USE OF OTOLITHS TO SEPARATE JUVENILE STEELHEAD TROUT FROM JUVENILE RAINBOW TROUT[1]

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ABSTRACT

Otolith nuclei were investigated as a means of separating juvenile steelhead trout, *Salmo gairdneri*, from juvenile rainbrow trout, *S. gairdneri*, in the lower Deschutes River, Oreg. An intensive recreational fishery necessitated development of a technique for differentiation so that impact of the fishery on each race could be assessed independently.

Investigations of adults and hatchery-reared young of *S. gairdneri* revealed that otolith nuclei of steelhead are significantly larger than those of rainbow, size of otolith nuclei does not change with growth of either race, and there are no differences in size of otolith nuclei due to sex or origin (wild vs. hatchery). Thus, size of otolith nuclei provides a means to differentiate effectively juvenile steelhead trout and juvenile rainbow trout regardless of sex or origin. Results also indicated that steelhead mature at a larger size than rainbow, egg size is directly related to body size of dam in both races, and size of otolith nuclei is likely determined by egg size.

This paper reports on an investigation of growth characteristics of the sagittae, the largest of the otoliths, as a means to separate juvenile steelhead trout, *Salmo gairdneri*, from juvenile rainbow trout, *S. gairdneri*. The technology for such differentiation is presently lacking but is necessary for independent management of the two races in streams where they coexist. In the lower Deschutes River, Oreg., for example, the most intensive fishery for rainbow trout occurs during the first week in May when most steelhead smolts migrate; consequently, the catch may be composed of 22-80% juvenile steelhead (King 1966; Wagner and Haxton 1968). Precise knowledge of this catch composition at various locations and times would allow fisheries managers to manipulate fishing pressure so that most steelhead smolts escape capture during migration.

Previously, otoliths have been used to differentiate stocks and races of salmonids. Kim (1963) found differences in the appearance and size of growth rings between spawning groups of sockeye salmon, *Oncorhynchus nerka*. The study most relevant to our investigation demonstrated that winter and summer races of steelhead trout can be separated on the basis of differences in size of the otolith nucleus (ON) (McKern et al. 1974).

The latter authors found that the otolith nucleus is formed early in steelhead trout embryos when all or a great part of nutrition comes from the yolk, and that ON size appears to be directly related to egg size. Also, egg size and fish length of salmonids are often directly related (McFadden et al. 1965; Bulkley 1967; Galkina 1970), and steelhead trout are generally larger than rainbow trout at maturity. Therefore, we hypothesized that steelhead ON are sufficiently larger than rainbow ON to permit separation of juveniles of both races.

We investigated this hypothesis via two series of observations. In the first, an indirect test of validity of the hypothesis, we compared measurements of ON of fish of known race and then determined whether these measurements changed with growth of fish or whether they were related to sex or origin. In the second series, we measured body size of adult fish, egg size of ripe dam, and ON size of fry hatched from these eggs, to determine if correlations between these variables logically accounted for differences in ON sizes. All investigations were conducted on adults and hatchery-reared fingerlings of summer steelhead trout and resident rainbow trout captured in 1971-73 from the lower Deschutes River.

METHODS AND MATERIALS

Study Area

The study area was the lower 100 miles of the

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Deschutes River in north central Oregon (Figure 1). The Deschutes River drains 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 average from 3,000 to 7,100 cfs. Important sport fish in the area include resident rainbow trout, summer steelhead trout, and chinook salmon, *O. tshawytscha*, (Montgomery 1971).

Collection of Samples

Otoliths were obtained from adult ($\geq$200 mm fork length [FL]) rainbow and steelhead (n = 101) sampled during routine Oregon Wildlife Commission creel censuses at Webb's access road (at Buck Hollow Creek) and near Maupin (Figure 1) during August and September 1971 and 1972. Otoliths were removed with a punch described by McKern and Horton (1970). Each fish was measured (FL) 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 a combination of coloration, relative size, and analysis of scales (Maher and Larkin 1954). In most cases sex was determined from jaw conformation and opercular coloration (steelhead only), and occasionally from fisherman's observations if the fish had been cleaned. To determine origin, we examined steelhead for hatchery marks; hatchery-reared rainbows were distinguished by worn or rounded fins, excessive number of missing scales, and other abnormalities.

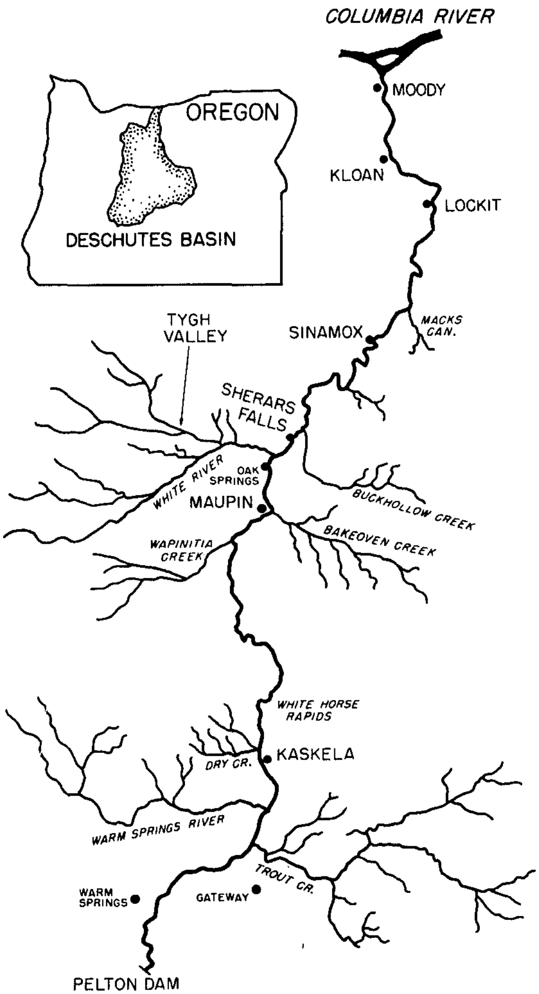

FIGURE 1.—Map of study area on the lower 100 miles of the Deschutes River, Oreg.

Other adult fish (n = 92) were collected by electrofishing near Maupin, below Pelton Dam, and in Bakeoven and Trout creeks (Figure 1) in April-June 1971 and August 1972. Each fish was measured (FL), 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 Wildlife Commission) on the Metolius River. These fish represented a random assortment of the offspring of ca. 150 females captured below Pelton Dam. Fork lengths were measured, and otoliths were removed by dissection.

To determine body lengths of mature steelhead trout and rainbow trout, specimens were obtained by electrofishing in the lower Deschutes River in 1972. Fork lengths were measured, and race was determined from hatchery marks or coloration (migrating summer steelhead are more silvery than resident rainbow).

For determination of ova size, adult steelhead were captured in late winter 1972 by trapping below Pelton Dam and were held in tanks until ripe. Twenty-two females were measured (FL), and a sample of eggs (ca. 100) was collected from each fish, fertilized, and allowed to water harden 8-22 h. 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. Male-female pairs were individually spawned, and, after water hardening, the eggs were transported to a laboratory 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 possible correlation between egg size and ON size of the hatched fry, we 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.

Storage and Treatment of Otoliths

The enveloping membrane (sacculus) was removed from each otolith prior to storage. Initially, otoliths were stored dry in coin envelopes before transfer to a clearing solution. Because this method led to breakage of otoliths, later samples were placed in a clearing solution immediately after removal from the fish. Otoliths were cleared from 1 to 21 mo 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, a 50:50 mixture of glycerin and water (McKern et al. 1974) was used for the remainder of the samples, but this solution tended to increase the opacity of the entire otolith. Neither burning these otoliths on an asbestos pad over a bunsen burner nor clearing the otoliths in oil of cloves increased contrast between the opaque and hyaline parts. Consequently, it was difficult to discern the nucleus.

Improved readings were obtained by applying drops of HCl to the medial surface of otoliths preserved in glycerin and water; this resulted in a dissolution of the medial lobes, a consequent thinning of the otolith, and clearer definition of density patterns. This method is quick (a few milliliters of HCl applied for 2-4 min for a large otolith) and is easily controlled by periodic inspection of the otolith during treatment. Because the edges of the otolith are dissolved, this method should not be used when age determinations are required.

Terminology and Examination of Otoliths

When viewed under reflected light on a black background, the ON of *S. gairdneri* is hyaline with a narrow opaque ring around the border (Figure 2). The metamorphic check is a narrow hyaline ring delineating the nucleus (Kim and Koo 1963). For examination, otoliths were placed lateral surface up on black Plexiglas[4] depression plates, illuminated with a beam of light at 45° and photographed on 35-mm film through a microscope at 50×. Panatomic-X film (ASA 32) was used, and the negatives were enlarged to 4×5 or 5×7 inches onto grade 3 or 4 (high contrast) paper. A stage micrometer was also photographed and enlarged at the same magnifications so that otolith measurements could be determined from the photographs. The length and width of the nucleus (Figure 2) was measured from the photographs by using a compass and the corresponding photograph of the micrometer.

RESULTS AND DISCUSSION

Size of Otolith Nucleus

The linear correlation between ON length and width was strong in both rainbow trout ($r = 0.838$)

[4]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

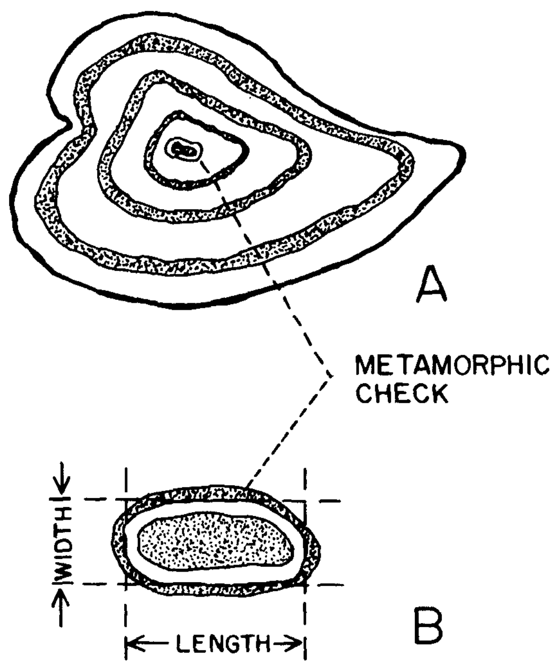

FIGURE 2.—Illustration of (A) otolith and (B) otolith nucleus of *Salmo gairdneri*, with notation of measurements and terminology used.

and steelhead trout (r = 0.916). Neither seemed easier to read. Primarily due to problems of developing methodology, 189 ON (29% of 641 examined) were not sufficiently distinct to permit measurement. Usually, the hyaline center of the nucleus was visible, but the metamorphic check could not be distinguished. Because measurement of the larger dimension would likely be more precise than widths, ON length was used in the following analyses.

The mean ON lengths of steelhead trout (0.354 mm; n = 114) and rainbow trout (0.245 mm; n = 145) differed significantly ($P \leq 0.001$). The length-frequency plot of these data (Figure 3A) demonstrates an overlap of lengths. Most unexpected in this plot are the nucleus 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 reared. Perhaps these fish are rainbow offspring which were inadvertently mixed with steelhead during hatchery operations or hybrids of the two species. The length-frequency plot of steelhead ON excluding those from Wizard Falls Hatchery increased the normality of the histogram (Figure 3B); the nadir at 0.46-0.48 mm is probably due to the small sample size of each interval. Therefore, although overlap of ON size of the two races occurs between 0.28 mm and 0.34 mm, the race of most juvenile *S. gairdneri* from the Deschutes River can be determined reliably on the basis of this measurement.

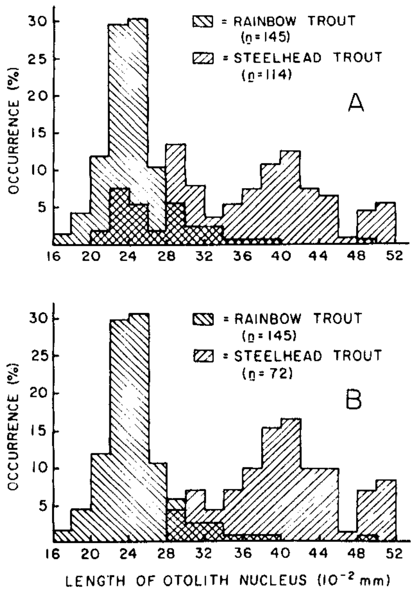

FIGURE 3.—Length-frequency distribution of otolith nuclei of (A) rainbow trout and all steelhead trout and (B) rainbow trout and steelhead trout excluding those from Wizard Falls Hatchery. All fish were captured from the lower Deschutes River, Oreg., 1971-73.

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 ON length of one adult rainbow was 0.48 mm. Although no hatchery marks were noticed, scale characteristics suggested a hatchery origin; because we have observed that almost all hatchery-reared rainbow succumb to *Ceratomyxa* before reaching maturity in the lower Deschutes River, this may have been a nonmigratory steelhead. In general, though, data from our samples do not support the suggestion of Wagner and Haxton (1968) that there may be a great number of such nonmigrants in the Deschutes River.

To determine whether size of ON changes during growth of fish, we regressed length of ON against FL of fish: 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 FL (504-762 mm) was tested; and if the relationship was strong it should have been noticeable in these data.

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

Mean length of ON of wild steelhead (0.395 mm; n = 52) was not significantly different ($P \geq 0.20$) from that of hatchery-reared steelhead excluding those from Wizard Falls Hatchery (0.405 mm; n = 20). A similar comparison between adult hatchery-reared and wild rainbow cannot be made since there are few, if any, adult hatchery-reared rainbow in the lower Deschutes River (as mentioned earlier, hatchery fish released in spring succumb to *Ceratomyxa* by summer).

Fish, Egg, and ON Size Relationships

The lengths of rainbow trout and steelhead trout from the lower Deschutes River are distributed into discrete size ranges (Figure 4). Although these fish are not necessarily ready to

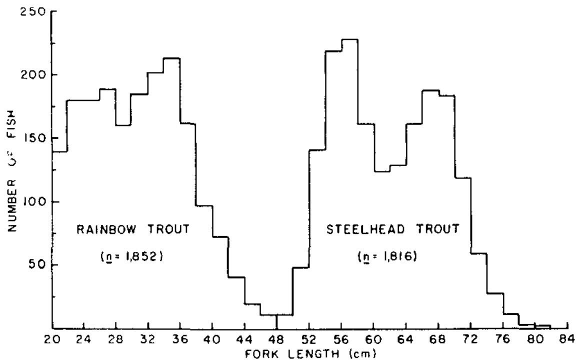

FIGURE 4.—Length-frequency distribution of mature (>20 cm) rainbow trout and steelhead trout from the lower Deschutes River, Oreg., 1972.

spawn, the data indicate low likelihood of significant overlap in length of mature 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). Also, mean egg size was strongly correlated with length of female (r = 0.829 and 0.791 for rainbow and steelhead trout, respectively) (Figure 5), although there was much variability of mean egg sizes between fish of a similar length and of egg sizes from any one female. For some fish, the largest egg was twice the size of the smallest.

The above r values between body size of dam and egg size are higher than those reported in many other investigations. Scott (1962) measured FL and egg weight of rainbow trout and found no significant correlation. Considering the narrow range of FL (231-264 mm) and the great variability of egg size within length classes, his

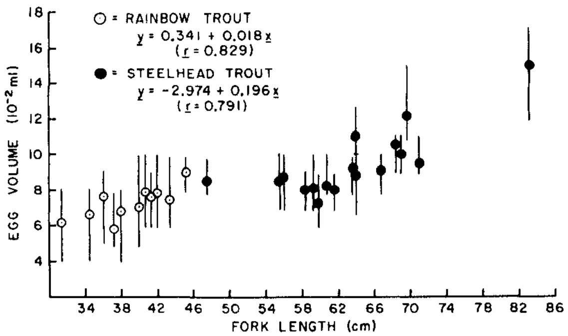

FIGURE 5.—Means and ranges of egg size plotted against length of dam for rainbow trout and steelhead trout from the lower Deschutes River, Oreg., 1972.

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). Although eggs of average size were found in all his females, the smallest eggs were obtained only from smaller females and vice versa. McFadden et al. (1965) found a higher correlation (r = 0.73) between egg size and length of brown trout, *S. trutta*. 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.

The presence or absence of any correlation between egg size and ON size of the hatched fish could not be determined directly for the rainbow trout groups reared in Corvallis (egg size had been measured for only four of the eight females whose offspring were available, and we considered this sample size too small). However, since there was a high correlation between egg size and length of dam (r = 0.829), this latter measurement was regressed against ON size of offspring from the eight matings (Figure 6). The r value of 0.489 and the overlap of ranges indicate the relationship is not strong; however, it is a positive correlation. Also, the small sample size (8), the narrow range of dam lengths (295-415 mm), the variation of egg size within any dam, and the substitution of FL for egg size are factors which may have obscured the real extent of the relationship of fish size to ON size.

In summary, we found that because steelhead ON are larger than rainbow ON, size of ON does not change with growth of either race, and correlations of ON size between dams and sires and between wild and hatchery-reared fish of either race are insignificant, ON size is an effective means of differentiating juvenile steelhead trout and juvenile rainbow trout regardless of sex or origin. We also concluded that because steelhead trout are larger at maturity than rainbow trout and because egg size is a direct function of body size, eggs of steelhead trout are larger than those of rainbow trout; and although we did not conclusively demonstrate that ON size is directly related to egg size, other evidence was offered to support the hypothesis that larger egg size was the mechanism responsible for larger ON size in steelhead trout as compared to rainbow trout.

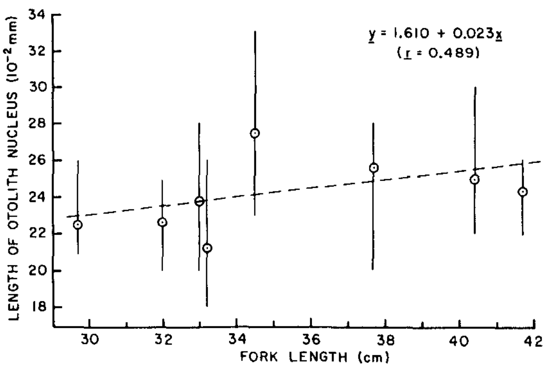

FIGURE 6.—Regression of length of rainbow trout dam from the Deschutes River on length of otolith nucleus of offspring cultured in Corvallis, Oreg., 1972. (Circles are means, and vertical bars are ranges of observation.)

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