

ENLARGED AND VACUOLATED NUCLEI IN THE LIVER
OF THE CISCO, Coregonus artedii LeSueur, OF LAKE GEORGE,
ESSEX AND WARREN COUNTIES, NEW YORK

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Abstract

Histology of the liver of ciscoes, Coregonus artedii LeSueur, taken in the spring of 1975 from Lake George, New York, shows a high incidence, in males, of vacuolated and herniated nuclei and disrupted muralia. Females, in contrast show relatively few aberrant nuclei and intact muralia. The cause of the liver abnormality is not determined.

Introduction

Earlier studies on several hundred specimens of the cisco, Coregonus artedii LeSueur, of Lake George, Essex and Warren Counties, New York, have shown an 85 to 95% preponderance of females (C. George, Unpublished data). This disparity, combined with observations of gonadal abnormalities and a well documented twenty year shift to increasingly larger average adult lengths, strongly suggests a reduction in population recruitment with differential sexual impact. Such observations have led us to consider application to this particular population of ciscos of several generalizations developed by Christian and others (Christian 1950, 1975; Brain, 1972) relevant to the influence of high population densities on population replacement. Prominent among these was the concept that high population densities induce increased adrenocorticotropin (ACTH) production with consequent increased synthesis and release of adrenocorticosteroids. The high level of circulating corticosteroids acts in turn to inhibit hypophyseal gonadotropin release, either directly or indirectly, and further, to inhibit the disease defense mechanisms of the body. Jointly these physiologic imbalances act to reduce population size.

Obvious manifestations of the process would be an increase in size of adult animals, some breakdown of reproductive processes and increased incidence of disease related morbidity and mortality. Increased incidence of histological indicators in liver could appear as part of the phenomenon and we thus began a study of the histology of this organ. The preliminary findings on hepatic structure in 9 male and 7 female ciscos is reported herein.

Methods and Materials

Specimens were collected on April 26-27 and June 10 of 1975 by means of overnight sets of demersal gill netting (Table 1). The April samples were taken near Burnt Point in the northern basin of Lake George. The June sample was provided by Ernest Lantiegne, Senior Aquatic Biologist, of the Warrensburg Offices of the New York State Department of Environmental Conservation, and was taken from the vicinity of Refuge Island and the Calf's Pen in the southern basin of Lake George, a site some 15 miles to the south of Burnt Point.

Specimens were transported to shore immediately upon removal from the water where they were weighed and measured for total length to the nearest mm. The livers were then removed and fixed in Bouin's solution.

Blocks of tissue were removed from comparable regions of each organ, processed by routine paraffin embedding and sectioned at six microns. The sections were then stained with hematoxylin and eosin. Measurements were made with a filar micrometer and routine bright field light microscopy. Diameters were measured to the nearest 0.1 micron.

At least four sections of each liver were used for counting and measurement. The scribings of the filar micrometer made possible definition of a rectangular area 80 X 26 microns and the circumscribed nuclei were then measured, counted and assigned to five classes relating to extent of vacuolization as observed at 1,000 magnification. The nuclei classes were defined as follows:

Class I: nuclei which did not show any vacuoles

Class II: nuclei which included vacuoles of a diameter less than the radius of the nucleus.

Class III: nuclei where the vacuole had a diameter greater than the nuclear radius but less than nuclear diameter.

Table 1. Capture data and characteristics of ciscoes taken in Lake George,
New York. (1975)

Specimen Number	Sex	Date of Capture	Location	Total Length (mm)	Body Weight (g)	I.C. ^a	Liver Weight (g)	% Body Weight
283-1	♂	April 26/27	Burnt Point	300	221	8.19	1.9	.9
287-6	♂	June 9/10	Refuge I.	338	377	9.76	2.9	.8
287-8	♂	June 9/10	Refuge I.	302	229	8.32	2.0	.9
287-10	♂	June 9/10	Refuge I.	79	164	7.55	1.4	.9
288-2	♂	June 9/10	Calf's Pen	229	178	14.82	1.4	.8
288-3	♂	June 9/10	Calf's Pen	283	171	7.54	1.2	.7
288-4	♂	June 9/10	Calf's Pen	266	161	8.55	.9	.6
288-9	♂	June 9/10	Calf's Pen	305	292	10.29	1.9	.7
288-10	♂	June 9/10	Calf's Pen	311	261	8.68	1.7	.7
282-1	♀	April 25/26	Burnt Point	320	268	8.18	6.5 ^b	2.4 ^b
287-1	♀	June 9/10	Refuge I.	313	280	9.13	3.3	1.2
287-2	♀	June 9/10	Refuge I.	343	344	8.53	3.5	1.0
287-3	♀	June 9/10	Refuge I.	317	277	8.69	1.9	.7
288-1	♀	June 9/10	Calf's Pen	233	159	12.57	1.4	.9
288-5	♀	June 9/10	Calf's Pen	275	178	8.52	1.1	.6
288-6	♀	June 9/10	Calf's Pen	309	228	7.73	2.0	.9

a. I.C. = index of condition, i.e. weight in grams divided by the cube of length in mm times 10^5 .

b. The liver of this fish is greatly enlarged due to the presence of a large epithelioid tumor.

Class IV: nuclei where the vacuole occupied the entire nuclear volume displacing the chromatin to the periphery.

Class V: nuclei where the vacuolated nucleus had assumed an irregular form or had ruptured leaving only a portion of the nuclear envelope intact.

Results

The cisco liver is an irregular hemispherical body situated in the anterior region of the peritoneal cavity. Its irregularly lobular, concave, posterior aspect partially enfolds the stomach and pylorus. The liver comprises about 0.8 percent of the total body of the total body weight in adult ciscoes examined (Table 1). The age of these fish as determined by counting scale annuli ranges from 4 to 8 years.

In gross aspect the muralia or cell sheets of male fish are disrupted and sinusoids are poorly defined. In contrast the muralia of females are distinct, having both unilamellar and bilamellar form, and sinusoids are coherent and well integrated with the vascular endothelium. The parenchymal cell margins are clearly set off from the non-staining intercellular space. Nuclei of the parenchymal cells are large relative to the cytoplasmic mass, occupying about 40% of the cell volume, and exhibit a granular and filamentous array of chromatin well dispersed in the nucleoplasm. Usually the nuclei are situated medially along the longer axis of each cell and may be in contact with the cell membrane along the shorter axis.

Female livers frequently contain scattered, roughly spherical clusters of large, extensively vacuolated cells with very pale cytoplasm and irregularly shaped, small densely stained nuclei. By comparison with routine biological

specimens the embedding and staining procedures employed here permit the conclusion that these cells normally contain globular lipid inclusions. Typically these lipoidal droplets range from 20 to 100 microns in diameter. Males, in contrast, did not show such clusters of vacuolated cells.

The average number of nuclei evident in the standard area ranged from 13.6 to 17.5 for males and 19.8 to 29.8 for females, establishing a larger size of the hepatocytes for male fish (Table 2).

The sizes of normal nuclei in male ciscoes are commonly, but not invariably, larger than those for the females. The Class I nuclei (N=905) of the livers from males exhibit an average diameter of 5.7 microns, as opposed to 5.4 microns for Class I nuclei (N=734) in the livers of females. True diameters may be slightly larger than those reported because not all nuclei measured are necessarily shown in a perfect equatorial plane of section.

In male ciscoes, nuclear vacuolization often progresses to such a degree that the nuclear membrane is distorted and, in some cases, herniated (Figs. 1 and 2). Enlargement of the nucleus seems to occur at the expense of the cytoplasm because cell size appears to remain relatively constant. Disruption of the cytoplasm is not evident, and rupture of the cell membrane is not noted. By direct contrast, the 7 females show nuclear vacuolization in only two cases and even in these vacuoles never occupy the full volume of the nucleus (Figs. 1 and 2).

The chromatin in nuclei with large vacuoles has lost its particulate character and has become a homogeneous, darkly staining periméter: (or limbus) of varying thickness. Simultaneously, the nuclear diameter is increased by about 12%, between classes I - III and IV - V (Table 3).

Table 2. Comparison of liver cell densities for male and female specimens of the cisco, Coregonus artedii LeSueur, from Lake George, New York (1975).

Males				Females			
Specimen No.	Number of Fields Counted	Number of Nuclei Counted	Number of Nuclei Per Standard Area	Specimen No.	Number of Fields Counted	Number of Nuclei Counted	Number of Nuclei Per Standard Area
283-1	6	95	15.83	282-1	5	99	19.80
287-6	6	105	17.50	287-1	4	104	26.00
287-8	7	110	15.71	287-2	4	96	24.00
287-10	6	94	15.67	287-3	5	101	20.20
288-2	6	100	16.67	288-1	4	119	29.75
288-3	7	95	13.57	288-5	4	113	28.25
288-4	7	113	16.14	288-6	4	102	25.50
288-9	6	93	15.50				
288-10	6	100	16.67				
Totals	57	905	15.88		30	734	24.47

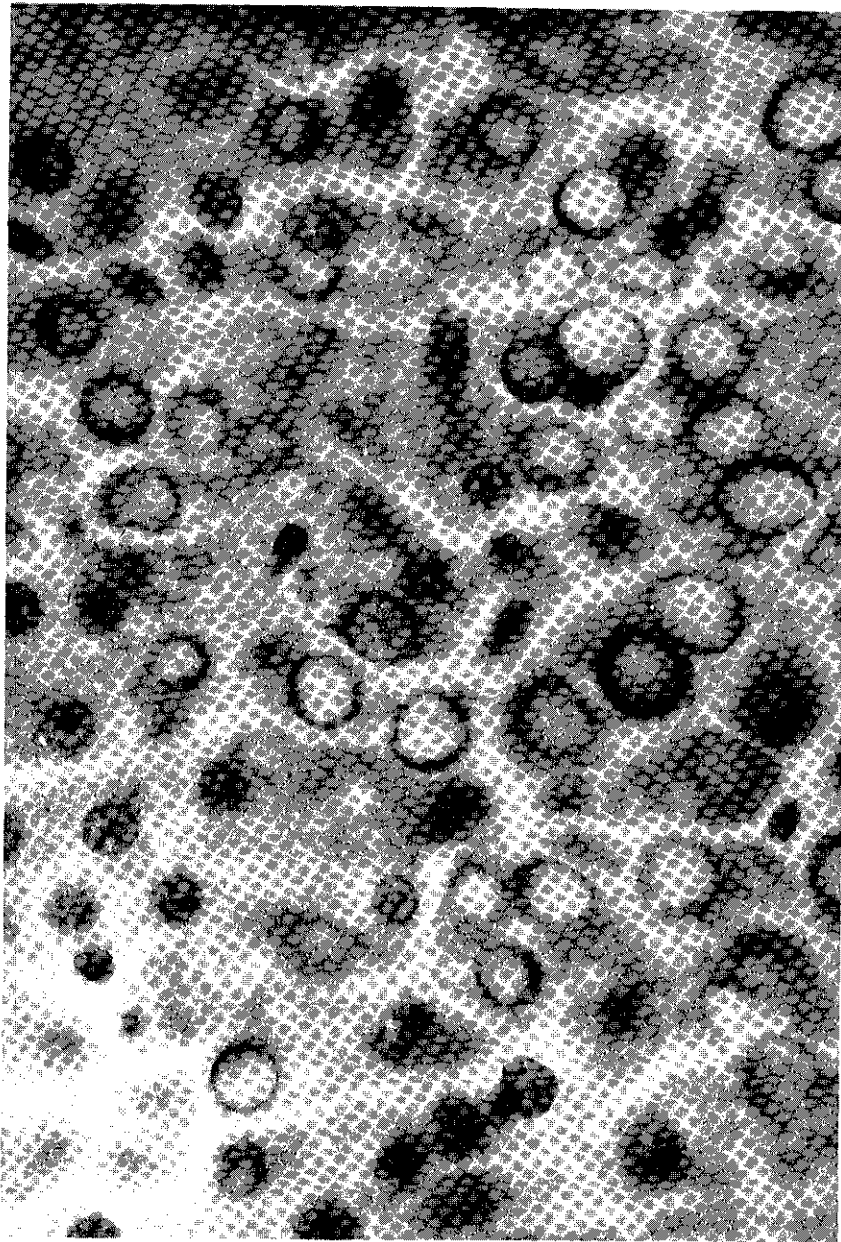


Fig. 1. The liver of a male cisco (LN 283-1), Coregonus artedii LeSueur, from Lake George, New York, showing enlarged, vacuolated nuclei with margined chromatin and other classes of nuclei as defined in the text. ib, inclusion body; I, Class I nucleus, etc. See text for definition of class types.

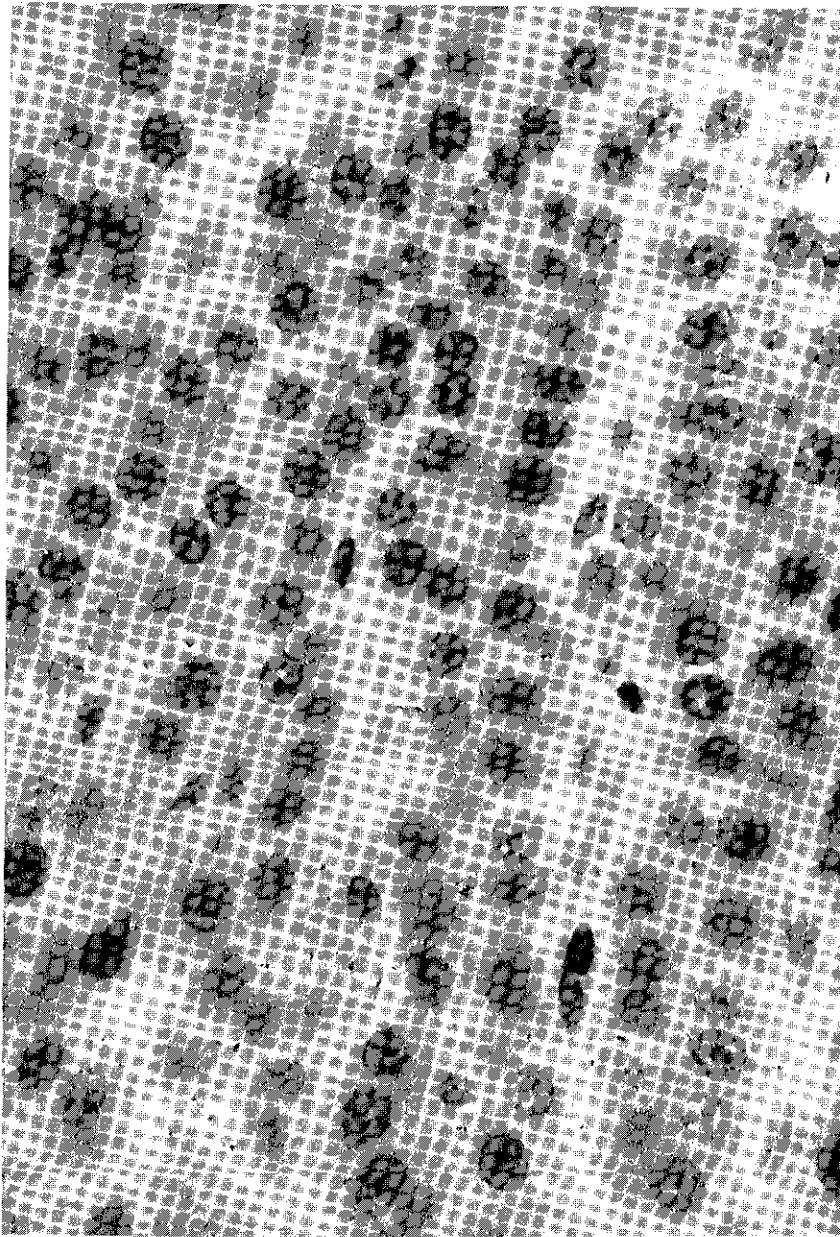


Fig. 7. The liver of a female cisco (LN 282-1) Coregonus artedii LeSueur, from Lake George, New York, showing the abundance of Class I parenchymal cell nuclei and intact muralia. I, Class I nucleus. See text for definition of class types.

Table 3. The average diameter in microns of liver cell nuclei, by sex and by class (see text), of the cisco, *Coregonus artedii*, in Lake George, New York. The percentage by class is also presented for each specimen. (1975)

	Male						Female					
	(N) Specm. No.	Class					(N) Specm. No.	Class				
		I	II	III	IV	V		I	II	III	IV	V
μ	(95)	6.0	6.5	6.5	7.9	8.9	(99)	5.6	-	-	-	-
%	283-1	35.8	7.4	4.2	43.2	9.5	282-1	100	-	-	-	-
μ	(105)	5.8	5.8	5.5	7.2	8.6	(104)	5.4	-	-	-	-
%	287-6	3.8	73.3	14.3	5.7	2.9	287-1	100	-	-	-	-
μ	(110)	5.6	5.8	5.8	-	-	(96)	5.6	-	-	-	-
%	287-8	50.9	40.0	0.9	0.0	0.0	287-2	100	-	-	-	-
μ	(94)	5.5	5.6	5.4	6.4	6.6	(101)	5.4	5.4	5.2	-	-
%	287-10	38.3	11.7	9.6	37.2	3.2	287-3	50.5	45.5	4.0	-	-
μ	(100)	5.8	6.0	-	7.0	-	(119)	5.3	5.5	-	-	-
%	288-2	56.0	38.0	0.0	6.0	0.0	288-1	96.6	3.4	-	-	-
μ	(95)	5.9	5.7	5.7	6.5	7.0	(113)	5.0	-	-	-	-
%	288-3	18.9	63.2	3.2	13.7	1.1	288-5	100	-	-	-	-
μ	(113)	5.9	5.7	6.3	6.1	7.2	(102)	5.6	5.6	5.5	-	-
%	288-4	28.2	54.0	1.8	15.0	1.8	288-6	25.5	68.6	5.9	-	-
μ	(93)	6.0	5.8	5.4	6.2	7.0						
%	288-9	22.6	66.7	6.5	1.1	1.1						
μ	(100)	5.2	5.4	5.2	5.8	5.7						
%	288-10	54.0	33.0	5.0	7.0	2.0						

The vacuolar contents are essentially non-staining or weakly basophilic except for refractile inclusions of uniform size which were occasionally aggregated centrally within the vacuole. These inclusions are eosinophilic, spherical and may form clusters several microns in diameter. Under phase contrast they are especially obvious as brightly illuminated bodies. Such inclusions are apparent in the vacuoles of male livers only and appear in less than 5 percent of all vacuolated nuclei. Nuclear diameters do not show a well defined increase from Class I through Class III. However, Class IV nuclei, where the vacuoles have reached the nuclear envelope, are consistently larger and show an average increase of 12 percent.

Sections with vacuolated nuclei treated with diastase and then periodic acid Schiff (P.A.S.) reagent exhibited a conspicuous positive reaction in the nucleoplasm of cells in stage III and IV, suggesting the presence of neuro-proteins or degradation products. The vacuoles were not P.A.S. positive in either the absence or presence of diastase.

Sections taken from the right posterior lobe of the liver were compared with those taken from the standard position on the anteroventral surface and were found to be similar, suggesting that nuclear characteristics were uniform throughout the liver in each specimen.

Discussion

Several generalizations emerge. Male ciscoes collected in two different basins nearly fifteen miles apart in Lake George, New York, in the spring of 1975 illustrate a high percentage of liver nuclei containing vacuoles of varying sizes, suggesting the lakewide extent of the phenomenon. In some cases these vacuoles appear to be related to an increase in nuclear volume and the eventual

rupture of the nuclear envelope. Our observations lead us to postulate that in certain hepatocytes, one or more small vacuoles form. Usually, these are adjacent to the nucleolus. These vacuoles enlarge and fuse, obliterating the nucleolus. The enlargement continues in the margination and dissolution of the chromatin. Upon contacting of the vacuole and nuclear envelope, the nuclear volume then increases to eventually cause the rupture of the nucleus. The vacuolar contents were generally translucent, but in a small percentage of vacuoles, highly refractile (using phase technique) oval to spherical bodies could be seen. Additional nuclei could have lost these inclusion bodies in the course of the microtechnique. We have no evidence as to their nature, however electron microscopy should provide additional information.

In contrast to the males, female ciscoes illustrated very few nuclear vacuoles and, when present, they never appeared to induce herniation of the nuclear envelope.

The influence of the vacuolation warrants some speculation. Even though grossly vacuolated and herniated nuclei were common, little or no indication of cytoplasmic breakdown was evident and macrophages were generally lacking. The general organization of the liver tissue was however, greatly disrupted when vacuolization was extensive and the typical, well defined mix of unilamellate and bilamellate muralia, defining the blood bearing sinusoids, was lacking. Coupled with this was the more diffuse packing of cells constituting the liver parenchyma of the males, i.e., females exhibited an average of 54% more nuclei per unit of cross sectional area. Such a condition seems grossly destructive to the orderly flow of blood essential for the proper functioning of this key organ and thus the vigor and survival of the organism.

The relative absence of males observed in the population may thus be linked to this condition. Extending upon this, we have searched for examples of male cisco dieoffs and cite the unpublished case for the Ashokan Reservoir, Ulster County, New York, where a mortality of only male ciscoes was found in late December early January of 1973-1974 and 1974-1975. We have not, however, observed such a phenomenon in Lake George and thus are led to suggest that the liver conditions results in a morbidity which facilitates capture by predators such as the lake trout and northern pike. A predominance of male ciscoes in the stomachs of such predators would support this idea.

To the best of our knowledge nuclear vacuolization has not yet been reported for the coregonine fishes, and, given the deterioration of their populations over the last 75 years, the observations made here may hold some importance. Unfortunately, and as is the case for several other commercially important species (e.g. menhaden, Cahn, 1975), a histological atlas does not exist for the cisco or any of its congeners and thus a baseline for comparison is unavailable. Works on a number of other salmonids are available, however, and permit a conclusion that the observed liver condition is indeed aberrant (Elias and Bengelsdorf, 1952; Weinesb and Bilsted, 1955; Simon et al, 1967; Anderson and Mitchum, 1974).

Sexual dimorphism of the liver has been reported in a number of cases and has been shown, in part, to be under hormonal control (Kobayashi, 1953; Egami, 1955). Following the implantation of estrone pellets in the loach, Misgurnus anguillicaudatus, the liver cells of males became smaller while the nuclei enlarged (Kobayashi, 1953). Furthermore, the observation is made that the female loach generally exhibits larger liver nuclei and nucleoli, but smaller liver cells than in males. Simon et al. (1967) have noted, to

the contrary, the consistently larger liver nuclei in male trout than in females. Our data support the observations of Simon et al., in general but not without exception. In 254 nuclei (Class I) measured among 8 male ciscoes, liver nuclei averaged 5.7 μ in diameter while 604 nuclei measured in 7 female ciscoes averaged 5.4 μ indicating that the liver nuclei of males were about 6 percent larger in diameter. The difference, though significant, may be seasonal and a year-round survey seems essential.

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References Cited

- Anderson, B. G. and D. L. Mitchum. 1974. Atlas of trout histology. Bull. No. 13, Wyoming Fish and Game Dept. 110 pp.
- Brain, Paul F. 1972. Mammalian behavior and the adrenal cortex - a review. Beh. Biol. 7: 453-477.
- Cahn, Phyllis H. 1975. The pathology of the liver and spleen in naturally stressed Atlantic menhaden In W. E. Ribelin and George Migaki (eds). The Pathology of Fishes. Univ. Wisc. Press, Madison. 1,004 p.
- Christian, John J. 1950. The adreno - pituitary system and population cycles in mammals. J. Mam. 31(3): 241-259.
- Christian, John J. 1975. Hormonal control of population growth, p. 205-274. In B.A. Eleftheriou and R.L. Sprott, Hormonal correlates of behavior, vol. 2, Plenum, New York.
- Egami, Nobuo. 1955. Effect of estrogen and androgen on the weight and structure of the liver of the fish Oryzias latipes. Annot. Zool. Japonensis 28(2): 79-85.
- Elias, H., and H. Bengelsdorf. 1952. The structure of the liver of vertebrates. Acta Anatomica 14: 297-337.
- Kobayashi, Hideshi. 1953. Effects of estrone upon the structure, weight and fat content of the liver of the fish, Misgurnus anguillicaudatus. Annot. Zool. Japonensis 26(4): 213-216.
- Simon, R. C., A. M. Dollar and E. A. Smuckler, 1967. Descriptive classification of normal and altered histology of trout livers. In Trout Hepatoma Research Conference Papers (J.E. Halver and I. A. Mitchell). U.S. Dept. of Health, Education and Welfare and U. S. Dept. of the Interior Research, Report #70, pp. 18-28.
- Weinreb, E. L., and N. M. Bilsted. 1955. Histology of the digestive tract and adjacent structures of the rainbow trout, Salmo gairdneri irideus Copeia 3: 194-204.