AN ABSTRACT OF THE THESIS OF

JAMES TODD RYBOCK (Name) for the MASTER OF SCIENCE (Degree)

in Fisheries Science (Major) presented on May 4, 1973 (Date)

Title: USE OF OTOLITHS TO DIFFERENTIATE JUVENILE STEELHEAD TROUT FROM JUVENILE RAINBOW TROUT IN THE LOWER DESCHUTES RIVER, OREGON

Abstract approved: Redacted for privacy

Howard F. Horton

Reported are results of a study of otolith nuclei as a means to separate juvenile steelhead trout (Salmo gairdneri) from juvenile rainbow trout (S. gairdneri) which co-exist in the lower Deschutes River, Oregon. An intensive recreational fishery necessitated development of a technique for separation so that the impact of the fishery on each race could be assessed independently.

Results revealed that steelhead trout mature at a larger size than rainbow trout, egg size in both races is directly related to body size of dam, and size of otolith nucleus is positively correlated with egg size in rainbow trout. Examination of adults demonstrated, in fact, that otolith nuclei of steelhead are significantly larger than those of rainbow. Size of otolith nucleus does not change with growth of either fish, nor are there differences due to

sex or origin. The validity and utility of this method to separate the races were confirmed.

Regressions of length and weight of otolith on length of fish demonstrated that otoliths of juvenile steelhead grow at the same rate as those of rainbow, whereas adult steelhead on their spawning migration are longer than rainbow for a given length or weight of otolith.

A list of methods used by others to facilitate the viewing of otoliths and a discussion of the formation of the otolith nucleus are included.

Use of Otoliths to Differentiate Juvenile Steelhead Trout from Juvenile Rainbow Trout in the Lower Deschutes River, Oregon

by

James Todd Rybock

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1973

APPROVED:

Redacted for privacy

Professor of Fisheries

Redacted for privacy

Head of Department of Fisheries and Wildlife

Redacted for privacy

Dean of Graduate School

Date thesis is presented May 4, 1973

Typed by Velda D. Mullins for James Todd Rybock

ACKNOWLEDGMENTS

This project was funded by the Oregon Agricultural Experiment Station, by the Research Division of the Oregon State Game Commission (OSGC), and primarily by my teaching assistantship in the Department of Fisheries and Wildlife.

I wish to thank those who contributed to this research. Dr. Howard Horton (Professor of Fisheries, Oregon State University) initiated the project and helped (via funding and advice) with its completion. James Fessler (Fishery Biologist, OSGC) aided greatly by contributing time, samples and advice. Francis H. Sumner (Scale Analyst, OSGC) helped with steelhead scale reading. Other personnel from the Game Commission, and especially Dr. Harry Wagner from the Research Division in Corvallis, were helpful. William Staeger (former graduate student, unemployed) thoroughly criticized the manuscript.

And, of course, I thank Shirley, whose mere presence demands the highest of gratitude, a blessing without words. Marysia, smile at ma-ma.

TABLE OF CONTENTS

Chapter	Page
INTRODUCTION	1
METHODS AND MATERIALS	5
Study Area	5
Organization of Research	5
Collection of Samples	8
Storage and Treatment of Otoliths	11
Terminology and Examination of Otoliths	13
RESULTS	17
Indirect Approach	17
Direct Approach	20
Effect of Sex, Origin and Size of Fish on Otolith Size	25
Relationship Between Fish Length and Otolith Size	26
DISCUSSION	30
Treatment of Otoliths	30
Formation of the Otolith Nucleus	32
Race Differentiation	33
Size of Entire Otolith	33
Size of Otolith Nucleus	34
LITERATURE CITED	38

LIST OF TABLES

Table		Page
1.	Mean lengths (mm) of otolith nuclei of wild and hatchery-reared steelhead trout from the Deschutes River, Oregon (1971-1973).	25
2.	Methods used to increase the readableness of otoliths.	31

LIST OF FIGURES

Figure		Page
1.	Lower 100 miles of the Deschutes River, Oregon.	6
2.	Research plan to investigate the otolith nucleus as a means of differentiating juvenile steelhead trout from juvenile rainbow trout.	7
3.	Photomicrographs (50X) of an otolith of Salmo gairdneri (A) before and (B) after HCl treatment.	13
4.	Illustration of (A) otolith and (B) otolith nucleus of Salmo gairdneri, with notation of measurements used.	15
5.	Length-frequency distribution of mature (>20 cm) rainbow trout and steelhead trout from the lower Deschutes River, Oregon (1972).	18
6.	Means and ranges of egg size plotted against length of dam for rainbow trout and steelhead trout from the lower Deschutes River, Oregon (1972).	19
7.	Regression of length of rainbow trout dam from the Deschutes River on mean length of otolith nucleus of offspring cultured in Corvallis, Oregon (1972). (Circles are means, and vertical bars are ranges, of observations.)	21
8.	Length-frequency distribution of otolith nuclei of rainbow trout and (A) all steelhead trout or (B) steelhead trout excluding those from Wizard Falls Hatchery. All fish were captured Aug. 1971-Jan. 1973 from the lower Deschutes River, Oregon.	23
9.	Regressions of otolith length on fish length for steelhead trout (adults) and rainbow trout in the lower Deschutes River, Oregon (1971-1973). Included are representative values for juvenile steelhead trout.	27

Figure | Page

10. Regressions of otolith weight on fish length for steelhead trout (adults) and rainbow trout in the lower Deschutes River, Oregon (1971-1973). Included are representative values for juvenile steelhead trout. 29

USE OF OTOLITHS TO DIFFERENTIATE JUVENILE STEELHEAD TROUT FROM JUVENILE RAINBOW TROUT IN THE LOWER DESCHUTES RIVER, OREGON

INTRODUCTION

The purpose of this investigation was to determine if growth characteristics of the sagittae, the largest of the otoliths, can be used to separate objectively juvenile rainbow trout (Salmo gairdneri) from juvenile steelhead trout (S. gairdneri). The nucleus of the otolith is formed early in steelhead trout embryos, and size of nucleus appears to be directly related to size of egg (McKern 1971). Because egg size and fish length of salmonids are directly related (McFadden et al. 1965; Bulkley 1967; Galkina 1970), and because steelhead trout are larger than rainbow trout at maturity, my hypothesis was that differences in size of the otolith nucleus could logically be used to separate juveniles of the two races. A second hypothesis was that regressions of length and weight of otolith on length of fish would reflect differences in growth rates sufficient to separate juveniles of the two races. These hypotheses were tested on fish captured in 1971-1973 from the lower Deschutes River, Oregon.

The ability to differentiate juveniles of the two races in their natural habitats would aid investigators studying their genetic and physiological differences. Although the two forms have been

considered separate species (Jordan 1905; Kendall 1920), recent workers have agreed that differences between forms are insufficient to justify such separation (Taft 1933). Neave (1944) concluded that the tendency of S. gairdneri to migrate or not is largely controlled by hereditary factors. Regarding salmonid anadromy, Behnke (1965) stated the following:

> Although the basis for anadromous or nonmigratory behavior is mainly genetic, the genetic difference must be slight and easily modified. The result is that no constant character can separate nonmigratory from anadromous populations.

However, this conclusion is not universally accepted.

> In watersheds where both sea-going and non-sea-going forms exist (which is over most of the rainbow's native range), it is still a moot question whether both occur mostly because of inherited differences, or mostly because of fortuitous environmental differences affecting young fish leading them either to migrate or to remain in the stream (Withler 1972).

The inability to separate these races also poses many problems in their independent management.

> If they [rainbow and steelhead] should prove to be distinct, that is, if freshwater rainbows always produce offspring that never go to sea and if steelhead always produce offspring that never remain in fresh water, then the problem of conservation involves a separate treatment for each of the types (Mottley 1936).

In the lower Deschutes River, the most intensive fishery for rainbow trout occurs during the first week in May, when most downstream movement of steelhead smolts occurs; consequently, the catch may be composed of 22-80% juvenile steelhead (Wagner

and Haxton 1968; King 1966). Since the magnitude of the sports fishery on the lower Deschutes River is high--in 1969, 14,438 anglers fished 68,854 hours on the river and caught 4,381 steelhead trout (Fessler 1971)--a means is needed to assess the impact of the spring sport fishery on the production of steelhead trout. In addition, large numbers of hatchery-reared steelhead may remain in the Deschutes River as a resident population (Wagner and Haxton 1968); whether this residual characteristic is caused by fish cultural practices or is inherent in the Deschutes River race of summer steelhead can be determined only when biologists can accurately identify wild juvenile steelhead. (Hatchery steelhead are easily identified by marks placed on the fish before liberation.)

Otoliths have been widely used as indicators of life histories (Hickling 1931; Martin 1941; Grainger 1953; Scott 1954; Kohler 1958, Watson 1964; Jensen 1970), in back calculations of length (Mina 1967; Holland 1969; McKern 1971), and in separation of species (Schmidt 1966; Fitch and Barker 1972). The advantages of employing these bony structures for the above information were reviewed by McKern (1971).

The use of otoliths in the differentiation of stocks and races of fish is a more recent development. Otoliths of herring (Clupea harengus) that spawn in summer-autumn are distinguishable from otoliths of winter-spring spawners (Einarrson 1951; Wood and

Foster 1966; Danielssen 1969; Messieh 1969). Altukhov and Mikhalev (1965) found significant differences in length-width ratios of otoliths from two races of Black Sea horse-mackerel. Kim (1963) found differences in otolith characteristics (appearance of hyaline rings and size of opaque rings) between spawning groups of red salmon (Oncorhynchus nerka).

Few investigations have dealt with the otolith nucleus. Sinoda and Jayashinghe (1971) were able to separate races of Glossanodon semifasciatus based on the degree of opacity of the nucleus. Messieh (1972) found that otolith nuclei of spring-hatched herring are smaller than those of autumn-hatched herring; he suggested that the shorter larval period of the former could account for this difference. The study most relevant to this investigation demonstrated that winter and summer races of steelhead trout can be separated on the basis of differences in the diameter of the otolith nucleus (McKern 1971).

METHODS AND MATERIALS

Study Area

The study area was the lower 100 miles of the Deschutes River in north-central Oregon (Fig. 1). The Deschutes River drains an area of approximately 10, 400 square miles, or nearly 11% of the land area of Oregon. Its western tributaries stem from the Cascade Mountains, while eastern tributaries drain Oregon's high plateau. Regulated river flows below Pelton Dam vary on the average from 3, 000 to 7, 100 cfs. The importance of the sport fishes in this section--resident trout, summer steelhead, and chinook salmon (Oncorhynchus tshawytscha)--was emphasized in 1970 when the lower 100 miles of the Deschutes River was placed under the protection of the Scenic Waterways Act (Montgomery 1971).

Organization of Research

The flow diagram in Fig. 2 was constructed to help clarify the experimental approach. Rectangles enclose conditional statements, i.e., that which must be investigated. Circled statements represent junction points which are equivalent to experimental objectives or results; the large circle surrounds the primary objective.

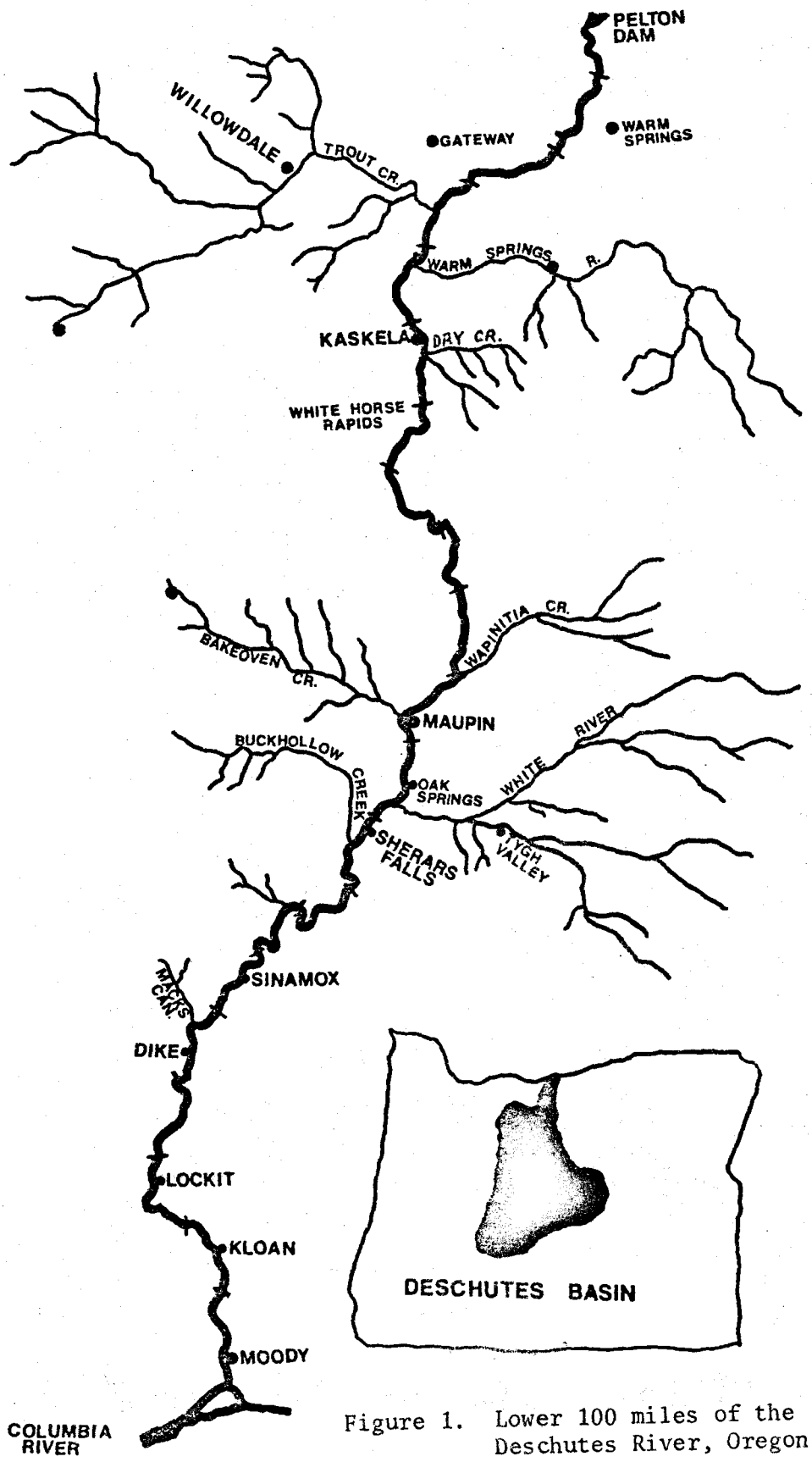
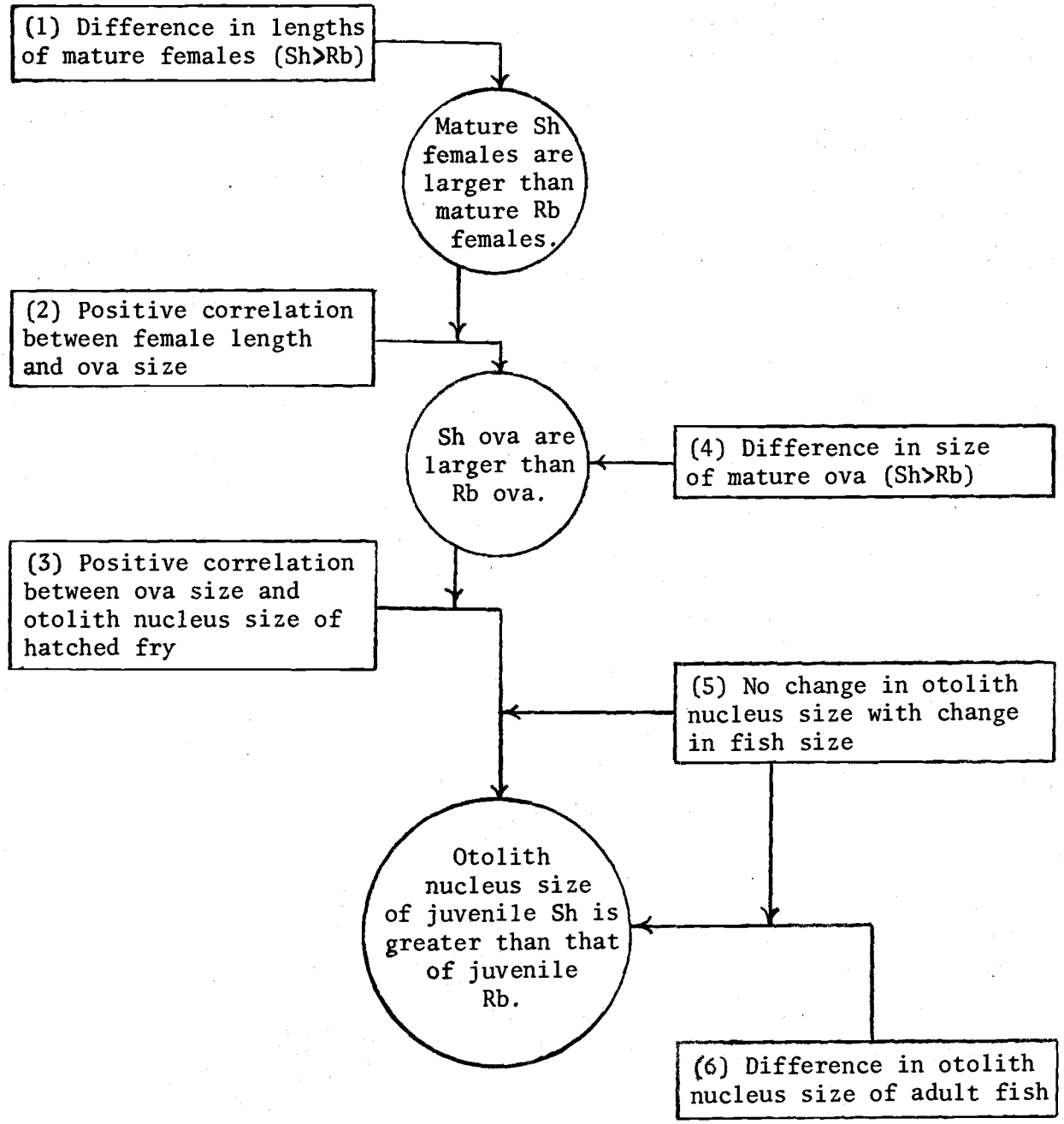


Figure 1. Lower 100 miles of the Deschutes River, Oregon.



Sh = Steelhead Trout
Rb = Rainbow Trout

Figure 2. Research plan to investigate the otolith nucleus as a means of differentiating juvenile steelhead trout from juvenile rainbow trout.

There are two major approaches to the primary objective. The first, the indirect or theoretical, is through examination of the conditional statements in Rectangles 1 to 5. The second, the direct or functional, is through examination of the statements in Rectangles 5 and 6. The results of either approach may serve as verification of the results of the other.

Collection of Samples

To determine body lengths of mature steelhead trout and rainbow trout (Rectangle 1), I used data collected by J. Fessler (Fishery Biologist, Oregon State Game Commission, Corvallis). In 1972, he obtained samples by electrofishing in the lower Deschutes River. Fork lengths were measured, and race was determined from hatchery marks or coloration, since migrating summer steelhead are distinguished from rainbow by their silvery appearance.

For determination of ova size (Rectangles 2 and 4), adult steelhead were captured in late winter 1972 by trapping below Pelton Dam and were held in tanks at Round Butte Dam (located immediately above Pelton Dam) until ripe (Fig. 1). Twenty-two females were measured (fork length), and a sample of eggs (ca. 100) was collected from each fish, fertilized, and allowed to water harden 8-22 hours. From 20 to 60 eggs from each pairing were then

measured volumetrically (10^{-2}ml) in a 25 ml burette.

Rainbow trout were captured in spring 1972 by electrofishing in the main stem of the Deschutes River. When ripe, male-female pairs were individually spawned. After water hardening, the eggs were transported to the laboratory of the Research Division of the Oregon State Game Commission in Corvallis to be hatched. Shortly after arrival, 20 eggs from each of 13 matings were measured as above. Fork lengths were later determined from the frozen dams and sires.

To obtain samples for determination of correlation between egg size and size of otolith nucleus of the hatched fry (Rectangle 3), I randomly selected 10 fingerlings from each of eight available matings of rainbow trout individually hatched and reared in Corvallis (above). Fork lengths were measured, and otoliths were removed by dissection.

To investigate the conditional statement in Rectangle 6, it was necessary to define "adult". Fessler (personal communication) measured fork lengths of 80 smolts from Bakeoven Creek (Fig. 1); the mean was 161 mm and the range was 141-217 mm. Based on these data and on Fessler's experience, any fish longer than 200 mm was probably either a resident rainbow (at or approaching maturity) or a steelhead on its spawning migration; consequently, I considered such fish adults.

Adult rainbow and steelhead (n=101) were sampled during routine Oregon State Game Commission creel censuses at Webb's access road (at Buckhollow Creek) and near Maupin (Fig. 1) during August and September, 1971 and 1972. Otoliths were removed with a punch described by McKern and Horton (1970). The fork length of each fish was measured, and scales (ca. 20) were removed from an area below the origin of the dorsal fin and just above the lateral line. Race was determined from coloration, size (see results), and analysis of scales (Maher and Larkin 1954). In most cases sex was determined from jaw conformation and opercular coloration (steelhead only), and from fishermen's observations if the fish had been cleaned. To determine origin, I examined steelhead for hatchery marks, while hatchery-reared rainbows were distinguished by worn or rounded fins, excessive number of missing scales, and other abnormalities (Fessler, personal communication).

Adult fish were also collected for sampling by electrofishing near Maupin, below Pelton Dam, and in Dry Creek, Bakeoven Creek and Trout Creek (Fig. 1) in April-June 1971 and August 1972. Each fish was measured, and race, sex, and origin were determined as above. Otoliths were removed by dissection.

In January 1973, 52 steelhead fingerlings were obtained from the stock of Deschutes River steelhead reared at Wizard Falls Hatchery (Oregon State Game Commission) on the Metolius River.

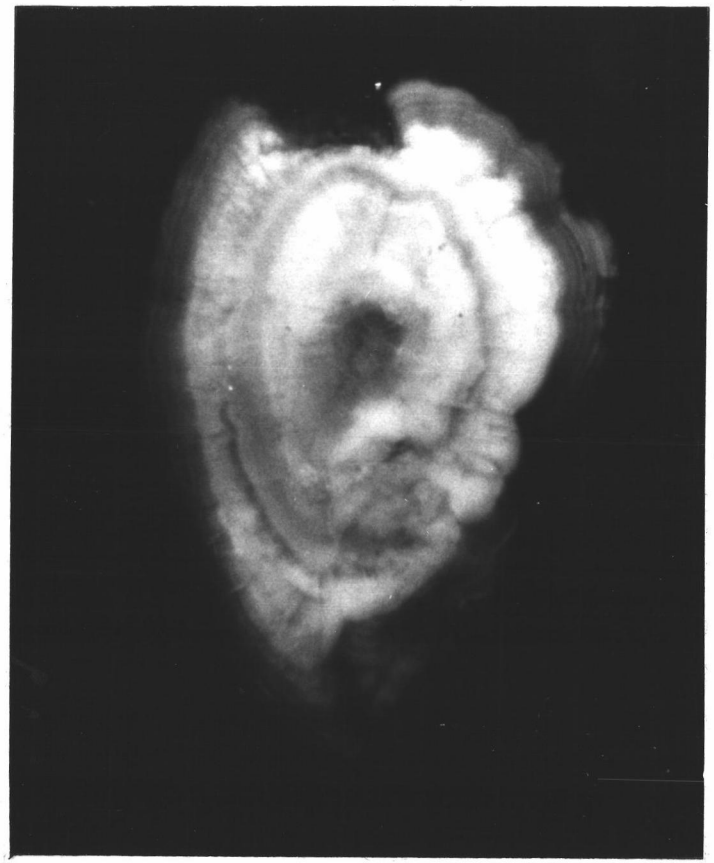
These fish represented a random assortment of the offspring of ca. 150 females captured below Pelton Dam. In addition to the 80 rainbow fingerlings obtained from the eight matings described above, 10 additional specimens were obtained from Oak Springs Hatchery (Oregon State Game Commission). (I discovered later that these fish do not represent the original Deschutes River stock but, rather, were spawned from hatchery stock descendant from fish taken from Roaring River, Oregon, in the 1930's. Since mean sizes of their otolith nuclei did not differ from the Deschutes River rainbow, these values were retained in the results.) In all cases, fork lengths were measured, and otoliths were removed by dissection.

Storage and Treatment of Otoliths

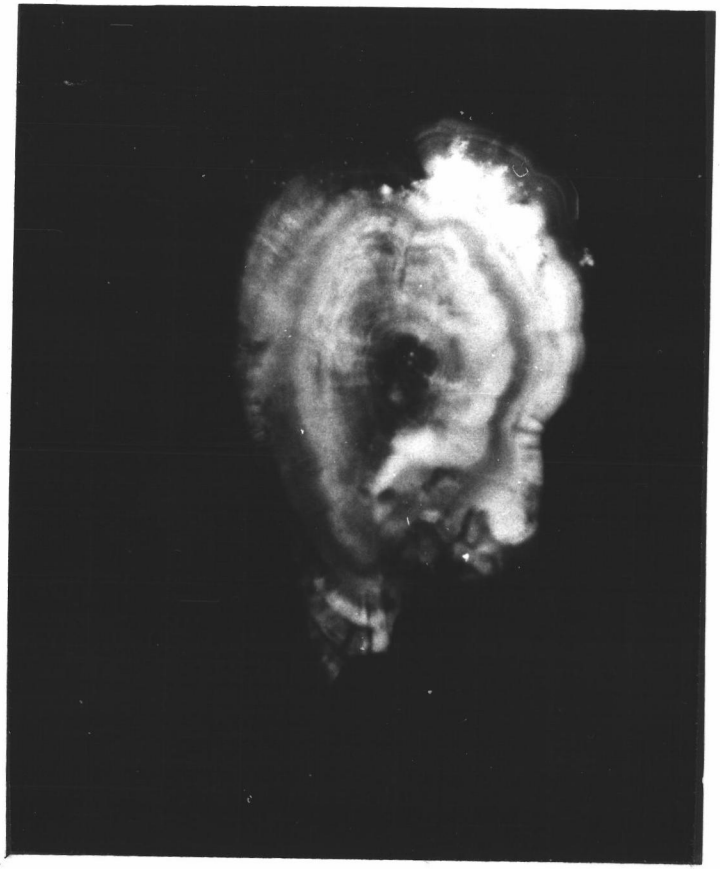
The sacculus was removed from each otolith prior to storage. Initially, otoliths were stored dry in coin envelopes before transfer to a clearing solution. Because they became brittle and prone to breakage, later samples were placed in a clearing solution immediately after removal from the fish. Otoliths were cleared from 1 to 21 months before examination; there was no apparent relationship between clearing time and readableness of the otolith.

Samples were initially cleared in methyl salicylate. Because some otoliths did not clear sufficiently, and because McKern (1971) obtained satisfactory results with a 50:50 mixture of glycerin and

water, this latter solution was used for the remainder of the samples. The glycerin solution tended to increase the opacity of the entire otolith, however, and it was judged less effective than methyl salicylate; therefore, I investigated other techniques to increase the readableness of the otolith. Neither burning the otolith on an asbestos pad over a Bunson burner nor clearing the otolith in oil of cloves increased the difference in contrast between the opaque and hyaline parts. The major problem in delineating the nucleus seemed to reside in the great opacity of the medial (convex) surface, which partially prevented light from passing through the hyaline structures. Grinding of this surface with an electric hand drill usually resulted in breakage. A satisfactory solution was reached by applying small drops of HCl to this medial surface; the results were a dissolution of the medial lobes, a consequent thinning of the otolith, and an increased readableness. This method is quick (a few ml of HCl applied for 2-4 min for a large otolith) and is easily controlled by periodic inspection of the otolith during treatment. Examination of a number of otoliths both before and after treatment demonstrated that this procedure does not alter the size of the nucleus; therefore, HCl treatment was used for all otoliths that had been cleared in the glycerin solution. Photographs of an otolith before and after treatment are presented in Fig. 3. Because the edges of the otolith are dissolved, this method should



A



B

Figure 3. Photomicrographs (50X) of an otolith of Salmo gairdneri (A) before and (B) after HCl treatment.

not be used when age determinations are required.

Otoliths were placed lateral surface up on black plexiglass depression plates, illuminated with a beam of light at 45°, and photographed with a 35 mm camera through a compound microscope at 50X. Panatomic-X film (ASA 32) was used, and the negatives were enlarged to 4 X 5 or 5 X 7 inches onto grade 3 or 4 (high contrast) paper. In most cases I printed the sample number on the back of each photo to reduce bias during reading of the otolith. A stage micrometer was also photographed and enlarged at the same magnifications so that otolith measurements could be determined from the photographs.

Terminology and Examination of Otoliths

An otolith of S. gairdneri, as seen under reflected light on a black background, is illustrated in Fig. 4. The nucleus is hyaline with a narrow opaque ring around the border; the metamorphic check is a narrow hyaline ring delineating the nucleus (Kim and Koo 1963).

If there was no breakage which would affect the measurements, total length and width (10^{-2}mm) and weight (10^{-2}g) of each otolith were determined prior to HCl treatment. The linear dimensions were measured with an ocular micrometer in a dissecting microscope; otoliths were blotted dry before being weighed on an

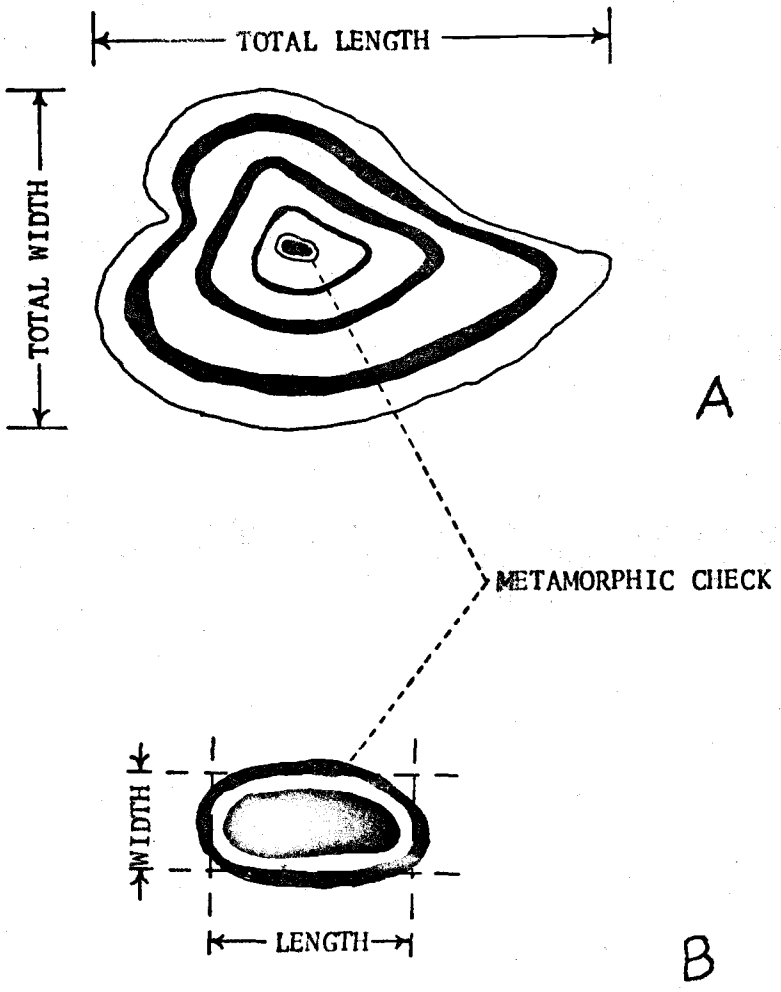


Figure 4. Illustration of (A) otolith and (B) otolith nucleus of Salmo gairdneri, with notation of measurements used.

analytical balance. The length and width of the nucleus was measured from the photographs by using a compass and the corresponding photograph of the micrometer. If there was uncertainty concerning size or position of the nucleus, measurements were not made.

RESULTS

Indirect Approach

The number and lengths of rainbow trout and steelhead trout captured by Fessler in the lower Deschutes River in 1972 are presented in Fig. 5. Discrete size ranges, evident in these data, were also demonstrated by the data collected for this study. Although these are not necessarily spawning fish, it seems obvious that there is no significant overlap in length of spawning rainbow and steelhead trout.

The mean egg size of steelhead (0.0936 ml) was significantly greater ($P \leq 0.001$) than that of rainbow (0.0727 ml). The means and ranges of egg size from each dam are plotted against fork length of dam in Fig. 6. Mean egg size is strongly correlated with length of female ($r = 0.829$ and 0.791 for rainbow and steelhead trout, respectively [Fig. 6]); however, there was much variability of mean egg sizes between fish of a similar length and of egg sizes within any one female. For some fish, the largest egg was twice the size of the smallest.

The presence or absence of any correlation between egg size and size of otolith nucleus of the hatched fish could not be determined directly for the rainbow trout groups reared in Corvallis;

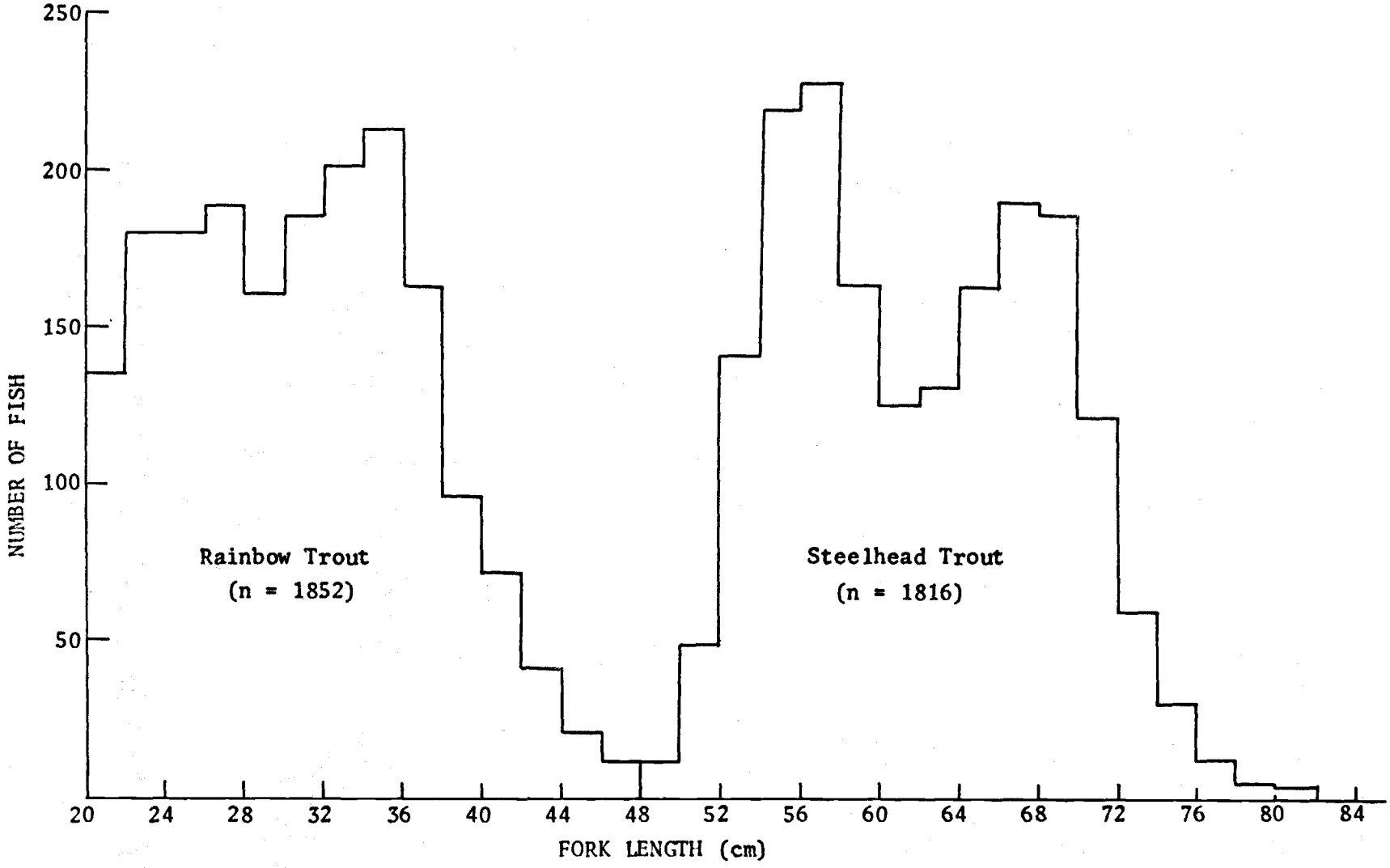


Figure 5. Length-frequency distribution of mature (>20 cm) rainbow trout and steelhead trout from the lower Deschutes River, Oregon (1972).

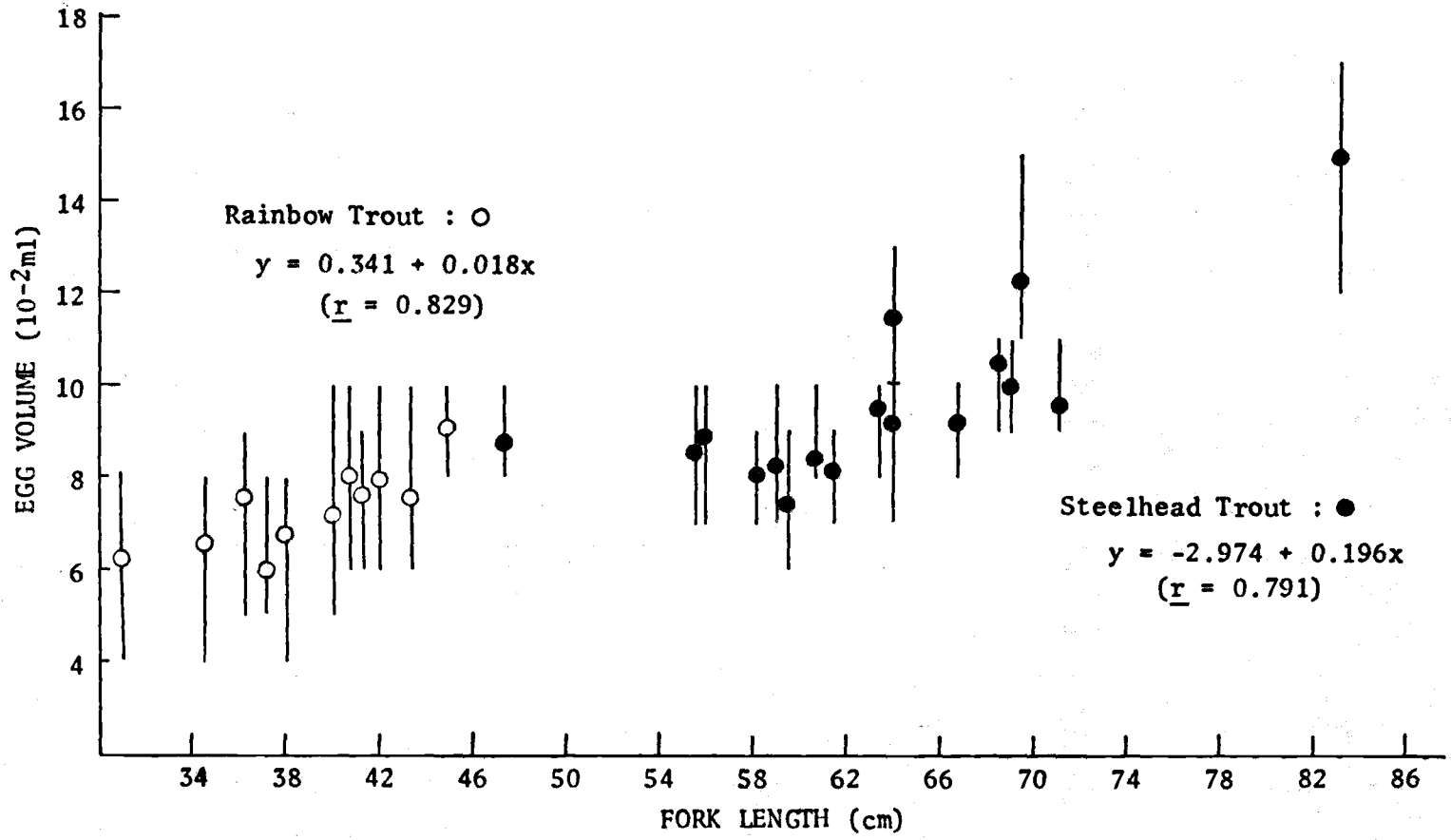


Figure 6. Means and ranges of egg size plotted against length of dam for rainbow trout and steelhead trout from the lower Deschutes River, Oregon (1972).

egg size had been measured for only four of the eight females whose offspring were available, and I considered this sample size too small. However, since there was a strong correlation between egg size and length of dam, this latter measurement was regressed against size of otolith nucleus of offspring from the eight matings (Fig. 7). The r value and overlap of ranges indicate the relationship is not strong; however, it is a positive correlation (unlike the regression of length of otolith nucleus of offspring on length of sire, where $r = -0.484$), and extraneous factors which may account for this relationship are discussed later.

In sum, these indirect results suggest that mean size of otolith nucleus of steelhead should be greater than that of rainbow; however, overlap of sizes is likely.

Direct Approach

Of 641 otoliths examined, nuclei of 189 (29%) were not sufficiently distinct to permit measurement. Usually, the hyaline center of the nucleus was visible, but the metamorphic check could not be distinguished. This may have been due to HCl treatment, which in some cases rendered visible groups of daily growth bands which previously were obscure; these bands were often confused with the metamorphic check.

For the 92 samples where both left and right otoliths were

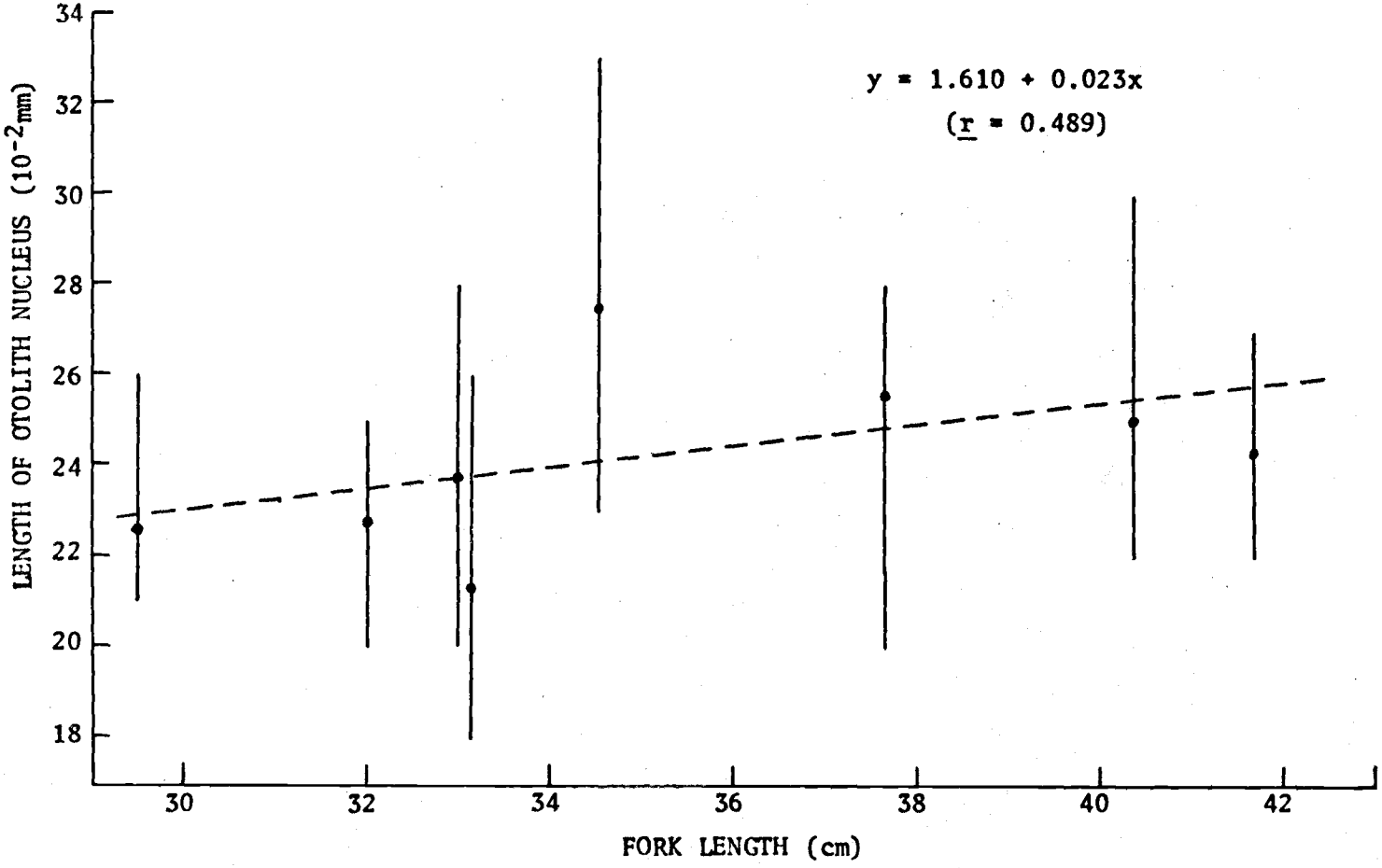


Figure 7. Regression of length of rainbow trout dam from the Deschutes River on length of otolith nucleus of offspring cultured in Corvallis, Oregon (1972). (Circles are means, and vertical bars are ranges, of observations.)

measured, I tested the hypothesis (paired t test) that neither of the pair was significantly larger than the other. With nucleus length $t = -0.874$, and with nucleus width $t = -1.451$; the null hypothesis cannot be rejected in either case ($P > 0.35$ and 0.15, respectively). Also, there was agreement between the two measurements of each pair. Means were computed for these 92 samples and are used in subsequent analyses; where only one otolith of a pair was readable, that single measurement is used.

The linear correlation between length and width of otolith nucleus was strong in both rainbow ($r = 0.838$) and steelhead ($r = 0.916$). Neither seemed easier to read. Since there possibly would be less percentage variation due to measurement when using the larger dimension, length of otolith nucleus is emphasized in the following.

The mean lengths of otolith nuclei of steelhead (0.354 mm) and rainbow trout (0.243 mm) differed significantly ($P \leq 0.001$). (The mean widths of otolith nuclei of steelhead [0.230 mm] and of rainbow [0.154 mm] also differed significantly [$P \leq 0.001$].) The length-frequency plot of these data (Fig. 8A) demonstrates an overlap of lengths. Most unexpected in this plot are the lengths (for steelhead) less than 0.26 mm. These values occur in direct proportion to the values for rainbow; also, these steelhead are from Wizard Falls Hatchery, where both rainbow and steelhead are

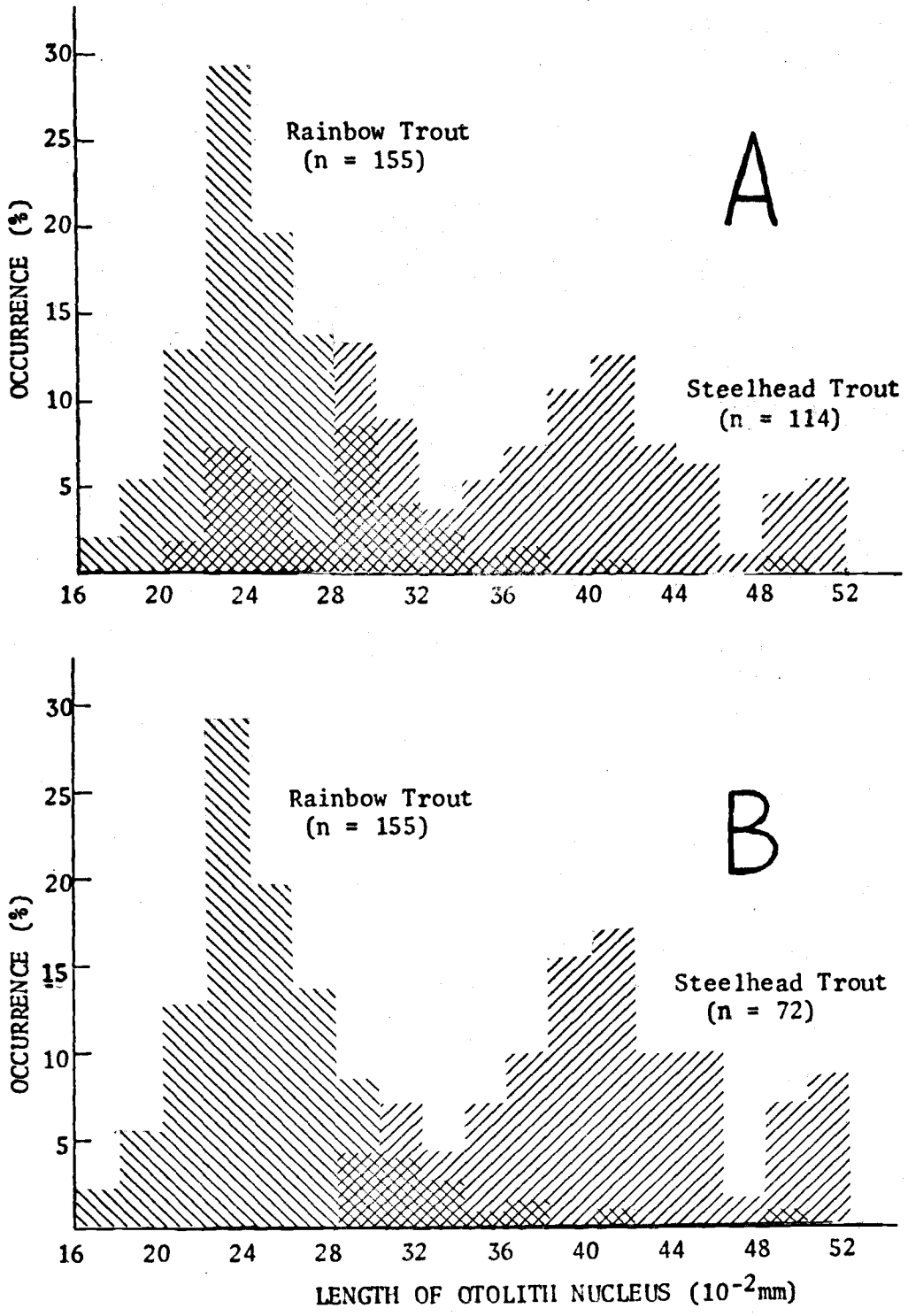


Figure 8. Length-frequency distribution of otolith nuclei of rainbow trout and (A) all steelhead trout or (B) steelhead trout excluding those from Wizard Falls Hatchery. All fish were captured Aug. 1971 - Jan. 1973 from the lower Deschutes River, Oregon.

reared. Perhaps these fish are rainbow offspring which were inadvertently mixed with steelhead or were spawned from rainbow identified as steelhead. The length-frequency plot of otoliths from steelhead excluding those from Wizard Falls Hatchery is presented in Fig. 8B. This exclusion has increased the normality of the histogram; the nadir at 0.46-0.48 mm is probably due to the small sample size of each interval.

The histogram for rainbow more closely approximates a normal distribution, probably the result of a larger sample size and of the many sources of variation operating within a more narrow size range of spawning fish. The length of the otolith nucleus of one adult rainbow was 0.48 mm. Although no hatchery marks were noticed, scale characteristics suggested a hatchery origin; because hatchery-reared rainbow may not reach maturity in the lower Deschutes River, this may have been a steelhead turned resident. In general, though, my data do not support the suggestion of Wagner and Haxton (1968) that there may be a great number of such reversions in the Deschutes River.

Regarding use of these data for identification of S. gairdneri from the lower Deschutes River, if juvenile fish with otolith nucleus lengths less than 0.28 mm are considered rainbow and those with lengths greater than 0.34 mm are considered steelhead, then most fish will be identified (the actual degree depends on the proportion

of juvenile rainbow to juvenile steelhead present in the river) and with a high degree of certainty (ca. 100% and 96%, respectively, using data from Fig. 8B).

Effect of Sex, Origin and Size of Fish on Otolith Size

Mean length of otolith nuclei was 0.339 mm for all females and 0.317 mm for all males; they are not significantly different ($P > 0.20$). Also, the data suggest no significant male-female difference within either race.

Mean length of otolith nuclei of wild steelhead was compared to that of hatchery-raised steelhead (Table 1). The difference becomes insignificant if the fish obtained from Wizard Falls Hatchery are

Table 1. Mean lengths (mm) of otolith nuclei of wild and hatchery-reared steelhead trout from the Deschutes River, Oregon (1971-1973).

Data used	Wild (n)	Hatchery (n)	P*
All fish	0.395 (52)	0.321 (62)	≤ 0.001
Excluding fish from Wizard Falls Hatchery	0.395 (52)	0.405 (20)	>0.200

* *t* test.

excluded. A similar comparison between hatchery-reared and wild rainbow cannot be made since there are few, if any, adult hatchery-reared rainbow in the lower Deschutes River; hatchery fish released in spring succumb to Ceratomyxa sp. by summer (J. Fessler, personal communication).

To determine whether size of otolith nucleus changes during growth of fish, I regressed length of otolith nucleus against fork length. For rainbow, $r = 0.060$. For all steelhead, $r = 0.694$; however, if Wizard Falls fish are excluded, $r = -0.018$. Even with this exclusion, a wide range of steelhead fork lengths (504-762 mm) was tested; therefore, if the relationship is strong, it should be noticeable in these data. The low r value is further evidence that some of the Wizard Falls fish may not be steelhead.

Relationship Between Fish Length and Otolith Size

Regressions of fork length on otolith length were developed for both rainbow and adult steelhead; plots of these equations, along with representative data points, are presented in Fig. 9. (Because the otolith is formed early in the fish embryo, the regressions were forced through the origin.) The usefulness of these regressions in estimating fish length from otolith length is supported by the high correlation coefficients. Also, although the otolith length-fish length relationship of juvenile steelhead is similar to

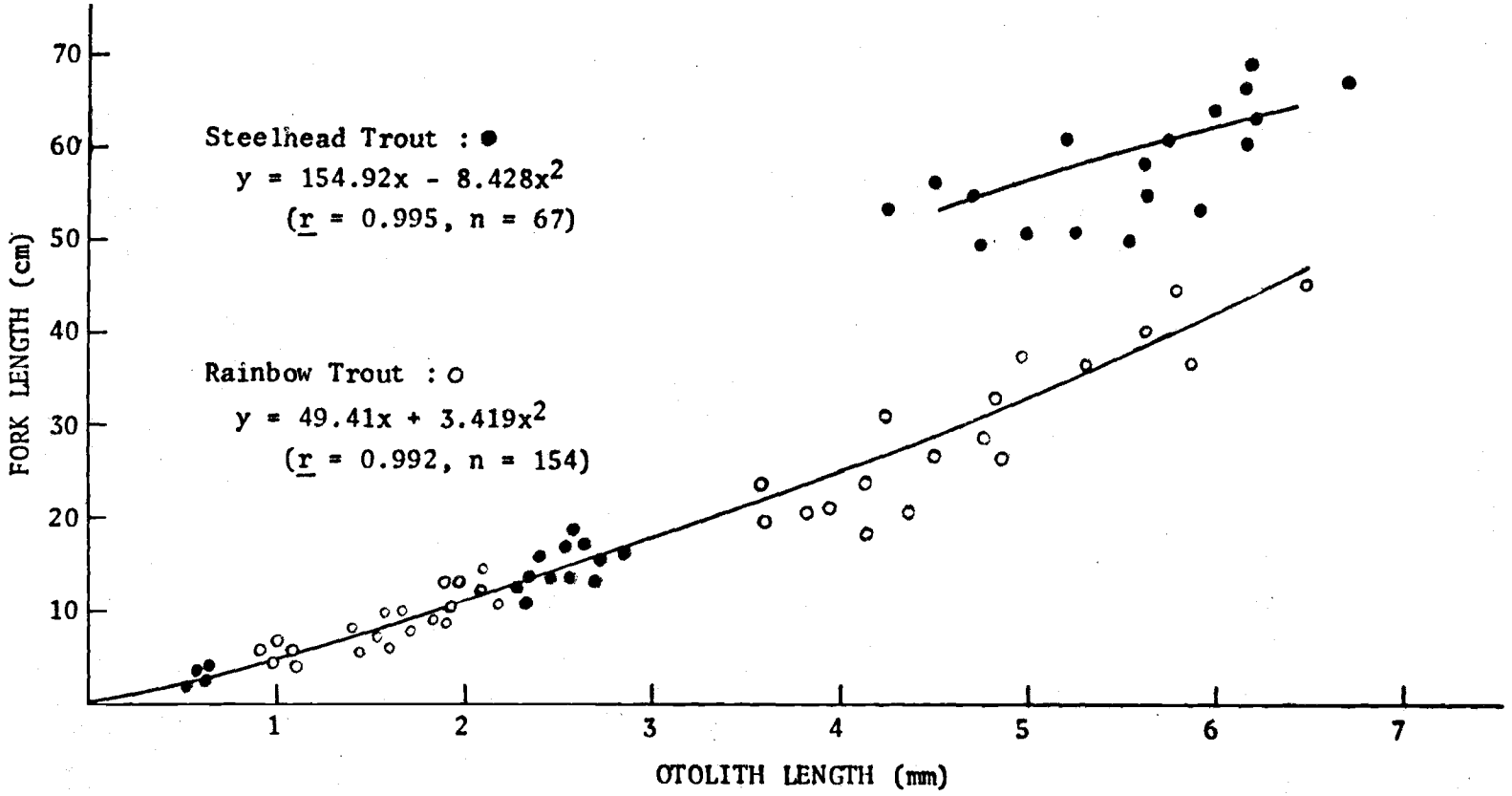


Figure 9. Regressions of otolith length on fish length for steelhead trout (adults) and rainbow trout in the lower Deschutes River, Oregon (1971-1973). Included are representative values for juvenile steelhead trout.

that of rainbow, adult steelhead are longer for a given otolith length.

The regressions of fork length on otolith weight for rainbow and adult steelhead (Fig. 10) also demonstrate high correlations. Also, data for juvenile steelhead closely approximates those for rainbow, and adult steelhead are longer than rainbow for a given otolith weight.

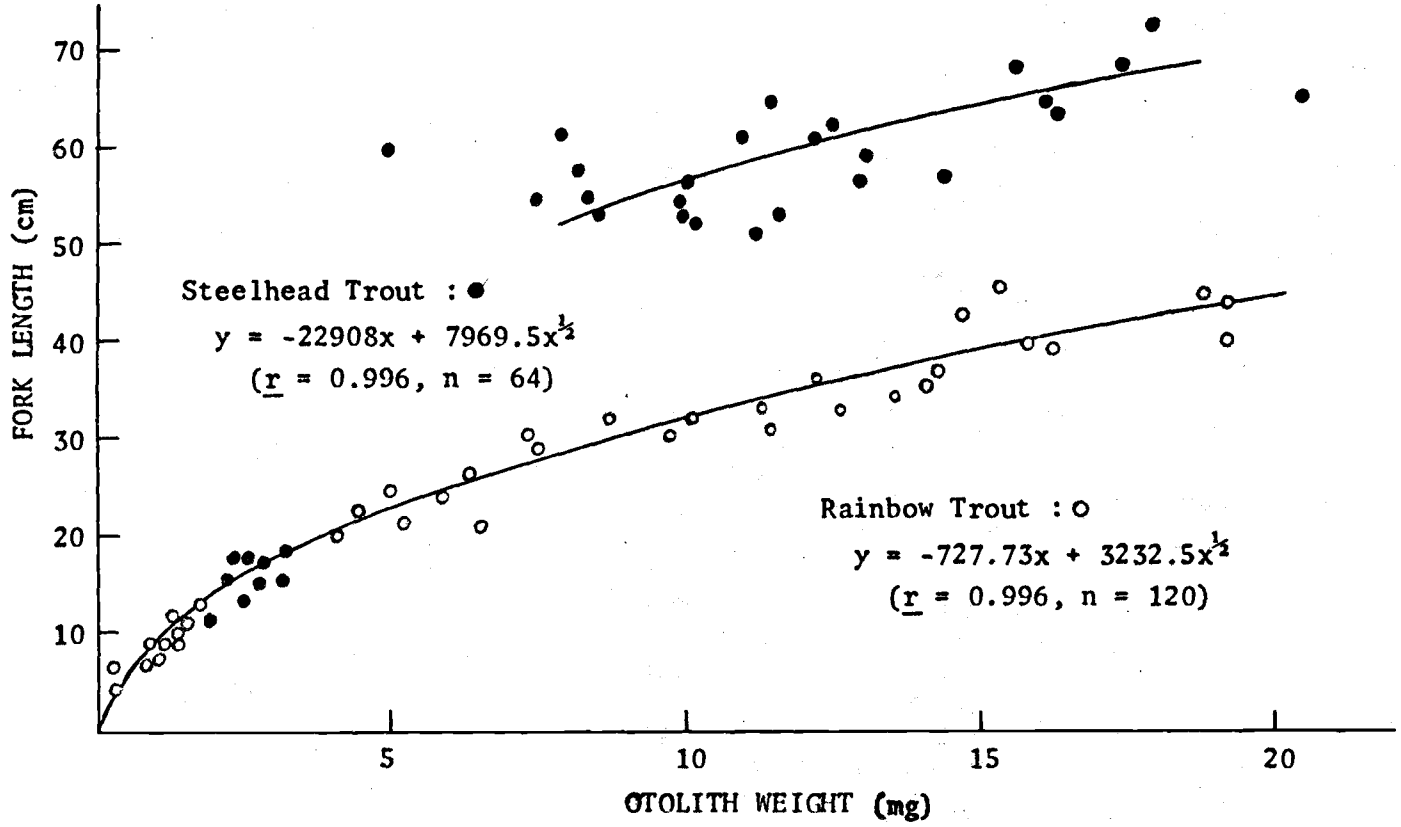


Figure 10. Regressions of otolith weight on fish length for steelhead trout (adults) and rainbow trout in the lower Deschutes River, Oregon (1971-1973). Included are representative values for juvenile steelhead trout

DISCUSSION

Treatment of Otoliths

When reading fish otoliths, the major problem is adequately distinguishing the various growth characteristics; in most cases the otolith must be subjected to some form of treatment. Many techniques have been reported, but there have been few comparative studies to determine which is best for otoliths from a particular group of fish. Johnston (1938) tested the effect of clearing agents on otoliths of various teleosts; aniseed and chloral hydrate produced the best results, whereas cinnamon oil and anilin were least effective in clearing. Clemens (1950) found that clearing burbot (Lota lota) otoliths in 3% tri-sodium phosphate and viewing them fresh, shortly after removal from the fish, were the most effective methods. He found the following clearing agents were relatively unsuccessful: 50% glycerin and water, potassium hydroxide, normal saline, acetone, alcohol. Except for placing otoliths in a storage or clearing solution, other techniques include burning (to char zones differentially), grinding and polishing (usually after the otolith has been split dorso-ventrally), and dyeing. The difficulties I encountered in clearing otoliths warrant further investigation. References which report commonly used techniques are listed in Table 2.

Table 2. Methods used to increase the readableness of otoliths.

Method	(Reference)
Stored or viewed dry	(Fitch 1951; Hagerman 1952; Grainger 1953; Mosher and Eckles 1954; Clutter and Whitesel 1956; Kohler 1958; Parrish and Sharman 1959)
Storage and clearing solutions	
30% sodium silicate	(Hickling 1933)
sodium hypochlorite	(Devereux 1967)
0.75% salt solution	(Hickling 1933)
3% tri-sodium phosphate	(Clemens 1950)
distilled water	(Watson 1964, 1965)
95% ethanol	(Kelly and Wolf 1959; Watson 1965)
70% alcohol	(Messieh 1969)
90 ethyl alcohol:10 glycerin	(Brigham and Jensen 1964)
50% glycerin	(Clemens 1951; Grainger 1953; Scott 1954; Kohler 1958; Lawler and McRae 1961; McErlean and Phillips 1961; Southward 1962; Moe 1969; Jensen 1970)
60% glycerin	(Nichy 1969)
xylol	(McMurrich 1913; Johnston 1938; Larson and Skud 1960; Kim 1963; Kim and Robertson 1968)
oil of cloves	(McMurrich 1913)
ammonia	(Chuganova 1963)
"hydrax"	(Kim and Koo 1963)
chloral hydrate aniseed creosote cinnamon oil anilin	(Johnston 1938)
potassium hydroxide normal saline acetone alcohol	(Clemens 1950)
Burning	(Lawler and McRae 1961; Chuganova 1963; Christensen 1964; Bayagbona 1966; Staples 1971)
Grinding, Breaking and Polishing	(Martin 1941; Irie 1955; Kelly and Wolf 1959; Mina 1967; Wiederman Smith 1968; Schott 1969; Staples 1971)
Dyeing	(Albrechtsen 1968)

Formation of the Otolith Nucleus

Using X-ray diffraction techniques, Degens et al. (1969) determined that the annual growth layers of otoliths are formed over an organic template composed of otolin, a high molecular weight (>150, 000) protein; the structure of this protein was similar among the 25 species of fish examined. During otolith growth, otolin is partially impregnated with $CaCO_3$ in the form of aragonite. During slow growth (when hyaline layers are formed), mineralization is more complete than during rapid growth (opaque layers). Thus, hyaline layers are almost exclusively $CaCO_3$, whereas there is a greater proportion of organic material in the opaque than in the hyaline layers (McMurrich 1913; Dannevig 1955; Erie 1955; Mugiya 1964, 1966; Mina 1965; Panella 1971).

Reibisch (1899, cited by McMurrich 1913), the first person to demonstrate that fish could be aged by annuli on otoliths, suggested that opacity is due to a higher ambient temperature at time of deposition. More recent studies indicate that the level of feeding may influence zone formation (Grainger 1953; Trout 1954). Also, an inherent physiological rhythm may be involved (many authors cited by Moe, 1969).

If these mechanisms operate in a similar manner during formation of the nucleus, then the following explanation is plausible.

During development of the trout embryo, little of the organic material in the yolk is available to the otolith due to the high energy demands of the growing embryo. Although the organic matrix of the otolith is formed, it is highly impregnated with $CaCO_3$; therefore, it is hyaline. A sudden change in physiology possibly induced by hatching may result in an increased supply of organic material to the otolith and formation of the opaque portion. The metamorphic check may be due to a temporal lag between absorption of the yolk and initial feeding or to a physiological change at time of yolk sac absorption, which account for a sudden decrease of available organics. Although McKern (1971) demonstrated that the otolith nucleus is present at the time of complete absorption of yolk, the sequential formation of nuclear zones has not been investigated. Further research, investigating the relationship between food consumption and nuclear zone formation, may provide information regarding the mechanism of annual zone formation.

Race Differentiation

Size of Entire Otolith

Otoliths of juvenile steelhead grow at the same rate (in relation to body size) as those of rainbow, whereas otoliths of adult steelhead show a different growth pattern. Extensive resorption of the steelhead otolith during the spawning migration could explain the

difference; however, as judged from the photographs, the actual degree of resorption was slight. While steelhead, in a marine environment, grow faster than rainbow, growth of otoliths in the two races appear to proceed at a similar rate in relation to time. Thus, mechanisms governing fish growth (e.g., food consumption) may not control growth of the annual zones of otoliths; rather, their growth may be controlled by factors more entrenched in phylogeny and less amenable by environmental change (unlike growth of the nucleus which appears to be governed by egg size). Certainly, such a suggestion needs further investigation; examination of otoliths from steelhead caught at sea and also comparisons between races within age groups are needed.

Size of Otolith Nucleus

Sizes of otolith nuclei of steelhead and rainbow trout are sufficiently different to allow for separation of these races. Steelhead are larger at maturity, and, since egg size is a direct function of body size, eggs of steelhead are larger. (Rass [1947, cited by Smirnov et al. 1970] stated that egg size constitutes a distinctive characteristic of species and well-defined subspecies.) If the amount of yolk is correlated with egg size, and since the otolith nucleus is formed when all or a great part of nutrition comes from the yolk, it is logical that size of otolith nucleus is a direct function

of egg size.

The egg size-otolith nucleus size relationship was not directly demonstrated in this investigation. There are two possible explanations. First, if this relationship is causal, then a significant correlation should be evident both between and within races; however, the correlation between length of rainbow dam and size of otolith nucleus of offspring was low ($\underline{r} = 0.489$). This may be due to the small sample size (8) and to the substitution of body length for egg size (where $\underline{r} = 0.829$); it is more likely that the low correlation is due to the narrow range of dam lengths (295-415 mm) combined with the great variation of egg size within any dam.

The second explanation is that this relationship is not causal - i.e., the difference in size of otolith nucleus between steelhead and rainbow is due (entirely or in part) to differences other than egg size. In the Deschutes River, for example, peak spawning time of steelhead occurs more than two months prior to that of rainbow (J. Fessler, personal communication). Since fertilized eggs of the former are exposed to colder water, the incubation period might be longer. Then, if otolith bands are deposited at the rate of one per day (Pannella 1971), and if the band widths are equal between races, the otolith nuclei of steelhead would be larger. However, such a mechanism probably could not explain why the otolith nuclei of

summer steelhead are smaller than those of winter steelhead (McKern 1971) since Everest (Fishery Biologist, Oregon State Game Commission) recently reported that summer steelhead spawn earlier than winter steelhead in the Rogue River (unpublished). Future work should examine the effects of environmental variables on incubation time and the relationship between otolith nucleus size and egg size of individuals rather than of means.

The r values between dam body size and egg size in this study are higher than those reported in many other investigations. Scott (1962) measured fork length and egg weight of rainbow trout and found no significant correlation. The range of fork lengths was narrow (231-264 mm), so that considering the great variability of egg size within length classes, his results are not surprising. Galkina (1970) found that length of rainbow trout (S. irideus) was not highly correlated with mean egg weight ($r = 0.48$); however, although eggs of average size were found in all females, the smallest eggs were obtained only from smaller females and the largest eggs were obtained only from larger females. McFadden et al. (1965) found a higher correlation ($r = 0.73$) between egg size and length of brown trout (S. trutta) dam. Blaxter (1969), Galkina (1970), and Lindsey and Ali (1971) cited numerous authors who examined this relationship in many species of fish; although most authors reported a wide range of egg sizes in females of similar

length, there is general agreement that a direct, and often high, correlation exists between egg size and dam size.

Whereas I was not able to read 29% of the otolith nuclei examined, McKern (1971) reported no problems regarding such measurements. This inconsistency may have been due not only to HCl treatment and subjective differences regarding delineation of the nucleus, but also to different otolith characteristics expressed in different stocks of fish. Further, I calculated mean width of otolith nuclei of Deschutes River summer steelhead to be 0.230 mm (excluding fish from Wizard Falls Hatchery, the mean width is 0.262 mm), whereas McKern reported such values for summer steelhead from four rivers in Oregon and Washington to range from 0.342 to 0.384 mm. Hopefully, further research will explain these differences.

LITERATURE CITED

Albrechtson, K. 1968. A dyeing technique for otolith age reading. J. Cons. int. Explor. Mer 32:278-280.

Altukhov, Yu. P., and Yu. A. Mikhalev. 1965. [Differences in the ratios of the dimensions of otoliths of "large" and "small" Black Sea horse-mackerel, which have been identified on the basis of heat tolerance of their tissues.] Ref. Zh. Biol., No. 4143. (Transl.) (Abstr.)

Bayagbona, E. O. 1966. Age determination and the Bertalanffy growth parameters of Pseudotolithus typus and P. senegalensis using the "burnt otolith technique." Pages 349-359 in Symposium on the oceanography and fisheries resources of the topical Atlantic. UNESCO, Paris.

Behnke, R. J. 1965. A systematic study of the family Salmonidae with special reference to the genus Salmo. Ph. D. Thesis, Univ. Calif., Berkeley. 259 pp.

Blaxter, J.H.S. 1969. Development: eggs and larvae. Pages 177-252 in W. S. Hoar and D. J. Randall, eds. Fish physiology. Vol. III. Academic Press, New York.

Brigham, R. K., and A. C. Jensen. 1964. Photographing otoliths and scales. Progr. Fish-Cult. 26:131-135.

Bulkley, R. V. 1967. Fecundity of steelhead trout, Salmo gairdneri, from Alsea River, Oregon. J. Fish. Res. Bd. Canada 24:917-926.

Christensen, J. M. 1964. Burning of otoliths: a technique for age determination of soles and other fish. J. Cons. int. Explor. Mer 29:73-81.

Chuganova, N. I. 1963. Age and growth studies in fish. Israel Prog. Sci. Transl., Nat. Sci. Found. 132 pp.

Clemens, H. P. 1950. The growth of the burbot Lota lota maculosa (LeSueur) in Lake Erie. Trans. Amer. Fish. Soc. 80:163-173.

Clutter, R. I., and L. E. Whitesel. 1956. Collection and interpretation of sockeye salmon scales. Inter. Pac. Salmon Fish. Comm., Bull. IX. 159 pp.

Danielssen, D. S. 1969. Investigations on herring Clupea harengus L. from the Norwegian Skagerak coast during the years 1963-64. FiskDir. Skr. Ser. Havunders 15:65-82. (Abstr.)

Dannevig, E. H. 1956. Chemical composition of the zones in cod otoliths. J. Cons. int. Explor. Mer 21:156-159.

Degens, E. T., W. G. Deuser, and R. L. Haednick. 1969. Molecular structure and composition of fish otoliths. Mar. Biol. 2:105-113.

Devereux, I. 1967. Temperature measurements from oxygen isotope ratios of fish otoliths. Science 155:1684-1685.

Einarsson, H. 1951. Racial analysis of Icelandic herring by means of otoliths. Rapp. P.-v. Reun. Cons. perm. int. Explor. Mer 128:55-74.

Fessler, J. 1971. Deschutes River summer steelhead ecology. Oregon State Game Comm., Res. Div., Prog. Rep. 26 pp.

Fitch, J. E. 1951. Age composition of the southern California catch of Pacific mackerel: 1939-40 through 1950-51. Calif. Dept. Fish and Game, Fish Bull. No. 83. 73 pp.

_____ and L. W. Barker. 1972. The fish family Moridae in the eastern North Pacific with notes on morid otoliths, caudal skeletons, and the fossil record. U.S. Fish Wildl. Serv., Fish. Bull. 70:565-584.

Galkina, Z. I. 1970. Dependence of egg size on the size and age of female salmon [Salmo salar (L)] and rainbow trout [Salmo irideus (Gib.)]. J. Ichthyol. 10:625-633.

Grainger, E. H. 1953. On the age, growth, migration, reproductive potential and feeding habits of the Arctic char (Salvelinus alpinus) of Frobisher Bay, Baffin Island. J. Fish. Res. Bd. Canada 10:326-370.

Hagerman, F. B. 1952. The biology of the Dover sole, Microstomus pacificus (Lockington). Calif. Dept. Fish and Game, Fish Bull. No. 85, 48 pp.

Hickling, C. F. 1931. The structure of the otolith of the hake. Quart. J. Microscop. Sci. 74:547-561.

_____ 1933. The natural history of the hake. Part IV. Age determination and growth rate. Fish. Invest., Lond. Ser. II, 13(2):1-120.

Holland, G. A. 1969. Age, growth, and mortality of races of English sole (Parophrys vetulus) in Puget Sound, Washington. Pacific Mar. Fish. Comm. Bull. 7:36-50.

Irie, T. 1955. The crystal structure of the otolith of a marine teleost Pseudosciaena. J. Fac. Fish. Anim. Husb., Hiroshima Univ. 1(1):1-8.

Jensen, A. C. 1970. Validation of ages determined from otoliths of Gulf of Maine cod. Trans. Amer. Fish. Soc. 99:359-362.

Johnston, M. 1938. Some methods of preparing teleost fish otoliths for examination. J. Roy. Microscop. Soc. 58:112-119.

Jordon, D. S. 1905. Guide to the study of fishes. Vol. II. Henry Holt & Co., New York. 599 pp.

Kelly, G. F., and R. S. Wolf. 1959. Age and growth of the redfish (Sebastes marinus) in the Gulf of Maine. U.S. Fish Wildl. Serv., Fish. Bull. No. 156. 31 pp.

Kendall, W. C. 1920. What are rainbow trout and steelhead trout? Trans. Amer. Fish. Soc. 50:187-199.

Kim, W. S. 1963. On the use of otoliths of red salmon for age and racial studies. M.S. Thesis. Univ. Wash., Seattle. 63 pp.

_____ and T.S.Y. Koo. 1963. The use of otoliths for age determination in red salmon. Fish Res. Inst., Univ. Wash., Seattle. Contrib. (147):17-19.

Kim, W. S., and K. Robertson. 1968. On the use of otoliths of sockeye salmon for age determination. Pages 149-168 in R. L. Burgner, ed. Further studies of Alaska sockeye salmon. Univ. Wash., Publ. Fish., New Ser. Vol. III.

King, D. N. 1966. Deschutes River summer steelhead. Oregon State Game Comm., Central Reg. Adm. Rep. No. 66-3. 41 pp.

Kohler, A. C. 1958. The validity of otolith age determinations for haddock (Melanogrammus aeglifinus L.) from the Lockeport, N.S., area. J. Fish. Res. Bd. Canada 15:1229-1238.

Larson, C. M., and B. E. Skud. 1960. Techniques for studying herring scales and otoliths. Progr. Fish-Cult. 22:85-86.

Lawler, G. H., and G. P. McRae. 1961. A method for preparing glycerin-stored otoliths for age determination. J. Fish. Res. Bd. Canada 18:47-50.

Lindsey, C. C., and M. Y. Ali. 1971. An experiment with medaka, Oryzias latipes, and a critique of the hypothesis that teleost egg size controls vertebral counts. J. Fish. Res. Bd. Canada 28:1235-1240.

Maher, F. P., and P. A. Larkin. 1954. Life history of the steelhead trout of the Chilliwack River, British Columbia. Trans. Amer. Fish. Soc. 84:27-38.

Martin, W. R. 1941. Rate of growth of the ling, Lota lota maculosa (LeSueur). Trans. Amer. Fish. Soc. 70:77-79.

McErlean, A. J., and R. C. Phillips. 1961. A technique for direct photography of translucent objects. Trans. Amer. Fish. Soc. 90:500-501.

McFadden, J. T., E. L. Cooper, and J. K. Anderson. 1965. Some effects of environment on egg production in brown trout (Salmo trutta). Limnol. Oceanogr. 10:88-95.

McKern, J. L. 1971. Steelhead trout otoliths for age, race, and stock analysis. M.S. Thesis, Oregon State Univ., Corvallis. 55 pp.

McKern, J. L., and H. F. Horton. 1970. A punch to facilitate the removal of salmonid otoliths. Calif. Fish and Game 56:65-68.

McMurrich, J. P. 1913. The life cycles of the Pacific coast salmon belonging to the genus Oncorhynchus as revealed by their scale and otolith markings. Roy. Soc. Canada, Ottawa, Proc. Trans. ser. 3, 6(sec. 4):9-28.

Messieh, S. N. 1969. Similarity of otolith nuclei in spring- and autumn-spawning Atlantic herring in the southern Gulf of St. Lawrence. J. Fish. Res. Bd. Canada 26:1889-1898.

_____ 1972. Use of otoliths in identifying herring stocks in the southern Gulf of St. Lawrence and adjacent waters. J. Fish. Res. Bd. Canada 29:1113-1118.

Mina, M. V. 1965. On the development of a method for objective evaluation of the structure of the zones of fish otoliths. (Transl. from Russian) Fish. Res. Bd. Canada, Transl. Ser. No. 805. 10 pp.

_____ 1967. Study of the relation between the weight of the otolith (sagitta) and the length of an individual in the cod population of the Barents and White Seas. (Transl. from Russian) Fish. Res. Bd. Canada, Transl. Ser. No. 990. 10 pp.

Moe, M. A. 1969. Biology of the red grouper. Fla. Dep. Natur. Resour. Mar. Res. Lab., Prof. Pap. Ser. 10:1-95.

Montgomery, M. 1971. The Deschutes fishery. Oregon State Game Comm. Bull. 26(3):3-5, 7.

Mosher, K. H., and H. H. Eckles. 1954. Age determination of Pacific sardines from otoliths. U.S. Fish Wildl. Serv., Res. Rept. No. 37. 40 pp.

Mottley, C. McC. 1936. The classification of the rainbow trout of British Columbia. Fish. Res. Bd. Canada, Prog. Rep. No. 27:3-5.

Mugiya, Y. 1964. Calcification in fish and shellfish. III. Seasonal occurrence of a prealbumin fraction in the otolith fluid of some fish, corresponding to the period of opaque zone formation in the otolith. Bull. Jap. Soc. Sci. Fish. 30:955-967.

Mugiya, Y. 1966. Calcification in fish and shellfish. VI. Seasonal changes in Ca and Mg concentrations of the otolith fluid in some fish, with special reference to the zone formation of their otolith. Bull. Jap. Soc. Sci. Fish. 32:549-557.

Neave, F. 1944. Racial characteristics and migratory habits in Salmo gairdneri. J. Fish. Res. Bd. Canada 6:245-251.

Nichy, F. E. 1969. Growth patterns on otoliths from young silver hake, Merluccius bilineatus (Mitch.). Int. Comm. Northwest Atl. Fish., Res. Bull. 6:107-117.

Panella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science 173:1124-1127.

Parrish, B. B., and D. P. Sharman. 1959. Otolith types amongst summer-autumn spawning herring in the northern North Sea. J. Cons. int. Explor. Mer 25:81-92.

Rass, T. S. 1947. Taxonomic significance of egg size in bony fishes (Teleostei) [in Russian]. Byull. Mosk. Ob-va Ispyt. Prir. (MOIP), Otd. Biol. 52(6).

Reibisch, J. 1899. Uber die Eizahl bei Pleuronectes platessa und die Alterbestimmung dieser form aus den otolithen. Wiss. Meeresunters. 4:233-248.

Schmidt, W. 1966. The otolith as a means for differentiation between species of fish of very similar appearance. Pages 393-396 in Symposium on the oceanography and fisheries resources of the tropical Atlantic. UNESCO, Paris.

Schott, J. W. 1969. A technique for producing and mounting otolith wafers for age determination. Calif. Fish and Game 55:86-88.

Scott, D. M. 1954. A comparative study of the yellowtail flounder from three Atlantic fishing areas. J. Fish. Res. Bd. Canada 11:171-197.

Scott, D. P. 1962. Effect of food quantity on fecundity of rainbow trout, Salmo gairdneri. J. Fish. Res. Bd. Canada 19:715-731.

Sinoda, M., and S. D. Jayashinghe. 1971. Possibility of race separation of "Nigisu" by means of otolith. Bull. Jap. Soc. Sci. Fish. 37:1140-1149.

Smirnov, A. I., M. S. Kamyshnaya, and Z. M. Kalashnikova. 1970. Dimensions, biochemical characteristics and caloric values of mature eggs of members of the genera Oncorhynchus and Salmo. J. Ichthyol. 8:524-530.

Southward, G. M. 1962. Photographing halibut otoliths for measuring growth zones. J. Fish. Res. Bd. Canada 19:335-338.

Staples, D. J. 1971. Methods of ageing red gurnard (Teleosti: Triglidae) by fin rays and otoliths. New Zealand J. Mar. Freshwat. Res. 5(1):70-79.

Taft, A. C. 1933. California steelhead trout problems. Calif. Fish and Game 19:192-199.

Trout, G. C. 1954. Otolith growth of the Barents Sea cod. Rapp. P.-v. Reun. Cons. perm. int. Explor. Mer 136:89-102.

Wagner, H., and J. Haxton. 1968. Observations on the migration disposition of summer steelhead reared at Oak Springs and Wizard Falls Hatcheries. Oregon State Game Comm., Res. Div., Prog. Rep. 5 pp.

Watson, J. E. 1964. Determining the age of young herring from their otoliths. Trans. Amer. Fish. Soc. 93:11-20.

_____ 1965. A technique for mounting and storing herring otoliths. Trans. Amer. Fish. Soc. 94:267-268.

Wiederman Smith, S. 1968. Otolith age reading by means of surface structure examination. J. Cons. int. Explor. Mer 32:270-277.

Withler, F. C. 1972. Research needs for intensive management of British Columbia steelhead. Fish. Res. Bd. Canada, Circ. No. 92. 41 pp.

Wood, M. J., and M. Foster. 1966. Studies on the Dunmore herring stock. 2. The otoliths of the Dunmore herring. J. Cons. int. Explor. Mer 30:222-236.