axis to produce a ratio >1 (i.e., long axis/short axis >1). A larger ratio indicates a more extensive elongation of the follicle and a further departure from the normal, spherical form.

Colloid content of the follicles was estimated and used as an indicator of the degree of hormonal exhaustion of the gland. The percentage of depletion of the colloid in a follicle was recorded, with values of 0 and 100% representing extremes of colloid-replete and colloid-exhausted follicles, respectively.

Epithelial cell heights were measured by ocular micrometer (in μm) and represent the most frequently used method of histologically assessing thyroid activity. Epithelial cell height is considered to be roughly proportional to the degree of response to endogenous stimuli; and if one assumes the classical mechanisms of pituitary-thyroid feedback regulation to operate in fish, it is therefore a measure of glandular activity. Nuclear diameter was measured and interpreted in a similar fashion.

Loss of follicular structure as an extreme thyroidal response to stimulation (in a nonneoplastic lesion) is a basic assumption which was made to incorporate overall glandular morphology in the index. Percentage afollicular morphology was estimated over the entire sectional field of the thyroid tissue. Follicular hyperplasia was also recorded as positive or negative and was intended to discriminate between a proliferative and a nonproliferative glandular response. The various criteria and interpretations used to quantify thyroid histology are summarized in Table 2.

Double-blind determinations of thyroid indices were made on 10 samples. The mean variation in the thyroid index was calculated to be approximately 15%. All histological samples were carefully scrutinized for evidence of neoplastic characteristics and, if present, were not used for calculation of indices.

The thyroid index for an individual animal consisted of 16 epithelial cell heights and 16 nuclear diameters measured from 4 randomly selected follicles (41, 59). The index also included 4 measurements each of follicle diameter (or follicle shape) and colloid content and an overall observation of percentage of afollicular morphology and presence or absence of follicular hyperplasia. A "collection site" index for 20 animals (for example) would thus contain 320 epithelial cell heights, 320 nuclear diameters, 80 follicular diameters (or follicle shape), 80 determinations of colloid content, and 20 observations each of afollicular morphology and hyperplastic response.

The range of values measured for the histological criteria was arbitrarily divided into an equally spaced scalar from 1 to 10, and an index value was assigned to all measurements on the basis of their position on this scale (Table 3). An overall thyroid index for each animal was determined to be the sum of the individual index values. Follicular morphology (e.g., follicular diameter and/or follicular shape) was considered to be a single criterion, and thus the 2 variables were combined. If the histological field examined contained only spherical follicles or only nonspherical follicles, then the morphology of the follicles was assessed on a scale from 1 to 10. If both types (shapes) of follicles were present in a single sample, then each type was assessed on a 1:5 basis, and the sum of these 2 assessments was considered to be the index value for follicular morphology. Hence, this criterion could contribute only a maximum value of 10 to the total thyroid index for a given fish.

Since the index was designed to reflect the relative degree of pathology of the gland according to predetermined assumptions regarding changes in individual criteria (Table 2), it follows that an index with a high value should indicate a more advanced thyroid pathology relative to a low index. The thyroid index...
would therefore provide a measure of both quantitative and qualitative differences. In order to test the validity of the assumptions regarding the criteria, correlations between different criteria and the thyroid index were examined (Charts 1 to 3). Since the index was used to compare coho and chinook salmon, both species are included in the correlation. However, the trends and correlation coefficients were similar when the 2 species are plotted separately.

Epithelial cell heights (Chart 1), nuclear diameter (Chart 2), and luminal colloid content (percentage depleted) (Chart 3) showed strong positive correlations with the thyroid index ($r = 0.781$; $r = 0.754$; $r = 0.853$) respectively, thus supporting the original assumptions regarding these criteria. However, follicular shape and afollicular morphology were only weakly correlated ($r = 0.221$ and $0.480$, respectively). Follicle diameter did not appear to contribute significantly to the index ($r = 0.075$). A large thyroid index value would therefore tend to reflect, with the exception of follicle diameter, high values for some or all of the individual criteria and suggests an advanced pathology for that sample.

Goiter frequency data were compared using the normal approximation of the binomial distribution with a Z test (49). All other data were compared using a one-way analysis of variance and the t test (52). Unless otherwise stated, the critical level of significance for testing hypothesis was chosen to be $p < 0.05$.

RESULTS

Occurrence of Goiter. Wide variations in goiter frequency occurred in the salmon collected from the Great Lakes. There was no significant difference in goiter frequency with regard to sex in any single collection; therefore, the data were combined for each collection site (Table 4). Interlake comparisons revealed highly significant differences in goiter frequency for fish collected at a similar stage in their spawning cycle. Lake Michigan coho salmon had a goiter frequency of 6.3%, which was significantly lower than that observed in collections from Lake Ontario (47.6 and 51.6%; $p < 0.05$) or Lake Erie (79.5%; $p < 0.05$). The range of observations showed a nearly 13-fold difference in goiter prevalence within the Great Lakes. Immature (collected in April) and prespawning coho salmon from Lake Ontario had lower goiter frequencies ($p < 0.05$) than did spawning fish from the same lake. In Lake Ontario coho salmon (spawning), the observed goiter frequencies of fish from United States and Canadian sources were similar ($p > 0.05$).

In 5 collections of chinook salmon from the Great Lakes, no grossly visible lesions were recognized.

Goiter Pathology. Overt goiters were found at the base of the second, third, and/or fourth gill arch in coho salmon. The nodules were generally firm to the touch and varied in color. Some goiters were pearly-white to gray-brown; others were bright red. The lesions were often highly vascularized and hypervascular, with large, engorged blood vessels visible near their surfaces. In some large lesions, focal areas of necrosis were evident.

The nodules ranged from 1 to 5 cm long, were usually located bilaterally, and were uniodular or multinodular in nature. Fish from those areas of high goiter frequency tended
to have a larger percentage of multinodular lesions compared to fish from areas of low frequency (Table 5).

The lesions appeared to originate as small areas of reddening or swelling at the base of the second and third gill arches.

Continued proliferation of thyroid tissue resulted in progressive enlargement in these areas, giving rise to distinct nodules. The nodules extended longitudinally along the isthmus and laterally along the gill arches replacing normal gill tissue. In extreme cases, large multinodular lesions had developed on all gill arches (Fig. 1), sometimes preventing full closure of the operculum. Some large goiters projected into the pharynx and were evident as bulbous masses on the floor of the mouth.

Signs of swelling or reddening at the base of the gill arches was considered to be the first visible evidence of diffuse (primary) thyroid hyperplasia. This assumption was later confirmed histologically and was important in discriminating an apparently "normal" fish from one in the primary stages of overt goiter development. Over 90% of the fish considered to be negative as regards overt goiter in the coho salmon pre-spawning and spawning adult collections showed gross signs of thyroid hyperplasia, whereas only 30% of the immature spring Lake Ontario coho salmon exhibited this characteristic. In all chinook salmon collections, over 80% of the fish displayed signs of primary hyperplasia, in spite of the fact that no nodular (overt) proliferations were observed.

Thyroid Histopathology. A wide variation in histological morphology was apparent between British Columbia (Pacific Ocean) and Great Lakes salmon. In British Columbia salmon, the thyroid was composed of a moderate number of spherical follicles scattered throughout the loose areolar connective tissue of the lower jaw, adjacent to the ventral aorta and the base of the gill arches. The follicular epithelium was a single layer of squamous or cuboidal cells with round, centrally located nuclei. The lumen was filled with relatively homogeneous, eosinophilic colloid material with little or no vacuolation. Although small deviations from this picture occurred in a few fish, the general morphology was typical of a normal, nonpathological gland (Fig. 2). In contrast, thyroid tissue collected from Great Lakes salmon was variable in appearance. In salmon showing no overt goiters, there was extensive follicular hyperplasia compared with the sample from British Columbia. At this stage, the follicles had departed from a normal spherical morphology to oblate and/or extremely elongate and irregular forms. The epithelium was one cell thick, composed of low to medium columnar type cells, with prominent, basally located nuclei. The colloid stained brightly with eosin and was relatively homogeneous, with extensive periph-
eral vacuolation (Fig. 3). There were papillary structures of hyperplastic follicular cells projecting into the lumina of some follicles. The hyperplasia was diffuse, displacing normal connective tissue, blood vessels, and nerves. Progressive enlargement of the lesion caused further follicular distortion leading to highly elongated, tubular (trabeculate) follicles. Budding of these larger follicles probably gave rise to the dense fields of aggregated microfollicles observed in numerous specimens (Fig. 4).

In this intermediate stage of development, there was increased epithelial hypertrophy, with the epithelial cells appearing markedly columnar. Cell nuclei were enlarged, were located basally, and had prominent nucleoli. Mitotic figures were absent or infrequent.

Colloidal material stained less brightly, had a granular appearance, and was sparse or completely absent from most follicles. The lumina appeared small because of the increasing formation of microfollicles coupled with the increasing epithelial cell height. This "intermediate stage" of thyroid proliferation was the most common histological type observed in both the spawning (United States) Lake Ontario (immature) and Lake Michigan coho. Erosion and replacement of bone and cartilage was a common finding, with ossification of the marginal borders of cartilage present in association with these areas (Fig. 5). Less common was the observation of small clusters or nests of follicles completely embedded in the bone or musculature (Fig. 6) adjacent to the lesion.

In a few specimens, an even more advanced lesion was observed. In these cases, there was a complete loss of follicular organization and normal thyroid structure. Only remnants of follicles could be seen and were used to identify the lesion as being of thyroid origin. The general picture was one of large masses of aggregated epithelial cells which had undergone partial desquamation (Fig. 7). At this stage, it was difficult to distinguish the lesion from a true neoplastic development, which suggests the possibility of neoplasia superimposed upon hyperplasia. These were not included in the thyroid index.

Intravascular foci (Fig. 8) were observed in 11 specimens (9 from Lake Ontario, 2 from Lake Erie); however, it was not clear whether this was evidence of metastasis or histological artifact. No evidence of ectopic thyroid tissue was found in the kidney, liver, or spleen samples from these fish; therefore, there was no direct evidence of metastasis. There was no sign of encapsulation of nodules, nor was there any significant inflammatory response.

The histological picture described above is representative of findings in both coho and chinook salmon from the Great Lakes. With few exceptions, histological examination revealed pathological glands from the Great Lakes collections compared to British Columbia coho. It was not possible to determine from histopathology which fish possessed overt goiters.

**Quantitative Histopathology.** There was a wide range of observations for the criteria measured, reflecting the degree of variability within individual criteria. The mean values of each criterion for the various collections are given for coho and chinook salmon in Tables 6 and 7, respectively.

British Columbia coho had a significantly lower thyroid index ($p < 0.05$) compared to all other Great Lakes collections of either coho or chinook salmon. The prespawning and spawning Lake Ontario coho had similar thyroid index values ($p > 0.05$) which were both significantly higher than those for the immature Lake Ontario coho ($p < 0.05$) or Lake Michigan coho ($p < 0.05$), and significantly lower when compared with the Lake Erie coho adults ($p < 0.05$). The thyroid index value in Lake Michigan coho was not significantly different from that in immature coho from Lake Ontario ($p > 0.05$) but was significantly lower than the corresponding index in Lake Erie coho ($p < 0.05$).

In chinook salmon, prespawning Canadian fish had a thyroid index value similar to that of their spawning counterparts ($p >...
Table 7
Mean values of histological criteria used to assess thyroid pathology and thyroid indices in chinook salmon from the Great Lakes

<table>
<thead>
<tr>
<th>Location and condition</th>
<th>Follicular shape [long axis (µm)/short axis (µm)]</th>
<th>Colloid content (% depleted)</th>
<th>Epithelial cell height (µm)</th>
<th>Nuclear diameter (µm)</th>
<th>Afollicular morphology (%)</th>
<th>Hyperplastic response (% positive)</th>
<th>Thyroid index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Ontario (spawning) (Canadian)</td>
<td>36.9 ± 3.3*</td>
<td>1.9 ± 0.1</td>
<td>45 ± 11</td>
<td>9.2 ± 1.3</td>
<td>5.0 ± 0.4</td>
<td>12.3 ± 8.4</td>
<td>42</td>
</tr>
<tr>
<td>Lake Ontario (spawning) (Canadian)</td>
<td>46.8 ± 6.8</td>
<td>2.3 ± 0.2</td>
<td>61 ± 9</td>
<td>13.1 ± 1.1</td>
<td>6.2 ± 0.3</td>
<td>13.6 ± 4.4</td>
<td>94</td>
</tr>
<tr>
<td>Lake Ontario (spawning) (United States)</td>
<td>53.0 ± 1.9</td>
<td>2.3 ± 0.4</td>
<td>72 ± 6</td>
<td>14.5 ± 1.3</td>
<td>6.2 ± 0.3</td>
<td>17.8 ± 9.2</td>
<td>100</td>
</tr>
<tr>
<td>Lake Michigan (spawning) (United States)</td>
<td>28.0 ± 1.3</td>
<td>2.3 ± 0.1</td>
<td>51 ± 8</td>
<td>7.7 ± 0.5</td>
<td>4.6 ± 0.2</td>
<td>17.6 ± 8.9</td>
<td>53</td>
</tr>
<tr>
<td>Lake Huron (spawning) (United States)</td>
<td>35.7 ± 2.3</td>
<td>2.6 ± 0.2</td>
<td>72 ± 7</td>
<td>13.2 ± 0.8</td>
<td>6.3 ± 0.3</td>
<td>2.0 ± 1.2</td>
<td>100</td>
</tr>
</tbody>
</table>
* Mean ± S.E.

Interspecies comparisons can be justified between parallel collections only with regard to lake site, stocking source, and stage of spawning. A significantly lower (p < 0.05) thyroid index was found in spawning Lake Ontario coho (United States source) compared with chinook salmon from the same site and source. Lake Ontario prespawning and spawning coho (Canadian source) had similar thyroid indices compared to prespawning and spawning chinook (Canadian source) from that same lake (p > 0.05). In addition, Lake Michigan coho were not significantly different from Lake Michigan chinook (p > 0.05). Also, from an interspecies standpoint, 2 collections of chinook salmon had thyroid indices as high as or higher than those of the largest coho index (i.e., Lake Erie), but no overt goiters were observed in the chinook salmon.

In Great Lakes coho salmon, goiter frequency was positively correlated with the thyroid index (r = 0.798, p < 0.05; Chart 4). The thyroid histopathology results indicated that interlake differences were more significant than interspecies differences of parallel collections.

Intraspecies Comparisons of Thyroid Hormone Determinations. Serum levels of triiodothyronine and thyroxine showed large variations between groups in both coho and chinook salmon (Table 8).

British Columbia coho salmon had significantly lower (p < 0.05) levels of thyroxine than those in all Lake Ontario collections of this same species. Levels of thyroxine in British Columbia coho were similar to those found in Lake Michigan coho (p > 0.05). Triiodothyronine levels in British Columbia coho were markedly lower (p < 0.05) than in all other collections, except Lake Erie, from which they were not significantly different (p > 0.05). Immature (spring) coho had thyroxine levels similar to those of both prespawning (p > 0.05) and spawning (p > 0.05) Canadian-stocked fish in Lake Ontario but slightly higher than those levels detected in spawning United States-stocked fish (p < 0.05). Triiodothyronine levels, on the other hand, were higher in these immature coho compared to all other groups of this species (p < 0.05). There were no differences in levels of either triiodothyronine or thyroxine between the prespawning and spawning Canadian-stocked fish in Lake Ontario. However, the spawning Canadian fish had significantly higher levels of both triiodothyronine and thyroxine compared to spawning United States-stocked fish (p < 0.05) in the same lake.

Both spawning Lake Ontario collections (i.e., Canadian and United States) had higher levels of triiodothyronine and thyroxine than did either Lake Michigan (triiodothyronine, p < 0.05; thyroxine, p < 0.05) or Lake Erie (triiodothyronine, p < 0.05; thyroxine, p < 0.05). Finally, coho from Lake Michigan had higher levels of thyroxine and triiodothyronine than did fish from Lake Erie (p < 0.05).

There were no significant differences in serum levels of either triiodothyronine or thyroxine between males and females from the same collection site or when comparing goitered versus nongoitered fish.

Canadian stocks of chinook salmon in Lake Ontario (Bronte Creek) had thyroxine levels that were significantly lower than in all other collections (p < 0.05). All collections of United States fish (i.e., Lakes Ontario, Michigan, and Huron) had similar levels (p > 0.05) of thyroxine, being nearly 2-fold greater than the levels in Bronte Creek fish. Lake Ontario...
Chinook salmon had thyroid indices as high as or higher than (Tables 6 and 7) the coho but did not exhibit any sign of the massive thyroid proliferation (i.e., overt goiter) observed in the coho. Using histological criteria, the thyroid in chinook salmon was commonly found to exhibit a condition similar to or more severe than that in coho salmon, and yet there was not the impetus for an extreme hyperplastic response in chinook. This possibly suggests a different mechanism of goitrogenesis in the 2 species, varying species sensitivities to evoking stimuli, or even different morphology of the lower jaw in chinook and coho salmon which may permit hyperplasia without goiter in the former species.

In both species, the glands resemble chronic iodide deficiency goiter (endemic) as described in humans (47) and that observed after goitrogen exposure in rats (9) and fish [Poecilia reticulata (45); Xiphophorus maculatus (1)]. This is not surprising in view of the fact that many goitrogens act either by blocking iodide uptake or suppressing organic binding within the thyroid (20), thus inducing a form of iodide insufficiency. There were no signs of the involutionary stages recognized during the early stages of goiter induced by intermittent stimuli (57), and the picture was one of a long-standing hyperplasia. This is intriguing in light of the rapid increase in goiter frequency observed in the Lake Ontario coho salmon. In less than 3 weeks, there was a 2-fold increase (i.e., from 23.7 to 47.6%) in goiter frequency, indicating very rapidly proliferating tissues. Sonstegard and Leatherland (53) reported similar findings in this same species from Lake Ontario. There appears to be no significant difference in thyroid histopathology (as determined by the thyroid index) between prespawning and spawning chinook salmon in Lake Ontario, suggesting that the chinook are not sensitive to the same stimuli which apparently affect the coho salmon during the course of the spawning cycle.

In coho and chinook salmon examined from marine populations, the thyroid gland showed histological evidence of a progressive decrease in activity during the spawning migration (8, 50). On the other hand, several investigators have reported an apparent increase in thyroid activity associated with the stage of spawning in fish (5, 25, 26, 33, 38, 46, 55, 56), perhaps reflecting an increased requirement for thyroid hormones. This added challenge to the thyroid may result in a further compensatory response as evidenced by the increased

**DISCUSSION**

The classification of thyroid lesions in fish has led to considerable confusion in diagnosis. The histological picture in the coho and chinook salmon examined here from the Great Lakes, while not unlike the follicular adenoma (35) (embryonal or trabecular type) described by Meissner and Warren (40) and Warren (58), is probably best classified as simple hyperplastic, parenchymatous goiter. The general histological type observed was similar to that reported in Lake Erie coho (4) and Lake Ontario coho (53) and is assumed to represent the same condition.

A wide variation in histopathology existed between the various collections of coho and chinook salmon from the Great Lakes and coho salmon from British Columbia. Although within a collection site it was not possible to distinguish by means of thyroid histology which fish possessed overt goiters, there was a significant correlation \( r = 0.798, p < 0.05 \) between the thyroid index and observed goiter frequencies in coho salmon (Chart 4). This suggests that coho populations having high goiter frequencies also have a more advanced histological picture of thyroid pathology. These data clearly show histological differences even between different goitered fish. This observation may hold true for coho salmon only, since a pattern of goiter was not evident in the chinook. It is surprising that the

### Table 8

<table>
<thead>
<tr>
<th>Collection site and condition</th>
<th>Origin of stock</th>
<th>Thyroxine (µg/dl)</th>
<th>Triiodothyronine (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coho</td>
<td>Lake Ontario (spawning)</td>
<td>United States</td>
<td>1.24 ± 0.24 (17)</td>
</tr>
<tr>
<td></td>
<td>Lake Michigan (spawning)</td>
<td>United States</td>
<td>0.60 ± 0.10 (20)</td>
</tr>
<tr>
<td></td>
<td>Lake Erie (spawning)</td>
<td>United States</td>
<td>0.18 ± 0.03 (20)</td>
</tr>
<tr>
<td></td>
<td>Lake Ontario (immature)</td>
<td>Canada</td>
<td>0.48 ± 0.04 (12)</td>
</tr>
<tr>
<td></td>
<td>Lake Ontario (spawning)</td>
<td>Canada</td>
<td>1.52 ± 0.17 (6)</td>
</tr>
<tr>
<td></td>
<td>Lake Ontario (spawning)</td>
<td>Canada</td>
<td>1.30 ± 0.13 (15)</td>
</tr>
<tr>
<td></td>
<td>Lake Ontario (spawning)</td>
<td>Canada</td>
<td>1.70 ± 0.24 (20)</td>
</tr>
<tr>
<td></td>
<td>Lake Ontario (spawning)</td>
<td>United States</td>
<td>0.93 ± 0.09 (15)</td>
</tr>
<tr>
<td></td>
<td>Lake Michigan (spawning)</td>
<td>United States</td>
<td>0.60 ± 0.10 (20)</td>
</tr>
<tr>
<td></td>
<td>Lake Huron (spawning)</td>
<td>United States</td>
<td>0.18 ± 0.03 (20)</td>
</tr>
<tr>
<td></td>
<td>Lake Ontario (spawning)</td>
<td>Canada</td>
<td>1.11 ± 0.05 (15)</td>
</tr>
<tr>
<td></td>
<td>Lake Ontario (spawning)</td>
<td>Canada</td>
<td>0.56 ± 0.04 (18)</td>
</tr>
<tr>
<td></td>
<td>Lake Ontario (spawning)</td>
<td>Canada</td>
<td>1.44 ± 0.11 (10)</td>
</tr>
<tr>
<td></td>
<td>Lake Michigan (spawning)</td>
<td>United States</td>
<td>1.24 ± 0.24 (17)</td>
</tr>
<tr>
<td></td>
<td>Lake Michigan (spawning)</td>
<td>United States</td>
<td>1.21 ± 0.21 (15)</td>
</tr>
</tbody>
</table>

a Mean ± S.E.
Numbers in parentheses, sample size.

As with the coho, there were no sex differences in triiodothyronine and thyroxine levels in any of the collections of chinook salmon.

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A wide variation in histopathology existed between the various collections of coho and chinook salmon from the Great Lakes and coho salmon from British Columbia. Although within a collection site it was not possible to distinguish by means of thyroid histology which fish possessed overt goiters, there was a significant correlation \( r = 0.798, p < 0.05 \) between the thyroid index and observed goiter frequencies in coho salmon (Chart 4). This suggests that coho populations having high goiter frequencies also have a more advanced histological picture of thyroid pathology. These data clearly show histological differences even between different goitered fish. This observation may hold true for coho salmon only, since a pattern of goiter was not evident in the chinook. It is surprising that the
Thyroid Hyperplasia in Great Lakes Salmon

goiter frequency. In addition, the cessation of feeding in salmon as they approach spawning may give rise to thyroid changes similar to those found in food-deprived mammalian species (7, 11, 30, 31, 51). Food deprivation in fish (37 to 40 days) lowered the thyroxine metabolic clearance rate, thyroxine degradation rate, thyroxine deiodination rate (to triiodothyronine), and plasma thyroxine levels (22, 24, 32, 43) and in sexually ripening sockeye salmon led to an apparent decrease (measured histologically) in thyroid activity (39). Thus, while the combined effects of changes in metabolism and added demands due to spawning may account for the increase in greater frequency concomitant with spawning migration within a given lake, they certainly do not explain the variations in goiter frequency between lakes. If the goiters are due entirely to these factors, one would not expect such a wide range of response (i.e., nearly a 13-fold difference in goiter frequency between coho populations in different lakes) to similar stress factors within a single species. The data, therefore, suggest that other factors must be involved in goitrogenesis in coho salmon and that the disease manifests itself in response to lake conditions and not entirely to the effects of spawning, migration, or nutritional deficiency per se. In addition, chinook salmon in Lake Michigan have a lower thyroid index than do Lake Ontario chinook, which inhabit lakes of low and high goiter frequencies, respectively, in coho salmon. Thus, although chinook salmon do not exhibit goiters (i.e., "overt" proliferations), they do show the same relative interlake response as do the coho, which further indicates that the factors involved in goitrogenesis are probably related to the lake rather than to the intrinsic physiology of the fish.

There exists a period of very rapid thyroid proliferation associated with the onset of the spawning migration. Examination of thyroid tissue from Lake Ontario fish caught in the spring, however, reveals that the condition exists in a less severe form at least 6 months prior to the reproductive phase of the life cycle, and the fish must therefore have been exposed to the evoking stimuli early in their life cycle. In support of this is the recent observation of a 44% goiter frequency in Lake Erie coho salmon collection in the spring (33). This may not necessarily be due to the same stimuli responsible for goiter induction in the adult, but in this condition the individual might be more sensitive to secondary exposures. It has been shown that the effects of early goitrogenic stimulation (i.e., during growth) in white Leghorn chickens can persist for up to 75 weeks after withdrawal of the goitrogen (34).

Most investigators agree that circulating hormone levels in combination with histological observations are probably a better indication of thyroid activity than is thyroid histology alone (19), particularly since several investigators found that histological evidence did not always correlate well with 

$[^{131}I]$Iodide uptake data (13–16). Thus, it is perhaps not surprising that serum levels of triiodothyronine or thyroxine do not correlate with the thyroid index values. Thyroid histology per se may therefore not be a good indicator of thyroid status. Moreover, the relative levels of free and bound serum thyroxine (and triiodothyronine) in fish from different Great Lakes was not known. There is now ample evidence to suggest that the level of free hormone is the important quantity, as it is this fraction which is biologically available to target cells (2, 3, 54). Because several factors, including malnutrition (31), are believed to alter the ratio of free to bound hormone (e.g., by lowering plasma levels of thyroxine-binding proteins), total hormone levels may not adequately reflect in vivo hormonal availability. Thus, an animal with low total triiodothyronine and thyroxine levels may remain clinically euthyroid by increasing the proportion of free (biologically active) hormone. This is known to occur in various forms of euthyroid goiter in humans (17). In addition, it has been shown in some fish that nutritional status can alter the peripheral conversion of thyroxine and triiodothyronine (22, 23) and therefore possibly maintain the fish in triiodothyronine euthyroidism in spite of low thyroxine levels [assuming that triiodothyronine is the active form of hormone at the cellular level (29)]. It is possible that the variation in thyroid hormone levels in salmon from different Great Lakes may reflect the amounts required to satisfy the free (unbound) serum thyroid levels. These differences in total thyroid hormone levels may therefore reflect interlake factors which affect hormone binding.

The levels of thyroxine and triiodothyronine measured in this investigation are highly variable between groups, with a 9-fold and 38-fold overall range, respectively. Although relatively few reports exist in the literature of thyroid hormone levels in teleosts, levels are generally less than 2 μg/dl and less than 1000 ng/dl for thyroxine and triiodothyronine, respectively (6, 21, 22, 28, 32, 42, 48, 53, 60). Similarly, coho salmon in Lake Ontario also had their highest levels of triiodothyronine in the spring collection with the lowest levels observed in the spawning adults (see Table 8), although thyroxine levels were not different between these groups. The serum thyroid hormone levels reported here agree in part with those of White and Henderson (60) but are at variance with data from coho collected from Lake Michigan and Ontario in previous years (12, 53). Lake Michigan coho in 1976 had lower levels of thyroxine than those reported in coho collected from Lake Michigan in 1971 (12), while Lake Ontario coho collected in 1976 had higher levels of both triiodothyronine and thyroxine than found in coho in 1975 (53). In general, direct comparisons of data from different laboratories are dubious at best, because these assay techniques are poorly standardized and because the fish studied are almost certainly in different physiological conditions. This argument does not hold for the data of Sonstegard and Leatherland (53), who used identical assay techniques and laboratory facilities. The data do not provide an explanation for the discrepancy with their data. In summary, little is known about the normal circulating levels of thyroid hormones in fish and even less about the effects of numerous physiological conditions (e.g., starvation, gonadal maturation, reproduction), and other factors (temperature, handling, stress, etc.). In light of these facts, it becomes extremely difficult to assess the practical significance of observed levels in a group of animals with obvious thyroid pathology (i.e., goiters). The lack of correlation of serum hormones with thyroid histology further complicates this problem.

The thyroxine levels of British Columbia coho salmon were markedly lower than those previously reported by Drongowski et al. (12) for adult coho salmon from Oregon. Considering that both of these collections are from Pacific Ocean stocks, the discrepancy in hormone levels is difficult to reconcile and may be due to sampling techniques (10), assay procedures (42), or even the natural range of variation. In addition, chinook salmon in the Great Lakes had as low or lower levels of these 2 hormones, compared to the coho salmon but failed to exhibit...
the extreme proliferative thyroidal response observed in this species. The important observation to be made from the hormone data is that both the coho (immature collection) and chinook (prespawning) salmon in the Great Lakes are capable of producing high levels of hormone compared to other species (see above for references). This fact is especially important because it implies that despite low ambient iodide levels in the Great Lakes compared with sea water (61) sufficient iodide is available in the lake environment for hormone production and that even a histologically exhausted gland is capable of hormone synthesis. Since lake levels of iodide are unlikely to vary seasonally, the high titers of serum thyroid hormones in Lake Ontario coho salmon in the spring are unlikely to be an artifact of excess iodide availability to the hyperplastic gland. This is supported by observations in spring-caught Lake Erie coho salmon which had markedly hyperplastic thyroids relative to comparable salmon from Lake Ontario but had significantly lower serum thyroxine and triiodothyronine concentrations (33). Thus, the difficulty in interpretation of the significance of absolute concentrations of total serum hormones and the apparent lack of correlation between hormone levels and histopathological findings may indicate that goiter frequency and/or thyroid histopathology are better criteria of thyroid dysfunction in these species than are hormone levels. There is clearly a need to determine the various factors which are responsible for altering circulating levels of thyroid hormones in fish.

Quantifying thyroid histopathology (thyroid index) has provided a means of morphologically staging the thyroid lesions observed here. Of considerable use has been the ability to compare goitered versus nongoitered fish of the same and different species from various lake environments. In the absence of overt goiter development, histological indexing is necessary to follow seasonal, annual, interspecies, and interlake variations in the severity of the lesions and has provided a numerical base line for future studies. The strong correlation of the thyroid index with the goiter frequency also indicates that histological assessment of a few fish might reveal the status of the disease without collecting large numbers of specimens for a goiter frequency analysis.

The findings have revealed that the pathological changes in the thyroids of these fish appear to reflect a response to heretofore unknown lake conditions other than or in addition to strict iodide deficiency. In addition, it appears that both species respond in a similar relative fashion; i.e., both coho and chinook salmon in Lake Michigan are less severely affected than are their Lake Ontario counterparts. Furthermore, the data indicate that the condition is a chronic one and does not develop purely with the onset of spawning, as the rapid increase in goiter frequency at this time in coho salmon might suggest (see also Ref. 53).

Although extensive attempts have been made to isolate extrinsic environmental factors as the cause of goiter, precious few explanations have been found for goiter endemias in lieu of iodide deficiency. This study has further substantiated our earlier claims (33, 44, 53) that environmental factors are involved in the etiology of the disease in coho and chinook salmon in the Great Lakes. Quantitative assessment of thyroid histopathology and serum hormones has helped to raise significant questions regarding lake water quality and should provide an essential monitor to study future alterations in the Great Lakes Environment.

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Fig. 1. Multinodular development of an advanced goiter. The first and second gill arches have been removed for clarity. H & E, x 175.

Fig. 2. Normal thyroid tissue from a British Columbia coho salmon. Note the low epithelial cell height and homogeneous appearance of the luminal colloid material. H & E, x 400.

Fig. 3. Early hyperplastic development in a nongoiteted coho salmon. Note the increased number of follicles, medium columnar epithelium, and vacuolation of the colloid. H & E, x 400.

Fig. 4. Intermediate stage of development of the hyperplastic gland. Note the dense field of aggregated microfollicles and reduced size of lumina. H & E, x 400.

Fig. 5. Erosion of bone and cartilage and marginal ossification adjacent to hyperplastic thyroid tissue. H & E, x 400.

Fig. 6. Nests of thyroid follicular epithelial cells embedded in muscle adjacent to the lesion. H & E, x 175.

Fig. 7. Mass of disoriented thyroid epithelial cells. Note distinct loss of normal thyroid architecture. H & E, x 400.

Fig. 8. Intravascular foci of thyroid follicles. H & E, x 175.
Quantitative Interlake Comparison of Thyroid Pathology in Great Lakes Coho (Oncorhynchus kisutch) and Chinook (Oncorhynchus tschawytscha) Salmon

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