FORMATION OF OTOLITH GROWTH INCREMENTS AND THEIR POTENTIAL FOR ASSESSING THE EARLY LIFE HISTORY OF CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA)

BY

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Formation of otolith growth increments and their potential for assessing the early life history of chinook salmon (Oncorhynchus tshawytscha)

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ABSTRACT

The applications of otolith microstructure examination in determining the freshwater and estuarine life history of juvenile chinook salmon (Oncorhynchus tshawytscha) were studied. A series of laboratory experiments were conducted to determine how otolith growth increment number and width reflect age and growth respectively in juvenile chinook salmon. The results of the experiments provided evidence that increments were formed at the rate of at least one/24 h and that the widths of increments were proportional to fish growth under different environmental conditions.

Increments were formed once every 24 h under laboratory conditions of 12:12 LD, 24 L or 24 D photoperiods and in constant water temperature regimes. Environmental events such as feeding frequency and water temperature fluctuations which recurred more than once every 24 h significantly increased the rate of increment production possibly by influencing fishes' activity periods. Modifications of photoperiod, ration, feeding frequency and water temperature regimes were generally reflected in a change in mean increment width. In some cases, experimental regimes were not measurably related to changes in the rate of fish growth in length or weight. Therefore, increment widths appear to have utility as indicators of short-term growth rate.

Regardless of the number of increments produced, the ratio of

fish size to otolith size in chinook salmon fry remained constant under all experimental regimes examined, except under conditions of food deprivation. A study of the embryonic development of salmonid otoliths showed that an allometry of fish size and otolith size also existed in recently-hatched alevins, and was related to the number and position of otolith primordia.

By examining otolith microstructure, the growth of juvenile chinook salmon in the Sixes River (Oregon) estuary was determined in relation to their freshwater life history, daily ration, estuary water temperature and population density. Population density was the most important factor affecting juvenile chinook salmon growth, and resulted in a growth rate depression in the mid-summer period. Late recruits to the estuary population were younger than early migrants in two of the three years studied, and late migrants showed no differential growth on entry into the estuary compared with earlier migrants.

Otoliths were obtained from adult chinook salmon returning to spawn in 1981-82. Fast growth as juveniles was negatively correlated with age of maturity of males. Fish which were large on entry into the estuary usually were larger than average at the formation of the first annulus. No evidence of size-selective mortality was found during the estuary residence period, although there was evidence of significant size-selective mortality associated with ocean residence.

ACKNOWLEDGEMENTS

I am almost embarassed to list the number of people who helped me with this thesis. However, without their contributions this work would not have been possible.

Dr. Glen Geen, my senior supervisor, provided excellent support in all phases of my thesis work. It is hard to imagine a better academic supervisor. The other members of my academic committee, Drs. M. Healey and B. McKeown, provided valuable input at many stages of the development of my thesis.

Excellent technical assistance was provided at various stages of my work by Mike Bradford, Tracey Crawford, Paul Higgins, Jeff Johansen and Barbara Puselja. Ron Long, photographer with the Department of Biological Sciences, helped prepare the photographic prints. Dr. Victor Bourne provided instruction on the use of the scanning electron microscope. Jim Oxton, Physics Department, demonstrated metallurgical grinding techniques, from which the methods described here are derived. Jim Vinnell, Vancouver Petrographics Ltd., developed the methodology further so that adult otoliths could be prepared for microstructure examination.

Eldon Stone of the Canada Dèpartment of Fisheries and Oceans Capilano Hatchery provided the chinook salmon used in the experimental

studies. Staff of the British Columbia Fish and Wildlife Branch, notably John Cartwright, Brian Chan and Bob Land, assisted with the provision of rainbow and steelhead trout from both the field and hatcheries. Oregon Department of Fish and Wildlife staff, in particular Dan Bottom and Dr. Paul Reimers, conducted the field collections of chinook salmon from the Sixes River. ODFW staff also contributed data in support of my analyses presented in Chapters 4 and 5.

Informal discussions of my work with fellow students Mike Bradford (SFU), Steven Campana (UBC) and Ken Wilson (UBC) were always useful. I must acknowledge Ken's contribution in particular, as his original work on sockeye otoliths stimulated my interest in the use of otolith microstructure examination for studying the life history of chinook salmon. Staff of the Canada Department of Fisheries and Oceans Pacific Biological Station, particularly Doris Chilton, were helpful in allowing me access to the facilities of the Groundfish Program Age Determination laboratory, where I was able to develop some of the methods used in my thesis.

In the customary manner, I should acknowledge the moral support of my wife Barbara. However, such an acknowledgement would be inadequate. I can only hope that I would be as supportive a spouse should she choose to undertake a graduate degree.

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GENERAL INTRODUCTION

Among the five species of Pacific salmon (Oncorhynchus sp.) occurring in North America, the chinook salmon (O. tshawytscha) has the most variable juvenile life history (Healey 1980). Chinook salmon juveniles may reside only a few days in freshwater, or at the other extreme more than two years. Similarly, the period of estuarine residence is variable although chinook salmon juveniles use estuary habitat to a greater extent than other Pacific salmon (Dorcey et al 1978). Discrete combinations of freshwater and estuarine residence periods have led some authors to suggest that several life history types may exist within a stock (Reimers 1973). The different life history strategies result in a wide range in size of downstream and ocean migrants. Smaller migrant coho (O. kisutch), chum (O. keta) and pink salmon (O. gorbuscha) are often subject to higher natural mortality rates (Chapman 1966; Healey 1982a and Parker 1971). While it is likely that similar phenomena occur in wild populations of chinook salmon, this has yet to be demonstrated.

This thesis was intended to address how juvenile growth of chinook salmon influences their subsequent growth and survival. On the basis of my examination of fish from the Sixes River, Oregon, I tested the following hypotheses:

1. Downstream migrants of different size and/or age grow at different rates in the estuary (Chapter 4).

2. Changes in fish growth rate in the estuary are explicable in terms of fish population density, prey abundance and water temperature (Chapter 4).

3. Growth as juveniles affects the size and age of fish at maturation (Chapter 5).

4. Smaller fish within a cohort are subject to greater natural mortality rates (Chapter 5).

A commonly used method of assessing residence period and growth at various life history stages of Pacific salmon has been examination of frequency and spacing of scale circuli. For example, Reimers (1973) based on his examination of the spacing of scale circuli of Sixes River chinook salmon, suggested that the growth rate of juvenile fish was reduced during the period of peak abundance in the estuary. Schlucter and Lichatowich (1977) used scale examination to determine that the size attained by chinook salmon at the end of their first year influenced subsequent growth and age at maturity. Healey (1982a) has demonstrated size-selective mortality of chum salmon juveniles on the basis of his examination of scale circuli spacing.

However, interpretation of age and growth from circuli, or any other features of bony parts of fish, is based on the assumptions that

the structures are formed at a known rate and that the distance between consecutive circuli is proportional to fish growth. In the case of scales however, several factors including feeding frequency and photoperiod are known to affect the rate of production and the distance between successive circuli (Bilton 1974) thus complicating or invalidating age and growth inferences.

The relatively new finding by Pannella (1971) and subsequent workers that many teleost fish deposit otolith growth increments with 24-h periodicity appeared to offer a method of assessing age and growth with greater accuracy and precision than with scales. Wilson and Larkin (1982) found that widths of otolith increments were correlated with fish growth in sockeye salmon (O. nerka). Some authors including Taubert and Coble (1977) and Campana and Neilson (1982) have suggested that growth increment production followed a circadian rhythm. If increment production is under the control of a biological clock, it may be that environmental variables have little effect on their production. The assumptions for interpretation of age and growth of regular frequency of formation and correlation with fish growth may therefore be better met by otolith microstructure than scale circuli in some species.

To examine the utility of otolith microstructure examination as a method for detailed study of chinook salmon age and growth, I conducted laboratory studies designed to test the following assumptions:

1. The development of otoliths is uniform and does not affect the interpretation of age and growth from otolith microstructure (Chapter 1).

2. The width of otolith increments is proportional to fish growth (Chapters 2 and 3).

3. The frequency of otolith increment formation and their widths is not affected by:
   a. photoperiod (Chapter 2)
   b. different non-cyclic temperature regimes (Chapter 2)
   c. cyclic water temperature regimes (Chapter 3)
   d. ration (Chapter 3)
   e. feeding frequency (Chapter 2 and 3)
   f. fish activity (Chapter 3).

The terms I use to describe fish of different age referred to in this thesis are defined below:

alevin - recently hatched fish, with yolk sac visible.

fry - yolk sac no longer visible, physiologically adapted for freshwater life. In the case of chinook salmon, generally less than 60 mm (fork length).

juvenile - used in Chapter 4 in reference to the underyearling chinook salmon caught in the Sixes River estuary. These fish ranged in size from 40 - 120 mm.

adult - sexually mature individual

The alpha (α) level for statistical tests of inference was 0.05. The exact significance of the test statistic is reported when determined with the aid of a computer. When computed by hand, the range of tabulated values encompassing the calculated value is given (i.e. $0.05 < p < 0.10$).

CHAPTER 1

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

INTRODUCTION

The early development of otoliths is poorly understood considering their potential to provide data on fish age and growth to the daily level of precision (Pannella 1971; Wilson and Larkin 1982). The interpretation of fish growth from otolith microstructure is based on the measurement of the width of bipartite growth increments formed concentrically around the nucleus. 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 (1982) in the estimation of fish growth from otolith growth could be identified.

Variability of otolith nucleus size and shape is also of concern in stock identification studies, since nucleus dimensions may be racial characteristics. Rybock et al (1975) have suggested a positive correlation of Salmo gairdneri otolith nucleus size and the mean egg size of the female which, in turn, was positively correlated to the size of the female. Their data on Deschutes River steelhead trout females, which were larger on average than females of the sympatric population of 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 Salmo gairdneri.

In this chapter, I describe development of sagittal otoliths of rainbow trout Salmo gairdneri (sea-run and freshwater resident) and chinook salmon (Oncorhynchus tshawytscha) and examine the effect of water temperature on otolith nucleus dimensions. These data permitted a re-examination 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 Salmo gairdneri, I obtained eggs from steelhead trout in the Deadman River, British Columbia 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 2-3 males of similar size and origin as the female. In total, eggs from 10 steelhead and 11 rainbow trout were used in this study.

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.

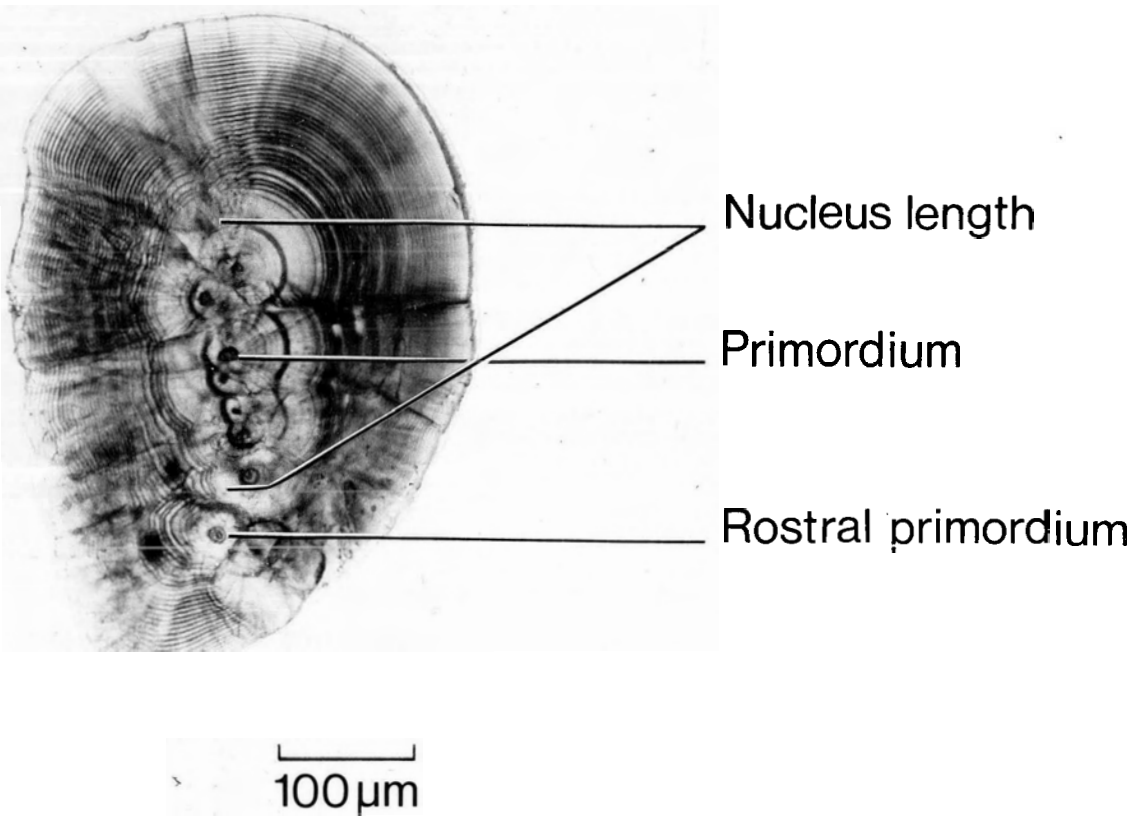
Chinook salmon eggs were taken from the fall, 1981 Capilano River stock and were incubated at 6°C under an approximate 12:12 LD photo-

period. Hatchery practice did not allow separate rearing of groups of eggs from individual females.

Otolith development in Salmo 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 non-calcified tissue transparent and amorphous compared with otoliths and other hard parts, permitted otolith examination with a transmitted light microscope at 400X. While I 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. 1-1). 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 Fig. 1-1.

Figure 1-1 Sagittal otolith from a Capilano River chinook salmon alevin showing the otolith nucleus, primordia and rostral primordium.


Nucleus length
Primordium
Rostral primordium
100 µm

Otolith nucleus length was measured from coded preparations with an ocular 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 from photographic enlargements (final magnification 9700X) with a vernier caliper. The frequency of increment formation was determined by 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 often greater in left-side than right-side sagittae, albeit not significantly so ($p=0.0811$, Wilcoxon Paired Sample Test).

During the course of this study, otoliths from 257 rainbow trout, 187 steelhead trout and 50 chinook salmon were examined.

RESULTS

To examine the hypothesis that egg size (a function of female fork length) influences otolith nucleus length in progeny, I examined the relationships of female fork length to egg dry weight and nucleus length in Salmo 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=0.0008, Fig. 1-2). However, there was no significant relationship between otolith nucleus length and female fork length (t-test, p=0.4511, Fig. 1-3), or egg dry weight (t-test, p=0.1141). I further investigated the utility of otolith nucleus lengths as a racial characteristic by calculating D^2, as 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 Salmo gairdneri. D^2 was 0.063 and was not significant (p=0.1858).

A major source of the variability in the otolith nucleus length-female parent length relationship (Figure 1-3) was apparently related to the ontogeny of otolith nuclei in the salmonid embryos. Otolith nuclei result from the fusion of primordia. Primordia, the first ossified structures to arise in Salmo gairdneri during embryonic development, appeared at 115-214 Centigrade degree-days. Individual primordia increase in size by concentric accretions, ultimately fusing with neighbouring primordia to form the nucleus of the otolith at

Figure 1-2 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 are rainbow trout from Pennask Lake, those 500-600 mm are rainbow trout from Mission Creek, and those greater than 700 mm are Deadman or Nicola River steelhead.

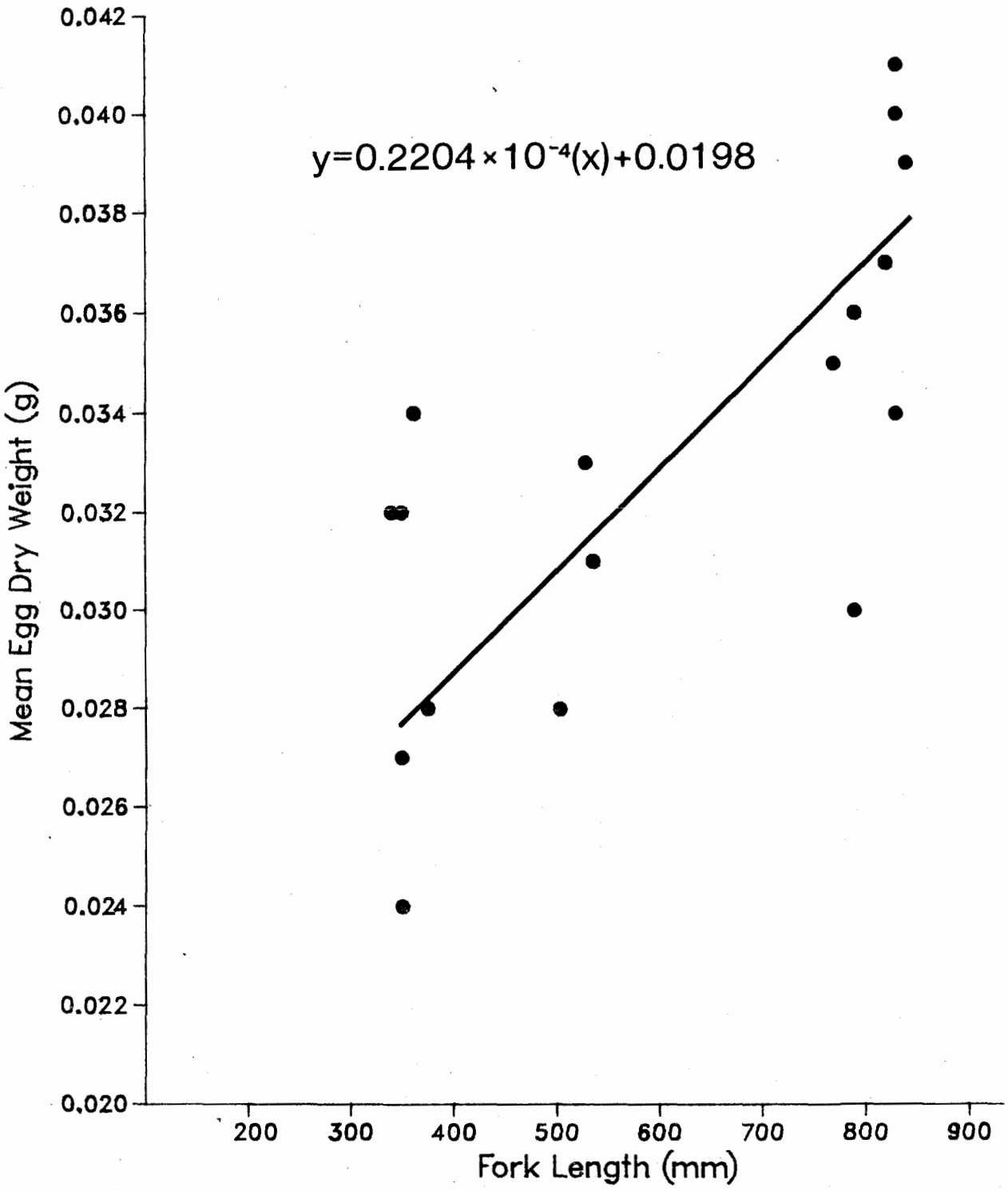
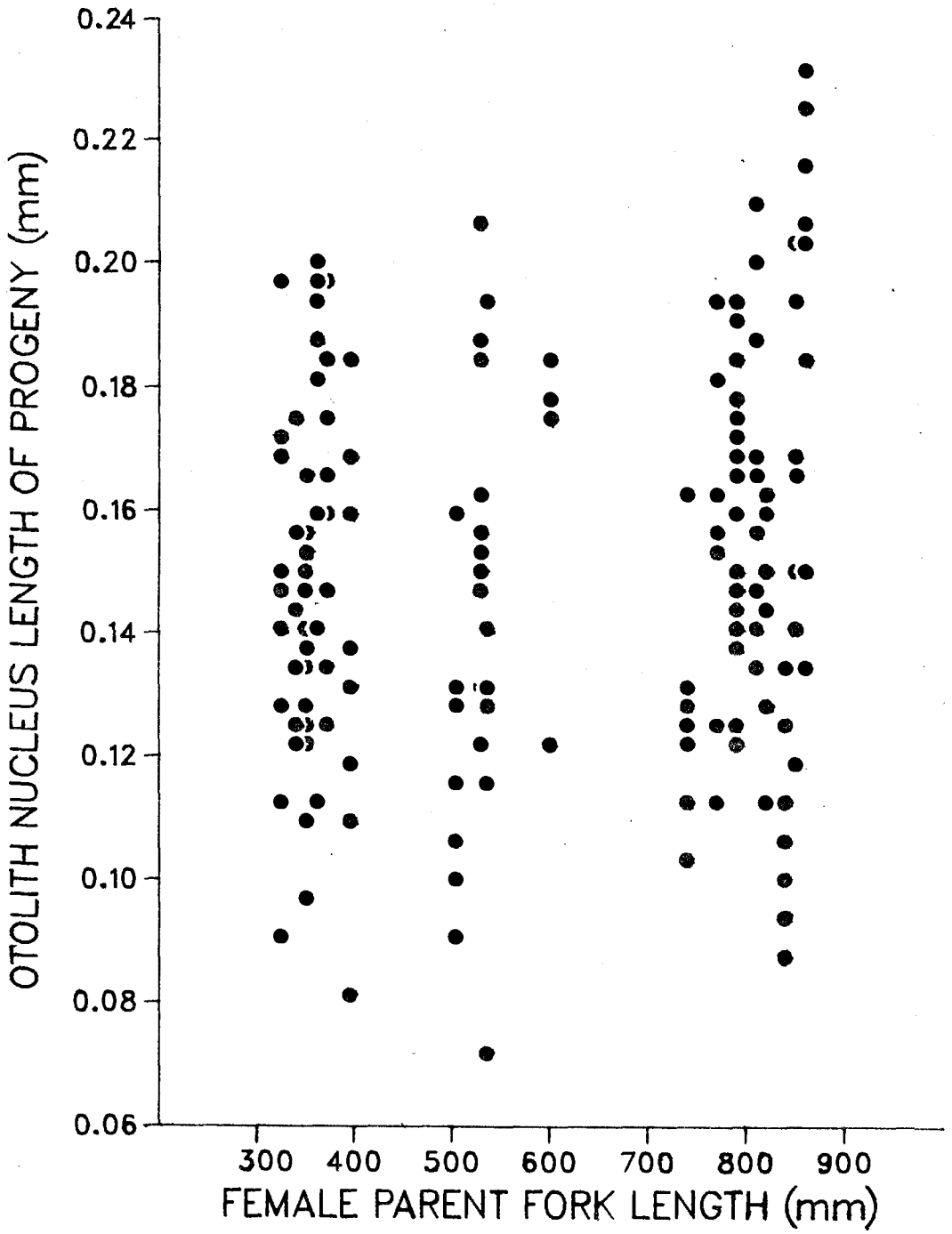

y=0.2204×10⁻⁴(x)+0.0198
Mean Egg Dry Weight (g)
0.042
0.040
0.038
0.036
0.034
0.032
0.030
0.028
0.026
0.024
0.022
0.020
200
300
400
500
600
700
800
900
Fork Length (mm)

Figure 1-3 Scatter plot of Salmo gairdneri female parent size on otolith nucleus length of progeny. The origin of adults is given in the caption of Figure 1-2.


0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
OTOLITH NUCLEUS LENGTH OF PROGENY (mm)
300
400
500
600
700
800
900
FEMALE PARENT FORK LENGTH (mm)

226-241 degree days (Fig. 1-4). Hatching occurred at approximately 320 degree-days. The pattern of nucleus development was similar in both rainbow and steelhead trout. Although I did not follow otolith development in chinook salmon, examination of their nuclei indicated that they also arose from fusion of multiple primordia. Deposition of growth increments commenced immediately after fusion.

The number of primordia fusing to form the nucleus in the salmonid species I 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 chinook salmon, 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 or the two stocks of steelhead trout examined (ANOVA, $p=0.2187$). Fig. 1-5 shows the relationship between the number of primordia deposited and otolith nucleus length.

The variable location of primordia within the nucleus also affects its 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. 1-6).

The mean nucleus length (mm ± 1 S.E.) of otoliths of S. gairdneri juveniles from the Deadman River was also affected by incubation

Figure 1-4 Deadman River steelhead trout sagittal otolith primordia before fusion (right, 214 degree-days) and after primordia fusion (left, 331 degree-days). Bar = 10 μm.

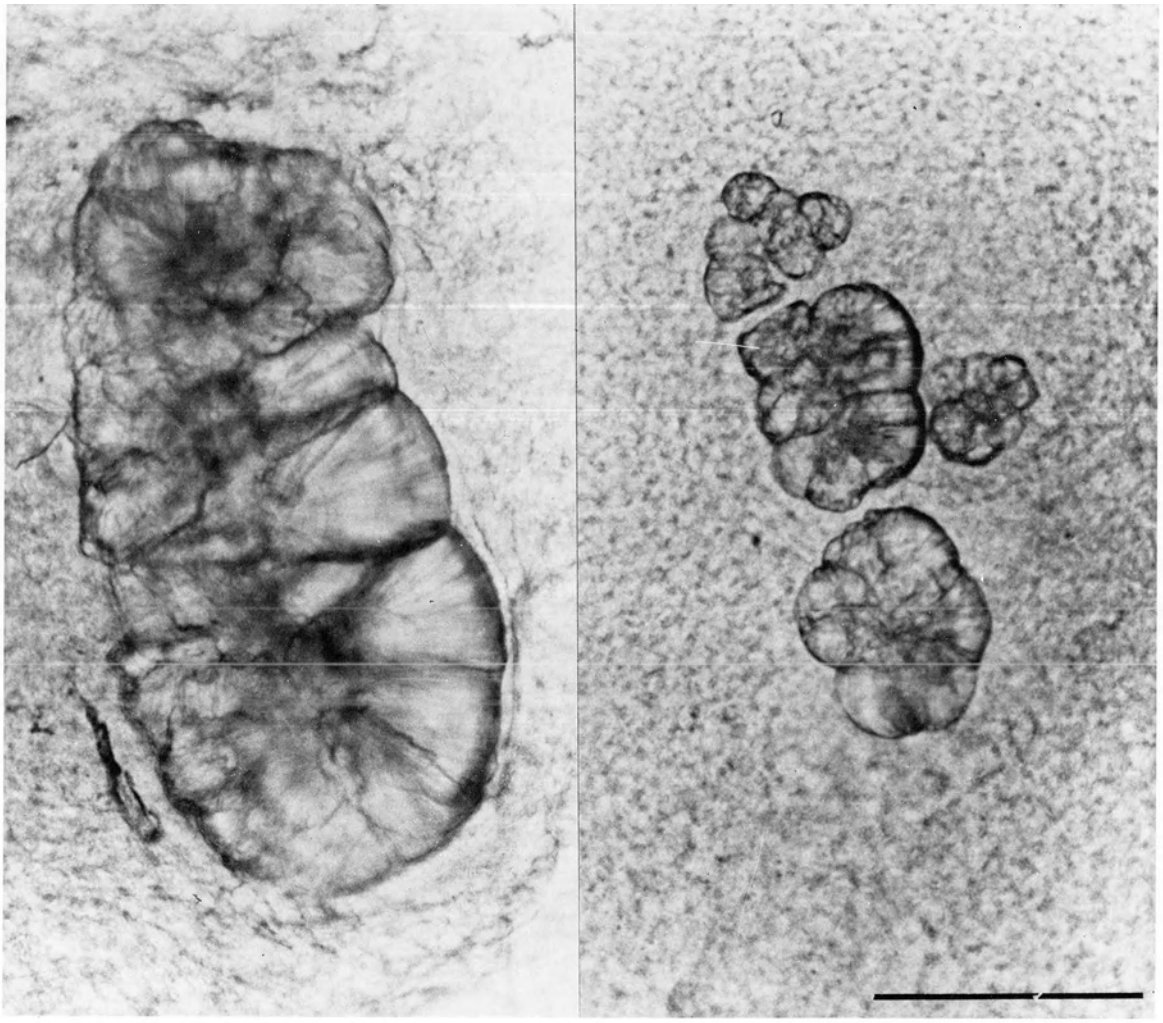

a

Figure 1-5 Geometric mean regressions of number of primordia per sagittal otolith on otolith nucleus length for steelhead trout (top), rainbow trout (middle) and Capilano River chinook salmon (bottom). Trout were incubated at 9.5°C, and salmon at 6°C.

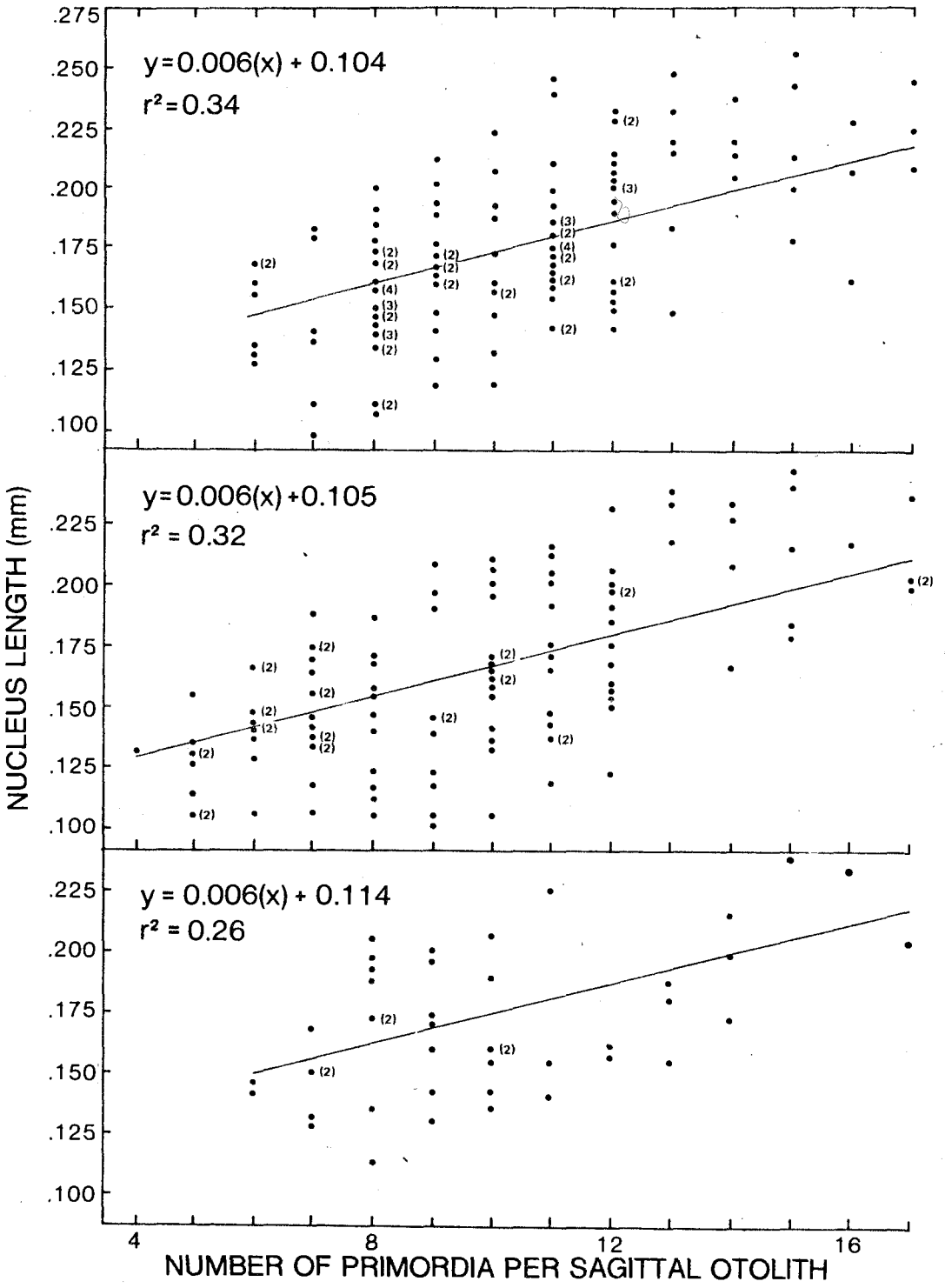
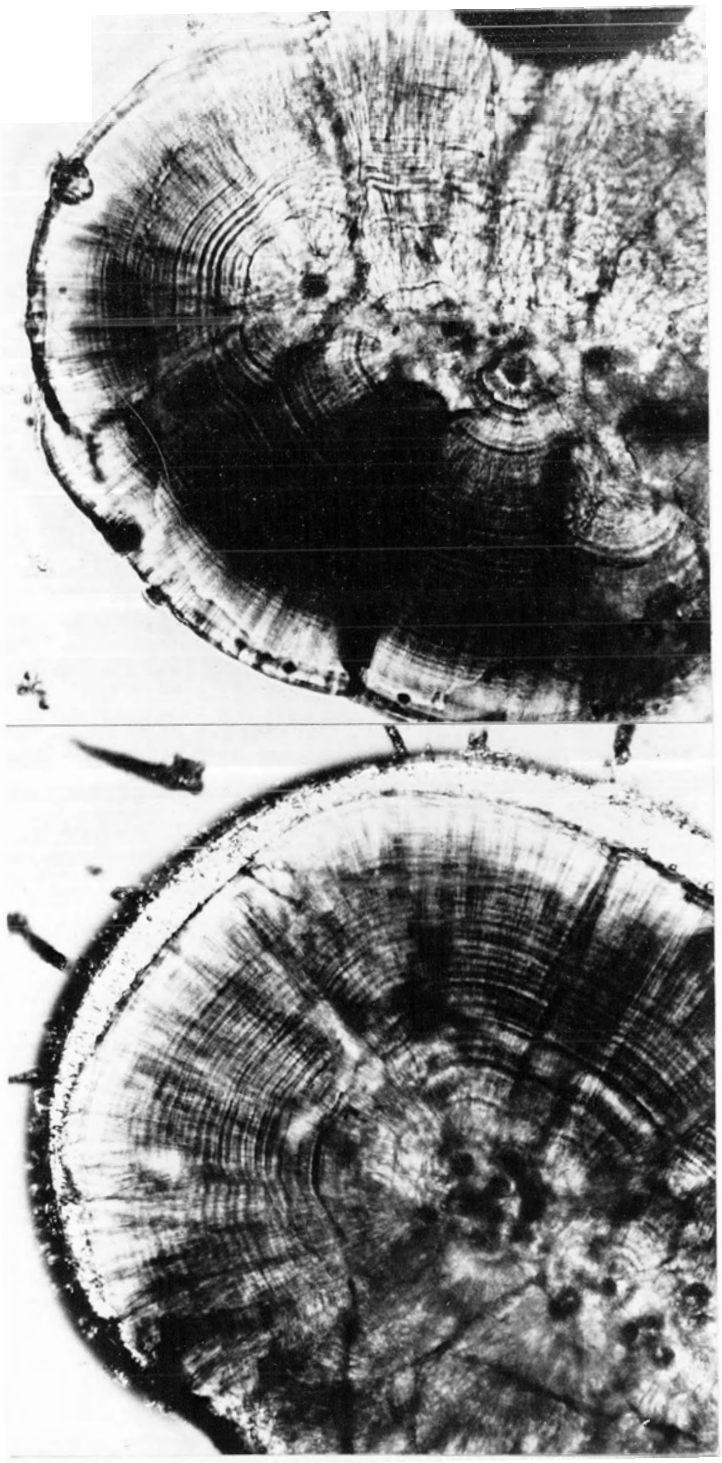

y=0.006(x) + 0.104
r²=0.34
y=0.006(x) +0.105
r² = 0.32
y = 0.006(x) + 0.114
r² = 0.26
NUCLEUS LENGTH (mm)
NUMBER OF PRIMORDIA PER SAGITTAL OTOLITH
.275
.250
.225
.200
.175
.150
.125
.100
4
8
12
16

Figure 1-6 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 post-rostral quadrant (terminology of Messieh 1975). Otoliths were from progeny of the same female.


100μm

temperature as shown below:

	Water Temperature		
	6.5°C	9.5°C	15.0°C
	Mean Nucleus Length (mm)		
Rainbow trout	0.142 ± .009	0.174 ± .009	0.172 ± .008
Steelhead trout	0.154 ± .004	0.197 ± .008	0.191 ± .005

One-way analysis of variance and the Student-Newman-Kuels test indicated that the mean otolith nucleus length in rainbow or steelhead trout reared at 6.5°C was significantly less ($p=0.0012$) than that at 9.5 or 15.0°C although no significant differences in otolith nucleus length ($p=0.5295$) 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 incubation temperature (analysis of variance, $p=0.4993$).

I determined possible effects 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 chinook salmon of similar size. I chose to report nucleus area in this case, as it may reflect nucleus dimension more precisely than one-dimensional measurements such as nucleus length. While nucleus

area and length are significantly correlated (p=0.0003), nucleus length accounted for only 47 and 52% of the variability in nucleus area in steelhead trout and chinook salmon respectively. The best correlations between nucleus area and subsequent otolith area were noted in the relatively small otoliths of recently-hatched alevins. The greatest degree of variability in otolith area also occurred during early stages of otolith development, up to 15 d after nucleus formation (Table 1-1).

I did not find any correlation between mean increment width through the various stages of development and nucleus area in either species (t-tests, p=0.4544 and 0.6211 for rainbow and steelhead trout respectively). 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=0.6756 and 0.5331 for both S. gairdneri and chinook salmon respectively).

TABLE 1-1

Coefficients of variability in otolith area at several stages of development and coefficients of determination for regressions of nucleus area on otolith area at several stages of development. N = 15 for both steelhead trout and chinook salmon. The initial size of the trout was 29-30 mm fork length, and the salmon 30-31 mm. Trout were reared at 9.5°C and chinook salmon at 6°C.

	Steelhead Trout		Chinook Salmon	
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 15d after nucleus formation	15%	.41**	14%	.62**
Otolith area 35d after nucleus formation	6%	$.21^{ns}$	10%	$.21^{ns}$
Otolith area 50d after nucleus formation	7%	$.16^{ns}$	11%	$.15^{ns}$

**= $p < 0.01$

ns= not significant ($p>0.05$)

DISCUSSION

Sagittal otoliths in Salmo gairdneri embryos arise by fusion of primordia, the first ossified 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 discuss any variability in their number or position. McKern et al (1974) did not describe primordia in their works involving the otolith nucleus in steelhead trout. Their results were based on the use of X-ray techniques. I was not able to detect primordia using this method.

The number and position of the primorida is variable, even within the progeny of a single female. This variation affects the extent of the otolith nucleus. In addition, I 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.

It is likely that the otoliths of any fish species are formed by the fusion of multiple primordia. This is apparently the case in all five species of Pacific salmon, rainbow trout, and the Pacific herring

(Clupea harengus). Radtke and Dean (1982) noted multiple primordia in masou salmon (Oncorhynchus masou), Arctic char (Salvelinus alpinus), brook trout (Salvelinus fontinalis) and the sculpin (Cottus nozawa).

In this study, eggs were fertilized with the pooled sperm of several males. It is possible that the observed variability in nucleus size was related to genetic 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. Any genetic effects influencing my 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 likely involved in fertilization of eggs of a single female in nature (Schroeder 1982; Gross, in press).

In developing a hypothesis to explain why otolith nucleus length could be used as a racial criterion, Rybock et al (1975) suggested that nucleus length was related to egg size, although no data were presented. While I 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. Furthermore, the variability of otolith nucleus dimensions in rainbow and steelhead trout from south-central B.C. made their measurement less useful for stock identification than has been suggested for S. gairdneri from the Deschutes River, Oregon (Rybock et al 1975). However, otolith nucleus dimensions did serve

to separate summer and winter races of steelhead fry (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 the water temperature during embryonic development. My data showed an increase of approximately 25% in nucleus length in otoliths of fish reared at 9.5 or 15.0°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, chinook salmon juveniles originating from the Canada Department of Fisheries and Oceans Quinsam Hatchery on the Campbell River had significantly greater otolith nucleus lengths ($p<0.01$) than wild Campbell River chinook salmon incubated in cooler waters (M. Bradford pers. comm.).

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 result in inconsistency in the measured dimensions. While I have defined the nucleus as lying within the first increment surrounding the primordia, several checks occur during early otolith development. Inconsistent use of these checks to describe the periphery of the nucleus would result in differences in measurements reported by the various investigators. For example, nucleus lengths of steelhead trout in this study were generally < 0.2 mm (Fig. 1-3). 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 of this magnitude may be racial in nature or may reflect differences in definition of the extent of the nucleus.

While both steelhead trout and chinook salmon otolith nucleus areas were variable, otolith areas in older fish (> 15 d after primordia fusion) were less so as indicated by the decreasing coefficient of variation of otolith area with increasing age (Table 1-1). The decreased variation probably reflects the development of otoliths from an indeterminate array of primordia to the otoliths of adult fish, the latter considered a species-specific characteristic (Fitch 1968; Morrow 1977). However, variation in otolith development in the juvenile salmonids studied here do not present difficulties for the

interpretation of microstructure as neither the number or width of growth increments is significantly affected by nucleus size variation. Having demonstrated that the variable development of salmonid otoliths does not significantly affect the formation of growth increments, I have satisfied the first requirement for the validation of otolith microstructure as a method for age and growth studies which I identified in the general introduction.

CHAPTER 2

DAILY GROWTH INCREMENTS IN OTOLITHS OF CHINOOK SALMON AND FACTORS INFLUENCING THEIR FORMATION

INTRODUCTION

Since Pannella (1971) described daily growth increments in marine fish otoliths, research on otolith microstructure has proliferated. Daily growth increments have been reported in several species of fish, usually juveniles less than 200 d old. Counts of daily growth increments were correlated with the age of the fish in days after hatching (Pannella 1971; Brothers et al 1976; Campana and Neilson 1982; Marshall and Parker 1982). As well as permitting improved resolution of age, otolith microstructure allows refined estimates of fish growth through measurements of increment width (Struhsaker and Uchiyama 1976; Methot and Kramer 1979; Wilson and Larkin 1982).

In Chapter 1, I demonstrated that variation in otolith nucleus development did not influence the number or width of growth increments that were formed subsequently. As noted in the general introduction, the further validation of conclusions based on otolith microstructure requires data on the effects of environmental variables such as photoperiod, temperature and feeding frequency on otolith increment formation. Recent evidence indicates, for example, that more than one growth increment may be formed each day under certain environmental conditions (Brothers 1979; Pannella 1980). Several environmental factors have been implicated in the formation of daily growth increments. Pannella (1980) suggested a close relationship between feeding frequency and number of growth increments formed, although no supporting data were

offered. Taubert and Coble (1977) stated that a 24-h light/dark cycle entrained a daily periodity in increment formation, while Brothers (1979) suggested that diel changes in water temperature may act as a zeitgeber, or time cue. Campana and Neilson (1982) found that the number of increments produced by starry flounders (Platichthys stellatus) was unaffected by photoperiod or temperature fluctuations. To date, research has largely been confined to the study of effects of environmental variables on numbers of otolith growth increments produced. Few authors have attempted to relate otolith increment width to environmental variables. In this chapter, I examine the effects of feeding frequency, photoperiod and constant temperature regimes on the number and width of otolith growth increments formed in juvenile chinook salmon (Oncorhynchus tshawytscha).

METHODS

Feeding Frequency

I used chinook salmon fry from the 1979 brood of the Canada Department of Fisheries and Oceans Capilano Salmon Hatchery, Vancouver, British Columbia. During the period from fertilization through yolk sac absorption, fish were held in Heath trays in a darkened room at an average of 4°C. The fish were transferred to Simon Fraser University approximately 90 d after hatching (free-swimming fry) and held in 28-L flow-through aquaria supplied with aerated and dechlorinated water. Fish were not fed prior to transfer, as yolk sacs were present until that time.

For experimental purposes, two groups of similarly-sized fish were held under a 12:12 LD light regime for 65 d. One group of 100 fish was provided with excess food (approximately 2.5 g Oregon Moist Pellets per feeding) by an automatic fish feeder at 6 h intervals. Excess food was siphoned from the aquarium at 1300 h each day. A second group of 100 fish was provided with approximately 10 g at 1200 h every day, with excess food removed after 30 min. During these experiments water temperature ranged from 10-12°C, and did not vary in any consistent diel fashion.

Temperature

Fish incubated at the Capilano Salmon Hatchery (1979 brood) were transferred as free-swimming fry 45 d after hatching to two outside holding tanks on hatchery premises. Prior to transfer to the holding

tanks, the fish had previously been kept in a darkened Heath Tray at 4°C until hatching, and at 12°C from the hatching to the free-swimming stage.

The experimental fish in the holding tanks were held at mean temperatures of 5.2 ± 1.6°C (± 1 S.D., range 2 - 9°C) or 11 ± 1.4°C (range 7 - 13°C). Although no diel fluctuations in water temperature were noted, mean daily temperature increased gradually over the 45-d duration of the experiment. Fish were fed to excess once every day.

Photoperiod

The role of photoperiod in modifying otolith growth increments at the earliest stages of development was examined. Two groups of 100 unfertilized eggs (1980 brood) were transported from Capilano Hatchery to Simon Fraser University and fertilized. One lot was placed in a darkened Weiss-type incubation funnel while a second lot was placed in a similar funnel and exposed to a 12:12 LD photoperiod. The water temperature through the incubation period (fertilization to yolk sac absorption) ranged from 7-9°C and did not vary in any consistent diel fashion. The experiment was terminated 80 d after fertilization (30 d after hatching).

The role of photoperiod in otolith increment formation in older fish was examined by holding a group of 90 d-old fry (1979 brood, Capilano Salmon Hatchery) under constant illumination from a fluorescent light source (200 lx) for 65 d. A second group of fry was held under a 12:12 LD photoperiod for 65 d. Light intensities were 200 and 40 lx during the light and dark periods, respectively. Both groups were fed approximately

2.5 g of Oregon Moist Pellets every 6 h. Water temperature ranged from 10-12°C over the duration of the experiment, and did not fluctuate with diel periodicity. The sensitivity of the recording thermograph was ± 0.5°C.

Preparation and Examination of Otoliths

The sagittae, the largest of the three pairs of otoliths in salmonids, were dissected from fish preserved in 95% ethanol and stored dry in individual wells of tissue culture plates until further processing. To prepare the otolith for microscopic examination, it was attached with the sulcus acusticus (a prominent depression on the proximal surface) side down to a standard glass microscope slide with "Crystalbond" (distributed by Aremco Ltd., New York), a thermosetting plastic resin. The otolith was ground and polished on its sagittal surface using a grinding jig (Neilson and Geen, 1981) and metallurgical lapping films whose grit size ranged from 0.3 - 30 μm. If the specimen was too opaque to allow examination using transmitted light microscopy after the first grinding, the slide was gently heated, the otolith removed and reset in the thermosetting plastic with the ground surface against the slide. The proximal side of the otolith was then ground and polished to reduce the thickness of the preparation.

If the otolith was to be examined using the scanning electron microscope, it was transferred to a SEM specimen stud, etched by immersing the stud and otolith in a 1% HCl bath for 90 s, and then rinsed with water.

The weak acid differentially etches various parts of the otolith, resulting in the growth increments having a three-dimensional structure, a prerequisite for scanning electron microscopic examination. After etching, the regularly recurring increments are bipartite, consisting of relatively narrow, deeply-etched zones and relatively wide, lightly etched zones. After drying, the stud and otolith were placed in a planetary specimen holder and transferred to a vacuum evaporator where the otolith was gold-plated. The otolith was then viewed using a Perkin-Elmer Autoscan SEM at 600-900X magnification.

Some otoliths, mainly those from the temperature experiment, were examined with a Zeiss photomicroscope at 800-1200X magnification. Light microscopy was most appropriate when examining otoliths from small fish (<50 d after hatching) since sufficient light was transmitted through the relatively thin otolith for examination.

Scanning electron and light micrographs were attached to a Tektronix 4954 digitizing table on which increment widths were measured. The digitizing table had a direct link to computer facilities where data were stored and analyzed further using standard statistical programs. The resolution of the digitizing table was 0.3 mm, representing about 3% of the average photographic enlargement of a 2 μm increment. Using a simple computer program, the data were transformed from scalar distances defined by increments along a radius of the otolith to increment widths. I measured increment width along a radius 30° from the long axis of the otolith in the quadrant of maximum growth, as suggested by Pannella (1980). The increments were typically widest along this radius.

I determined which increments were to be digitized by locating checks in the otherwise regular pattern of increment deposition caused by events such as hatching, transport stress or abrupt temperature shifts. Such checks were common to all fish examined. After confirming that the checks corresponded to an event of known date, they were used as dated references for counts or increment width measurements.

Error of the digitizing table operator in recognizing and recording increment boundaries was assessed by digitizing a series of fifty increments on four duplicate SEM micrographs. The mean increment widths for each of the four trials did not significantly differ ($p=0.5972$), and the same number of increments was recorded.

Measurements of increment width might be affected by any deviation in the angle of grinding and polishing from the preferred plane (the plane of maximum area on a longitudinal axis). I simulated this effect by tilting the SEM stage through 15°, which was assumed to be the maximum deviation of the grinding plane. Micrographs of a SEM preparation were taken at 5° intervals through this arc, and digitized. The error in measurement of otolith increment width through this arc was <1% in the 600-900X magnification range used and is not considered a serious source of error.

The results obtained using light and scanning electron microscopy were compared by making a reference mark on five otoliths with a carbide-tipped stylus. This mark, visible under light and scanning electron

microscopes, served as the starting point for counts and increment width measurements. Counts and increment widths obtained using either type of microscope did not differ significantly ($p=0.7938$). Thus, etching the otolith surface with weak acid prior to SEM examination did not affect increment width or number.

Fifty pairs of otoliths from alevins were examined to determine whether counts or otolith increment widths varied according to the side of the fish from which the otolith originated. Using a Wilcoxon Paired-Sample test, I detected no difference in counts obtained from either side of the fish ($p=0.8911$). However, otoliths removed from the left side of the fish were significantly larger (maximum length) than those from the right side. Data reported here are from left side otoliths only.

Some authors (Brothers 1978; Campana 1983) have attempted to develop criteria for distinguishing daily from sub-daily increments. Such distinctions were often based on subjective appraisals of increment continuity and appearence when viewed with a light microscope. In chinook salmon otoliths, no such differences were noted among growth increments. Moreover, as the purpose of this study is to determine the periodicity of increment formation as a basis for detailed study of fish growth, the subjective interpretation of increments as daily or sub-daily was not necessary. To determine if microstructure features reflect fish growth, it was sufficient to determine the frequency of formation of increments and the relationship between increment width and growth.

RESULTS

Feeding Periodicity

Fish fed once daily deposited an average of one growth increment per 24 h, whereas fish fed 4x/24 h formed more than one growth increment per day (Fig. 2-1 and 2-2). The slopes of the two regressions (0.98 and 1.35 for 1 feeding/24 h and 4 feedings/24 h respectively) are significantly different (t-test, $0.01<p<0.05$). The slope of the regression for 1 feeding/24 h does not significantly differ from unity ($0.10<p<0.20$), indicating one increment was formed every 24 h. The slope of the regression for fish fed 4x/24 h was significantly greater than unity ($0.01<p<0.05$). I inferred that total food assimilated by fish in the two groups was comparable since there were no significant differences in fork length at the conclusion of the experiment (analysis of variance, $p=0.2129$).

Mean increment widths during the 14-30 d period of the experiment were 1.52 ± 0.10 and 1.30 ± 0.09 μm for fish fed once and four times every 24 h respectively. During the 31-54 d period, mean increment widths were 2.27 ± 0.39 and 1.25 ± 0.25 μm for 1 feeding /24 h and 4 feedings /24 h respectively (Fig. 2-3). Mean increment widths were significantly different during both periods (analysis of variance, $0.01<p<0.05$ and $0.001<p<0.01$ for d 14-30 and 31-54 respectively).

The slopes of regressions of otolith size on fish size did not significantly differ for each feeding regime (analysis of covariance, $p=0.1102$) nor were they different from the slope of the regression

Figure 2-1 Relationship of number of otolith increments formed in chinook salmon fry fed 1x/24 h or 4x/24 h. Points on the regressions are means of counts from 5-10 otoliths (1 feeding/24h) and 4-14 otoliths (4 feedings/24 h), and 1 SD is indicated by the error bars.

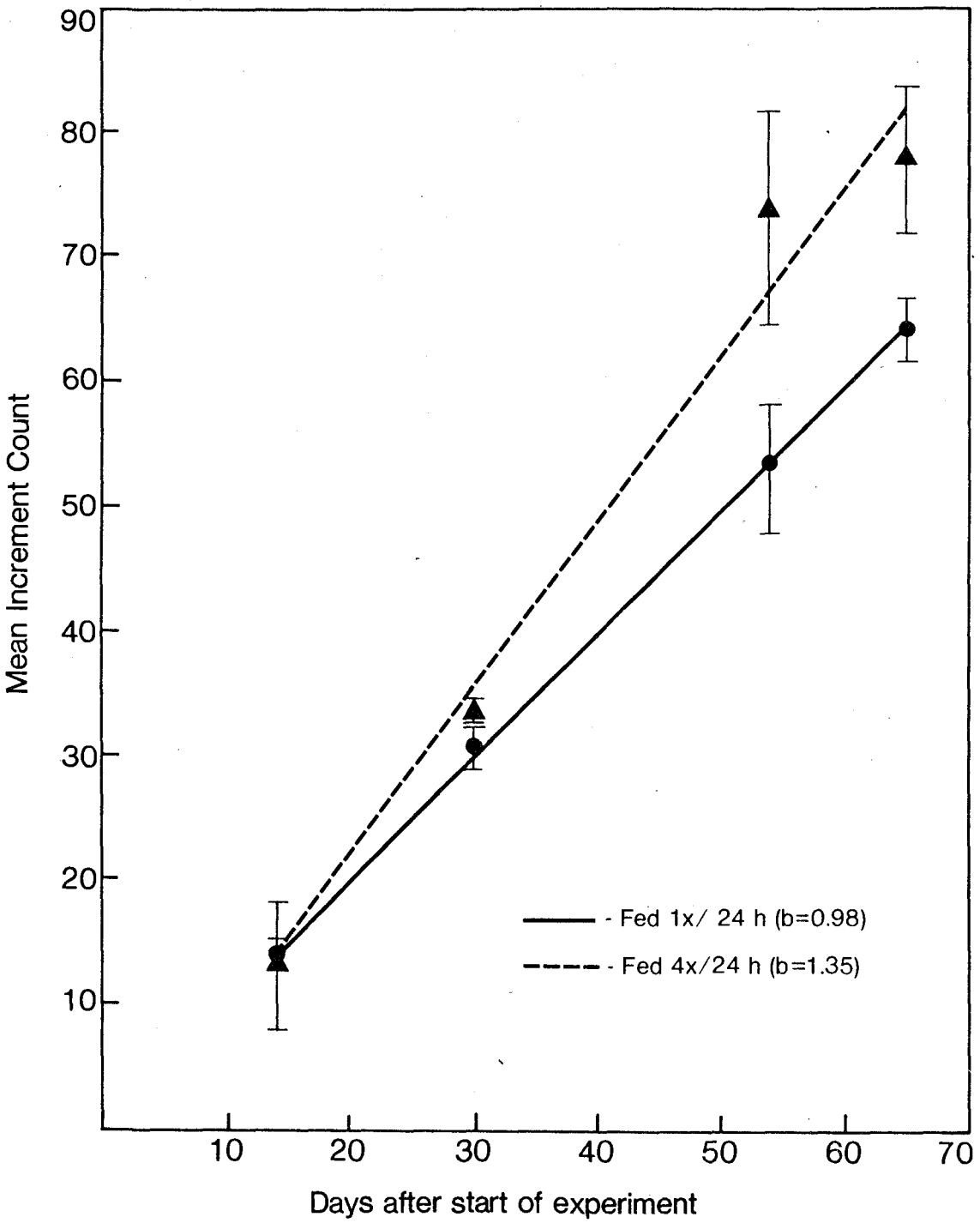

90
80
70
60
50
40
30
20
10
Mean Increment Count
10
20
30
40
50
60
70
Days after start of experiment
- Fed 1x/ 24 h (b=0.98)
- Fed 4x/24 h (b=1.35)

Figure 2-2 Scanning electron micrographs of ground and etched chinook salmon otoliths taken from fry fed 4x/24 h (top) or 1x/24 h (bottom). Fish were sacrificed at d 65 of the feeding frequency experiment.

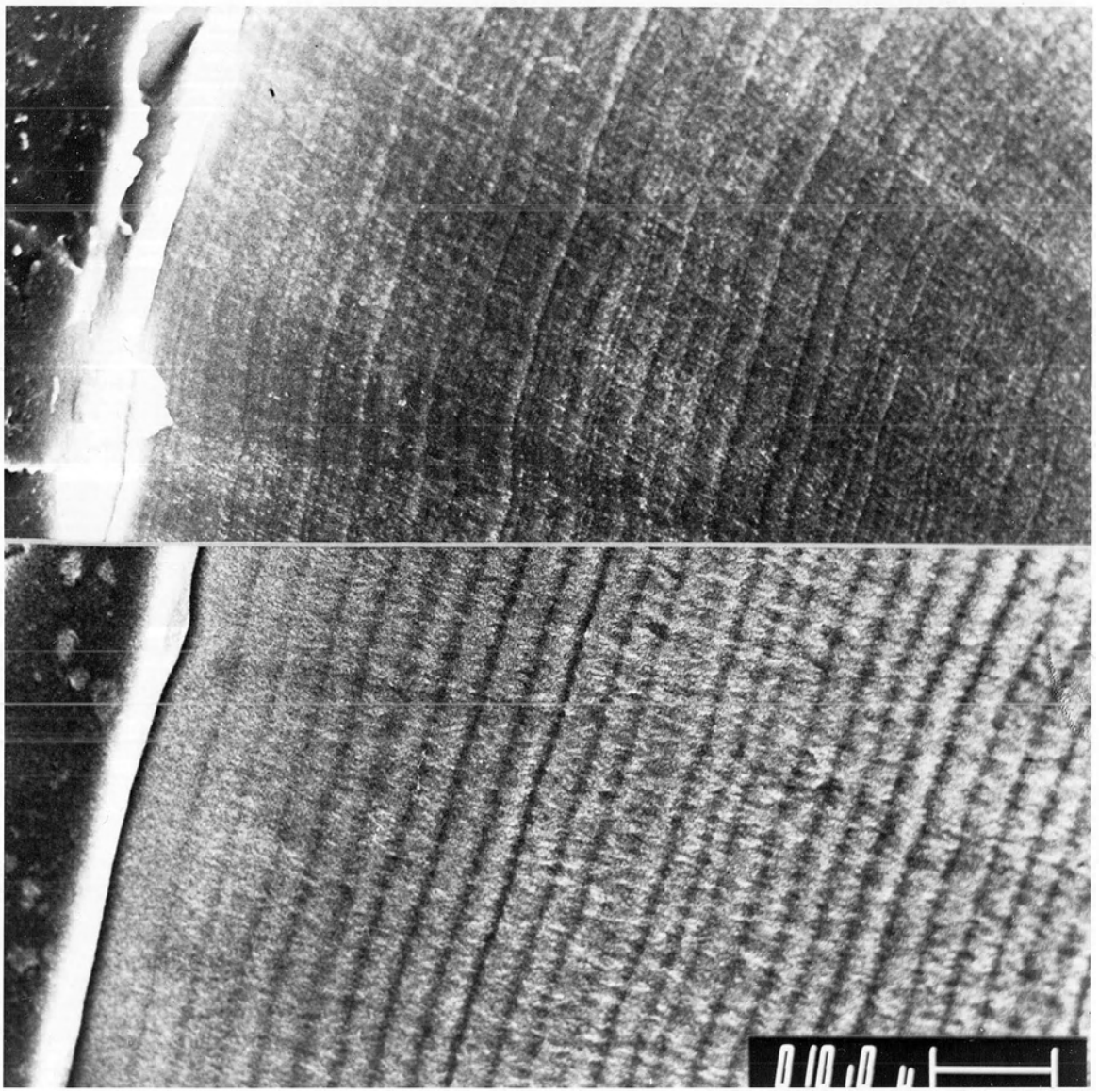
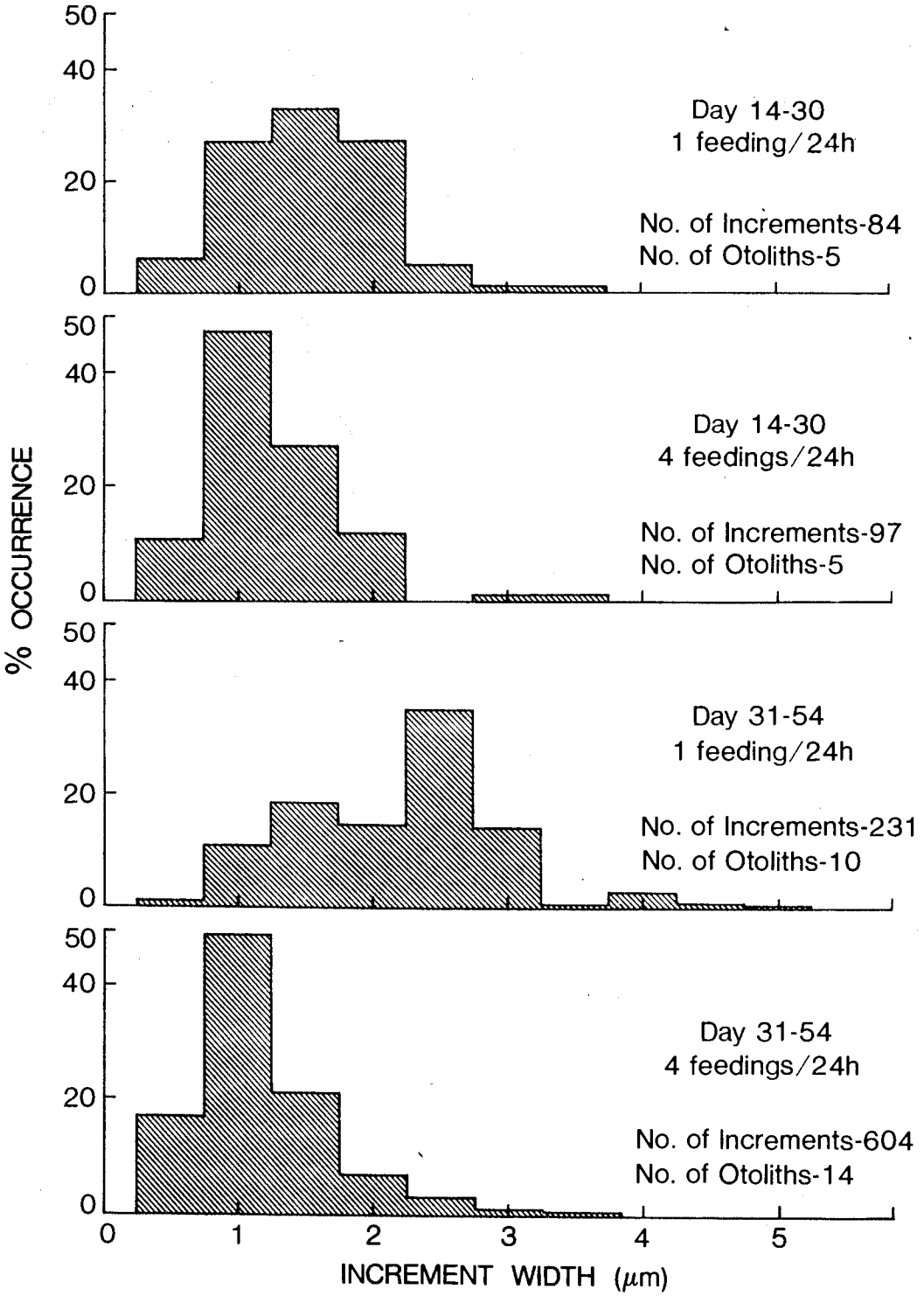


Figure 2-3 Distribution of chinook salmon fry otolith increment widths through d 14 - 30 and d 31 - 54 of the feeding frequency experiment.

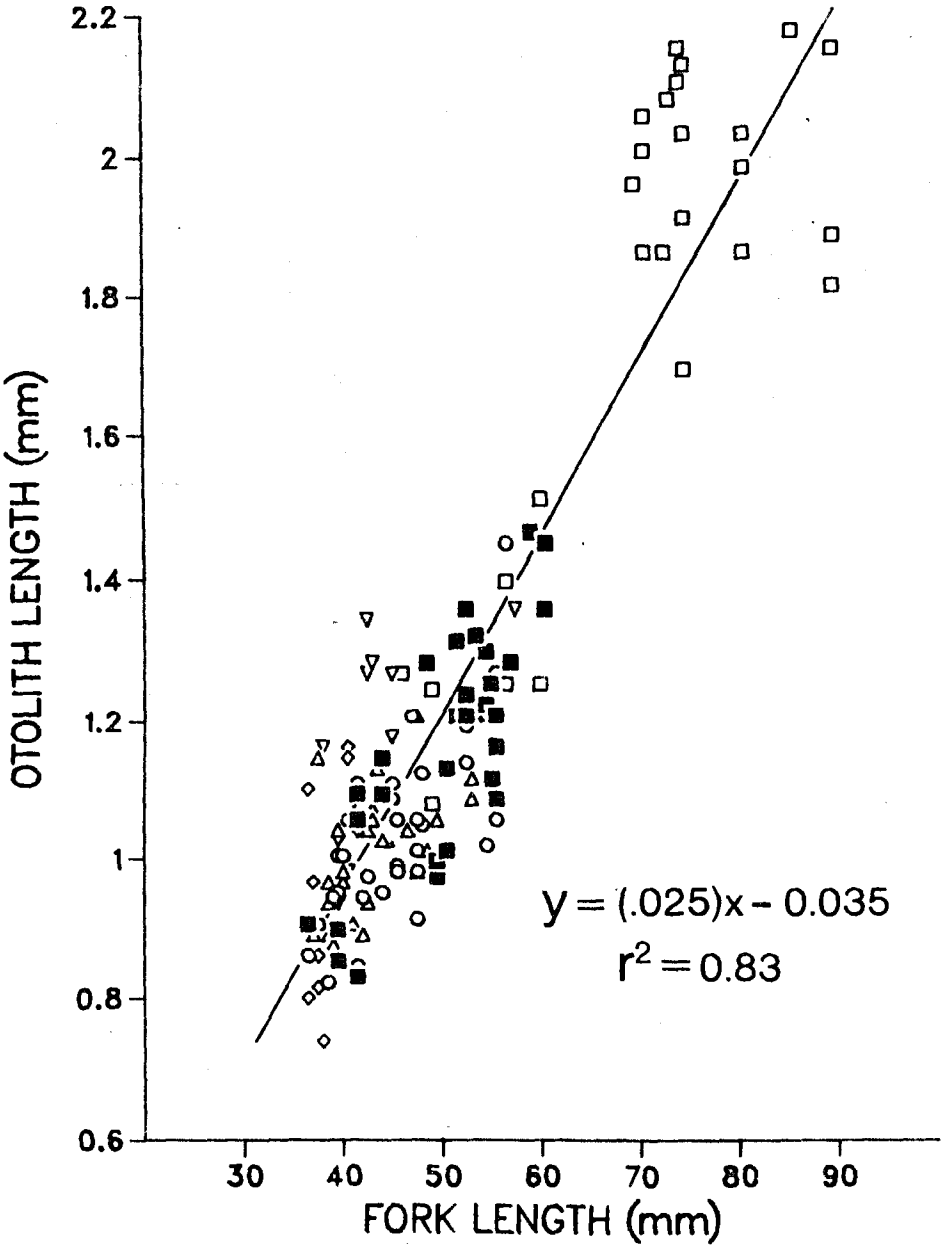

% OCCURRENCE
50
40
20
0
Day 14-30
1 feeding/24h
No. of Increments-84
No. of Otoliths-5
Day 14-30
4 feedings/24h
No. of Increments-97
No. of Otoliths-5
Day 31-54
1 feeding/24h
No. of Increments-231
No. of Otoliths-10
Day 31-54
4 feedings/24h
No. of Increments-604
No. of Otoliths-14
0
1
2
3
4
5
INCREMENT WIDTH (μm)

experimental treatments (Fig. 2-4, analysis of covariance, p=0.2994). Since both groups of fish grew at the same rate, and the length of the otolith relative to fork length remained the same, the increase in otolith size results from deposition of fewer, wide increments (1 feeding/24 h) or more, narrow increments (4 feedings/24 h).

Temperature

The mean number of increments produced in free-swimming fry reared under the warmer and colder temperatures for 45 d were 42.50 $\pm$ 4.87 and 42.40 $\pm$ 3.57, respectively and were not significantly different (analysis of variance, p=0.3451, n=10). Mean width of increments formed during this 45-d period were 1.54 $\pm$ 0.32 μm (n=10) and 1.11 $\pm$ 0.12 μm (n=10) for fish reared in warmer and colder water respectively and were significantly different (analysis of variance, p=0.0012). At the end of the 45-d period, mean fork lengths of fish were 39.0 $\pm$ 1.2 mm and 53.2 $\pm$ 4.5 mm under the lower and higher temperatures, respectively, and were significantly different ($0.001<p<0.01$). Slopes of regressions of otolith size on fish size did not significantly differ (analysis of covariance, p=0.3936, Fig. 2-4) reflecting isometric increases in otolith size and fish length. In this instance age in days was reflected by the number of increments, and growth rate indicated by increment width.

Figure 2-4 Relationship of otolith length versus fish length for fish fed 4x/24h (▽), 1x/24h (△), reared in warm water (□), reared in cool water (◇), reared under constant illumination (○), and reared under 12:12 LD (■). The regression is calculated from data pooled from all treatments.


OTOLITH LENGTH (mm)
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
30
40
50
60
70
80
90
FORK LENGTH (mm)
y = (.025)x - 0.035
r² = 0.83

Photoperiod

Photoperiod did not influence the number of increments produced by fish during the alevin stage. Slopes of regressions of mean increment counts on days after start of treatment did not differ significantly between treatments, or from unity (t-test, $0.10<p<0.20$). This indicates that in fish feeding endogenously, one growth increment was formed on average every 24 h, regardless of photoperiod.

Although increment widths were not examined in this phase of the work, the mean total length of otoliths of fish held under 24-h darkness was significantly greater (analysis of variance, $p=0.0011$, $n=12$) than that of fish held under a 12:12 LD regime. This indicates that increment width was greater in fish reared in darkness.

In the photoperiod experiment with 90-d fry, SEM examination of otoliths taken from fish held under constant illumination and the 12:12 LD regime did not reveal any significant differences in the number of increments between the two treatments (t-test, $0.10<p<0.20$). Fish held under constant illumination laid down growth increments whose mean width was significantly less ($0.01<p<0.05$, analysis of variance) than that of fish from the 12:12 LD treatment (1.03 ± 0.16 µm and 1.25 ± 0.25 µm respectively). However, no significant difference (t-test, $0.10<p<0.20$) was found between mean fork lengths of fish held in these photoperiod regimes. Slopes of regressions of otolith size on fish size also did not

differ significantly, nor were they different from the slope of the regression representing pooled data from all experimental treatments (analysis of covariance, p=0.2179 Fig. 2-4).

DISCUSSION

Of the environmental variables examined, only feeding frequency affected both increment number and width. Young chinook salmon fed more than once per day produced more than one increment every 24 h, while one feeding per day was associated with the average production of one increment every 24 h. My results are in agreement with those of Panella (1980) for Tilapia sp. Since several workers have indicated that more than one peak in feeding activity occurs in temperate fish populations (Keast and Welsh 1968; Elliott 1970; McDonald 1973), there is reason to expect formation of >1 increment/24 h in many fish species. However, Marshall and Parker (1982) felt that foraging was not an important factor in otolith increment production as formation began prior to first feeding and continued when fish were starved. The possibility that environmental cues such as photoperiod may act as zeitgebers in the absence of exogenous feeding opportunities was not examined.

The frequency of feeding may not be the ultimate factor determining the number and width of increments. Feeding undoubtedly affects fish activity patterns. The manner in which increment width or number might be affected by activity has not been established although it might relate to activity-induced changes in calcium metabolism. This possibility is examined further in Chapter 3.

The linear relationship between fish size and otolith size (Fig.

2-4) for all experimental conditions studied here indicates that otolith increment width is inversely related to the number of increments deposited. While otolith growth is proportional to fish growth during a given period, the change in otolith size under different feeding frequencies is given by the number of increments deposited multiplied by their average width.

My results showed that rearing temperature did not influence the number of otolith growth increments deposited and that increments were deposited daily under constant temperature. My laboratory results do not support Brothers' (1979) work on temperate stream-dwelling fish which implicated diel temperature fluctuations in otolith increment production. Further, although I found daily increment production at 4°C, Marshall and Parker (1982) showed that increment formation in juvenile sockeye salmon ceased at 5°C.

Water temperature affected fish growth which was in turn reflected in differences in mean increment width. Irie (1960) also described a relationship between temperature and otolith growth and showed that deposition of calcium (the major constituent of the otolith) was directly related to both water temperature and feeding. However, Irie did not assess temperature effects on otolith growth independent of food consumption rates.

My results from experiments on eggs, alevins and free-swimming fry suggest that a 12:12 LD photoperiod is not necessary for otolith incre-

ment formation. Free-swimming fry reared in constant illumination and alevins incubated in darkness from time of fertilization produced at least one growth increment every 24 h. Campana and Neilson (1982) also found that production of daily increments in otoliths of starry flounders (Platichthys stellatus) was independent of a diel photoperiod. Taubert and Coble (1977) and Tanaka et al (1981) found that otolith growth in juvenile fish is primarily controlled by an endogenous rhythm synchronized to photoperiod. In pre-emergent chinook salmon, however, photoperiod is not likely responsible for the entrainment of daily growth increments. Fertilized chinook eggs may be deposited in 30 cm of gravel, well beyond the vertical extent of light penetration in gravel typically used for spawning (Heard 1964). Therefore, developing eggs and alevins are probably not subject to photoperiod as a possible zeitgeber.

I inferred that mean increment width in otoliths of alevins held in darkness was greater than that of alevins held in a 12:12 LD photoperiod since otoliths of alevins reared in darkness were larger. Direct measurements of otolith increment width from free-swimming fry showed that mean width was less in otoliths from fish held under constant illumination than in those held under a 12:12 LD photoperiod. Thus, mean increment width in these experiments can be summarized as follows: constant illumination < 12:12 LD < constant darkness. As the rate of otolith growth is proportional to fish growth, this means that growth was reduced under the 12:12 LD photoperiod and constant illumination. Eisler (1961) also presented results showing greater growth in salmonids held in darkness. His results and those presented here may be explained by the

photonegative behaviour of salmonid alevins (Dill 1969, Dill 1977). Chinook alevins exposed to the 12:12 LD photoperiod probably expended energy to avoid light, leaving less energy available for growth than in alevins held in total darkness. The photonegative response may continue beyond emergence and yolk sac absorption, albeit in a weakened form (Mason 1976). Hence, the decreased growth (as reflected by increment width) in chinook alevins and fry exposed to light in these experiments may be due to a light-induced increase in activity.

While I found significant differences in otolith increment widths for fry held under 12:12 LD and continuous illumination, no difference in fish fork length was noted. This may be due to comparatively imprecise measurements of preserved fish relative to increment width measurements and the short duration of the experiment relative to the age of the fish.

Some of the comparability of the experimental results presented here may be reduced since fish of somewhat different ages were used in experiments. For example, 90-155 d old fish were used for the feeding frequency experiments while 45-90 d old fish were used in the temperature experiment. This is probably not a serious problem since the age differences are slight. However, it may be inappropriate to extrapolate my results to other life history stages, since chinook salmon experience several different environmental factors in the estuarine and oceanic environments which could affect increment formation. Furthermore, the utility of otolith microstructure studies for life history stages of

temperate fish other than underyearlings is not clear. With the onset of winter, changing environmental conditions including reduced water temperature and prey availability may act to modify the pattern of increment deposition.

No one environmental variable appeared to be a critical determinant of production of otolith growth increments. The production of one increment/24 h in otoliths of alevins held in total darkness lends credence to the suggestion that an endogenous rhythm is responsible for increment production. Other workers, including Brady (1979), have described a circadian rhythmicity in laboratory-reared animals which had never experienced environmental rhythms of 24 h periodicity. Deviations from the one increment/24 h relationship such as was seen when the chinook salmon received 4 feedings/24 h may result from an interaction between an endogenous rhythm and some recurring exogenous event such as feeding activity. Considerable latitude probably also exists for modification of increment width through interactive effects of environmental variables, as each variable examined singly in this chapter affected increment width. In Chapter 3, I describe the interactive effects of feeding frequency and diel water temperature regimes.

Otolith microstructure permits greater resolution of age and growth in young fish than was previously possible using scales or other bony parts of fish, and possibly offers a means of assessing past environmental conditions that affect growth. However, some recent

studies have treated growth increments as daily in their occurrence without corroboration. In a few cases, these growth increments have provided the basis for back-calculation of growth. Data presented here indicate that while the relationship between otolith size and fish size remains constant under a variety of experimental treatments, both number and width of otolith increments can be modified by environmental variables. Therefore, the derivation of back-calculated growth estimates and other inferences based on otolith microstructure should only be undertaken after the frequency of formation of growth increments and the role of environmental variables in their formation is understood.

CHAPTER 3

EFFECTS OF FEEDING REGIMES AND DIEL TEMPERATURE CYCLES ON OTOLITH INCREMENT FORMATION IN JUVENILE CHINOOK SALMON

INTRODUCTION

In the previous chapter, I presented evidence that feeding frequency affected both increment number and width in otoliths of chinook salmon fry. I suggested that feeding activity (or some other periodic event affecting fish activity) might modify the rate of otolith increment production, perhaps through activity-induced changes in calcium metabolism. In this chapter, the influence of ration, feeding frequency and fish activity on otolith increment formation is examined in more detail.

The effect of constant water temperature regimes on otolith increment formation was considered in Chapter 2. In this chapter, the influence of diel water temperature cycles on increment formation in chinook salmon alevins and fry is described. Diel water temperature cycles have received little consideration as a possible modifier of the rate of increment formation or their width. Brothers (1978) suggested that diel temperature cycles were responsible for cyclic increment production in temperate stream-dwelling fish, although no data were presented. This gap in our understanding is significant, as diel water temperature cycles are common features of aquatic environments. I also present data on the effects of interactions of water temperature regimes and feeding frequency on increment formation in chinook salmon fry.

METHODS

Alevins

Chinook salmon used in the experiments described below originated from the 1981 brood of the Canada Department of Fisheries and Oceans Capilano Hatchery. Eggs were transferred to incubation facilities at Simon Fraser University at the "eyed" stage of development, corresponding to 347 Centigrade degree-days. Prior to transfer, the eggs were held under a 12:12 LD photoperiod and at a constant 8°C water temperature. The eggs were held for 5 d at 8.5°C before exposure to diel water temperature regimes.

Two lots of 100 fish were exposed as eggs and later, as alevins over a 69-d period to a water temperature regime whose diel amplitude averaged 2° and 4°C (range 1.8-2.4 and 3.0-4.5) above a minimum temperature averaging 8.5°C. These ranges of temperature were consistent with my observations of diel temperature cycles in the Deadman River, a southern British Columbia stream supporting a chinook salmon population. At d 39, 20 alevins were transferred from the 4°C diel temperature treatment to a warm/cool temperature cycle with a 4°C amplitude and 12-h period for 30 d. A fourth group was held at a constant 8.5°C. The constant water temperature corresponded to that of the cool period of the diel water temperature regimes. Eggs or alevins (n=10) were sampled 19, 40, 55 and 69 d after the start of the experiment, following 100% hatch at d 23, 26 and 29 for the 4°C, 2°C

cycles and the constant temperature regime respectively. Sagittal otoliths were removed and prepared following the methods of Neilson and Geen (1981). Otolith sections were examined using light or scanning electron microscopes as described in Chapter 2.

Fry

Chinook salmon fry used in these experiments were approximately 90-d old post-hatch and originated from the Capilano River hatchery stock. Prior to transfer to 25-L aquaria at Simon Fraser University, fish were held under natural light at a constant 8°C and fed once every 24 h. After transfer to SFU, fry were held for 2 wk in flow-through aquaria supplied with aerated and dechlorinated water at 6°C before experiments commenced. During this period the 50 fish in each aquarium were fed once/24 h and exposed to a 12:12 LD photoperiod.

Experimental feeding and temperature regimes to which fry were exposed are summarized in Table 3-1. The amplitude of daily temperature fluctuations was 4°C (range 3.6-4.4) above the average minimum of 6°C. The diel temperature cycle in relation to photoperiod and feeding events is shown in Fig. 3-1. The activity of one group of fish was artificially increased to examine the effects of activity on otolith increment formation. These fish were forced to evade a slowly moving aquarium net for 10 min beginning at 1900 h daily. The induced activity level appeared similar to that associated with feeding.

Table 3-1

List of abbreviations denoting experimental regimes to which chinook salmon fry were exposed in 1982. Percent ration (% of body weight offered every 24 h) is given and the water temperature at time of feeding during the diel cycle, if applicable, is indicated in brackets. Refer to Figure 3-1 for details of feeding, temperature and photoperiod regimes.

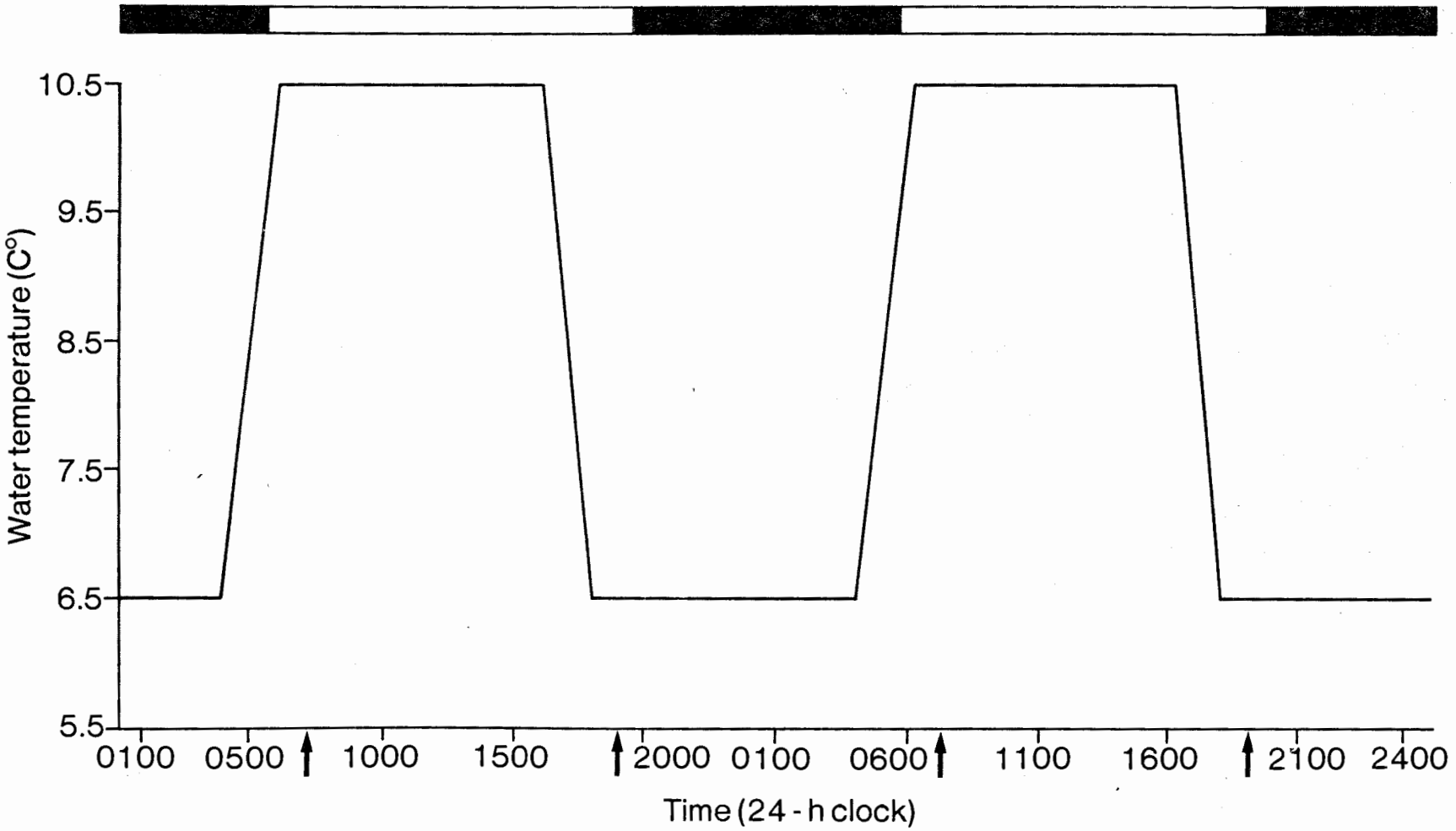
Treatment	Time of Feeding
8% (warm)	0700 h
8% (cool)	1900
8% (constant)[1]	0700
4% (warm)	0700
4% (cool)	1900
4% (constant)[1]	0700
2 x 4%[2]	0700 & 1900
2 x 2%[2]	"
4% + activity[3]	0700
Starvation	n/a

[1]Fish in these treatments were held at constant temperature.

[2]Fish in these treatments received two feedings/24 h.

[3]Fish in this treatment were exposed to a 10-min bout of forced activity at 1900 h every day.

Figure 3-1 Diel water temperature cycle in relation to photoperiod and feeding events (↑) for chinook salmon fry. Light and dark periods are indicated by the open and solid bars respectively.


10.5
9.5
8.5
7.5
6.5
5.5
Water temperature (C°)
0100
0500
1000
1500
2000
0100
0600
1100
1600
2100
2400
Time (24 - h clock)

Ration provided to experimental lots of fish was maintained as a constant proportion (4 or 8%) of average fish dry weight by adjusting total food offered as fish grew or were sampled. Every third day, excess food was removed from aquaria within 30 min of offering, weighed and corrected consumption estimated.

On d 26, I exposed chinook salmon fry for 30 min to a hypertonic solution of 1 ppt sodium chloride and 40 mg/l oxytetracycline hydrochloride. The tetracycline was incorporated into the otolith and provided a time-marker which fluoresced under ultraviolet light. All fry were successfully marked by this method. An attempt at d 1 to mark otoliths of fish with an intra-peritoneal injection of oxytetracycline hydrochloride/neutral saline following the methods of Campana and Neilson (1982) was unsuccessful in this case.

Originally, I had intended to sample 15 fish at d 10, 20, and 40. However, an accidental interruption of the dechlorinated water supply on d 19 resulted in the mortality of some fish in treatments 4% (cool), 4% (constant), 2 x 4% and 4% + activity. Complete mortality of starved fish occurred at that time. To ensure an adequate ($N \geq 10$) sample on experiment completion, samples were not taken at d 20 for the above 4 treatments. Even so, a sample of only 5 fish was obtained at d 40 for the 4% (cool) treatment.

Fork lengths were determined immediately after sacrifice.

Fish were then oven-dried to a constant weight (60°C for 48 h) in individual labelled containers and weighed. After rehydrating the fish in a glycerol/water solution, sagittal otoliths were removed, weighed with a Cahn electrobalance and prepared as described in the previous chapter for examination with the SEM or a light microscope.

RESULTS

Eggs and alevins

Chinook salmon embryos and alevins produced 1 otolith increment/ 24 h on average, whether the water temperature was constant or followed a diel cycle (Table 3-2, analysis of variance; $0.10<p<0.20$). However, the appearance of the daily growth increments differed between treatments. Otoliths of fish subject to a diel cycle in temperature were characterized by more regular and easily-observed growth increments than those subject to constant temperature (Fig. 3-2).

Examination with a scanning electron microscope at 1000X revealed that the bipartite nature of otolith growth increments differed between the temperature regimes. After etching with a weak acid (part of the standard SEM preparation procedure) the relatively deeply-etched portion of the bipartite growth increment (corresponding to the opaque portion of the bipartite structures when viewed with transmitted light microscopy) comprised a larger average fraction of growth increments ($p=0.0041$) in otoliths of fish subject to a diel cycle in temperature than those of fish subject to constant temperature. The deeply-etched portion of daily growth increments did not differ significantly between fish held in 2° and 4° C diel temperature regimes (analysis of variance and the Student-Newman-Keuls test, $p=0.2125$).

Table 3-2

Summary of chinook salmon otolith increment counts for alevins held under various temperature regimes.

Experiment Day	Increment Count		
	Constant Temperature	2°C Fluctuation	4°C Fluctuation
19	17.8 ± 2.6[1]	18.5 ± 1.2	17.4 ± 1.0
40	-	38.0 ± 2.4	39.5 ± 2.1
55	51.8 ± 2.8	54.1 ± 1.9	53.3 ± 3.0
69	68.4 ± 5.6	68.4 ± 4.1	70.2 ± 4.6

[1] ± 1 standard deviation indicated, n = 10.

Figure 3-2 Comparison of chinook salmon alevin (length 30-31 mm) otolith microstructure in fish held for 69 d in diel water temperature regimes of 4°C amplitude (left), 2°C amplitude (middle) and constant temperature (right).


50 µm

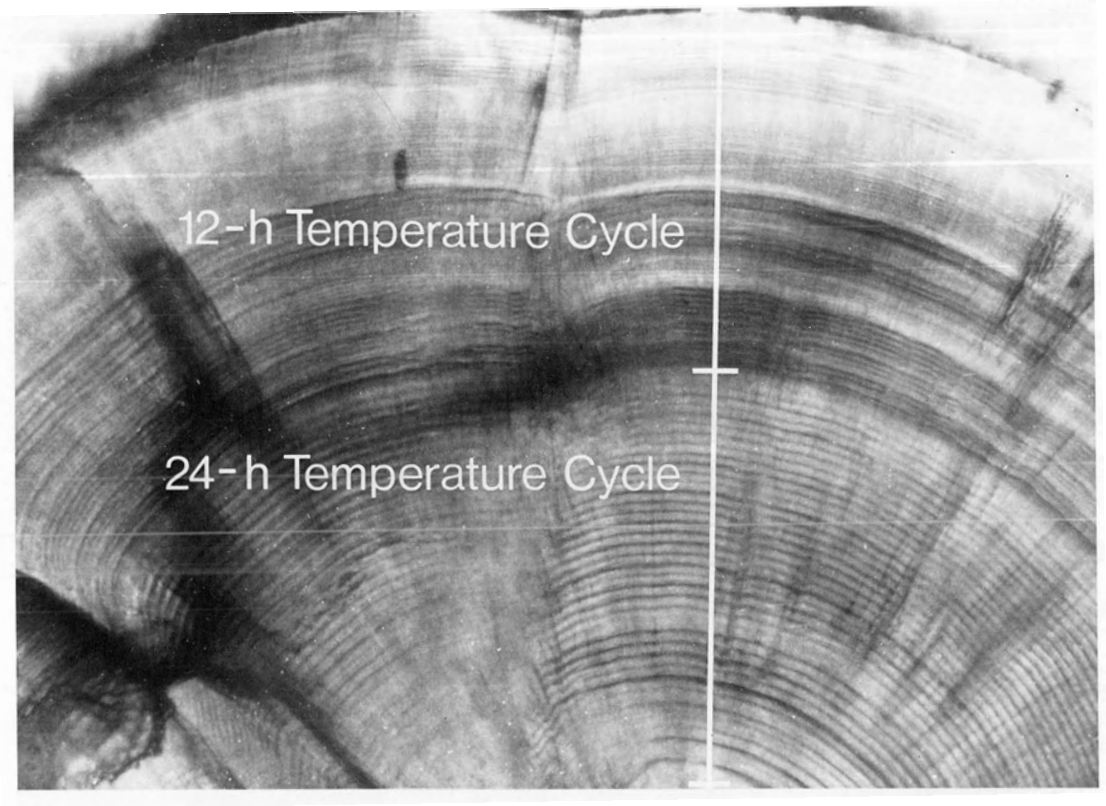
Chinook salmon transferred from a 4°C diel temperature regime (24-h period) to a 12-h regime of similar amplitude produced an average of 1.56 increments/24 h. The slope of the regression of mean increment counts on experiment day differed significantly from unity ($p=0.0019$). An example of an otolith from a fish exposed to the 12-h period cyclic temperature regime is shown in Fig. 3-3 and illustrates the narrower increments associated with the 12-h cycle.

Fry

Slopes of arithmetic mean linear regressions of fish dry weight on experiment day indicated that the average rate of growth of fish fed 8% body weight/24 h at the beginning of the warm portion of the diel temperature cycle was significantly greater than that of fish fed at the beginning of the cool portion or at the constant water temperature (analysis of covariance and the Student-Newman-Keuls test, $p=0.0034$). Similar analyses among treatments where fish were fed 4% B.W./24 h (4% (warm), 4% (cool), 4% (constant)) or received two feedings or an enforced bout of activity (2x4%, 2x2%, 4% + activity) indicated no significant differences in growth rate ($p=0.4147$).

To determine whether otolith growth-fish growth relationships were similar among treatments, I calculated otolith weight-fish dry weight regressions for data from all experimental regimes. Analysis of covariance indicated that the slopes of the regressions among

Figure 3-3 Change in otolith microstructure in a chinook salmon alevin transferred from a 24-h temperature cycle (4°C amplitude) to a 12-h temperature cycle (4°C amplitude).


12-h Temperature Cycle
24-h Temperature Cycle
50 μm

groups of fish fed 8% B.W./24 h did not significantly differ from each other ($p=0.2511$). Nor were there significant differences among treatments with fish fed twice/24 h or those exposed to the enforced 10-min bout of activity ($p=0.6870$). The slope of the regression representing the otolith weight-fish weight relationship for those fish receiving a ration of 4% B.W./24 h on the cool portion of the diel cycle was significantly greater than the slopes of regressions representing fish fed 4% B.W./24 h ($p=0.0034$, analysis of covariance and the Student-Newman-Keuls test). However, the regression is based on only 20 data points, whereas others are based on at least 40 observations each.

Otolith growth increments were formed at the rate of one every 24 h under all experimental regimes (t-tests, $n \geq 20$ for all treatments $p > 0.05$) except those groups receiving 2 feedings/24 h or 1 feeding and one bout of activity (t-tests, $p < 0.01$). Arithmetic mean regressions of increment counts on experiment day for the latter treatments are given below:

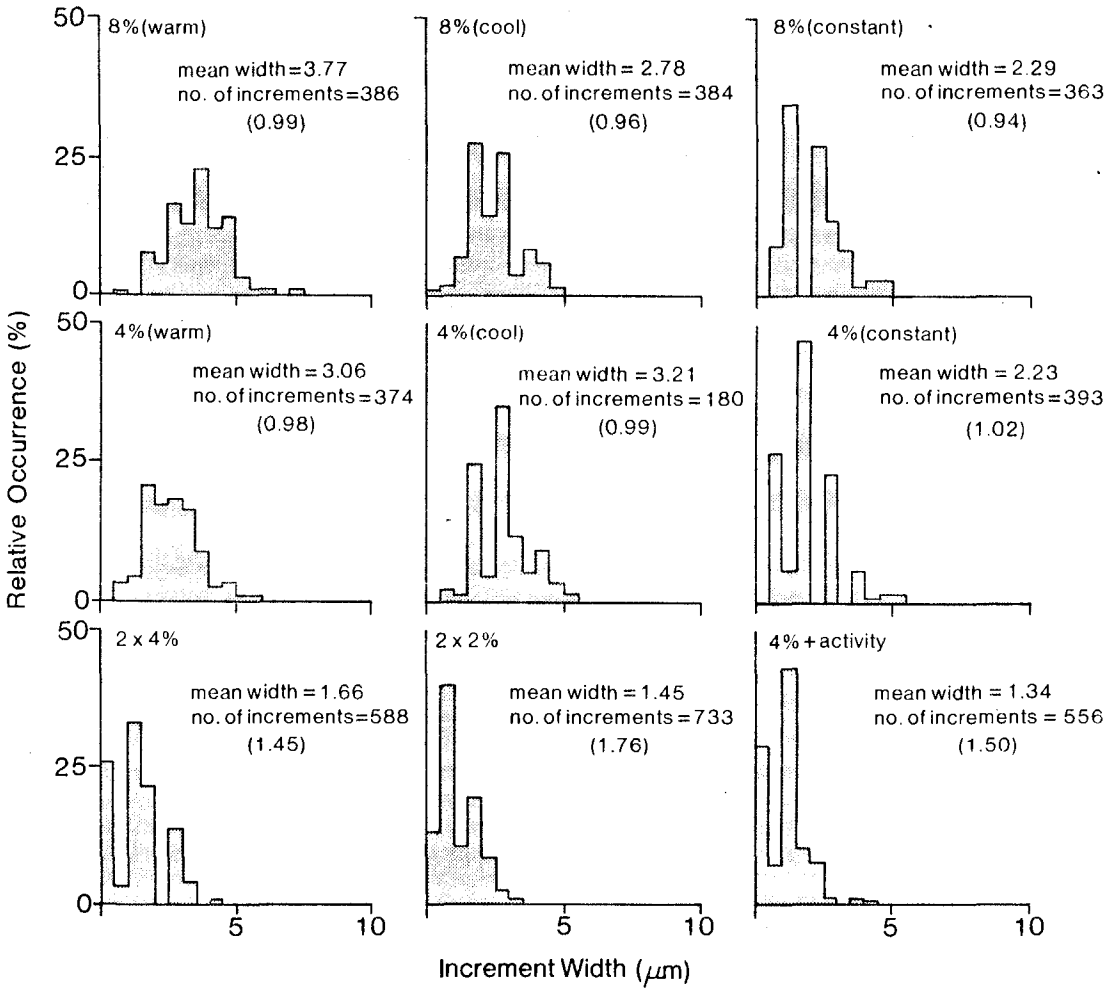
Treatment	Regression Equation	r^2
8% B.W. ration fed twice/ 24 h	$y=1.45(x) + 1.58$	0.91
4% B.W. ration fed twice/ 24 h	$y=1.76(x) - 1.40$	0.98
4% B.W. ration and one 10-min bout of forced activity	$y=1.50(x) - 0.80$	0.93

In the above treatments slopes did not significantly differ from each other (analysis of covariance, $p=0.2191$).

The distributions of increment widths for the fed groups of fish are shown in Fig. 3-4. One-way analysis of variance was completed on each of the two upper horizontal strata. The Student-Newman-Keuls test indicated that among treatments with fish receiving a ration of 8% B.W./24 h in one feeding, mean increment widths significantly differed between groups ($p=0.0264$). Among fish receiving a ration of 4% B.W./24 h in one feeding, mean increment widths did not differ significantly in fish receiving the ration either during the cool or warm portion of the diel temperature cycle (Student-Newman-Keuls test, $p=0.3211$). However, fish receiving 4% B.W./24 h under constant water temperature produced growth increments whose mean width was significantly less than those of fish held in the diel water temperature regimes ($p=0.0094$, Student-Newman-Keuls Test).

The top two horizontal strata of Fig. 3-4 constitute a 3 x 2 factorial design, and were examined with a two-way analysis of variance. The effects of time of feeding with respect to the diel temperature cycle, ration level and their interaction was examined in relation to mean otolith increment width. The effect of time of offering with respect to the diel temperature cycle on mean increment width was significant ($p<0.0001$) whereas ration level was not ($p=0.0905$). The interaction of time of offering in relation to the

Figure 3-4 Distributions of otolith increment widths under the experimental regimes. Treatments are identified by numbers in the top-left corners of histograms, and correspond to treatments listed in Table 1. The average rate of increment formation per 24 h is shown in brackets.


8%(warm)
mean width=3.77
no. of increments=386
(0.99)
8%(cool)
mean width = 2.78
no. of increments = 384
(0.96)
8%(constant)
mean width = 2.29
no. of increments=363
(0.94)
4%(warm)
mean width = 3.06
no. of increments=374
(0.98)
4%(cool)
mean width = 3.21
no. of increments = 180
(0.99)
4%(constant)
mean width = 2.23
no. of increments=393
(1.02)
2 x 4%
mean width = 1.66
no. of increments=588
(1.45)
2 x 2%
mean width = 1.45
no. of increments = 733
(1.76)
4% + activity
mean width = 1.34
no. of increments = 556
(1.50)
Relative Occurrence (%)
50
25
0
5
10
Increment Width (μm)

diel temperature cycle and ration level on otolith increment width was also significant (p=0.0003).

Fish in treatments receiving 2 feedings/24 h or 1 feeding and 1 10-min bout of activity produced growth increments whose average widths were significantly less than those of fish in treatments receiving the same level of ration with one feeding/24 h. Treatments where fish received either rations of 8% or 4% with one or two feedings/24 h comprise a 2 x 2 factorial design, and were analyzed with a 2-way ANOVA. Increased feeding frequency significantly reduced mean increment width (p=0.0001), although ration level did not (p=0.4561). The interaction of feeding frequency and ration level was not significant (p=0.5543).

Widths of otolith increments formed when fish were fed 4% B.W./24 h and subjected to a 10-min bout of activity were not significantly different from widths of increments in fish which received 2 feedings equivalent to the 4% B.W./24 h ration level (p=0.4261). However, fish fed 8% B.W./24 h with two feedings produced increments whose average width was significantly greater than the latter two treatments (analysis of variance and the Student-Newman-Keuls test, p=0.0004). Mean increment widths in fish from the treatment receiving 4% B.W./24 h with a constant water temperature regime were significantly greater than increment widths in fish receiving the same ration plus a 10-min period of forced activity (t-test, p=0.0002). A summary of the

comparisons of increment widths among treatments is provided in Fig. 3-5.

Production of the narrower growth increments associated with 2 feedings/24 h or 1 feeding and induced activity did not occur immediately upon commencement of the experimental regimes. A period of transition in otolith microstructure was evident corresponding to the initiation of the experimental feeding regime (Fig. 3-6). Fig. 3-6 shows the decrease in increment widths with time in fish fed 8% B.W./24 h over two feedings compared with those that received an 8% ration once each day. The slope of the regression of increment width on date for the latter treatment did not differ significantly from zero ($p=0.6112$), whereas the former did (t-test, $p=0.0001$).

Starved chinook salmon fry continued to produce one otolith increment every 24 h. However, the growth increments were faint when observed with a transmitted light microscope. That portion of otolith growth formed under starvation conditions was more transparent than the portion of otolith growth produced when fish were fed. Growth increment diel periodicity was also more pronounced during the portion of otolith growth corresponding to that period when fish were fed (Fig. 3-7).

To confirm that increment widths were proportional to fish growth, I plotted instantaneous growth in dry weight against average

Figure 3-5 Summary of Student-Newman-Keuls or t-test ($\alpha = 0.05$) comparisons of mean increment widths in chinook salmon fry held under the various experimental regimes. Arrow heads pointing left or right signify "less than" and "greater than" respectively.

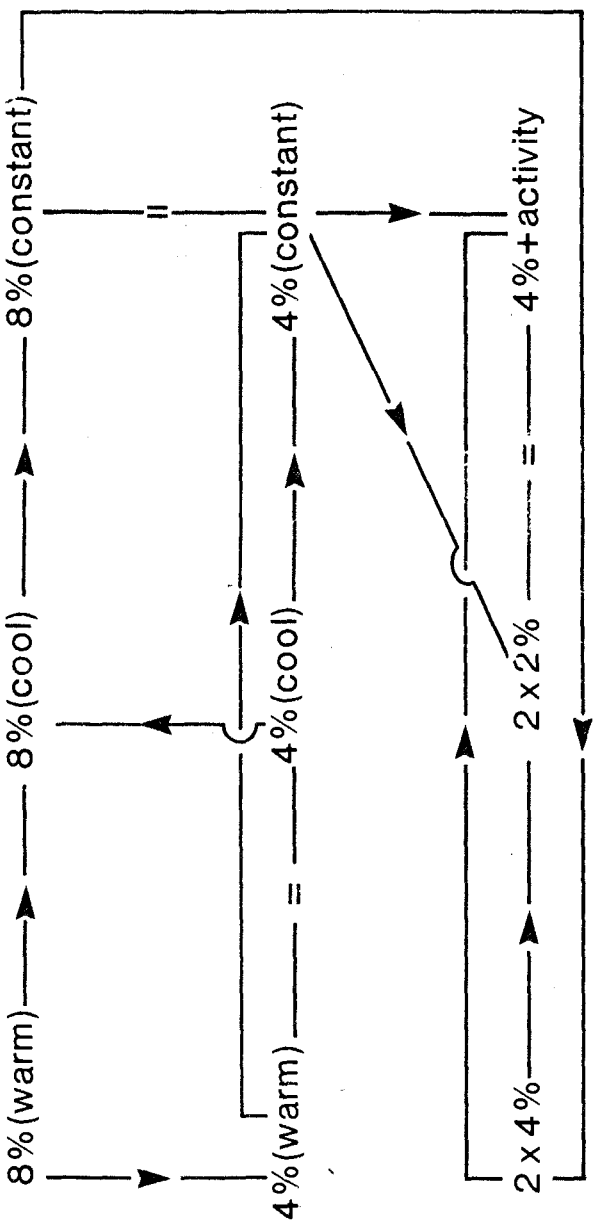

8%(warm)
8%(cool)
8%(constant)
=
4%(warm)
=
4%(cool)
4%(constant)
2x4%
2x2%
=
4%+activity

Figure 3-6 Mean otolith increment widths for chinook salmon fry from the 2x4% experimental feeding regime over d 1-40 (○, regression line shown). Prior to d 1, fish were fed once every 24-h. Also shown are mean increment widths (■) of fish from the 8% (constant) feeding regime, where fish received only one feeding every 24 h.

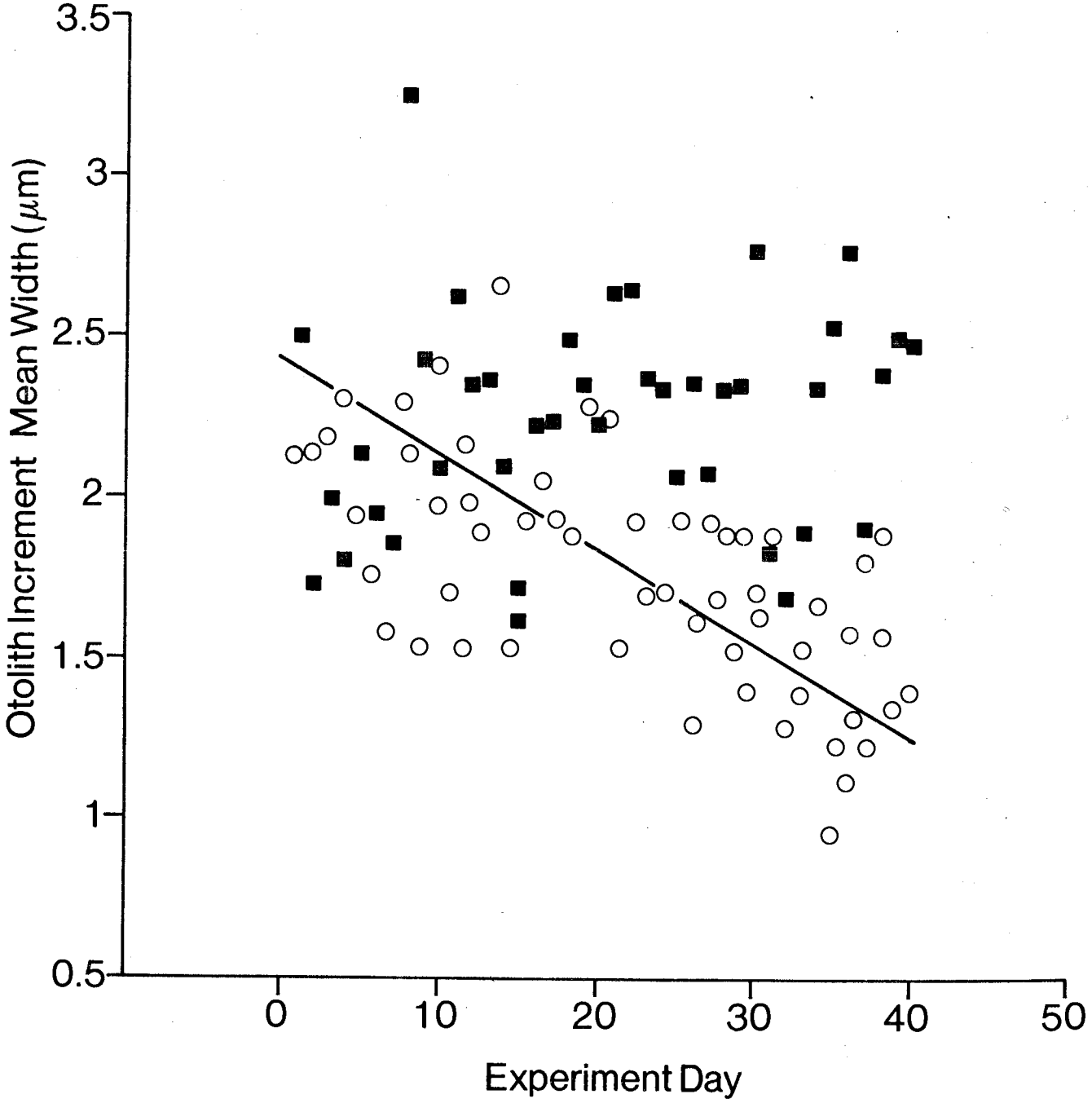
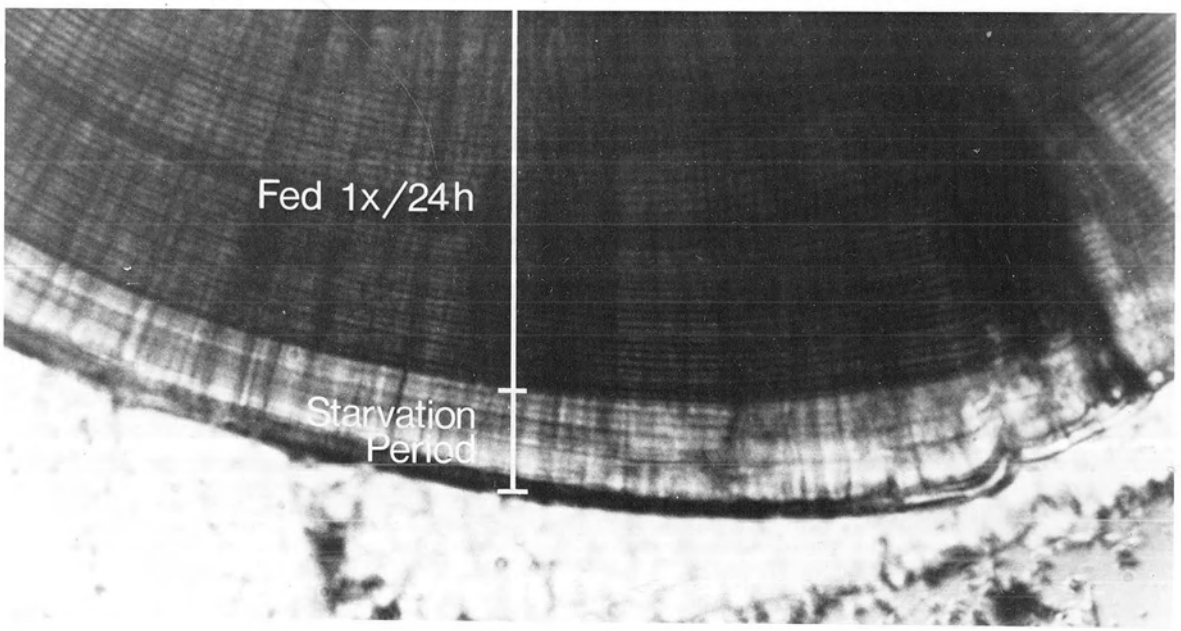
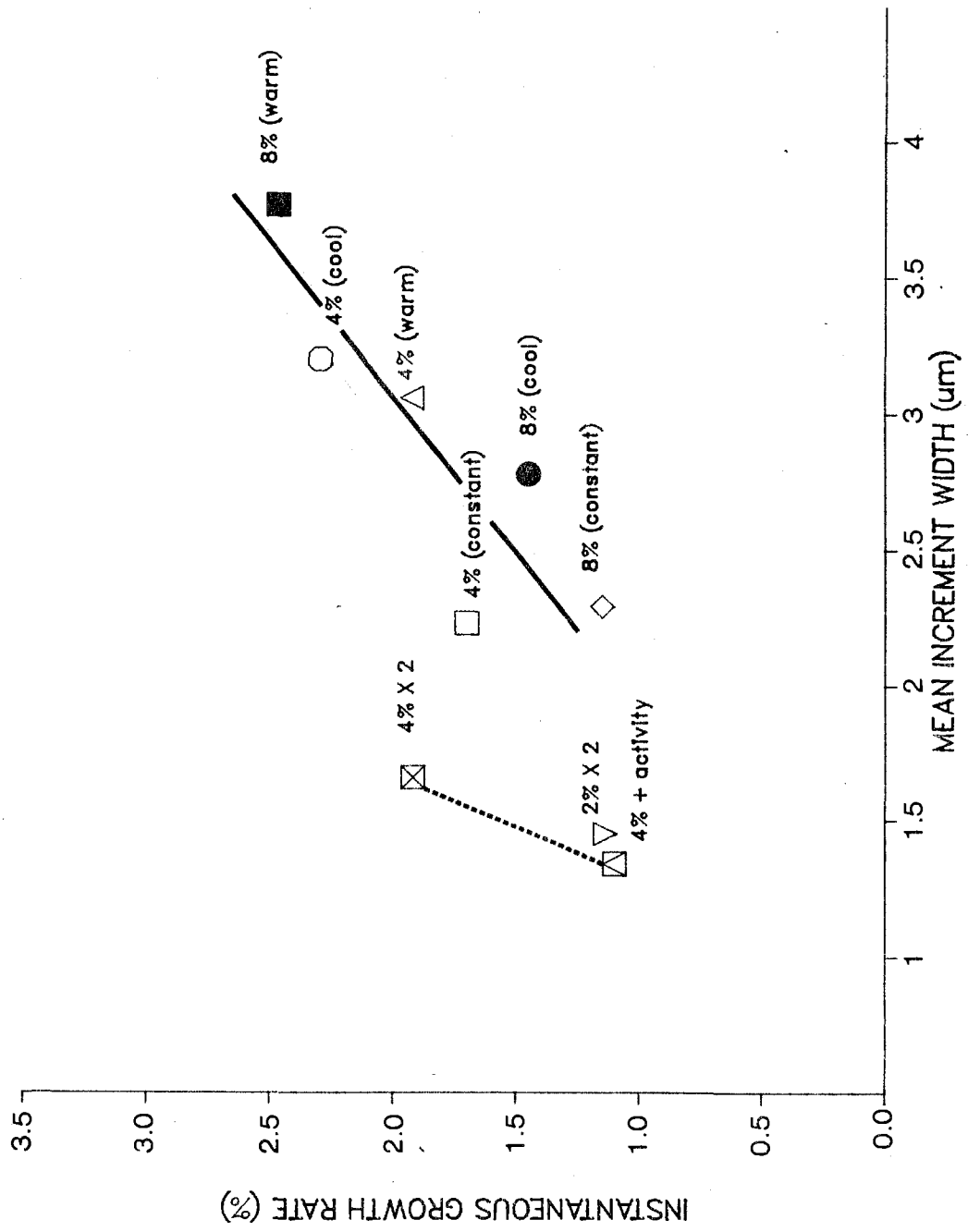

3.5
3
2.5
2
1.5
1
0.5
Otolith Increment Mean Width (μm)
0
10
20
30
40
50
Experiment Day

Figure 3-7 Example of otolith microstructure from a starved chinook salmon fry when viewed under transmitted light microscopy. The relatively transparent region near the otolith periphery corresponds to the starvation period.


Fed 1x/24h
Starvation
Period
50μm

increment width for all treatments except the starved group (Fig. 3-8). The coefficient of determination (r^2) associated with those treatments in which fish formed one growth increment every 24 h was 0.735, and the slope of the regression was significantly different from zero ($p=0.0084$). Note that points associated with treatments in which fish formed more than one increment every 24 h lie considerably above that regression. The regression of these data differs significantly in both slope and y-intercept (analysis of covariance and t-test, $p<0.0001$) as from that of fish fed once per day.

Figure 3-8 Regressions of mean otolith increment width versus instantaneous growth rate (dry weight) for the various experimental regimes. Solid line represents groups where fish produced 1 increment every 24 h on average and the dashed line represents treatments where fish produced significantly more than one increment every 24 h. The geometric mean regressions are $y = 0.8524(x) - 0.6249$ and $y = 2.8814(x) - 2.6917$, respectively.


INSTANTANEOUS GROWTH RATE (%)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
1.5
2
2.5
3
3.5
4
MEAN INCREMENT WIDTH (um)
8% (warm)
4% (cool)
4% (warm)
4% (constant)
8% (cool)
8% (constant)
4% X 2
2% X 2
4% + activity

DISCUSSION

Under most environmental conditions studied here and reported in the previous chapter, otolith growth increments were formed with diel periodicity giving support to the hypothesis that an endogenous rhythm influences growth increment formation. In the previous chapter, I also found that multiple feedings every 24 h resulted in the formation of > 1 increment/24 h and suggested that this resulted from the interaction of an endogenous diel rhythm of increment production and some regularly recurring environmental event. Data presented here are consistent with that view, as increased feeding frequency, the 12-h period warm/cool temperature cycle (Fig. 3-3) and fish activity all were associated with an increased rate of increment formation. The effects of at least some environmental events on otolith microstructure may be mediated through activity-induced modification of fish metabolism, which often follows a circadian rhythm (Matty 1978). If otolith growth increment production follows a circadian rhythm that is sometimes overlain by environmental events, it seems reasonable to assume that while fish may produce one or more growth increments every 24 h, they do not produce less than one every 24 h. In my experimental studies, chinook salmon alevins and fry produced one or more growth increments every 24 h. This agrees with the results of the majority of studies reported by other workers or other species, even when fish were exposed to stimuli with a period > 24 h (Campana and Neilson 1982).

When fish previously exposed to a cyclic event (temperature or feeding) of 24-h duration were transferred to an environment with 12-h periodicity or less, the transition in otolith microstructure did not occur immediately (Figs. 3-6 and results in Ch. 2). In my relatively short-term (<65 d) experiments, otoliths of fish exposed to a cyclic phenomena with periods <24 h exhibited increment counts that were less than expected had the transition in otolith microstructure occurred immediately. Aschoff et al (1975) in reviewing similar findings, noted that the time required for entrainment to the new external cue is negatively correlated with the degree of influence the new zeitgeber has on the biological rhythm and positively correlated with the magnitude of the zeitgeber period change.

While diel water temperature fluctuations are not necessary for otolith increment production in chinook salmon, they enhance structural differences between each portion of bipartite otolith growth increments. The proportion of the total increment width comprised by the deeply-etched portion of daily growth increments is significantly greater in otoliths of fish taken from a diel water temperature regime than those from fish held in water of constant temperature. On the basis of examination of otoliths from goldfish (Carassius auratus), Mugiya et al (1981) concluded that the deeply-etched portion is composed of calcium carbonate and protein, the latter in relatively greater concentration. The postulated increase in percent of protein in the increment might explain the greater contrast of daily growth increments produced under a diel

water temperature regime, if it was assumed that less light is transmitted through proteinaceous material than calcium carbonate.

Degens et al (1969) suggested that the deposition of the organic matrix is a conservative process, not readily modified by environmental events. Therefore, of the two major constituents of the otolith, it is probably the rate of calcium carbonate deposition, not protein, which changes in response to diel water temperature cycles. However, as mean increment width reflected fish growth under a variety of water temperature regimes (Fig. 3-8), I conclude that the total width of a given growth increment reflects the rate of fish growth during its formation, regardless of water temperature regime and related effects on growth increment structure.

On the basis of my examination of otolith weight-fish weight regressions and data presented in the previous chapter, I conclude that otolith growth of chinook salmon fry is closely coupled to fish growth under a variety of experimental conditions, ranging over what might be expected to occur in natural environments. Marshall and Parker (1982) also reported that differences in ration and water temperature to which sockeye salmon fry (Oncorhynchus nerka) were exposed did not significantly affect slopes of otolith size - fish size regressions among fed groups. Exceptions to the isometric growth relation between fish size and otolith size have only been observed in recently-hatched salmonid alevins, as noted in Chapter 1, and in starved chinook salmon fry. Chinook salmon fry deprived of food for

19 d continued to form daily growth increments. Assuming fish dry weight did not change over this period, then the slope of the otolith weight-fish weight regression would probably be greater than for fed fish. Marshall and Parker (1982) reported similar results over a 2-wk starvation period of sockeye salmon (Oncorhynchus nerka). Evidently continued otolith growth in starved fish resulted from the metabolism of stored energy reserves.

Interactions between ration level and time of feeding with respect to the 24-h temperature cycle affected mean increment width. Ration level as a single factor influencing increment width was not significant. However, the interaction between temperature and ration on increment width was significant, suggesting that calcium carbonate deposition on the otolith was higher under elevated temperatures at time of feeding. Estimates of food consumption indicated that fish fed 8% B.W./24 h in conjunction with the diel water temperature cycle consumed significantly more food per g of fish when the food was offered during the warm period (t-test, $p<0.01$). Differences in food consumption were not noted for fish receiving a ration of 4% B.W./24 h on either the warm or cool portion of the diel water temperature regime (t-tests, $p>0.05$). With the lower ration level (4% B.W./24 h) it is possible that fish were not satiated, regardless of water temperature regime. With the higher ration, fish were satiated even when the food was offered during the cool period of the water temperature cycle, and additional consumption occurred only if offered during the warm portion of the diel temperature cycle. English (1981)

also found that increment widths in chinook salmon reflect water temperature more closely than food abundance. However, his conclusions were based on a small sample (n = 6) and possible interactions were not reported.

In a two-way comparison with ration level and feeding frequency (water temperature was constant), increment width was affected by feeding frequency and not by ration level. This result is expected, as the rate of increment production is affected by feeding frequency as was shown earlier, and in the previous chapter.

The increased growth rate of salmonids exposed to daily temperature cycles compared with those in a constant water temperature regime (equivalent to the mean of the cyclic temperatures) has been noted by Hokanson et al (1977) and Biette and Geen (1980), and is comparable to the results presented here for fish receiving a ration of 8% B.W./24 h. Increased growth under daily cyclic temperature fluctuations may be related to the metabolic advantage postulated by McLaren (1963) where reduced metabolic costs are associated with the cool portion of daily temperature cycle, leaving more energy available for growth. Brett (1979) noted that the effect of cyclic temperatures positively affects fish growth when the fluctuation encompasses a temperature range at or below the optimum for a given ration level. My results satisfy this criterion since the experimental temperature range is on the left-hand limb of the dome-shaped function of satiation ration versus water temperature described for chinook salmon by

Brett et al (1982). However, the similarities of my results to those previously reported are somewhat overstated because the constant water temperature regime in my experiments corresponded to the daily minimum of the fluctuating regime, not the mean as in the work of Hokanson et al (1977) and Biette and Geen (1980). In addition, the mean growth rate and otolith increment width associated with the 4% (cool) treatment was higher than expected. I believe this may have resulted from the interruption of the water supply mentioned earlier, with only larger fish surviving.

Given that both water temperature and food consumption are considered the most important aspects of fishes' environment affecting their growth (Paloheimo and Dickie 1966), it is not surprising to find that water temperature regimes and ration levels influence otolith growth increment production. On the basis of my findings and those presented by English (1981) it seems likely that interpretation of prey abundance and feeding success from otolith microstructure may be masked by relatively small changes in water temperature. Workers attempting to quantify fish growth with respect to ration size through examination of otolith microstructure should be aware of the effects of water temperature documented here, and design studies accordingly.

CHAPTER 4

THE ESTUARINE GROWTH OF JUVENILE CHINOOK SALMON AS DETERMINED FROM OTOLITH MICROSTRUCTURE

INTRODUCTION

Juveniles of three Pacific salmon species (chinook, Oncorhynchus tshawytscha, coho, O. kisutch, and chum, O. keta) reside in estuaries for significant periods prior to ocean migration. Estuaries provide opportunities for sea-water acclimation and feeding prior to entry into the oceanic environment. Chinook salmon juveniles, although not obligate estuary dwellers, utilize estuaries to the greatest extent (Dorcey et al 1978; Healey 1982b) and often spend several months in the estuary before migrating to the ocean. Levy and Northcote (1981) suggested that the duration and quality of estuarine residence might be a determinant of subsequent marine survival of chinook salmon. Rapid growth during the period of estuarine residence confers advantages, as the size attained by juvenile Pacific salmon prior to entering the marine environment is considered critical by many workers. Larger salmon smolts adapt better to higher salinities (Hoar 1976) and are thought to be less vulnerable to predation (Parker 1971). Schlucter and Lichatowich (1977) found that the size attained by juvenile chinook salmon after estuarine residence and by the end of their first year influenced subsequent growth and age at maturity. In a review of the importance of estuaries to Pacific salmon, Simenstad et al (1982) suggested that salmonids use estuaries as refugia from predators, for optimum availability of preferred food organisms promoting rapid growth and as a physiological transition area. Those authors noted, however, that the quantitative significance of such factors to the ultimate survival of adults is not well established.

One of the few studies which attempted to quantify the significance of estuarine residence was completed by Reimers (1973). He found that of the five juvenile life history types thought to exist among Sixes River (Oregon) chinook salmon, those that spent 3-4 mo in the estuary following a freshwater residence of similar duration comprised over 90% of returning adults. The percentage of fish which had spent an extended period in the estuary was considerably greater in mature adults than that expected based on the relative proportions of different life history types that occurred in juveniles, possibly indicating a greater rate of survival to maturation.

Reimers (1973) also suggested that a reduction of juvenile chinook salmon growth rate occurred in years of high population abundance. He hypothesized a density-dependent growth reduction related to prey availability. To test Reimers' hypothesis, Oregon Fish and Wildlife staff have collected several years of data on Sixes River juvenile chinook salmon population abundance. In 1980, prey availability, quantity of prey consumed, and estuarine water temperature data were also obtained. The substantial data record on Sixes River chinook salmon populations also provides an opportunity to assess other factors which might affect estuarine growth of chinook salmon.

In this chapter, I describe the use of otolith microstructure to assess of Reimers' hypothesis of density-dependent growth related to

prey abundance. In addition, examination of otolith microstructure within the population permitted an assessment of the variability in the time at which estuarine residence commences and estuarine growth in relation to previous freshwater life history, including duration of freshwater residence, freshwater growth rate and size at entry to the estuarine environment.

Although scale circuli were used by Reimers (1973) to assess patterns of estuarine residence and growth, otolith microstructure offers greater resolution and precision due to the greater frequency with which regular structural features are formed (Pannella 1971). In addition, regression models of fish length on otolith size often give better fits than do models of fish length and scale radii for salmonid data (Jonsson and Stenseth 1977). This chapter represents one of the first applications of otolith microstructure to interpretation of field data which go beyond a more detailed description of age and/or growth. Moreover, confidence in the interpretation of otolith microstructure is increased as data in the previous chapters have provided a better appreciation of the effect of various photoperiod, feeding and temperature regimes on otolith increment production.

METHODS

Description of the Study Area

The estuary of the Sixes River (Fig. 4-1) is located 80 km south of Coos Bay, Oregon, and 110 km north of the Oregon-California border (42° 58'N, 124° 33'W). The river drains 340 km^2 of the coastal and Klamath mountains, dropping approximately 290 m to sea level over a distance of 56 km (Bottom et al 1983). Mean daily discharge ranges from 0.5 m^3/sec in the summer to more than 200 m^3/sec in the winter (Reimers 1973).

Tidewater extends about 4 km upstream from the mouth of Sixes River. However, most of the research has been confined to the broad, shallow embayment in the lower 1 km of the river (Fig. 4-1). Sediments in this area are primarily gravel, or sand and gravel. Maximum depth at high tide is approximately 5 m, although most of the lower estuary is only 1-2 m deep during seasonal low flows.

Reduced outflows and strong northwest winds cause a shallow sand sill to build at the mouth of the Sixes River during the summer. When the sill is fully developed, ocean waters enter through a narrow gap in the sill only near the high tide; there is a net outflow from the estuary 75% of the time. Throughout most of the summer the estuary is stratified during the period of outflow with as much as 10 to 25 o/oo

difference in salinity between surface and bottom layers of the shallow bay (Reimers 1973). During flood tides, water mixes rapidly near the mouth and saltwater moves slowly upstream along the bottom.

Two species of tube-dwelling amphipod, Corophium salmonis and C. spinicorne, and the gammarid amphipod Eogammarus confervicolus represent 71-92% of the total number and 62-78% of the total biomass of prey consumed by juvenile chinook salmon in the Sixes River estuary through 1978-1980 (Bottom et al 1983). The standing crop of Corophium peaks in late June and declines in abundance through the summer.

Sixes River chinook salmon are a "fall" race, with the adults entering freshwater in the fall of each year, and spawning from December through March. After about 3 months, fry and a smaller number of yearlings move down into the estuary beginning in May or June and are the most abundant salmonids in the Sixes River estuary through the summer. Population estimates of juvenile chinook salmon over the past 15 years have ranged from 59,000 to 269,000 during the midsummer period. Young-of-the-year reside in the estuary until October or November, ranging in size from approximately 40 mm in the spring to 110-130 mm in mid-October. Size at migration to the sea from the estuary varies with population abundance and the productivity of the Sixes River estuary in a particular year (Reimers 1973).

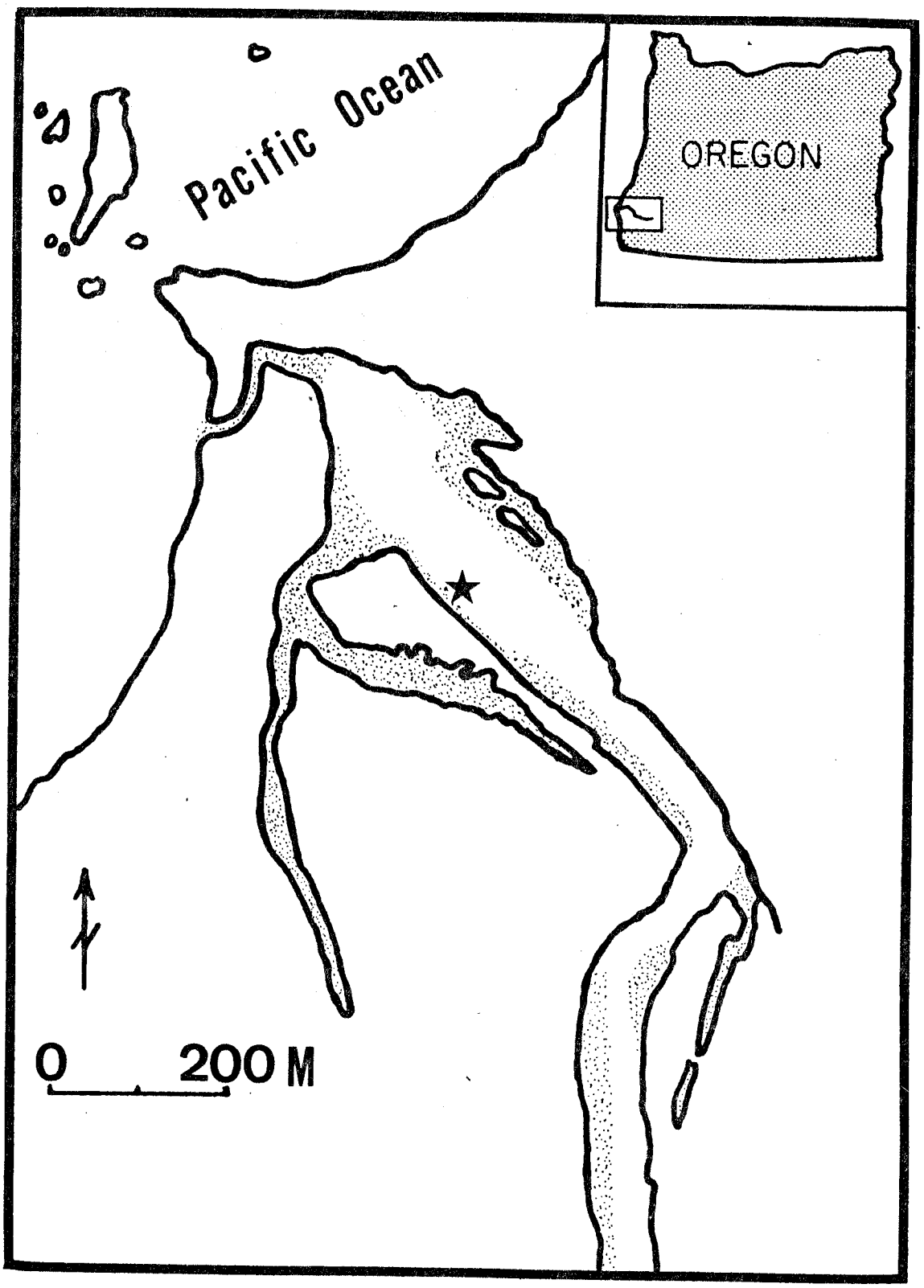
Field Sampling Program

Juvenile chinook salmon were sampled by Oregon Department of fish and Wildlife (ODFW) personnel in the Sixes River Estuary in 1979, 1980, and 1981. Samples of fish (n=15) used for otolith microstructure examination were collected from seine hauls at approximately biweekly intervals from May through September. The sampling location is shown in Fig. 4-1. The daily ration was determined (1980 only) and otoliths were retained for microstructure examination. Water temperature was recorded continuously in 1980 by thermographs located at the surface and bottom of the estuary near where fish were captured.

Population size was estimated four times in 1980 using the Bailey-modified Peterson single census mark-recapture procedure (Ricker 1975), and catch per unit effort (CPUE) statistics. Fish were collected with seines over a 2 to 3-d period and marked with a brand cooled with liquid nitrogen. The use of different brands for successive population estimates allowed ODFW personnel to estimate the population abundance at each time period. However, no correction for loss of tagged fish through mortality or emigration was made, a procedure which may significantly inflate the population estimate.

Estimates of daily ration were obtained by ODFW personnel in 1980 using Elliot and Persson's (1978) method. A sample of 15 fish was obtained every 2 h over eight 24 h periods from the end of May through

Figure 4-1 Sixes River estuary showing the sampling location (★) in 1980. Insert shows the location of the Sixes River in Oregon. Figure supplied courtesy of Oregon Department of Fish and Wildlife.


Pacific Ocean
OREGON
0 200 M

September. Once captured, fish were anaesthetized with MS-222 to prevent regurgitation of gut contents. They then injected with 7.6% formaldehyde to halt digestion. Fish were stored in 7.6% formaldehyde for 1 wk and then transferred to 80% ethanol until examination. The stomach contents for each fish were weighed after blotting and a 10-min period of air drying. Consumption (C_t) was estimated using the equation:

$$C_t = 24 \bar{s} R_t$$

where

$\bar{S}$ = mean amount of food in the stomach over a 24-h period

R_t = rate of gastric evacuation

During each daily ration experiment, R_t was estimated by regression analyses, assuming that logarithmic decreases in mean stomach content weight represented periods of non-feeding. In later experiments, periods of non-feeding were simulated by holding captive fish in barrels supplied with aerated and filtered estuary water. Every few hours, fish were sacrificed and the logarithmic decrease in stomach contents provided a second estimate of R_t. The results of the experiments with captive fish were comparable to those obtained with fish caught in the estuary (Bottom et al 1983). However, results presented here are based on the latter method.

Preparation of Otoliths and Data Analyses

Otoliths were prepared for microstructure examination following the methods outlined by Neilson and Geen (1981). Where otolith

increment widths are reported, the data were derived by digitizing increment widths along a standard radius on enlarged scanning electron microscope photographs. Details are given in Chapter 2. Preparations were coded and examined in a random sequence to avoid bias in the interpretation of otolith microstructure.

Back calculations of lengths at age were made from otoliths of juvenile chinook salmon using Lee's method as described by Carlander (1981). Length at age is given by the equation:

$$\hat{L}_i = a + \frac{L_c - a}{S_c} S_i$$

where

S_c = the otolith length at capture,

S_i = the otolith length at age i,

L_C = the length of the fish at capture,

$\hat{L}_i$ = the length of the fish at age i,

and

a = is the y-intercept of the fish length-otolith length regression.

In developing the fish length-otolith length relationship, I used otolith length as the independent variable rather than otolith radius. The presence of multiple otolith precursors, or primordia, in chinook salmon (Chapter 1) created difficulty in locating the centre of the otolith for radius measurements. I used geometric mean regressions for the fish size-otolith size relationships as natural

variability was associated with both axes (Ricker 1973). Measurements of otolith length were made from images projected on a microfiche viewer. I made two independent sets of measurements on the coded series of preparations. In cases where measurements differed by > 10%, the preparation was excluded from further examination. Using this criterion, 14 of the preparations were rejected.

I used a multiple regression model to examine the interrelations between the mean otolith increment width of fish in the estuary, and daily ration, estuarine bottom water temperature and juvenile chinook salmon population density. The model was determined using the methods described by Bowerman and O'Connell (1979) and Bails and Peppers (1982). The steps consisted of identifying the functional relationships (i.e. linear, quadratic, etc) between the dependent and independent variables, by examination of plots of the independent terms on increment width. In all three cases, there was evidence of non-lineary, warranting the inclusion of second and third-order terms for possible selection in the regression model. I tested for lagged effects between water temperature and increment width using a cross-correlation and as none were found, water temperature was included as a non-lagged variable only. This step was completed for bottom temperature only, as time-series of other independent variables were derived from interpolations between point estimates and as such, were inappropriate for examination by cross-correlation. Bottom water temperature was considered for inclusion in the regression model rather than surface temperature, as best correlations with daily

ration were noted with the former variable. This approach seemed reasonable as Sixes River chinook salmon consume epibenthic prey in most cases (Bottom et al, 1983). Of the two measures of population size available, the mark-recapture estimates and catch per unit effort indices, the former measure of abundance was used in the regression model (Fig. 4-2). I did not use the catch per unit effort data as observed schooling of juvenile chinook salmon (D. Bottom, pers. comm.) might cause biased estimates. Moreover, the CPUE data were obtained from single locations in the estuary whereas each mark-recapture estimate was based on comparatively intensive sampling through out the estuary.

The final components of the regression model were interaction terms including ration and population abundance and ration and temperature. Interaction terms were only included when there were a priori grounds for doing so. For example, a clear relationship (r^2=0.623) existed between water temperature and ration (Fig. 4-3) and hence a multiplicative interaction term was included.

The variables to be included in the regression model were then selected using a stepwise variable elimination computer routine. The variable elimination routine adds independent variables to the regression model based on a user-specified level of significance for their inclusion (α = 0.10). The variable added at each step was that with the highest partial correlation coefficient with respect to the

Figure 4-2 Chinook salmon population abundance in the Sixes River estuary in 1980, estimated from Bailey-modified mark-recapture with confidence intervals shown and catch per unit effort statistics.

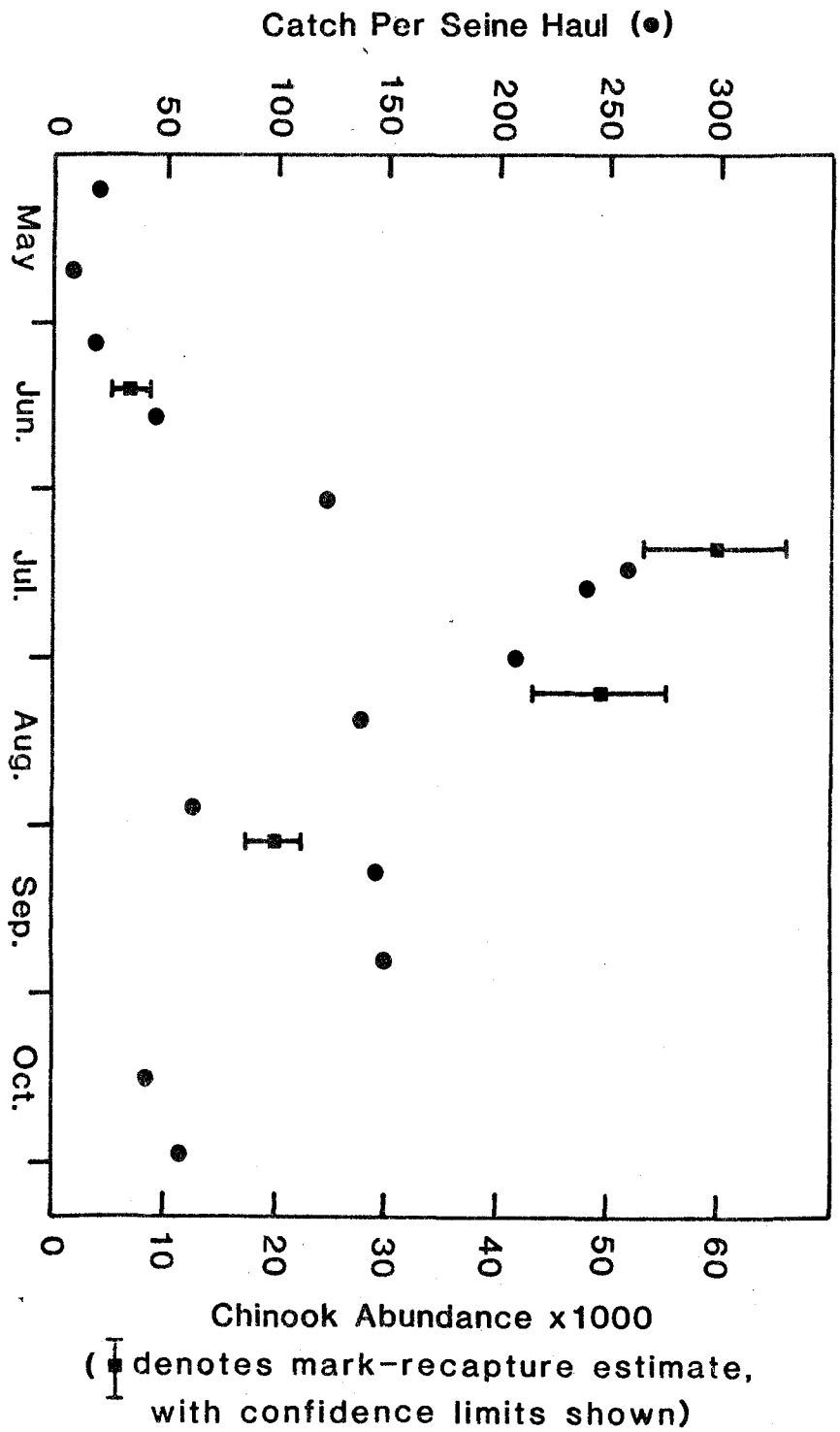
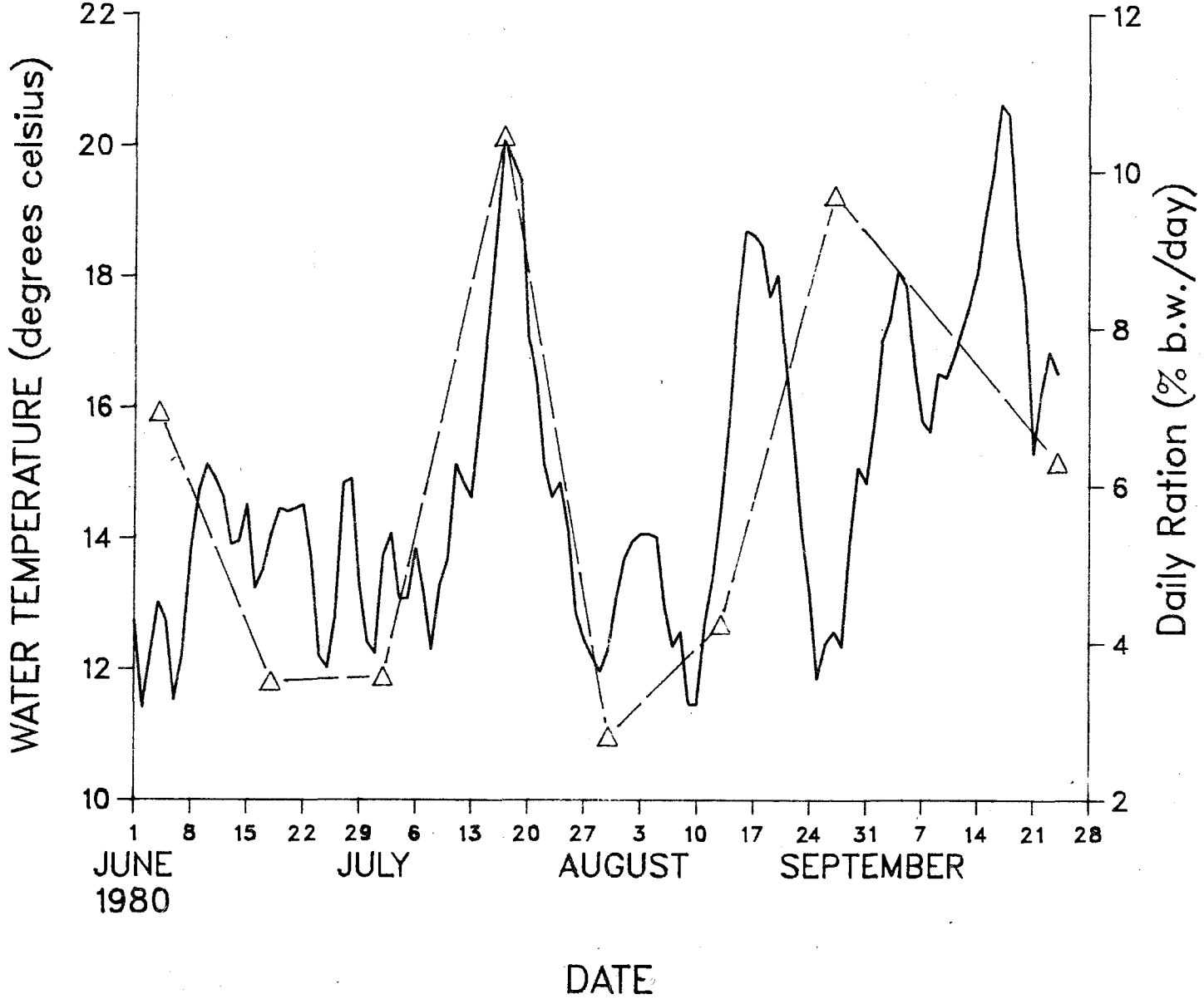

Catch Per Seine Haul (●)
0
50
100
150
200
250
300
May
Jun.
Jul.
Aug.
Sep.
Oct.
0
10
20
30
40
50
60
Chinook Abundance x1000
(▪ denotes mark-recapture estimate,
with confidence limits shown)

Figure 4-3 Relationship between daily ration of juvenile chinook salmon (---) and mean daily water temperature (——) (measured by a thermograph located near the estuary bottom) in the Sixes River estuary, 1980.


WATER TEMPERATURE (degrees celsius)
Daily Ration (% b.w./day)
22
20
18
16
14
12
10
12
10
8
6
4
2
1 8 15 22 29 6 13 20 27 3 10 17 24 31 7 14 21 28
JUNE
1980
JULY
AUGUST
SEPTEMBER
DATE

dependent term (increment width). The regression was recomputed as each variable was added, until no variables were left with the required level of significance. The time series of increment widths ranged from June 1 to September 25, 1980, a total of 116 d.

A total of 255 left-side sagittal otoliths were examined with the microfiche viewer during this study. Of that total, 60 were examined with a scanning electron microscope for determination of increment widths.

RESULTS

To study estuarine growth using otolith microstructure, I first described the relationship between fish size and otolith size and determined the frequency of otolith growth increment formation. The relationships between fish length and otolith total length are adequately represented by geometric mean linear regressions and are shown below:

Year of Sample	n	Regression Equation	Coefficient of Determination(r^2)*	Significance *
1979	87	y=0.021(x)+0.515	0.77	< 0.0001
1980	69	y=0.033(x)-0.280	0.78	< 0.0001
1981	99	y=0.032(x)-0.125	0.81	< 0.0001

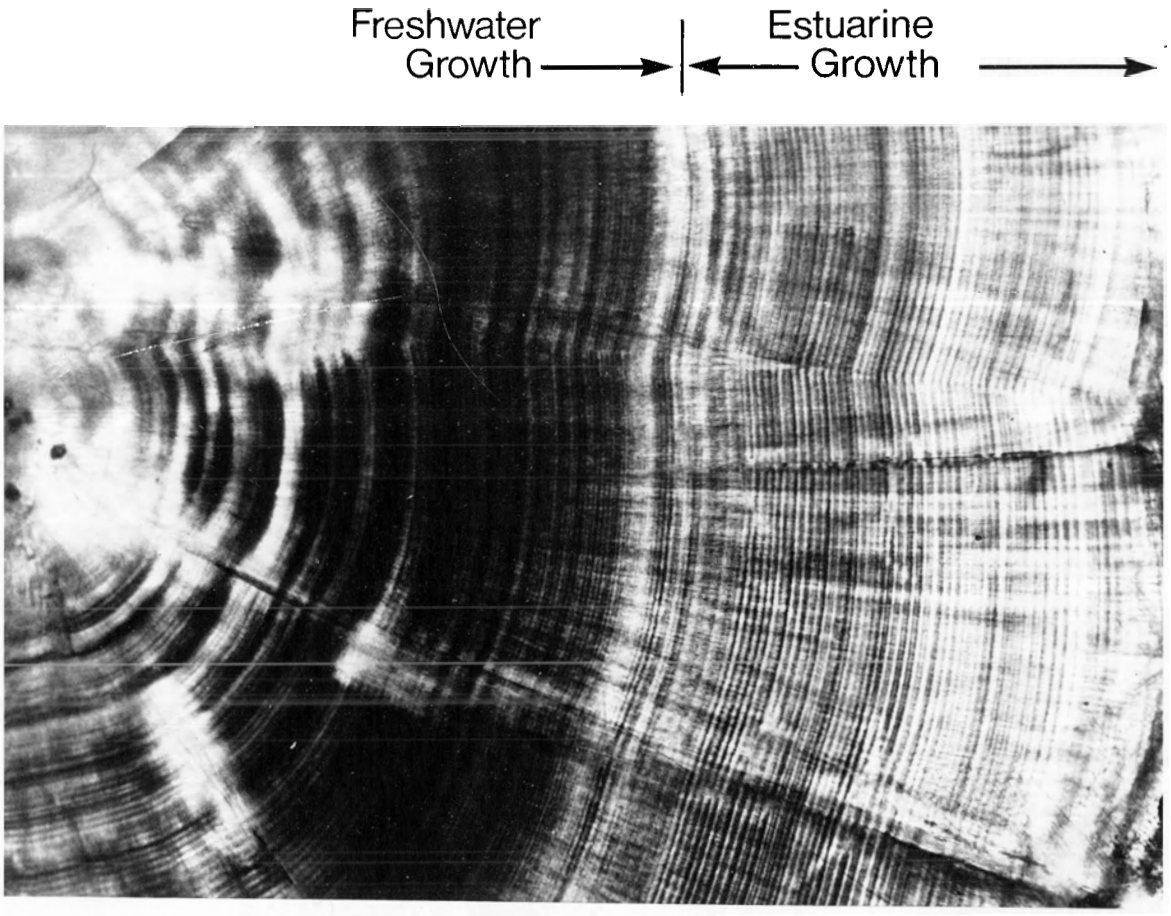
*Calculated using the arithmetic mean procedure

The equations are given in the form y = otolith length and x = fish length to facilitate comparisons with results in Chapters 2 and 3. However, back-calculations of size were based on regressions of fish length (y) on otolith length (x). Analysis of covariance (using the arithmetic mean regressions) and the Student-Newman-Keuls test indicated that the slope of the regression of otolith length-fish length data in 1979 samples was significantly less ($p<0.0001$) than the slopes of the 1980 and 1981 regressions. For that reason, separate regressions were retained for back-calculations of fish length at age.

The rate of growth of juvenile chinook salmon downstream migrants increased markedly upon entry into the estuarine environment. That was reflected in greater otolith increment widths (Fig. 4-4). The criterion for determining the beginning of estuarine residence was the point at which increment width increased 25% or more relative to the mean of the previous 10 increments, and was sustained over at least 10 consecutive increments or to the otolith periphery. Increments formed in freshwater averaged < 3 μm in width, whereas the estuarine growth increments formed in early summer were > 4 μm. The close correspondence between increased increment width and the migration from freshwater to the estuary is supported by data from the biweekly fish collections from the Sixes River estuary. In 1981, the first migrants in the estuary were taken on May 4, and had 0-14 (x=4.5) estuarine growth increments. Examination of otoliths from fish caught above the estuary provided a means of characterizing known freshwater growth increments and verified my ability to differentiate between increments formed in freshwater and in the estuary.

Estuarine growth increments appeared to be formed with daily periodicity. I determined the frequency of formation by comparing the mean observed increase in estuarine increment counts versus the expected increase in increment counts assuming a daily frequency of formation in consecutive bi-weekly samples. To calculate the expected number of growth increments, I took the Julian date of first capture each year and subtracted 14 d to account for the interval between biweekly samples. That date was assumed to be the first day of

Figure 4-4 Polished section of a juvenile chinook salmon sagittal otolith showing microstructure differences associated with movement from freshwater to the estuary.


Freshwater
Growth
Estuarine
Growth
50μm

estuarine residence. Because of the way in which I calculated the date of commencement of the estuarine rearing period, increment counts were generally less than expected, although not significantly so. The slope of arithmetic mean regressions of observed increment counts on expected increment counts did not significantly differ from unity for the 1979, 1980 or 1981 data (t-tests, $0.05<p<0.10$) indicating that one otolith growth increment was formed every 24 h, on average. Data for 1981 are shown in Fig. 4-5.

As I did not have sequential samples of chinook salmon from freshwater, evidence that otolith increments were formed once every 24-h in freshwater was indirect. Averages of 72.4 ± 15.9, 86.6 ± 20.3 and 72.4 ± 19.3 freshwater growth increments were counted in otoliths from juvenile chinook salmon samples from 1979, 1980 and 1981 respectively (± one standard deviation indicated). The counts were reasonably consistent with the number of days elapsed from peak emergence to peak numbers of fish migrating downstream into the estuary (approximately 75 d) plus approximately 10 d as otolith increment production begins before hatching and emergence (Chapter 1).

Estuarine Growth in Relation to Freshwater Growth and Residence

The back-calculated size distributions of chinook salmon migrants entering the Sixes River estuary in 1979, 1980 and 1981 are shown in Fig. 4-6. The calculated mean size of migrants entering the estuary did not differ significantly between years (analysis of variance,

Figure 4-5 Arithmetic mean regression of counts (± 1 S.E.) of estuary growth increments versus expected number of increments for otoliths of Sixes River juvenile chinook salmon.

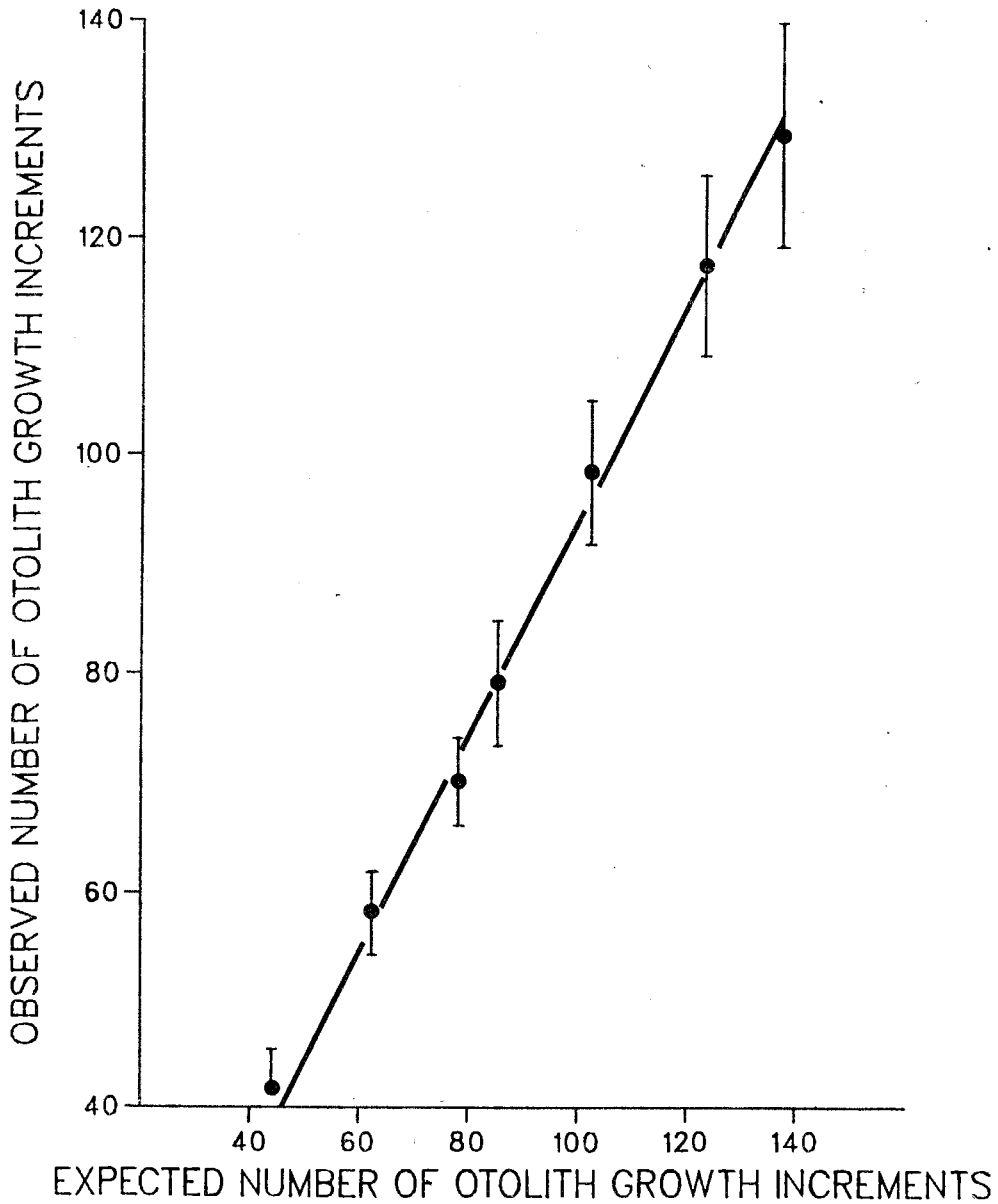
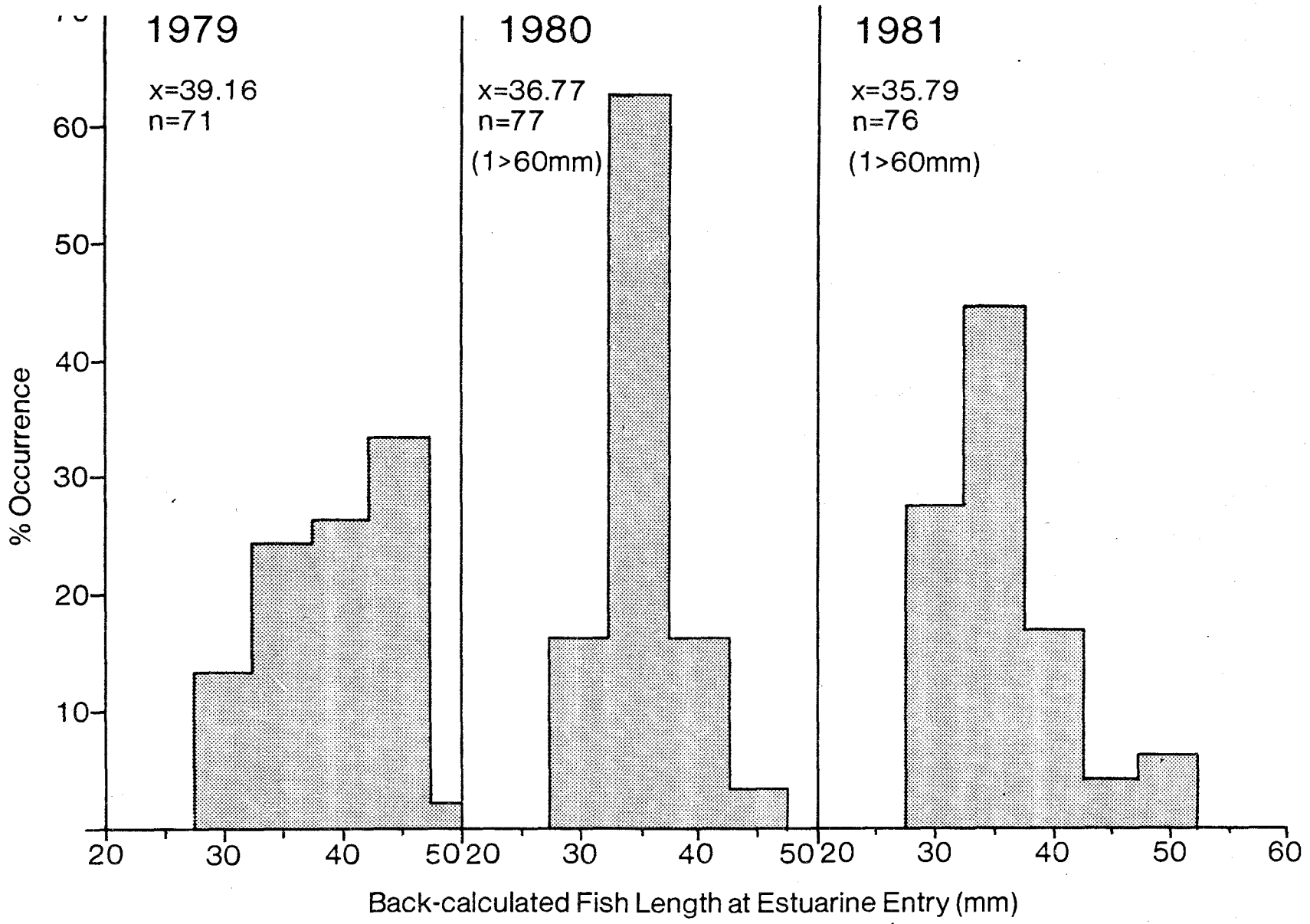

OBSERVED NUMBER OF OTOLITH GROWTH INCREMENTS
140
120
100
80
60
40
40
60
80
100
120
140
EXPECTED NUMBER OF OTOLITH GROWTH INCREMENTS

Figure 4-6 Size-distributions of juvenile chinook salmon entering the Sixes River Estuary in 1979, 1980 and 1981 determined by back-calculation from otolith microstructure and size at capture.


1979
x=39.16
n=71
1980
x=36.77
n=77
(1>60mm)
1981
x=35.79
n=76
(1>60mm)
% Occurrence
60
50
40
30
20
10
20
30
40
50
20
30
40
50
20
30
40
50
60
Back-calculated Fish Length at Estuarine Entry (mm)

p=0.0538), nor did the average date of estuarine entry vary significantly (Fig. 4-7, analysis of variance, p=0.1007) as indicated through otolith microstructure examination. Regressions of back-calculated size at estuarine entry on date of estuarine entry (Julian day) were not significant (p=0.1129, 0.7129 and 0.2615 respectively). Regressions of freshwater age (number of increments) on date of estuarine entry (Julian day) were significant in 1979 and 1981 (p=0.0044 and 0.0030 respectively) and not significant in 1980 (p=0.1836). Regression equations for the 1979 and 1981 data are given below:

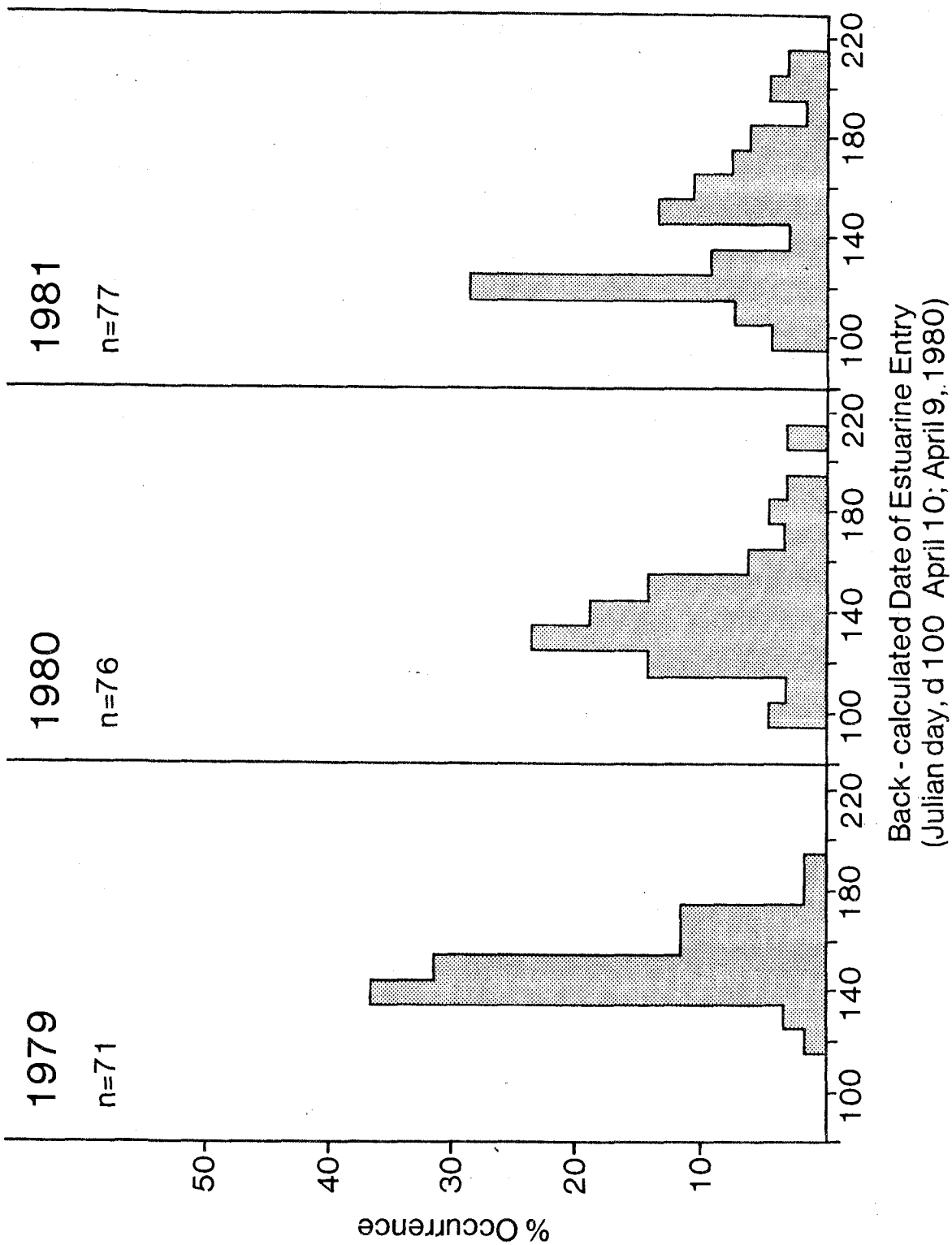
$$(1979)\ y = -1.30(x) + 267.1$$

$$(1981)\ y = -0.68(x) + 170.3$$

where 'y' is number of increments formed in freshwater and 'x' is Julian day. The negative slopes of the geometric mean regressions indicated that as summer progressed in 1979 and 1981, migrants entering the estuary were younger on average.

To assess whether late migrants into the estuary showed different trends in growth from earlier migrants, I randomly selected 10 fish that arrived in the estuary before May 24, 1981 (the mean date of estuarine entry) and compared the pattern of estuarine growth as indicated by otolith microstructure with that of 10 fish which had entered the estuary after May 24. The mean date of entry into the estuary was May 17 and June 25 for the samples of the "early" and "late" migrants

Figure 4-7 Date of entry into the estuary for chinook salmon migrants in 1979, 1980 and 1981 as determined from otolith microstructure.

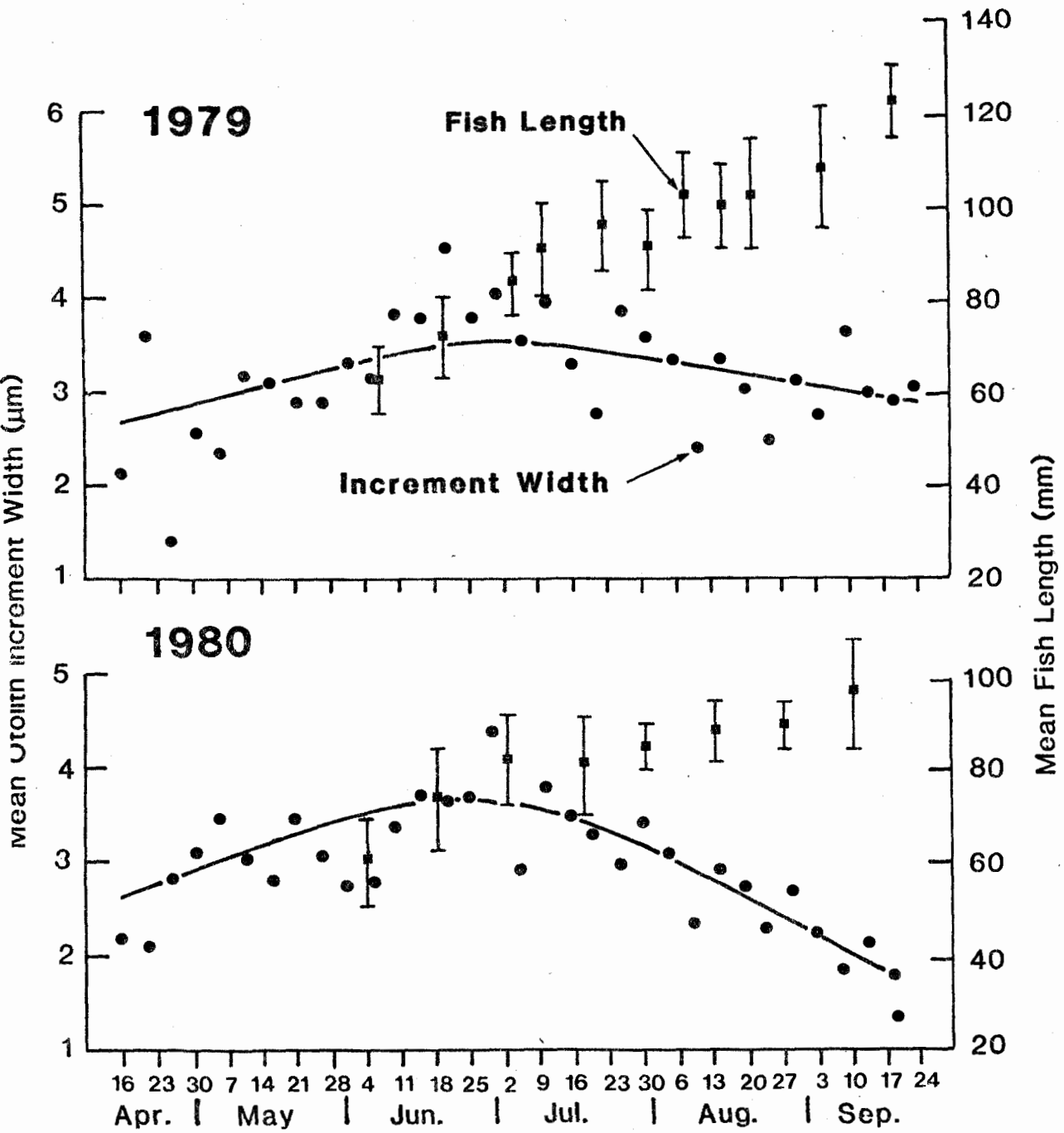

1979
n=71
1980
n=76
1981
n=77
% Occurrence
10
20
30
40
50
100
140
180
220
100
140
180
220
100
140
180
220
Back - calculated Date of Estuarine Entry
(Julian day, d 100 April 10; April 9, 1980)

respectively. After the deposition of a series of relatively wide increments assumed to correspond with the beginning of estuarine residence, increment widths gradually declined. Neither the peak increment width (t-test, $0.05<p<0.10$) nor the rate of increment width decline differed significantly (analysis of covariance, $0.10<p<0.20$), indicating that no differential growth was associated with different times of entry into the estuary.

Estuarine Growth in Relation to Environmental Factors

The average growth rates of fish expressed as mean otolith increment width in 1979 and 1980 are shown in Fig. 4-8. Mean increment width increased markedly in May and June during the period corresponding to estuarine entry. Thereafter a gradual reduction in increment width was observed, an indication of declining growth rate. That trend was observed in all three years. The reduction in otolith increment width was more noticeable in 1980 than in 1979, as analysis of covariance for data from June 1 to September 24 indicated that the slope of the regression of mean increment width on time was significantly greater in 1980 than in 1979 (analysis of covariance, $p=0.0020$). The reduction in increment width in 1980 reflected a slower growth rate of fish. Note that increment width maxima coincide approximately with the beginning of reduced fish growth. The instantaneous daily growth rate in June, 1980 averaged approximately 5% based on fork length, corresponding to increment widths of about 3.5 μm (Fig. 4-8). By late August, instantaneous daily growth was 2% or

Figure 4-8 Mean increment width (•) versus time as determined from otoliths of juvenile chinook salmon caught in the Sixes River estuary, late September, 1979 and 1980 (n = 10 for each year). Curves are fitted using the method of cubic splines with delta (a root-mean-square weighting factor) equal to 0.5. Average size (± 1 standard error) of juvenile chinook salmon caught in biweekly seine hauls are also indicated (■).


1979
Fish Length
Increment Width
1980
Mean Otolith Increment Width (µm)
Mean Fish Length (mm)
16 23 30 7 14 21 28 4 11 18 25 2 9 16 23 30 6 13 20 27 3 10 17 24
Apr. | May | Jun. | Jul. | Aug. | Sep.

less, corresponding to increment widths of about 2 μm. While data shown are for fish caught at the end of the estuary rearing period, fish caught earlier in the summer also showed an increasing trend upon entry into the estuary which declined as the summer progressed.

I attempted to relate the observed pattern of otolith growth to environmental conditions in the estuary in 1980, the year for which the most complete data were available. I completed a stepwise multiple regression of population density, daily ration and bottom water temperature with the dependent variable, otolith increment width. A summary of the data used in the regression model is provided in Table 4-1.

The results of the multiple regression analysis are shown in Table 4-2. Population density (the linear term) was the independent variable accounting for the most variability (40%) in the dependent term, mean increment width. Other significant independent variables included ration, ration^3 and the population abundance-ration interaction term. These independent terms accounted for a further 12, 10 and 3% respectively of the observed variance in mean increment width.

TABLE 4-1

Summary of data used in a multiple regression describing otolith increment widths in Sixes River juvenile chinook salmon, May-September, 1980

	n	$\bar{x}$	Range	Standard Deviation
Increment width (um)	116	3.00	1.30-5.20	0.86
Mean daily water temperature (°C)[1]	116	14.83	11.30-20.60	2.30
Daily Ration (% body weight/ 24 h)	8	6.12	2.18-10.45	2.17
Population abundance[2]	4	39810	6762-59233	14226

1. Mean of hourly observations from recording thermograph placed near the estuary bottom.

2. Estimates determined by the mark-recapture technique (see text).

TABLE 4-2

Stepwise regression analysis of population density, water temperature and daily ration on mean otolith increment width, Sixes River juvenile chinook salmon, 1980.

	Partial correlation coefficient	Slope	Standard error of slope	t-statistic	Cumulative Coefficient of determination (r^2)	Significance
Variables selected:						
Ration	-0.34968	-0.99739	0.99546×10^{-1}	-10.019	0.12227	0.0009
Ration^3	0.33575	0.21142×10^{-2}	0.70326×10^{-3}	3.006	0.22122	0.0016
Population Abundance-Ration Interaction	0.21258	0.15483×10^{-4}	0.14245×10^{-5}	10.869	0.25641	0.0508
Population Abundance	-0.75812	-0.09545×10^{-4}	0.90673×10^{-5}	-10.527	0.68378	0.0000
Not selected:						
Temperature	-0.08458					0.4471
Population Abundance^2	-0.02384					0.8306
Population Abundance^3	0.00039					0.9972
Temperature^2	-0.09665					0.3847
Temperature^3	-0.10708					0.3353
Ration^2	0.17654					0.1104
Temperature-Ration Interaction	-0.07252					0.5147

N.B. Superscripts appended to variables indicate the power to which terms were raised to better reflect possible non-linearities in the relationships with the dependent term.

DISCUSSION

Of the variables influencing otolith increment width, population density was the most important (Table 4-2). Lower average increment widths (and hence fish growth rates) were associated with higher population densities. The mechanism responsible for reduced growth rate was probably food limitation. Evidence for food limitation was given by the relatively high significance of the ration-population abundance interaction and ration terms in the stepwise regression model (Table 4-2). Density-dependent growth in Sixes River juvenile chinook salom has been suggested in previous studies (Reimers 1973; Bottom et al 1983). However, previous workers hypothesizing density-dependent growth rate reductions in the Sixes River were not able to dismiss the possibility that observed mean growth rate reductions were artifacts resulting from inclusion of recently recruited smaller fish in the samples. Based on examination of otolith microstructure my analyses provided growth records for individual fish and gave further support to Reimers' (1973) hypothesis that density-dependent growth rate reductions occur during chinook salmon residence in the Sixes River estuary. Bottom et al (1983) provided further evidence of food limitation, noting that Corophium standing crop and productivity were less in 1980 than in 1979. The mean biomass and number of Corophium in chinook salmon stomachs was also less in 1980. Finally, the smaller average size of Corophium in the stomachs of fish sampled in 1980 may have reflected a greater rate of removal associated with a smaller food resource.

Healey (1982b) has reviewed evidence for growth rate reductions of juvenile Pacific salmon in more northern estuaries. The only other systems where growth rate reductions were thought to occur were the estuaries of the Squamish River, B.C. and the Yaquina River, Oregon. However, given the apparent variability of growth rate of juvenile chinook salmon in the Sixes River from year to year, density-dependent growth rate reductions in juvenile salmon rearing in other systems not closely studied on an annual basis may be a more common occurrence than is currently thought.

In my multiple regression model, no temperature term was significant in accounting for variability in mean otolith increment width. This finding was somewhat counter-intuitive, since English (1981) and results in the previous chapter indicated that water temperature could affect mean increment width, and mask the effect of other environmental variables such as ration. However, the temperature range through the 1980 period (11.9-20.1°C) differed from that of my lab experiments (6°C + 2-4°C diel temperature fluctuations) and English's study in Patricia Bay, B.C. (≈10.5-12.5°C). Brett et al (1982) have shown that the growth rate of chinook salmon when fed to satiation varied little with respect to water temperature through the range 13-21°C (2.9 - 3.3% dry weight/day). While it is unlikely that Sixes River fish were feeding to satiation given the average stomach content weight (1-2% of wet body weight; Bottom et al 1983) compared with other published data such as Healey's (1980) Nanaimo River study (up

to 5% of body weight), it may be that chinook salmon growth is relatively insensitive to the range of temperatures observed in the Sixes River estuary in 1980.

The temperature-ration interaction term was also not significant. However, I may have underestimated the degree of association between water temperature and ration. On June 4 and August 27 (Fig. 4-3), daily ration was greater than expected, assuming a positive relationship between water temperature and daily ration. On both days the estuary was highly stratified, with mean bottom temperatures less than 13.5°C. During periods of stratification it is possible that the relatively thin layer of seawater measured by the thermograph was not representative of the average water temperature experienced by the chinook salmon juveniles.

A difficulty with the interpretation of the multiple regression model was that the degrees of freedom were somewhat inflated due to the inclusion of time series of daily ration and population abundance data determined by interpolation between point estimates. The question of how many degrees of freedom are appropriate for the model when the degrees of freedom of the independent terms vary has not received attention from statisticians (S.Smith, pers. comm.). However, several statisticians considered the methodology used here as adequate (C. Vilegas, S. Smith, A. Sreedharan pers. comm.). Given the difficulty of obtaining data such as daily ration and mark-recapture population abundance estimates, it seems unlikely that more frequent

collections of data yielding higher degrees of freedom could occur under most circumstances.

English (1981) examined otolith microstructure in juvenile chinook salmon and proposed a multiple regression model of mean increment width using the independent terms water temperature and prey abundance. However, his regression model explained only 14% of the observed variation in otolith increment width. My model accounts for 68% of the variation in mean increment width. Possible drawbacks of English's model included a small sample size (n=6) and no consideration of non-linear relationships between increment widths and the independent variables.

It has been argued that incautious use of multiple regression computer programs has led to erroneous conclusions (Geary and Leser 1968; Cramer 1972). Indeed, a drawback of the multiple regression approach is that no unique "best" model exists for a given situation, and different procedures for deriving the model may yield conflicting results (Zar 1974). However, when I completed the multiple regression following the stepwise variable deletion procedure instead of the variable addition method used previously, the same variables were selected with a total R^2 of 0.66. Therefore, my results appear unaffected by the choice of method.

The back-calculated sizes of fish on entry into the estuary underestimated their observed size. This was particularly apparent

in 1981, when the mean back-calculated fork length was 35.8 mm. Among fish caught on May 4, 1981, those which by my criterion of otolith microstructure had entered the estuary that day averaged 40.0 mm fork length (range 38-41 mm, n=5). The bias could have been caused by an inappropriate back-calculation procedure or by a misinterpretation of the beginning of estuarine residence from examination of otolith microstructure. However, the Lee method of back-calculation employed here is widely used and generally considered appropriate (Carlander 1981). The linear otolith length - fork length relationships also seemed adequate as examination of residual plots of the unexplained variance gave no indication of curvilinearity. The possibility of misinterpretation of the beginning of the estuarine residence period is not readily dismissed, as no independent confirmation such as tetracycline marking (Chapter 3) were available. However, as the bias towards underestimating the observed size seems systematic, my conclusions are robust for comparative purposes. Other authors who have noted systematic errors in back-calculated lengths from otolith measurements include Hickling (1933), Halliday (1969) and Reay (1972).

In the absence of artificially induced time-markers there was some uncertainty regarding the frequency of increment formation. While my comparison of observed versus expected increment counts indicated that one increment was formed every 24 h in fish caught in the estuary, the technique was not precise since fish were recruited to the estuary population over a long period of time. However, the

increment widths were consistent with those formed in Chapters 2 and 3. Brothers (pers. comm.) and English (1981) also found that juvenile chinook salmon form one increment every 24 h in marine environments. In the field, the frequency of increment formation could be determined by examining otoliths of chinook salmon held in pens located in the estuary. However, even if increments were not formed once every 24 h in Sixes River chinook salmon, it was still likely that increment widths were proportional to fish growth and had utility for growth studies, if the frequency of increment formation was assumed constant. For example, the model proposed here describing the relationships between fish growth and environmental variables employed time series of data. With such an approach, knowledge of the frequency of increment width data must be precisely correlated with environmental data collected over discrete time intervals.

The marked visual contrast which aided identification of estuarine growth increments may have reflected the greater amplitude of water temperature fluctuations in the Sixes River Estuary relative to temperature fluctuations in the river. In Chapter 3, I showed that diel temperature fluctuations of 2-4°C amplitude resulted in increased visual contrast of growth increments, compared with otoliths from fish reared in a constant temperature regime. In the Sixes River diel temperature fluctuations, while present, were irregular in occurrence and amplitude, compared with the estuary. River temperatures ranged through 3.5 ± 1.5°C (mean daily amplitude measured ≈20 km upstream of

the estuary, ± 1 S.D. indicated) in May 1979, the only year in which temperature data were available for the early summer period. During a corresponding period in 1980, mean daily fluctuations in the estuary were 5.9 ± 1.0°C. Fish might also be exposed to daily temperature fluctuations if they exhibited a diel vertical migration through the thermally stratified water column (up to 10°C) of the estuary. Volk et al (1983) have also noted a similar transition in otolith microstructure as juvenile chum salmon leave freshwater as has Bradford (pers. comm.) in a preliminary study of otoliths of Yakoun River pink salmon (O. gorbuscha).

I have compared my findings for Sixes River chinook salmon to the laboratory (Chapters 2 and 3) from which I suggested that multiple feedings or water temperature fluctuations increased the rate of otolith increment production and thereby could create possible difficulties in interpretation of age and growth data. Sixes River juvenile chinook salmon, however, did not show significant deviations from the 1 increment/24 h rate of increment production. Twice daily temperature cycles, as might be expected under tidal influence, did not occur in the estuary although there were marked diel fluctuations. Similarly, while some diel periodicity in gut contents were noted, they were weak and irregular in their occurrence. The absence of any regularity in feeding pattern or temperature fluctuations may have precluded formation of more than one growth increment every 24 h such as were noted in the laboratory experiments.

I examined the effects of different freshwater life history patterns on timing of estuarine entry and subsequent growth. I found a negative correlation between date of estuarine entry and freshwater age in 2 of 3 years studied. The earlier emigration of fish developing from eggs deposited relatively late in the spawning season may have been due to increased river temperatures, a mechanism suggested by Reimers (1973). Alternately, part of the life history strategy associated with progeny of late spawners may include an abbreviated period in freshwater with the result that fish benefit from better growth while in the estuary. However, my examination of otolith microstructure indicated that while otolith increment widths in fish arriving in the estuary relatively late in 1980 increased upon entry into the estuary, the rate of growth inferred from increment widths did not differ significantly from that of early recruits to the estuary population. It may be that late migrants leave the estuary at a smaller average size than earlier recruits to the estuary, assuming no differential duration of residence in the estuary.

Many workers have commented upon the importance of estuaries as rearing habitat for Pacific salmon juveniles. However, prior to the use of otolith microstructure examination as a tool for detailed study of age and growth, it has been difficult to quantify fish growth in estuaries relative to other habitats without extensive mark-recapture programs. This chapter has identified the relationships between fish growth rate, expressed through otolith mean increment width, and

environmental conditions in the estuary. I present evidence that growth of juvenile chinook salmon in the Sixes River estuary is density-dependent and limited by food availability. Among possible alternative hypotheses that might explain the apparent decline in growth rate, two of the most likely are size-selective mortality or emigration. While data presented in the next chapter do not support the occurence of size-selective mortality in the estuary, I cannot discount the possibility that size-selective emigration existed.

It is difficult to comment on the applicability of my results to other stocks as the feeding options available to Sixes River chinook salmon differ significantly from those available in other estuaries. As noted by Bottom et al (1983), the food web of the Sixes River estuary is simple and comprised of relatively few fish or invertebrate species. Sixes River chinook salmon do not feed on the infauna found throughout the estuary, but consume only the large epibenthic crustaceans. In contrast, chinook salmon in larger Oregon estuaries consume a greater variety of prey, including insects and larval fish (Forsberg et al 1977; Myers 1980). In British Columbia estuaries, chinook salmon diets are variable, but adult insect and decapod larvae are frequently important (Sibert and Kask 1978; Levy and Northcote 1981; Healey 1982b). Although the feeding habits of chinook salmon vary significantly among estuaries, the chinook salmon-amphipod food chain in Sixes River estuary may be comparable to the food chain of larger, more diverse estuaries, where salmon production is also primarily detritus based (Sibert et al 1978; Levings 1980; Healey 1982b).

CHAPTER 5

THE EFFECTS OF FIRST-YEAR GROWTH RATE ON SIZE-SELECTIVE MORTALITY AND AGE AT MATURITY OF SIXES RIVER CHINOOK SALMON AS DETERMINED FROM OTOLITH MICROSTRUCTURE

INTRODUCTION

Several workers have noted that growth rate during the first year affects the subsequent survival, growth and maturation of Pacific salmon (Oncorhynchus sp.). Parker (1971) and Healey (1982a) have presented evidence of size-selective mortality in the first year of life in pink (O. gorbuscha) and chum salmon (O. keta) respectively. Parker's conclusions were based on examination of a series of length-frequency plots of pink salmon fry as the cohort grew older, while Healey examined the widths between successive circuli of chum salmon scales. In the latter case, slower-growing fish had narrower circuli spacing on average. The loss of the smaller, slower-growing fish to predators resulted in wider average circulus spacing among the survivors. Hager and Noble (1976) and Bilton (1982) have also suggested that based on experimental releases of coho salmon (O. kisutch) from hatcheries, juvenile males which grow faster generally mature at an earlier age.

Otolith microstructure provides a tool for examining age and growth of juvenile Pacific salmon (Wilson and Larkin 1982) and also for determining size-selective mortality as has been done with scales. More precise estimates of the timing and extent of mortality may be possible with otoliths given the greater frequency of formation of growth increments compared with scale circuli. In this chapter, I examine the evidence for size-selective mortality of juvenile Sixes River (Oregon) chinook salmon (O. tshawytscha) while rearing in the

estuary and during later ocean life.

On the basis of my examination of otoliths from returning Sixes River chinook salmon, I was also able to test whether fast growth as juveniles was related to early maturation and better survival in a wild population. The work described in this chapter represents the first application of otolith microstructure examination to adults of a temperate fish species.

METHODS

Oregon Department of Fish and Wildlife personnel collected otoliths from 320 carcasses following the 1980-81 spawning run of chinook salmon to the Sixes River. Otoliths were removed using the punch described by McKern and Horton (1974) and stored dry in paper envelopes. An attempt was made to sample size-classes in proportion to their abundance.

Lengths of adult fish were recorded as the "MEPS" length, a measurement which extended from the middle of the eye to the most posterior scale on the caudal peduncle. Changes in body structure associated with sexual maturation did not appear to affect the MEPS measurement (Reimers 1970). However, in order to generate a fish length - body length regression based on the same unit of measurement for data from both the juvenile and adult fish collections, it was necessary to convert the MEPS measurements to fork length equivalents. To do this, I used the regression:

$$FL = 1.2451\ (MEPS) - 1.4804$$

$$r^2 = 0.994,\ N=233$$

(Reimers 1970)

Examination of adult otoliths necessitated the development of a specialized preparation technique. Chinook salmon otoliths are convex on the sulcus side of the sagittal plane, with the degree of convexity increasing in larger fish. The zone of freshwater and estuarine

growth is skewed with respect to the adult otolith. The long axis of the portion of the otolith formed during the juvenile phase was not parallel to the long axis of the adult otolith and was often displaced up to 30°. Because of the size and structural complexity of adult chinook salmon otoliths, the simple methods used for preparing juvenile otoliths such as handheld (Wilson and Larkin 1980) or jig-assisted (Neilson and Geen 1982) grinding and polishing did not consistently provide adequate sections through juvenile growth zones. Attempts to obtain otolith sections using jewellers' saws or microtomes were also unsuccessful.

Satisfactory results were obtained using petrographic techniques for grinding and polishing hard mineral samples. Otoliths were attached to the head of labelled roofing rails with a thermosetting plastic, sulcus (proximal) side up, and aligned with the long axis of the otolith parallel with the surface of the nail head. A coarse grind was performed at 400 rpm on a concentric grooved cast iron grinding wheel using a slurry of 240 grit silicon carbide and water. The nail was held vertical to the abrasive surface, otolith down, and lowered until the otolith made contact. Light pressure was exerted as grinding proceeded quickly. During the grinding, frequent checks were made to ensure that the grinding didn't proceed beyond the desired plane of the otolith. The coarse grind was stopped just before the groove of the sulcus was no longer apparent.

A fine grind was performed on a glass plate using a slurry of 100 grit silicon carbide and water. The nail was held in the same manner as during the coarse grind. Using light pressure and a figure eight motion, the otolith was ground by hand for 2 to 3 min. The nail-mounted otoliths were then placed in an ultrasonic cleaner for 30 s to remove any residual abrasive after which specimens were placed on a hot plate and heated to 100°C to melt the thermosetting plastic. Otoliths were removed and carefully reattached, sulcus side down, to numbered 26 x 46 mm petrographic slides. The distal surface of the otolith was then coarse and fine-ground to approximately 120 μm thickness.

After the preparation was again cleaned in the ultrasonic bath, the final polish was completed on a Buehler Ecomet II polisher using the Buehler microcloth and Alumina A slurry (0.3 micron). Polishing duration was 3 min, with a load weight of 4.5 kg applied to the preparation. After each run sections were examined with a microscope to determine clarity of growth increments. Polishing was repeated until the desired otolith thickness (approximately 100 μm) was achieved. An example of an adult chinook salmon otolith preparation is shown in Fig. 5-1.

Measurements of total otolith length were made using a vernier caliper (± 0.05 mm) prior to the grinding and polishing treatment. Measurements of otolith total length on entry to the estuary and at

Figure 5-1 Micrograph of a polished sagittal section of an otolith from a Sixes River adult chinook salmon, with the freshwater and estuarine zones of growth identified.


Estuary and
Marine Growth
Freshwater
Growth
Nucleus
100μm

the first annulus were determined from images projected on a microfiche reader. Otolith growth increments formed during freshwater and estuarine residence were differentiated using the criteria described in Chapter 4. The first annulus was evident as a distinct dark band when viewed with a transmitted light microscope, and measurements were made along the long axis of the otoliths to the midpoint of the band. All preparations were coded to avoid bias. I made two independent sets of measurements on the coded series of preparations. In cases where measurements differed by > 10%, the preparation was excluded from further examination. Back-calculations of size-at-age were made using the Lee formula described in Chapter 4. Only left-side otoliths were used in this study.

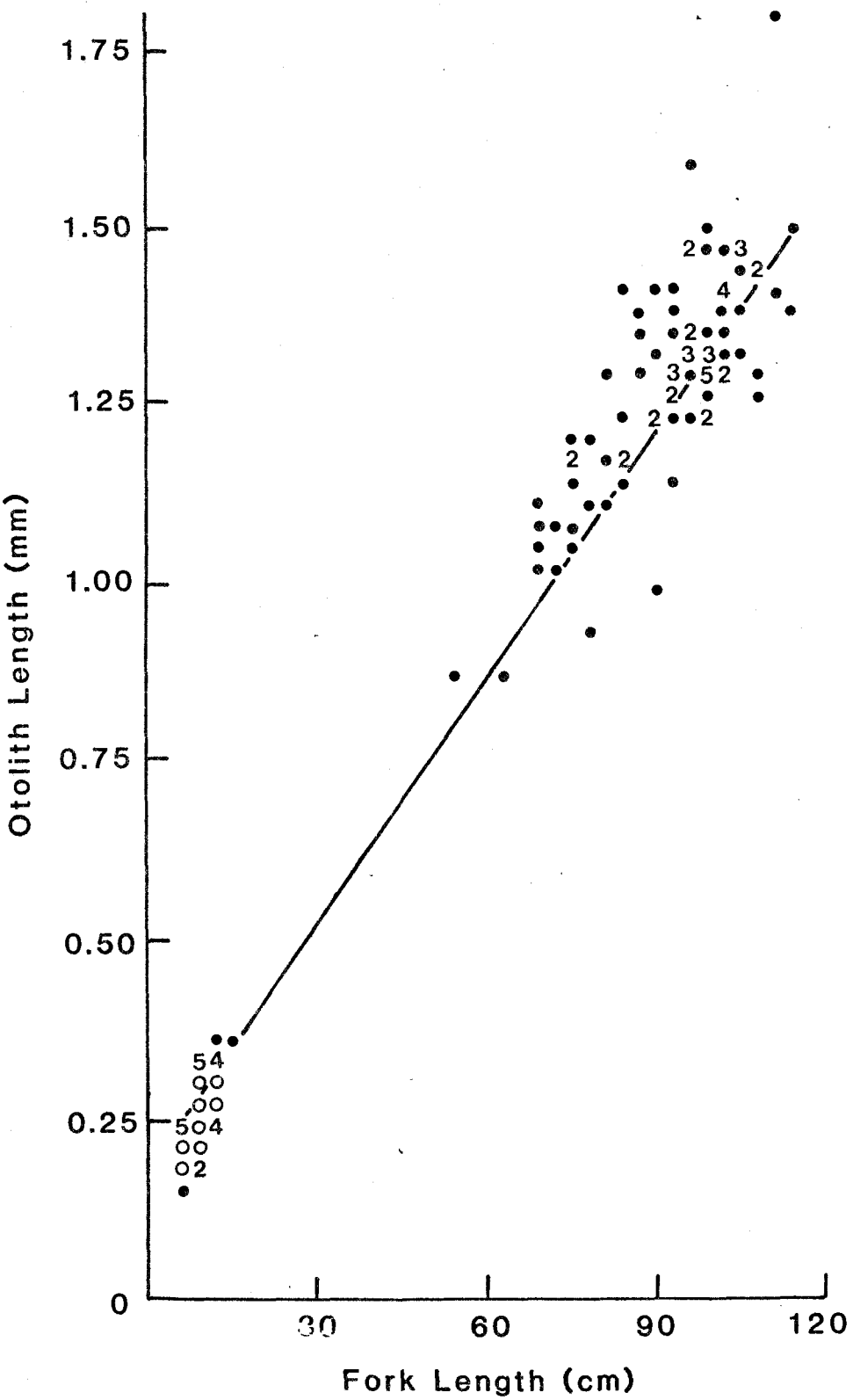
RESULTS

Of the 320 adult fish sampled for otoliths, 117 were male and 203 were female. The mean size of males was 75.55 ± 13.43 cm and of females, 78.47 ± 6.96 cm. The average size of females was significantly greater than males (analysis of variance, p=0.0039). The age composition of the spawning adults, as determined by Oregon Department of Fish and Wildlife personnel from examination of scales from 452 fish, was 2.8% 2-year-olds, 14.3% 3-year-olds, 12.5% 4-year-olds, 64.7% 5-year-olds and 5.7% 6-year-olds. Only 8 (1.8%) had overwintered in freshwater.

I randomly selected 200 otoliths for microstructure examination. Problems developing an adequate methodology for grinding and polishing adult otoliths reduced the total considered suitable for interpretation of juvenile growth patterns to 102 (51%). The fish examined had spent some time in the estuary but had migrated from freshwater before formation of the first annulus.

The relationship between fish size and otolith size for Sixes River juvenile and adult chinook salmon is shown in Fig. 5-2. The equation of the geometric mean regression of otolith length on fish length was $y = 0.0123(x) + 0.1481$ ($r^2 = 0.973$) and was used for back-calculations of size-at-age from the otoliths of the adult fish. Although fish size - otolith size data were not available for

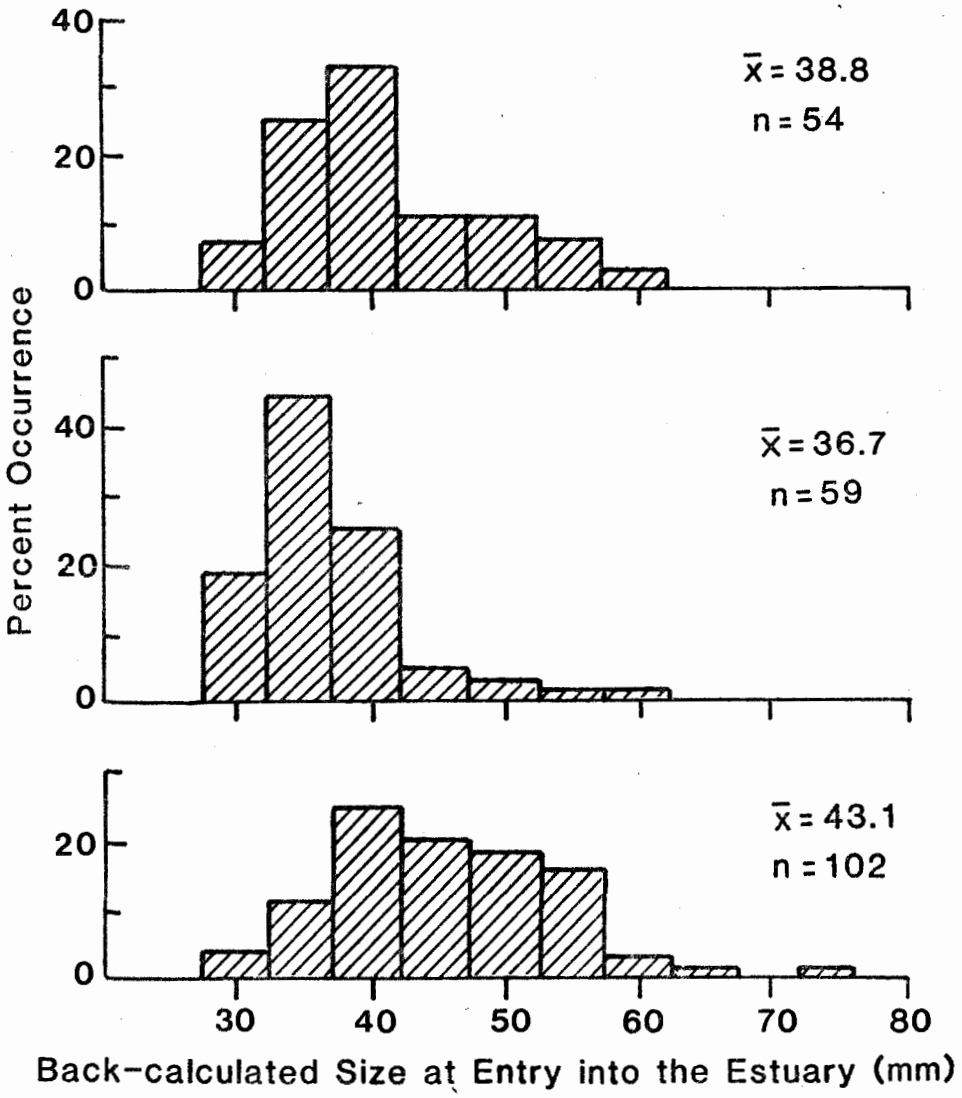
Figure 5-2 Geometric mean regression of left sagittal otolith length on fork length for Sixes River chinook salmon. n = 581. In cases where more than nine points are superimposed, the symbol 'O' is plotted.


1.75
1.50
1.25
1.00
0.75
0.50
0.25
0
Otolith Length (mm)
30
60
90
120
Fork Length (cm)

juveniles in the estuary in 1975-1978 (4 of the 5 brood-years comprising the 1980-81 run of adults) I pooled data from the 1979-81 juvenile fish collections reported in the previous chapter to develop the relationship shown in Fig. 5-2. As noted in the previous chapter, while the average date and size of fish at entry into the estuary did not vary significantly from year to year, the slopes of the otolith length - fish length regressions did. I therefore concluded that pooling the three years of data from the collections of juvenile chinook salmon would provide the most representative otolith length - fish length relationship.

I compared the distribution of back-calculated sizes at entry into the estuary for chinook salmon juveniles caught in the Sixes River Estuary in May and June of 1979-81 (the beginning of estuary rearing of chinook salmon - Chapter 4) to that of fish caught in September and October, 1979-81 (the end of the estuary rearing period). These distributions were also compared with the distribution of sizes of fish at entry into the estuary as back-calculated from the adult otoliths (Fig. 5-3). While the average size at entry into the estuary did not differ significantly between the two juvenile collections ($0.05<p<0.10$), the average back-calculated size at estuarine entry was significantly larger when determined from otoliths of returning adults (analysis of variance and the Student-Newman-Keuls test, $p<0.05$). It was not possible to comment on the size of fish when they left the estuary, or the total duration of estuarine residence as there was no

Figure 5-3 Histogram of back-calculated size at entry into the estuary of juvenile chinook salmon caught in the Sixes River estuary in May-June of 1979-81 (top histogram) and September-October (middle) of 1979-81; and from adults returning in 1980-81 (bottom).


40
20
0
$\bar{x}$ = 38.8
n = 54
40
20
0
$\bar{x}$ = 36.7
n = 59
20
0
$\bar{x}$ = 43.1
n = 102
Percent Occurrence
30
40
50
60
70
80
Back-calculated Size at Entry into the Estuary (mm)

clear demarcation between estuarine and oceanic growth evident from examination of otolith microstructure.

The larger juvenile chinook salmon at time of entry into the estuary remained large compared with the rest of the cohort on average, until at least the formation of the first annulus (Fig. 5-4). The slope of the regression differs significantly from zero ($p<0.0001$).

The fork length of males at the time of first annulus formation is a significant predictor of age of maturation. The larger fry in the estuary returned to spawn earlier on average ($p=0.012$, Fig. 5-5). However, no relationship was found between the size of the juveniles at entry into the estuary and age of maturation of the males ($p=0.2196$). Similarly, no relationship was found between either size at estuarine entry or first annulus and age of maturity in female chinook salmon ($p=0.3991$).

Figure 5-4 Geometric mean regression of back-calculated size at entry into the estuary on size at formation of the first otolith annulus for Sixes River chinook salmon returning to spawn in 1980-81, n = 102.

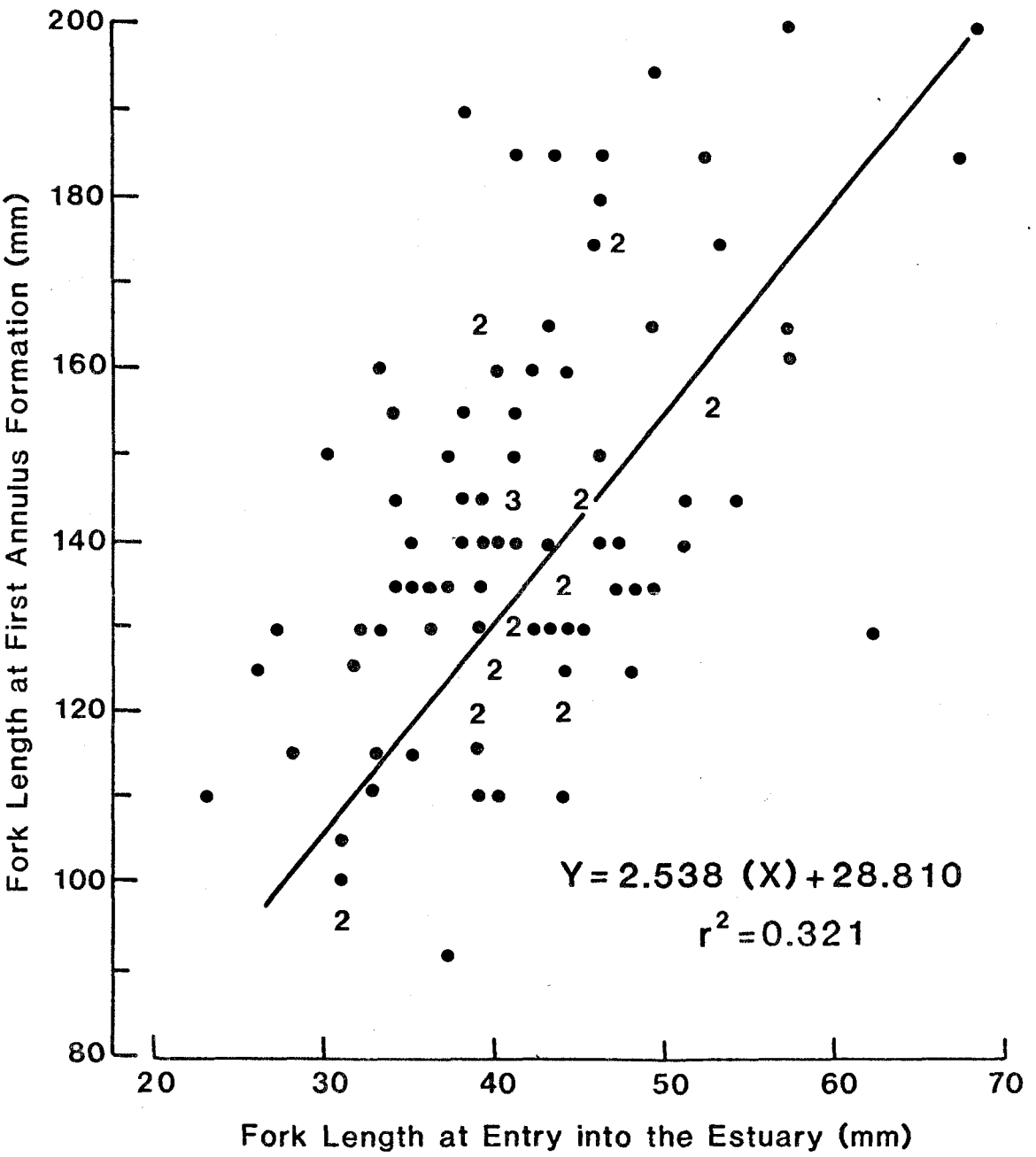
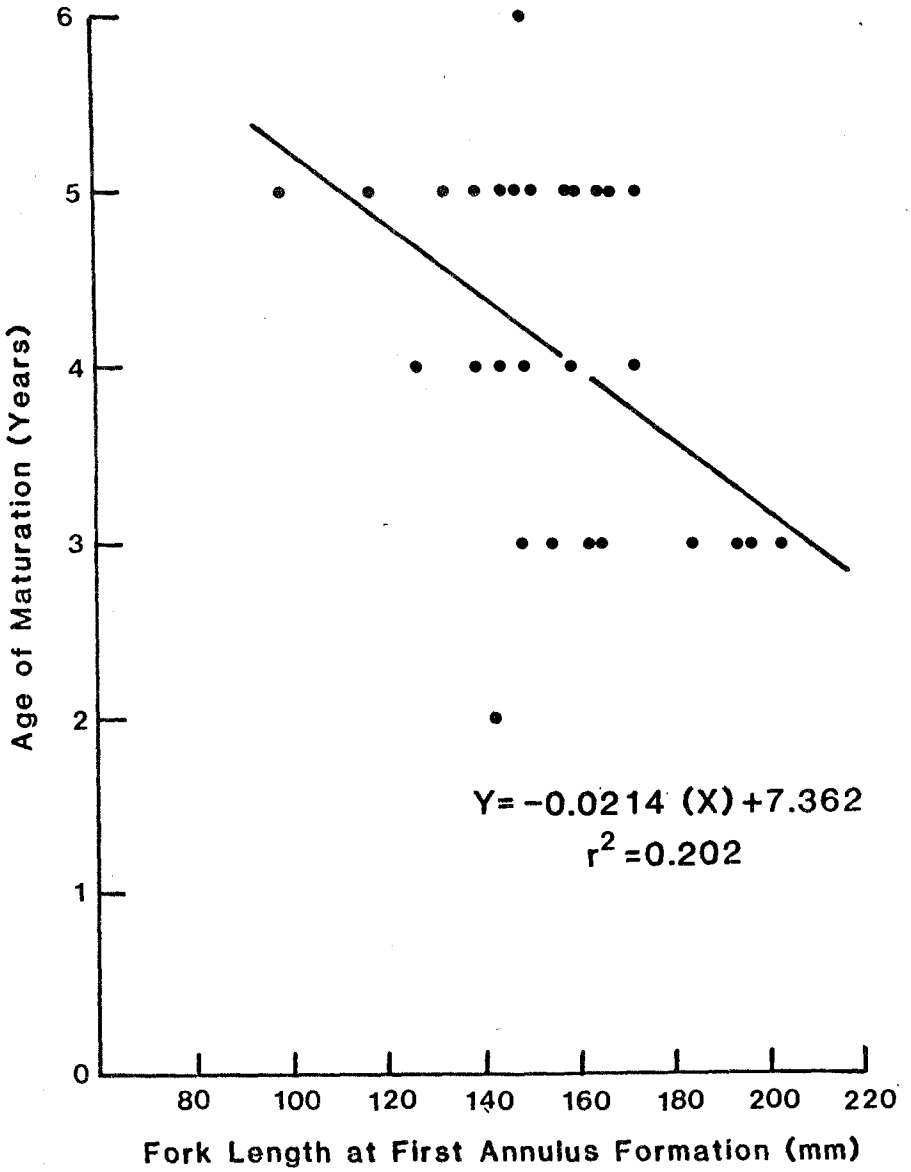

Fork Length at First Annulus Formation (mm)
200
180
160
140
120
100
80
20
30
40
50
60
70
Fork Length at Entry into the Estuary (mm)
Y = 2.538 (X) + 28.810
r² = 0.321

Figure 5-5 Geometric mean regression of age at maturity on back-calculated size at formation of the first otolith annulus for male Sixes River chinook salmon, n = 38.


6
5
4
3
2
1
0
Age of Maturation (Years)
80
100
120
140
160
180
200
220
Fork Length at First Annulus Formation (mm)
Y= -0.0214 (X) +7.362
r² =0.202

DISCUSSION

The size-distributions in Fig. 5-3 are indicative of negative Lee's phenomenon (Ricker 1969): lengths of fish entering the estuary back-calculated from older fish are larger than those calculated from otoliths of younger fish. To provide evidence that Fig. 5-3 reflects size-selective mortality (one explanation offered by Ricker for Lee's phenomena) requires that the two alternate explanations, biased sampling or an incorrect otolith length-fish length relationship do not apply in this case. An incorrect fish length-otolith length relationship seems unlikely since the sample size was large (n = 581, Fig. 5-2) and the geometric mean regression was used (Ricker 1973). However, the slope of the regression in Fig. 5-2 was less than those of regressions calculated in Ch. 4 on the basis of data for juvenile fish only. The inclusion of the adult fish data increased the y-intercept of the regression and hence also increased the back-calculated lengths. It may be that a better fit to the data could have been obtained through use of a power function or a polynomial regression as done by West (1983) using otoliths of juvenile sockeye salmon (O. nerka). However, the form of the non-linear relationship was unknown as no data existed for fish intermediate in size between the juveniles and adults. While I cannot completely dismiss the possibility of biased sampling of returning adults, ODFW personnel endeavored to sample size-classes of carcasses in proportion to their abundance. Therefore, I concluded that the distributions shown in

Fig. 5-3 are consistent with the hypothesis of size-selective mortality although this cannot be shown conclusively. Smaller fish apparently were removed at a greater rate during oceanic or late estuarine life. Size-selective mortality during ocean life has also been noted by Healey (1982a) for chum salmon and by Parker (1971) for pink salmon, with predation suggested as the most likely source of mortality. However, the possibility of size-selective fishing mortality exists as a troll fishery takes place near the mouth of the Sixes River (Bottom, pers. comm.).

There was no evidence of size-selective mortality during the estuary rearing period (Fig. 5-3) although my data do not allow me to quantify total mortality to all size-classes, which may be proportionate across all sizes. High mortality due to predation seems unlikely however, since the only potential predators of chinook salmon in the Sixes River estuary were steelhead trout (Salmo gairdneri, abundant only in May-June) and sea-run cutthroat trout (Salmo clarki, Bottom et al 1983).

The mean size of fish entering the estuary back-calculated from adult otoliths was considerably more variable (coefficient of variation = 27.5%) than that calculated from otoliths of juveniles (coefficients of variation were 21.8 and 11.1% for early and late recruits to the estuary respectively). The increased variability was unexpected, as size-selective mortality alters the variability of the back-calculated length-frequency distributions only slightly or not at all

(Ricker 1969). Identification of freshwater and estuarine otolith microstructure was somewhat more difficult in adult otoliths than in juveniles as a result of greater opacity and may therefore constitute a measurement error in the back-calculation procedure. Alternately, it may be that the five different juvenile life history types thought to occur in Sixes River chinook salmon (Reimers 1973) are represented to a greater extent in returning adults than in juveniles collected in the estuary. For example, fish which went directly to sea a few weeks after emergence (Reimers' Type 1 life history) might have been underestimated in the samples of juveniles caught in the estuary. However, the existence of the Type 1 life history is hypothetical, as no examples have been found either by Reimers or myself. Fish which had overwintered in freshwater and migrated directly to the ocean might also contribute to the variance in back-calculated sizes shown in Fig. 5-3. However, as indicated earlier, only 8 of the 452 adults whose ages were determined by ODFW personnel had overwintered in freshwater as juveniles. Straying of adults of other stocks to the Sixes River stock could also be responsible for the results shown in Fig. 5-3. While some chinook salmon from the Elk River are known to stray into the Sixes River, their number is considered insignificant (Bottom, pers. comm.).

On average, fish which were large on entering the estuary remained larger relative to the rest of the cohort until formation of the first annulus. Given the possibility of size-selective predation once fish have left the estuary, larger size at entry to the estuary may then be a predictor of future survival. The consistency with

which fish retain their position within the size hierarchy of the cohort (Fig. 5-4) suggests that little or no differential growth was evident among juveniles in the estuary during their first year. West (1983) also found that the rank of a sockeye salmon fry within the size heirarchy of the cohort was retained on average, as the fish grew from emergence through to smoltification.

Male chinook salmon juveniles which grew quickly in the estuary and ocean up to the formation of the first annulus matured earlier, on average. Schlucter and Lichatowich (1977) also found that on the basis of scale examination of Rogue River (Oregon) chinook salmon, the size attained at the end of the first year influenced subsequent growth and age at maturity. In terms of life-history strategy, early-maturing males appear to be subject to a lower mortality rate due to their larger size as juveniles and the duration of the period of vulnerability to natural mortality prior to reproduction is less. However, small males may be at a disadvantage when spawning, as observations by Hanson and Smith (1967) indicated that jack sockeye salmon (O. nerka) are often incapable of defending a single spawning female from other males, while large males may defend several females at once. In summary, it seems that different rates of growth as juveniles promote different ages at maturity, a feature which probably helps ensure the long-term survival of the stock (Schaffer 1974).

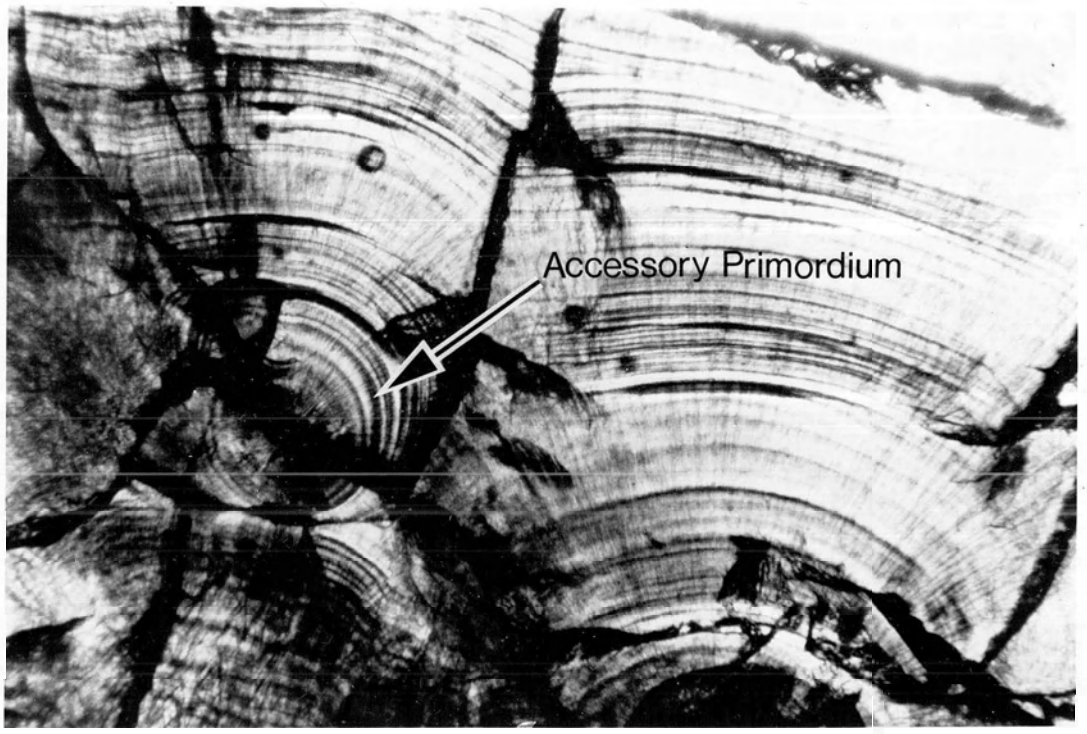
A possible long-term consequence of the size-selective mortality postulated here might be that the fish which grew quickly as juveniles

were at a selective advantage. In addition, as fast-growing males reach sexual maturity sooner on average, the period of vulnerablity to natural mortality prior to reproduction is shorter. However, reproductive success once at the spawning grounds is another important aspect of fishes' fitness and smaller fish may be at a disadvantage in that regard. Hanson and Smith (1967) indicated that jack sockeye salmon are often incapable of defending a single spawning female from other males, while large males may defend several females at once. Gross and Van Den Berghe (in press) have also noted that the lifespan of jacks on the spawning grounds is significantly shorter than that of the older males. While it is difficult to quantify survival to reproduction and reproductive success as factors affecting fishes' fitness, the persistence of jacks and older males as alternate life history strategies suggests that the fitness associated with each is equal. Possible reasons why faster-growing females do not return to spawn earlier as do males might include the inability of smaller fish to construct redds of sufficient depth. Alternatively, as smaller females are known to produce smaller eggs, the resulting smaller fry might be subject to higher mortality (Ricker 1978).

Examination of otolith microstructure allows greater resolution of age and growth and related problems in chinook salmon than was previously possible using scales. However, it appears that applications of otolith microstructure for detailed study of age and growth are limited to the period prior to the formation of the first

annulus. During that period, new centres of otolith growth which I term accessory primordia arise (Fig. 5-6), resulting in a variable and often intersecting array of growth increments. The value of otolith microstructure for interpretation of fish growth beyond the first year of life may therefore prove minimal in species with similar otolith development.

Figure 5-6 Micrograph of a polished sagittal section of an otolith from a Sixes River adult chinook salmon, showing centres of otolith growth (accessory primordia) that arise at or before the time of the formation of the first annulus.


Accessory Primordium
50 μm

GENERAL DISCUSSION

A major portion of my thesis research was concerned with determining how environmental factors influenced otolith increment production. The chemical composition of increments or biochemical processes responsible for their formation therefore were not significant topics of my research. However, a review of those topics of my research is useful to identify plausible pathways of increment formation which may be studied furthered.

When otoliths of chinook salmon were viewed with the SEM, a 24-h sequence of increments usually consisted of a relatively wide, lightly etched zone followed by a narrow, deeply-etched zone. Under high magnification (>1000X), the crystal structure was apparent, with the long axis of the crystals oriented perpendicularly to the growth increments. The crystalline structure of the lightly-etched zone is thought to be largely calcium carbonate in the aragonite configuration (Mugiya et al 1981). Dunkelberger et al (1980) have shown that the narrow, deeply-etched zone is rich in a protein matrix. The matrix structure is sheet-like, with tightly-packed fibers 80 Å in diameter. As discussed in Chapter 3 and by Mugiya et al (1981), the calcium and protein dominant zones may be alternately deposited as a result of a diel rhythm of calcium deposition.

The cyclic nature of Ca^{++} deposition has been elucidated by workers using the radioisotope ^{45}Ca. Mugiya (1974) showed that macular cells secreted ^{45}Ca into the endolymphatic fluid surrounding

parts of the otolith was proportional to the concentration of adjacent macular cells. Mugiya et al (1981) demonstrated that the rate of calcium deposition on goldfish (Carassius auratus) otoliths slowed around sunrise, possibly as a result of reduced macular cell secretion.

Given that $CaCO_3$ is the major constituent of the otolith, aspects of fishes' calcium metabolism and its control should affect otolith growth. In higher vertebrates, calcium metabolism is controlled through the action of parathyroid hormone, vitamin D and calcitonin. Of the three compounds, only Vitamin D and calcitonin are known to be present in fish. While the role of these compounds is not known (Simkiss 1973), it seems clear that calcium homeostatis is under endocrinological control (Fleming 1967). Simkiss (1973) has speculated that scale growth may closely reflect the level of growth hormone. Such relationships have not yet been advanced for otoliths. Indeed, parallels between scale and otolith growth are difficult to identify. For example, scale growth ceases under conditions of food deprivation and in cases of severe stress, resorption may occur. Similar phenomena have not yet been documented for otoliths. Otoliths continue to grow under conditions of food deprivation (Marshall and Parker 1982; Volk et al in press; Chapter 3). Fish stressed by exertion (Campana 1983) or by exposure to low pH (Geen et al unpublished) did not show evidence of otolith resorption. It is possible that seperate pathways of calcium accretion exist for the two structures.

Pannella (1980) summarized the knowledge of calcification processes affecting periodic growth patterns in fish otoliths and came to the following conclusions: (1) although chemically different from bones and scales, otoliths appear to be affected by periodic variation in diffusible calcium in a similar manner to that of bones and scales; (2) an organic precursor is necessary for calcium deposition in fish skeletal tissues incuding otoliths; (3) endolymph physiochemical changes control the deposition of organic and inorganic components of otoliths and (4) feeding activities appear to affect endolymph Ca^{++} concentration and therefore the growth of otoliths, a conclusion supported by my laboratory studies in Chapters 2 and 3. In Chapter 3, I suggested that there may be a relationship between increment formation and cyclic periods of activity associated with periodic feeding events. Pannella (1980) speculated that a close relationship existed between the sharpness of increment boundaries and the nature of the physiological transition between cycles of activity and rest. Pannella further indicated that in those species known to be almost constantly active, increment separations are faint or indistinct. These observations are consistent with my observations regarding the effects of activity on increment production.

That otolith size reflects fish size so closely serves to underscore its utility for growth studies relative to other parts such as scales. The conservative nature of otolith growth compared with scale growth may reflect the functions of each: the otolith as an

organ of equilibrium presumably must maintain a precise configuration with respect to other parts of the fishes' inner ear whereas the scales' function as part of the integument may permit greater latitude in functioning as part of the calcium reserves.

In addition to providing better indicators of fish growth in some salmonid species (Jonsson and Stenseth 1977), the effects of environmental variables on growth of otoliths appear better understood than is the case for scales (Table 6-1). This probably reflects the convenience of studying phenomenon with daily periodicity rather than monthly or longer, as in scale circuli. However, scales will probably continue to be an important source of age and growth data in the future. The advantages of use of scales include ease of preparation and examination relative to otoliths and fish need not be sacrificed to obtain them.

A concern regarding the application of otolith microstructure examination to wild populations is the apparently large number of environmental stimuli which affected increment number and width under laboratory conditions (Chapter 2 and 3). In nature, it seems likely that many cyclic phenomena of various periods exist which could cause deviation from the one increment every 24 h relationship. Yet juvenile chinook salmon in the Sixes River estuary formed increments every 24 h, on average, possibly in response to a circadian rhythm of calcium carbonate deposition. It may be that while there is a variety of possible zeitgebers in the environment, the endogenous rhythm of

TABLE 6-1

Summary of Effects of Selected Variables on Formation of Otolith Increments and Scale Circuli in Pacific Salmon

Response to:	Ration and Feeding Frequency	Water Temeperature	Photoperiod	Stress	Age-related Effects
Formation of Otolith Increments	Otolith increments are formed through short-term periods of food deprivation (Ch. 3; Marshall and Parker 1982); Feeding frequency positively correlated with rate of increment production (Ch. 2 and 3).	Increment width reflects fish growth in response to water temperature. Cyclic water temperature regimes not necessary for daily increment production, although more than one fluctuation every 24 h increased the rate of increment formation (Ch. 3).	Cyclic photoperiod not necessary for increment production, although varying photoperiod may influence increment width (Ch. 2).	No evidence of cessation of increment production or resorption (Campana 1983; Geen, Bradford and Neilson unpub.), reduced increment width appears to reflect reduced growth of stressed fish (Geen, Bradford and Neilson unpub.).	Complex patterns o increment depositi in fish older than approximately one year renders their interpretation difficult.
Formation of Scale Circuli	Number and spacing of circuli positively correlated with ration level. During periods of food deprivation, scale circuli are not formed or resorbed (Bilton 1974)	Not known in Pacific salmon.	Changing photoperiod influences rate of circulus formation (Bilton and Robins 1971).	Resorption may occur as mature fish return to freshwater, a particular problem in chinook salmon (Y. Yole, pers. comm.).	Scales not present in fry of some species, but useful for later life history stages.

the animal may only register as zeitgebers cylic cues which have a certain period, amplitude or phase. From an energetic perspective, it may be advantageous to be in phase with the most constant zeitgeber to allow fish to concentrate feeding behaviour, for example, at critical times of the day. If circadian rhythms serve primarily to concentrate appropriate behavior at certain times of the day, cyclic cues not in phase with the optimum activity periods might not influence fish. Such a phenomenom is known as frequency demultiplication (Marler and Hamilton 1967; Aschoff et al 1975). Frequency demultiplication might explain why apparently only one increment was formed every 24 h in the otoliths of Sixes River chinook salmon, despite the possibility of zeitgebers with a period less than 24 h. However, as discussed in Chapter 4, the presence of such zeitgebers is questionable. Temperature fluctuations in the estuary were irregular and fish appeared to be feeding more or less continuously. In the former case, the irregular period of the temperature cue disqualifies it as a potential zeitgeber entraining a regular endogenous rhythm. As there was no periodicity in feeding detected comparable to the laboratory regimes, it may not be surprising that no difference in otolith microstructure was apparent between fish receiving one feeding every 24 h in the lab and fish caught in the Sixes River estuary.

In conclusion, otolith microstructure examination has particular utility for detailed study of the age and growth of juvenile Pacific salmon. The validation of this method offers a new tool for fisheries

managers. Some possibilities might include detailed assessment of enhancement measures such as lake fertilization or control of predators and/or competitors. Examination of increment widths before and after the management measures were enacted would indicate whether measures resulted in improved fish growth. The importance of certain habitats such as estuaries may now be determined with greater precision. In general, growth during the first year of life of Pacific salmon, a phase considered critical to subsequent survival, may be assessed in more detail than was previously possible.

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