VARIABILITY IN DIMENSIONS OF SALMONID OTOLITH NUCLEI: IMPLICATIONS FOR STOCK IDENTIFICATION AND MICROSTRUCTURE INTERPRETATION

JOHN D. NEILSON,[1] GLEN H. GEEN,[2] AND BRIAN CHAN[3]

ABSTRACT

Sagittal otoliths in rainbow trout, *Salmo gairdneri*, and chinook salmon, *Oncorhynchus tshawytscha*, arise by fusion of otolith precursors (primordia) before hatching. Size of the otolith nucleus exhibited considerable variability even in the progeny of a single female. Otolith nucleus length was directly related to the number and position of the primordia and water temperature at which the eggs were incubated. This variability limits the utility of nucleus dimensions as criteria for separating sympatric populations of juvenile steelhead and rainbow trout. Variability in otolith nucleus dimensions also accounted for a significant error in otolith size-fish size relationships in recently hatched alevins.

The early development of otoliths is poorly understood considering their potential use in stock identification (Postuma 1974; Rybock et al. 1975) and in the provision of data on fish age and growth to the daily level of precision (Pannella 1971; Wilson and Larkin 1982). Variability of otolith nucleus size and shape is of particular concern in stock identification studies since nucleus dimensions may be racial characteristics. Rybock et al. (1975) have suggested a positive correlation of the rainbow trout, *Salmo gairdneri*, otolith nucleus size and the mean egg size of the female which, in turn, is positively correlated to the size of the female. Their data on Deschutes River steelhead trout (the sea-run form of *S. gairdneri*) females, which were larger, on average, than females of the sympatric population of freshwater resident rainbow trout, led to the suggestion that otolith nucleus dimensions would differ significantly and provide a basis for racial identification of juveniles. This hypothesis was of particular significance since no other meristic or morphometric trait is known which permits identification of juvenile sea-run and freshwater resident *S. gairdneri*.

Nucleus dimensions might affect the widths of concentrically formed daily growth increments deposited around the otolith nucleus. Bipartite daily growth increments consist of alternating protein and calcium rich zones (Brothers 1981), and their widths are proportional to fish growth during the period of increment formation (Wilson and Larkin 1982). If increment width and number vary as a function of nucleus size and shape, then a source of the 15% error described by Wilson and Larkin in the estimation of fish growth from otolith growth could be identified.

In this paper, we describe development of sagittal otoliths of *S. gairdneri* (sea-run and freshwater resident) and chinook salmon, *Oncorhynchus tshawytscha*, and examine the effect of water temperature on otolith nucleus dimensions. These data permit a reexamination of the hypothesis of Rybock et al. (1975). Finally, the implications of variability in otolith nucleus size on otolith microstructure and its interpretation are considered.

METHODS

To study otolith nucleus development in *S. gairdneri*, we obtained eggs from steelhead trout in the Deadman River, British Columbia (B.C.), in 1981 and from the Nicola and Deadman Rivers in 1982 (Thompson River tributaries). Rainbow trout eggs were taken from the Deadman River in 1981, and from stocks in Mission Creek and Pennask Lake in south-central B.C. in 1982. Prior to fertilization, samples of eggs (n = 20) were taken for dry weight determination (17 of 18 fish collected in 1982). In all cases, eggs were fertilized with pooled sperm from 2 to 3 males of similar size and origin as the female. In total, eggs from 10 steelhead and 11 rainbow trout were used in this study.

[1]Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6; present address: Marine Fish Division, Canada Department of Fisheries and Oceans, Biological Station, St. Andrews, N.B., Canada E0G 2X0.
[2]Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6.
[3]British Columbia Fish and Wildlife Branch, Ministry of Environment, Kamloops, B.C., Canada V2C 5Z5.

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The fertilized eggs of each female were incubated in separate compartments in Heath Trays at Abbotsford and Loon Lake trout hatcheries. In 1981, fertilized eggs from two female steelhead and one female rainbow trout were subdivided into three lots and held at 6.5°, 9.5°, and 15.0°C until yolk-sac absorption. In 1982, all fish were held at 11°C. An approximate 12:12 LD photoperiod was maintained through incubation and rearing. Samples of steelhead and rainbow trout eggs or alevins were taken at biweekly intervals in 1981. Alevins only were sampled in 1982.

Oncorhynchus tshawytscha eggs were taken from the 1981 Capilano River stock and were incubated at 6°C under an approximate 12:12 LD photoperiod. Hatchery practice did not allow separate rearing of groups of eggs from individual females.

Otolith development in *S. gairdneri* embryos was studied by dissecting the embryo from the egg, clearing it with carbol xylol, and then squashing the embryo between two microscope slides. This treatment, which made noncalcified tissue transparent and amorphous compared with otoliths and other hard parts, permitted otolith examination with a transmitted light microscope at 400×. While we also examined embryos with X-ray and xeroradiographic techniques, satisfactory results were obtained more simply with the carbol xylol treatment.

Examination of the nuclei of otoliths from alevins required that otoliths be ground and polished following the method of Neilson and Geen (1981). The extent of the otolith nucleus in both embryos and alevins was delimited by the first growth increment encircling all central otolith precursors or primordia (Fig. 1). The first growth increment encircling the central primordia generally appeared dark when viewed with a transmitted light microscope. The only primordium outside the nucleus was in the anterior-ventral quadrant and was associated with the formation of the rostrum, the pointed anterior extremity of the otolith shown in Figure 1.

To avoid bias, otolith nucleus length was measured from coded preparations with an ocular

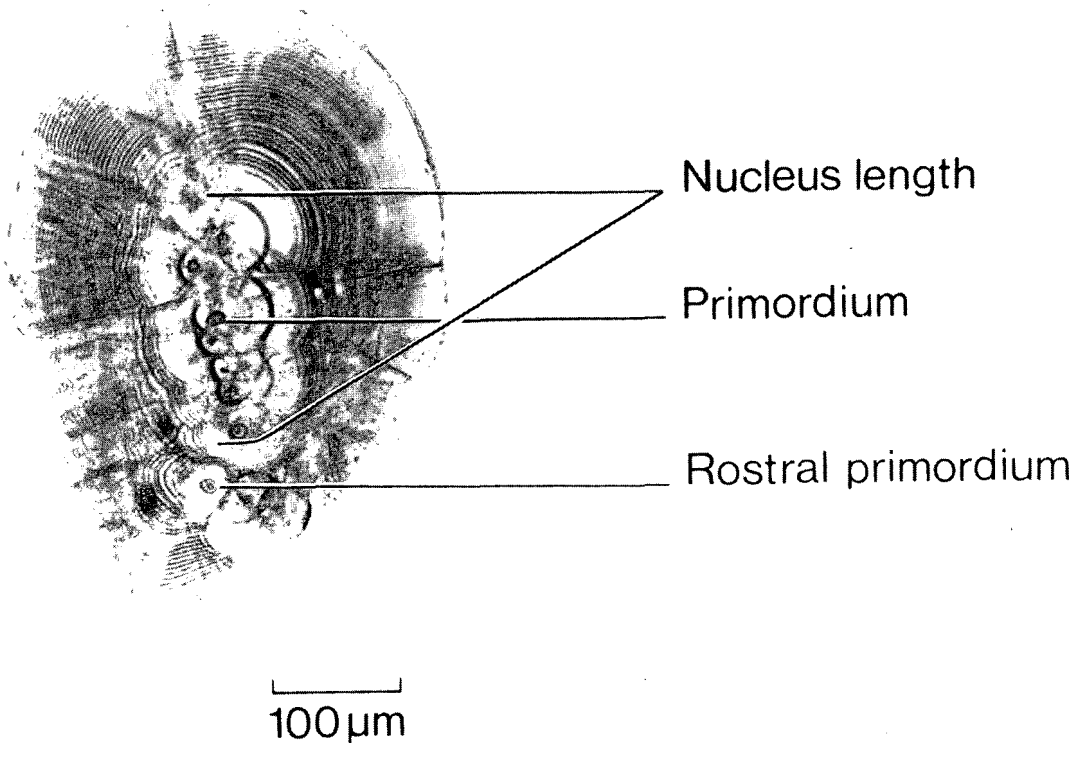

FIGURE 1.—Sagittal otolith from a Capilano River chinook salmon, *Oncorhynchus tshawytscha*, alevin showing the otolith nucleus, primordia, and rostral primordium.

micrometer along the longest axis through the nuclear zone. The area of the otolith nucleus was measured from photographic enlargements with a polar planimeter. Increment widths were measured with a vernier caliper from photographic enlargements (final magnification 9700×). The frequency of increment formation was determined from slopes of regressions of increment counts from otoliths of fish of known age.

Nucleus measurements and primordia counts are only reported for otoliths removed from the fishes' left side as nucleus lengths were significantly greater in left-side than right-side sagittae, albeit at a low level of significance ($P < 0.10$, Wilcoxon Paired Sample Test).

During the course of this study, otoliths from 257 rainbow trout, 187 steelhead trout, and 50 *O. tshawytscha* were examined.

RESULTS

To examine the hypothesis that egg size (a function of female fork length) influences otolith nucleus length in progeny, we examined the relationship of female fork length to egg dry weight and nucleus length in *S. gairdneri*. The dry weight of steelhead and rainbow trout eggs was positively correlated with the size of the female from which the eggs originated ($r^2 = 0.54$, $P < 0.001$, Fig. 2). The slope of the geometric mean regression shown

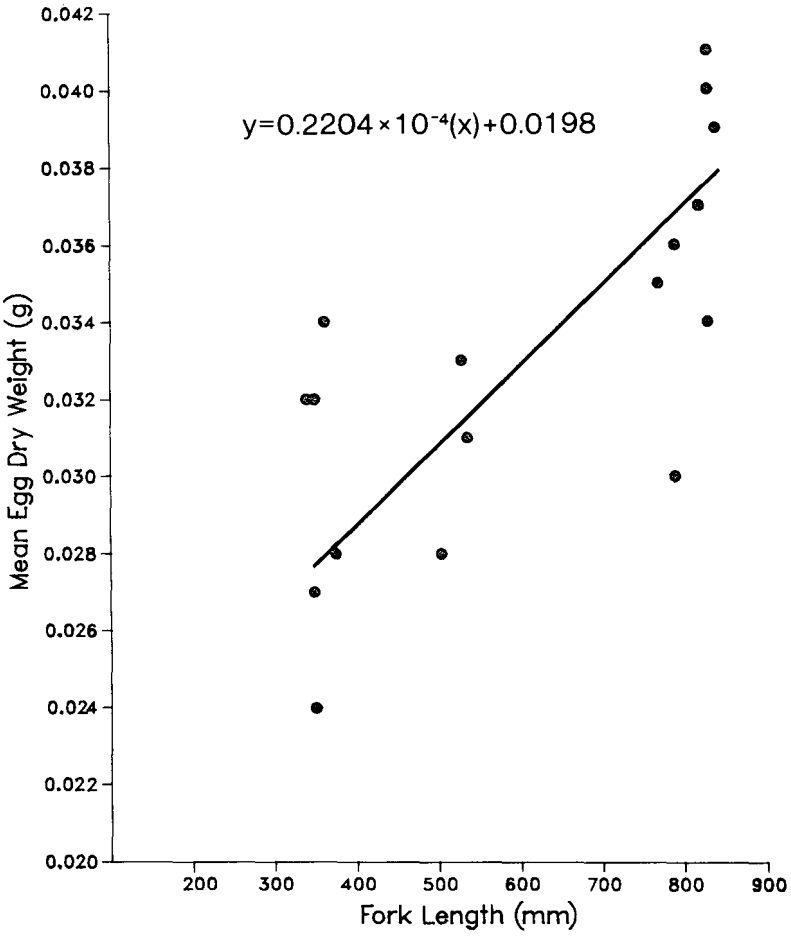

FIGURE 2.—Geometric mean regression of mean unfertilized egg dry weight on fork length of female *Salmo gairdneri* from which eggs were obtained. Each point is the mean of 20 eggs from each female. Fish in the 300-400 mm size interval were rainbow trout from Pennask Lake, those 500-600 mm were rainbow trout from Mission Creek, and those >700 mm were Deadman or Nicola River steelhead.

in Figure 2 differed significantly from zero (t-test, $P < 0.001$). However, there was no significant relationship between otolith nucleus length and female fork length (t-test, $P > 0.05$, Fig. 3), or egg dry weight (t-test, $P > 0.10$). We also investigated the utility of otolith nucleus lengths as a racial characteristic by calculating D^2, a part of a discriminant function analysis. In this instance, D^2 is a measure of the power of discrimination of nucleus length in separating juvenile sea-run and freshwater *S. gairdneri*. D^2 was 0.063 and was not significant ($P > 0.1$).

A major source of the variability in the otolith nucleus length-female parent length relationship (Fig. 3) was apparently related to the ontogeny of otolith nuclei in the salmonid embryos. Otolith nuclei result from the fusion of primordia. Primordia, the first calcified structures to arise in *S. gairdneri* during embryonic development, appeared at 115-214 Centigrade degree-days. Individual primordia increase in size by concentric accretions, ultimately fusing with neighboring primordia to form the nucleus of the otolith at 226-241 degree-days (Fig. 4). Hatching occurred at about 320 degree-days. The pattern of nucleus development was similar in both rainbow and steelhead trout. Although we did not follow otolith development in *O. tshawytscha*, examination of their nuclei suggested that they also arose from fusion of multiple primordia. Deposition of growth increments commenced immediately after fusion.

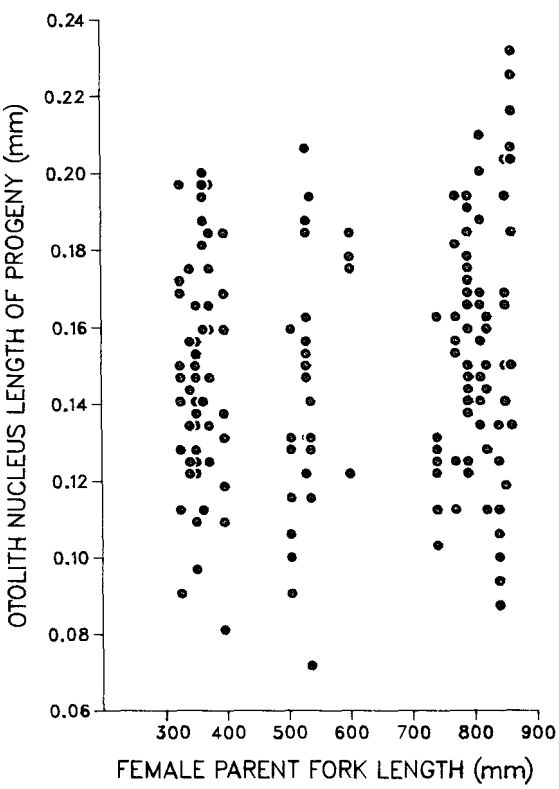

FIGURE 3.—Scatter plot of *Salmo gairdneri* female parent size on otolith nucleus length of progeny. The origin of the adults is given in the caption of Figure 2.

The number of primordia fusing to form the otolith nucleus in the salmonid species we examined was variable, even within the progeny of a single female. In rainbow trout, there was an average of 8.2 ± 2.7 primordia (±1 standard deviation indicated). In steelhead trout and *O. tshawytscha* numbers of primordia averaged 10.7 ± 2.4 and 10.1 ± 2.7, respectively. There were no significant differences in mean primordia counts among the three stocks of rainbow trout or the two stocks of steelhead trout examined (analysis of variance, $P > 0.05$). Figure 5 shows the relationship between the number of primordia deposited and otolith nucleus length.

The variable location of primordia within the nucleus also affects nuclear dimensions and further increases variability. In some instances (<5%), primordia were formed at the periphery of the nucleus, resulting in a local distortion of otherwise regular growth increments (Fig. 6).

Otolith nucleus length (mm) ±1 SE in *S. gairdneri* from the Deadman River was also affected by incubation temperature as shown below:

	Water temperature		
	6.5°C	9.5°C	15.0°C
	----------Mean nucleus length (mm)----------		
Rainbow trout	0.142 ± 0.009	0.174 ± 0.009	0.172 ± 0.008
Steelhead trout	0.154 ± 0.004	0.197 ± 0.008	0.191 ± 0.005

One-way analysis of variance and the Student-Newman-Keuls test indicated that the mean otolith nucleus length in rainbow or steelhead trout reared at 6.5°C was significantly less ($P < 0.01$) than at 9.5° or 15.0°C, although no significant differences in otolith nucleus length ($P > 0.05$) existed in fish reared at the two higher temperatures. The number of primordia formed in both Deadman River steelhead and rainbow trout was independent of the water temperature at which the eggs and alevins were incubated (analysis of variance, $P > 0.05$).

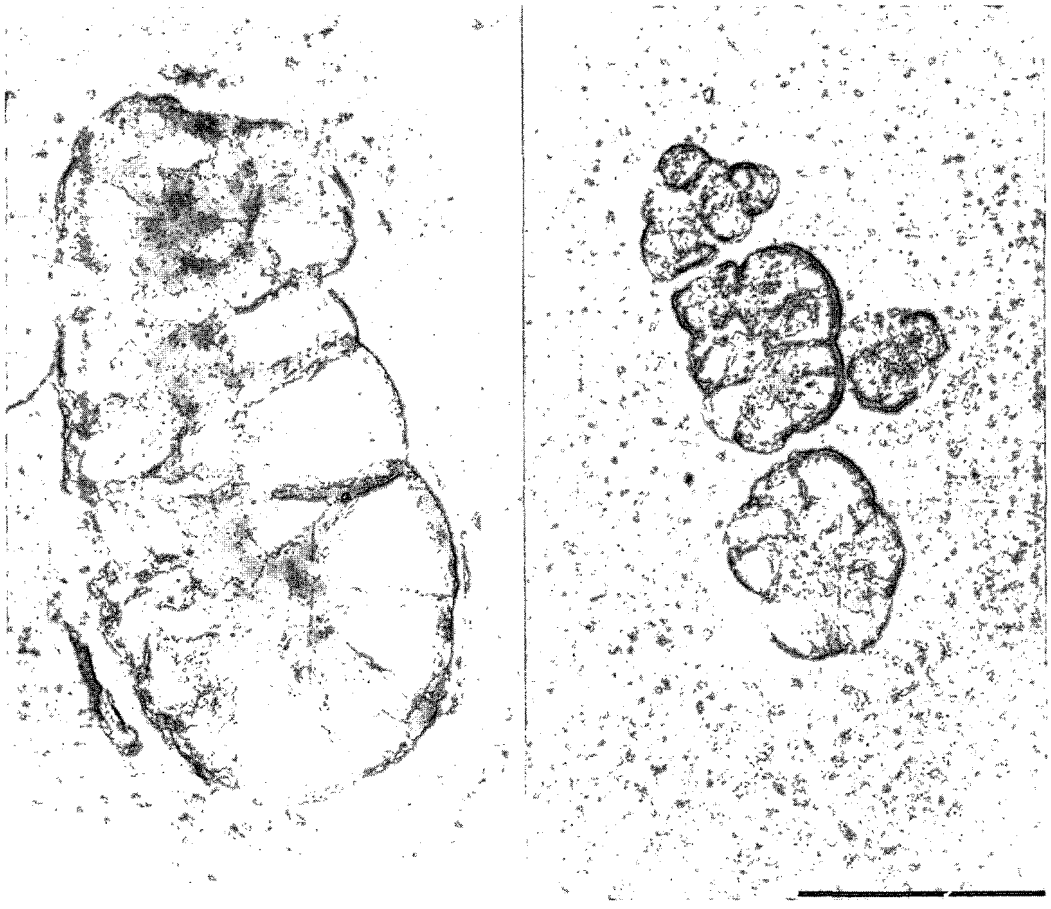

FIGURE 4.—Deadman River steelhead trout sagittal primordia before fusion (right, 214 degree-days) and after fusion (left, 331 degree-days). Bar = 10 μm.

We determined the effect of nucleus size variation on otolith size by examining correlations between nucleus area and otolith area at several stages of development of steelhead trout and *O. tshawytscha* of similar size. We chose to report nucleus area in this case, as it reflects nucleus dimension more precisely than one-dimensional measurements such as nucleus length. While nucleus area and length are significantly correlated ($P < 0.001$), nucleus length accounted for only 47 and 52% of the variability in nucleus area in steelhead trout and *O. tshawytscha*, respectively. The best correlations between nucleus area and subsequent otolith area were noted in relatively small otoliths of recently hatched alevins. The greatest degree of variability in otolith area occurred up to 15 d after nucleus formation (Table 1).

TABLE 1.—Coefficients of variability in otolith area at several stages of development, and coefficients of determination for regressions of otolith area at several stages of development. N = 15 for both steelhead trout and *Oncorhynchus tshawytscha*. The steelhead trout were 29-30 mm FL, and *O. tshawytscha* 30-31 mm. Trout were reared at 9.5°C and *O. tshawytscha* at 6°C.

	Steelhead trout		*O. tshawytscha*	
Stage of otolith development	Coefficient of variation in otolith area (%)	Coefficient of determination (r^2) when regressed on nucleus area	Coefficient of variation in otolith area (%)	Coefficient of determination (r^2) when regressed on nucleus area
Otolith area at nucleus formation	33	n/a	23	n/a
Otolith area 15 d after nucleus formation	15	0.41**	14	0.62**
Otolith area 35 d after nucleus formation	6	0.21 NS	10	0.21 NS
Otolith area 50 d after nucleus formation	7	0.16 NS	11	0.15 NS

** = $P \leq 0.01$.
NS = not significant ($P > 0.05$).

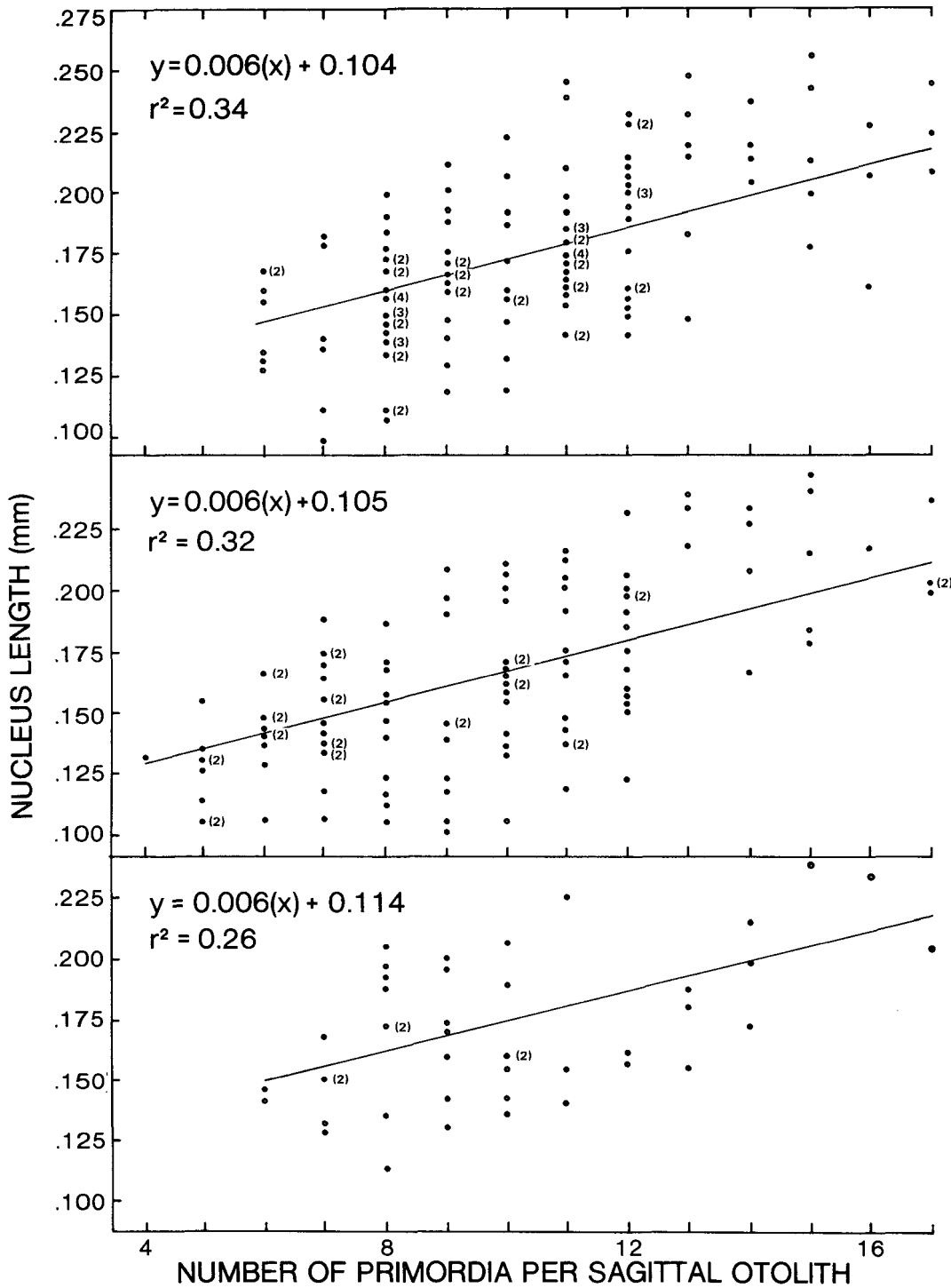

FIGURE 5.—Geometric mean regressions of number of primordia per sagittal otolith on otolith nucleus length for steelhead trout (top), rainbow trout (middle), and Capilano River *Oncorhynchus tshawytscha* (bottom). Trout were incubated at 9.5°C and salmon at 6°C.

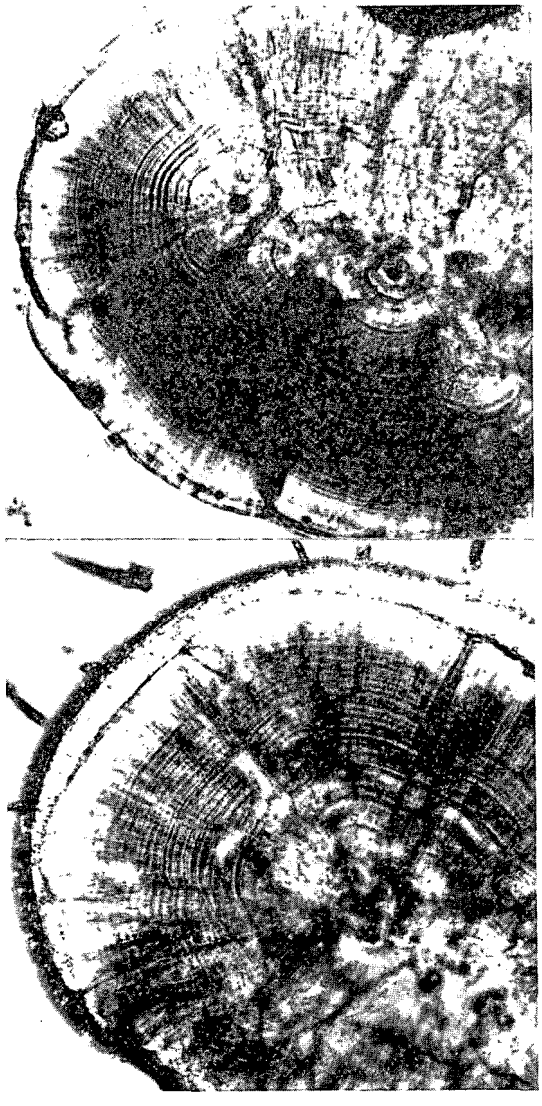

FIGURE 6.—Development of a steelhead trout otolith nucleus resulting from a peripheral primordium (top) and the typical pattern of nucleus development (bottom). Note compression of otolith growth increments in the postrostral quadrant. Otoliths were from progeny of the same female parent.

We did not find any correlation between mean increment width through the various stages of development and nucleus area in either species (t-test, $P > 0.05$). In addition, examination of regressions of increment counts on nucleus area indicated that the frequency of increment formation did not vary as a function of nucleus dimension ($P > 0.10$ for both *S. gairdneri* and *O. tshawytscha*).

DISCUSSION

Sagittal otoliths in *S. gairdneri* embryos arise by fusion of primordia, the first calcified structures to appear during development (McKern et al. 1974). Radtke and Dean (1982) reported similar results for mummichogs, *Fundulus heteroclitus*, and also noted that the otolith nucleus was first apparent as an amorphous gel-like mass in the area of the labyrinth in the developing larvae. Calcified primordia appeared later although Radtke and Dean did not describe any variability in their number or position.

The number and position of the primordia were variable, even within the progeny of a single female. This variation affected the extent of the otolith nucleus. In addition, we observed that water temperature influenced nucleus size. The observed variation in nucleus size limits the utility of this feature as a criterion for stock identification. However, differences in nucleus size did not affect the number of growth increments subsequently formed and had no significant influence on their width.

In our studies eggs were fertilized with the pooled sperm of several males. It is possible that the observed variability in otolith nucleus size was related to the differences between the male parents. There was little difference in the size of the males used, either within the group or relative to the females. We cannot rule out genetic differences between males as a factor affecting variability in nucleus size. However, any genetic effects influencing our results would be no greater than would be expected in natural populations. The numbers of males from which sperm was pooled was usually three, a number frequently involved in fertilization of eggs of a single female in nature (Schroeder 1982; Gross in press).

In developing a hypothesis to explain the basis for use of otolith nucleus length as a means of distinguishing races, Rybock et al. (1975) suggested that nucleus length was related to egg size, although no data were presented. While we found that greater nucleus lengths were associated with larger eggs on average, and larger eggs originated from larger female parents, the slope of the regression of nucleus length on egg weight was not significant (Fig. 3). Furthermore, the variability of otolith nucleus dimensions in rainbow and steelhead trout from south-central B.C. made their measurement much less useful for stock identification that has been suggested for *S. gairdneri* from the Deschutes River, Oreg. (Rybock

et al. 1975). However, otolith nucleus dimensions did serve to separate summer and winter races of steelhead trout (McKern et al. 1974). Workers proposing to use otolith nucleus dimensions as stock identification criteria should consider rearing fish under controlled conditions to establish the extent of nucleus size variability in the stocks in question.

Otolith nucleus length is also influenced by water temperature during embryonic development. Our data showed an increase of about 25% in length in fish reared at 9.5° or 15°C relative to that observed in fish incubated at 6.5°C. The sensitivity of otolith nucleus length to water temperature may allow separation of selected fish stocks whose eggs are incubated at different water temperatures. For example, *O. tshawytscha* juveniles originating from Campbell River stock reared in the Canada Department of Fisheries and Oceans Quinsam Hatchery on Campbell River had significantly greater otolith nucleus lengths ($P < 0.01$) than wild Campbell River *O. tshawytscha* incubated in cooler waters (M. Bradford pers. commun.[4]). Increased water temperature may influence nucleus length through a greater rate of accretion of the calcium/protein matrix around primordia, reflecting a faster rate of embryonic development.

The definition of otolith nucleus suggested here can be consistently applied. With relatively simple preparation techniques, otolith nucleus dimensions can be measured from micrographs or by using a light microscope equipped with an ocular micrometer. Previous workers have delimited the otolith nucleus in relation to metamorphic or nuclear checks. Such terms are ill-defined and should be avoided since they imply that otolith checks result from important developmental events. While it seems likely that such events may result in growth interruptions or checks, causal links have not yet been demonstrated.

The imprecise definition of the periphery of the otolith nucleus may reduce the comparability of measured dimensions derived in various studies. While we have defined the nucleus as lying within the first increment surrounding the primordia, several checks occur during early otolith development. Use of one of these checks to define the periphery of the nucleus would result in inconsistency between various investigations. For example, nucleus lengths of steelhead trout used in this study were generally <0.2 mm (Fig. 3). The mean diameter of the otolith nucleus of summer and winter steelhead reported by McKern et al. (1974) were 0.348 and 0.436 mm, respectively. Differences between studies of this magnitude may be racial in nature or may reflect differences in definition of the extent of the nucleus.

Data on variation in primordia number and location have not been reported previously although the existence of primordia was described by Radtke and Dean (1982) in mummichogs. McKern et al. (1974) did not describe primordia in their work involving the otolith nucleus in steelhead trout. Their results were based on the use of X-ray techniques. We were not able to detect primordia using this method.

It is likely that the otoliths of many fish species are formed by fusion of multiple primordia. From our observations, this is apparently the case in all five species of Pacific salmon and the Pacific herring, *Clupea harengus pallasi*. Radtke and Dean (1982) noted multiple primordia in masou salmon, *O. masou*; Arctic char, *Salvelinus alpinus*; brook trout, *S. fontinalis*; and the sculpin, *Cottus nozawa*.

While both steelhead trout and *O. tshawytscha* otolith nucleus areas were variable, otolith areas in older fish (longer than 15 d after primordia fusion) were less so as indicated by the decreasing coefficient of variation of otolith area with increasing age (Table 1). The decreased variation probably reflects the development of otoliths from an indeterminant array of primordia to the otoliths of adult fish, the latter considered a species-specific characteristic (Fitch 1968; Morrow 1979). However, variation in otolith development in the juvenile salmonids studied here do not present difficulties for the interpretation of microstructure as neither the number nor width of growth increments is significantly affected by nucleus size variation.

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[4]M. Bradford, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6, pers. commun. November 1983.

lation of the D^2 statistic. Barbara Puselja and Tracey Crawford assisted with otolith preparation and examination. Ron Long, Department of Biological Sciences, Simon Fraser University, prepared the photographs. This work was supported by a Graduate Research Engineering and Technology Award to G. H. Geen from the British Columbia Secretariat of Science, Research and Development, and a Canada Department of Fisheries and Oceans Subvention Grant.

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