THE USE OF OTOLITH DAILY INCREMENT WIDTHS TO ESTIMATE THE SIZE AND GROWTH OF JUVENILE CHINOOK SALMON (<u>ONCORHYNCHUS</u> <u>TSHAWYTSCHA</u>)

by

Michael J. Bradford

B.Sc. Simon Fraser University 1981

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
in the Department
of
Biological Sciences

SIMON FRASER UNIVERSITY

November 1985

APPROVAL

NAME:	Michael J. Bradford
DEGREE:	Master of Science
TITLE OF THESIS:	The use of otolith daily growth increments to estimate the size and growth of juvenile chinook salmon (*Oncorhynchus tshawytscha*).
EXAMINING COMMITTEE:	
Chairman:	Dr. L.M. Dill
	Dr. G.H. Geen, Senior Supervisor
	Dr. R.M. Peterman
	Dr. C. Levings, Fisheries & Oceans
	Dr. E.B. Hartwick, Public Examiner

Date approved 18 November 1985

Title of Thesis/Project/Extended Essay

The use of otolith daily growth increments to estimate the size and growth of juvenile chinook salmon (*Oncorhynchus tshawytscha*).

Author:

(signature)

Michael J. Bradford

(name)

Nov. 28 1985

(date)

ABSTRACT

Daily growth increments of fish otoliths are potentially very useful in the study of age and growth of larval and juvenile fishes. I assessed the utility of otolith daily increment widths in estimating the size and growth of juvenile chinook salmon (*Oncorhynchus tshawytscha*). For fish reared in the laboratory under constant environmental conditions back-calculations of length made at 10-20 day intervals over a 120-day experiment were accurate on 8 of 9 occasions. Growth of individual fish over 7-15 day intervals was not accurately reflected by increment widths. Over 50 days, however, estimated and observed growth rates of individual fish were highly correlated.

In a second series of experiments fish were reared under changing conditions of temperature and ration to determine if increment widths could be used to estimate growth in more realistic growth conditions. Otolith growth responded independently of fish growth to changes in water temperature. The effects of reduced ration on increment widths were lagged and resulted in poor estimates of growth. Increment widths reflected both past and present growth rates and environmental conditions.

In the final section the growth of chinook salmon rearing in the Campbell R., British Columbia estuary was examined with the techniques developed in the laboratory experiments. Indirect evidence indicated that one increment was formed per day.

Back-calculated lengths revealed no evidence of size-selective mortality acting on the estuary rearing population. Increment width followed changes in both temperature and invertebrate abundance but was not related to fish density.

These results indicate that very detailed examinations of growth may not be possible because of the conservative nature of otolith in relation to fish growth. Increment widths should be examined with environmental factors as the literal interpretation of increment widths as measures of growth may be inaccurate because of the interactive and lagged effects of environment and fish growth on otolith growth.

ACKNOWLEDGEMENTS

I would like to thank my senior supervisor, Dr. Glen Geen, for originally suggesting this project, and for providing scientific, editorial, and moral support throughout its completion. I also thank the members of my committee for their assistance. The field portion of this work would not have been possible without the generosity of Dr. Colin Levings in allowing me to take part in the Campbell River project. Dr. Randall Peterman provided analytical and editorial assistance and allowed me to complete the writing while I was under his employ.

I thank Dr. C. MacAllister, Dr. S. MacDonald, T. Brown, and M. Kotyk of the Canada Department of Fisheries (DFO) and the staff of the C.R. Lodge for assistance in the field. I thank the staff of the West Vancouver lab for their help in running the laboratory experiments.

This thesis would not have been possible without the research of Dr. John Neilson, and I am grateful for his tutelage on technical and analytical aspects. Collette O'Reilly prepared most of the otoliths, and Dr. V. Bourne was helpful with microscopic and photographic matters. The financial support of DFO is gratefully acknowledged.

This thesis is dedicated to Louise Fockler whose patience and support kept me going. I also thank the B9219 gang and the Farsiders for the frequent injections of levity that have kept me (somewhat) sane over the years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

The processes which determine the survival of a brood of salmon are usually thought to occur in their first year of life (Parker 1968). Growth is an important factor during this period, as survival has been shown to be positively related to size at smolting (Bilton *et al*. 1982) and during the first few months of marine life (Healey 1982).

Accurate estimates of growth during the first year are sometimes difficult to obtain using traditional methods. Size-frequency analysis is often inaccurate because of biasses due to the presence of multiple cohorts, and size-dependent migrations and mortality (Healey 1980a; Volk *et al*. 1984). Back-calculations using annular structures can only provide information about size at the first year; details of growth growth during the first year are not possible.

The recent discovery of daily increments in teleost otoliths provides considerable potential (Panella 1971; Campana and Neilson 1985) as a tool for the analysis of the size and growth of juvenile salmonids. Teleost otoliths are acellular bones composed of a protein matrix overlain with calcium carbonate crystals in the aragonite form (Degens *et al*. 1969). Otolith growth results from the deposition of protein and calcium secreted from the surrounding macular cells onto the surface of the otolith. Daily growth increments are visible in the microstructure of otoliths because the two major constituents of the otolith, calcium and protein, are deposited at different rates. A daily growth increment consists of a calcium-rich "incremental" zone, and a protein-rich "discontinuous zone" (Campana and Neilson 1985). Calcium deposition rates have been shown to be higher during the day, when the incremental zone is formed, and decrease at night when the discontinuous zone is produced (Mugiya *et al*. 1981). There are three pairs of otoliths in the inner ear of teleost fish; throughout this thesis 'otolith' will refer only to the sagittae, the largest of the three pairs.

A prerequisite to the application of any ageing technique is to determine the periodicity of formation of the structure and the robustness of that periodicity to various environmental conditions (Beamish and MacFarlane 1983). For juvenile salmon reared in the laboratory under most conditions of photoperiod, temperature and ration, one increment is formed per day (Neilson and Geen 1982,1985; Campana 1983; Volk *et al*. 1984). More than one increment can be produced per day if fish are subjected to cycles of higher frequency temperature, activity or feeding (Neilson and Geen 1982,1985). In some cases these "subdaily" increments can be distinguished from daily increments by their appearance (Campana 1983). In the field, Neilson *et al*. (1985a) were able to demonstrate in a population of estuary rearing chinook salmon (*Oncorhynchus tshawytscha*) one increment was formed per day.

Two types of data are available from otolith microstructure examination; counts of increments, and measurements of increment widths (Brothers 1981). For species where the otolith forms near hatching, increment counts can be related directly to fish age (e.g. Lough *et al.* 1982). In juvenile salmonids, however, the otolith is formed well before hatching so that total increment counts are not reflective of fish age unless the date of first increment formation is known. (Neilson *et al.* 1985b). In addition, in field populations the first increments are formed during the winter months when water temperatures are low and are often very narrow and faint, making them difficult to interpret (Bradford unpubl. data). Meaningful estimates of age are possible if an identifiable mark on the otolith corresponds to a known life history event; for example Neilson *et al.* (1985) used an estuarine entry mark on the otolith to determine the duration of estuary rearing in juvenile chinook salmon.

Otolith increment widths can be used to back-calculate growth rates and fish sizes using techniques analagous to those used for annular structures. Wilson and Larkin (1982) back-calculated the weights of sockeye salmon fry (*O. nerka*) over a 54-day experiment and observed an average 15% error in the back-calculated sizes of individuals compared to their observed sizes. Other workers have used this technique without verification.

The allometric relationship between fish size and otolith size (Neilson and Geen 1982; Wilson and Larkin 1982 and others) also implies that increment width should be, on average, proportional to daily fish growth rate. Mean increment widths have been correlated with growth rates for fish reared under constant environmental conditions over >30 day experiments (Volk et al 1984; Neilson and Geen 1985). Campana (1984) correlated increment widths of juvenile starry flounder otoliths (*Platichthys stellatus*) to environmental factors over a 60-day period. Fish growth was not, however, compared to increment widths, to assess the accuracy of growth rate estimates. Whether increment width can be used to estimate growth when environmental factors continuously change has not been determined.

As noted by Campana and Neilson (1985), there is still considerable uncertainty over the accuracy of growth rates and back-calculated sizes estimated from otolith increment widths. In the first two chapters of this thesis I describe laboratory experiments that attempted to define the accuracy of growth rates and back-calculated sizes derived from the increments widths juvenile chinook salmon otoliths. Specifically the experiments were designed to determine:

1. If accurate back-calculations of length can be made from microstructure measurements;
2. If increment widths accurately reflect growth rates of individual fish over short time intervals;
3. If increment widths can provide accurate estimates of growth rates when growth is varied by changing environmental factors.

Using the results of these experiments as guidelines, in the final chapter I examined the size and growth of chinook salmon fry in Campbell R., B.C. estuary. My objectives were to examine the factors which regulate growth, and determine if density-dependent growth or size-selective mortality was occurring. In addition I compared my laboratory results to the field data to assess the applicability of the laboratory studies.

CHAPTER 1: SIZE AND GROWTH OF JUVENILE CHINOOK SALMON BACK-CALCULATED FROM OTOLITH GROWTH INCREMENTS

Introduction

Otolith daily increment widths have the potential to provide estimates of daily growth. While the increment deposition rate has been confirmed as daily in some species, the reliability of estimates of short-term growth rates or back-calculations of size-at-age based on otolith microstructure data have rarely been tested. If increment widths are to provide information about growth rates during the early life of fish, a close coupling of fish growth and otolith growth will be required. To date, increment widths have only been related to average growth rates for groups of fish measured after longer-term experiments (>40 days, Volk et al. 1984; Neilson and Geen 1985). Few studies have assessed the accuracy of size back-calculations, although Wilson and Larkin (1982) reported a 15% error in the back-calculated weights of individual juvenile sockeye salmon.

In this chapter I report experiments which enabled me to examine the accuracy and precision of lengths back-calculated from otolith increment measurements of juvenile chinook salmon. In addition, I compared observed growth rates of individual fish to estimates derived from otolith increment widths to determine if these microstructure features accurately reflected the growth of individual fish over shorter time intervals.

Methods and Materials

Chinook salmon fry (mean fork length 60 mm) from the 1982 run to Campbell River, British Columbia were transported from the Quinsam Hatchery to the Canada Department of Fisheries and Oceans West Vancouver Laboratory on March 25, 1983. They were placed in 400-liter tanks supplied throughout the experiments with 10-11° C aerated well water. A constant ration of 8% of dry body weight of Oregon Moist Pellets was provided once per day. After 5 days fry were divided into two groups:

Treatment 1: 180 fish kept for 120 days. Approximately 40 fish were sacrificed on days 30,60,90 and 120.

Treatment 2: 40 fish held for 67 days, and sacrificed at the end of the experiment. Fish in this group were individually marked on day 1 with wire brands cooled in liquid nitrogen.

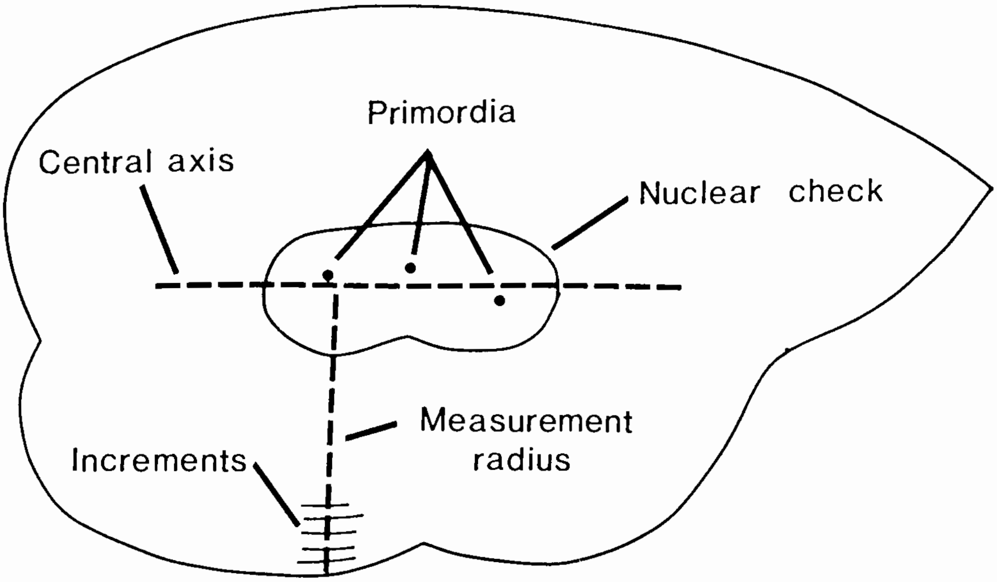
On day 1, and at approximately 14-day intervals thereafter, all fish from both groups were anaesthetized with MS 222 and their fork lengths measured.

Left sagittal otoliths were removed from the fish which had been sacrificed and were prepared for examination as described by Neilson and Geen (1981). Otolith radius and increment widths were measured along a standard radius, 90° from the long axis of the otolith on the pararostral side (Fig. 1.1). Otolith radius was measured from the cental axis of the nuclear area to the outer edge of the otolith, using a compound microscope equipped with an ocular micrometer (Fig. 1.1). Photomicrographs were taken along the standard radius. The resulting negatives were projected on a screen and the location of the increments were marked on a paper strip. Increment widths were then measured on a digitizing tablet as described by Neilson and Geen (1982). Increments were measured from the edge of the otolith inward until they became unreadable due to preparational problems.

I did not test the rate of increment formation in these experiments because I did not have a time marker on the otolith to make counts from. I assumed increments were formed daily, based on the experiments of Neilson and Geen (1982,1985), who found an average of one increment produced per day for juvenile chinook salmon reared under similar conditions.

A predictive relationship of the form $L = a + bR^2$ was fitted to the fork length (L) and total otolith radius (R) data from fish in Treatments 1 and 2. To back-calculate fish length, the otolith radius on the measurement dates was calculated by summing increment widths from the edge of the otoliths inward to the increments formed on previous measurement dates. That sum was subtracted from the total radius to give the radius of the otolith on the measurement dates. For each fish back-calculated mean length was estimated at each measurement date by inserting the otolith radius into the otolith radius-fish size regression equation. For Treatment 1 individual back-calculated fish lengths were averaged and compared to the mean lengths of all fish in the tank at each measurement date.

Figure 1.1: Diagram of a prepared chinook salmon otolith, showing the central axis and the radius along which increment measurements were made.


Primordia
Central axis
Nuclear check
Measurement radius
Increments

The marking of individual fish in Treatment 2 allowed the comparison of growth rates and lengths back-calculated from increment measurements to observed lengths and growth rates of individual fish, rather than the group means that was done in Treatment 1. Back-calculated and observed lengths of individual fish were compared with paired t-tests. Observed growth rates between measurement dates were calculated from the measured lengths. Daily growth in length was estimated from otolith measurements as the product of the derivative of the otolith radius-fish length relationship and increment width: $G = 2bRI$, where G is fish growth as mm/day, b is the coefficient of the fish length-otolith radius regression, R is the otolith radius at which the increment measurement was made, and I is the width of the increment. Daily growth estimates were averaged over the intervals between measurement dates and compared with observed growth rates using correlation analysis.

Results

Although the curvilinear relationship used to fit the otolith radius-fish length data was not significantly better than a simple linear regression (F test, P>0.1), the curvilinear did lower the the error sum of squares of the regression (Fig. 1.2). Data from the 4 samples of Treatment 1 and the single sample of Treatment 2 were pooled, as the range of fish lengths in any single sample was insufficient to form a regression.

The number of increments measured from otoliths of the four Treatment 1 fish samples was greater than the number of days between samples so that otolith radius data were available for more than one sample on 7 of 9 measurement dates. Because the samples were drawn randomly from the tank I expect that the mean radii of the samples would not be different from each other at the measurement dates. Significant differences might indicate that errors in reading or interpretation of the increments had occurred. No significant differences existed in any of these comparisons (t-tests, all P>0.10); consequently data from all samples were pooled to back-calculate mean fish length (Fig. 1.3).

Back-calculated lengths were not significantly different from the observed mean lengths on 8 of 9 occasions in Treatment 1 (Table 1.1). Back-calculated length was different than observed length at the end of the experiment when there was a rapid increase in fish growth. The back-calculated size-at-time

Figure 1.2: Relationship between fish fork length and otolith radius for all chinook salmon used in the experiments. Equation fitted was $Y=42.2+0.000138X^2$, $N=141$, $r=0.87$: ● Treatment 1 fish, ◻ Treatment 2 fish.

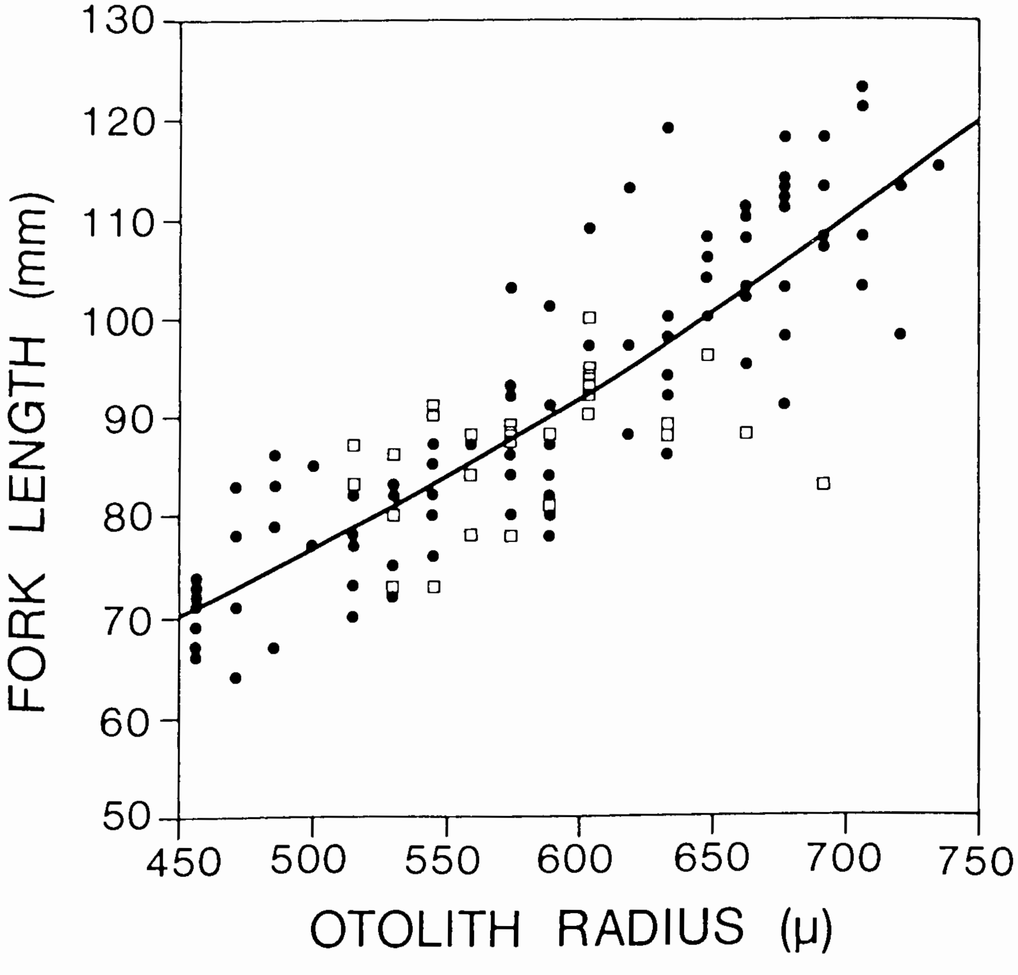

FORK LENGTH (mm)
130
120
110
100
90
80
70
60
50
450
500
550
600
650
700
750
OTOLITH RADIUS (μ)

Table 1.1: Back-calculated and observed mean fork lengths (mm) of juvenile chinook salmon in Treatment 1. Back calculated data are from all available otoliths, observed sizes are from all fish in the treatment. Significant difference between observed and back calculated lengths indicated as * (P<0.05).

	Back-calculated			Observed		
Expt. day	N	Length	SE	N	Length	SE
0	22	61.8	1.15	83	64.9	0.54
15	35	66.8	0.85	149	67.9	0.45
30	36	71.5	0.85	149	71.5	0.49
45	24	78.0	1.59	120	76.8	0.59
60	37	82.8	1.18	118	80.3	0.64
81	37	90.1	1.17	69	89.1	0.88
91	38	93.4	1.15	60	94.9	0.94
105	24	98.2	1.18	33	98.9	1.16
120	24	103.9	1.15	33	109.8*	2.28

curve was less variable than the actual size curve (Fig. 1.3).

The mean difference between back-calculated and observed fork lengths of individual fish in Treatment 2 were not significantly different from 0 on any of the 6 measurement dates (Table 1.2, all $P>0.1$) The difference between back-calculated and observed lengths of individual fish in Treatment 2 ranged from -8 to +19 mm, with an overall standard deviation of 5.64 mm. The 95% confidence limits (±2SD) of 11.3 mm on the back-calculated length of individual fish represents a 9-19% error over the size range of fish used in this experiment.

Growth rates calculated from increment widths and observed growth rates of individual fish were not significantly correlated in 4 of 5 intervals of Treatment 2, although the r^2 values were positive in 4 of 5 intervals (Table 1.3). Fish growth rates in these correlations ranged from 0 to 0.86 mm/day.

I examined the relationship between mean increment width and growth of individual fish over a longer time interval by comparing the calculated rates against the observed values averaged over the last 4 intervals of the experiment (51 days). The regression was significant ($P<0.001$, $n=24$) with the slope of 0.93 not significantly different from 1.0, the expected slope if increment width accurately reflected fish growth (Fig. 1.4).

Variability in growth rates calculated from increment widths among individual fish in Treatment 2 within each interval was much less than was observed for actual rates (coefficient of

Table 1.2: Back-calculated and observed mean fork lengths (mm) of juvenile chinook salmon in Treatment 2. BCL: back- calculated length, OL: observed length, SD: standard deviation of the difference between back-calculated and observed lengths of individual fish.

Day	N	BCL	OL	Difference	SD
0	6	67.2	66.1	1.13	6.21
15	14	72.8	71.8	1.04	6.36
30	25	77.0	75.4	1.63	4.17
45	30	81.7	80.0	1.72	5.37
60	30	86.2	82.8	3.37	5.87
67	30	88.3	88.3	-0.04	5.58

Table 1.3: Correlations between observed and increment width derived-growth in length of juvenile chinook salmon in Treatment 2. Last column is the range of observed values. Sample size (N) is smaller in earlier intervals because not all otoliths had useable increment records over the whole experiment. Significant correlation indicated, *P<0.05.

Interval	Days	N	r^2	Range
1	0-14	12	0.39	0.00-0.33
2	15-29	25	0.30	0.07-0.67
3	30-44	30	0.24	0.13-0.60
4	45-59	30	0.51*	0.21-0.71
5	60-67	30	-0.22	0.10-0.86

Figure 1.3: Back-calculated (●) and observed (◻) mean fork lengths of laboratory-reared juvenile chinook salmon in Treatment 1. Back-calculated lengths are from data pooled from the 4 samples taken over the experiment. Error bars indicate ±2 SE.

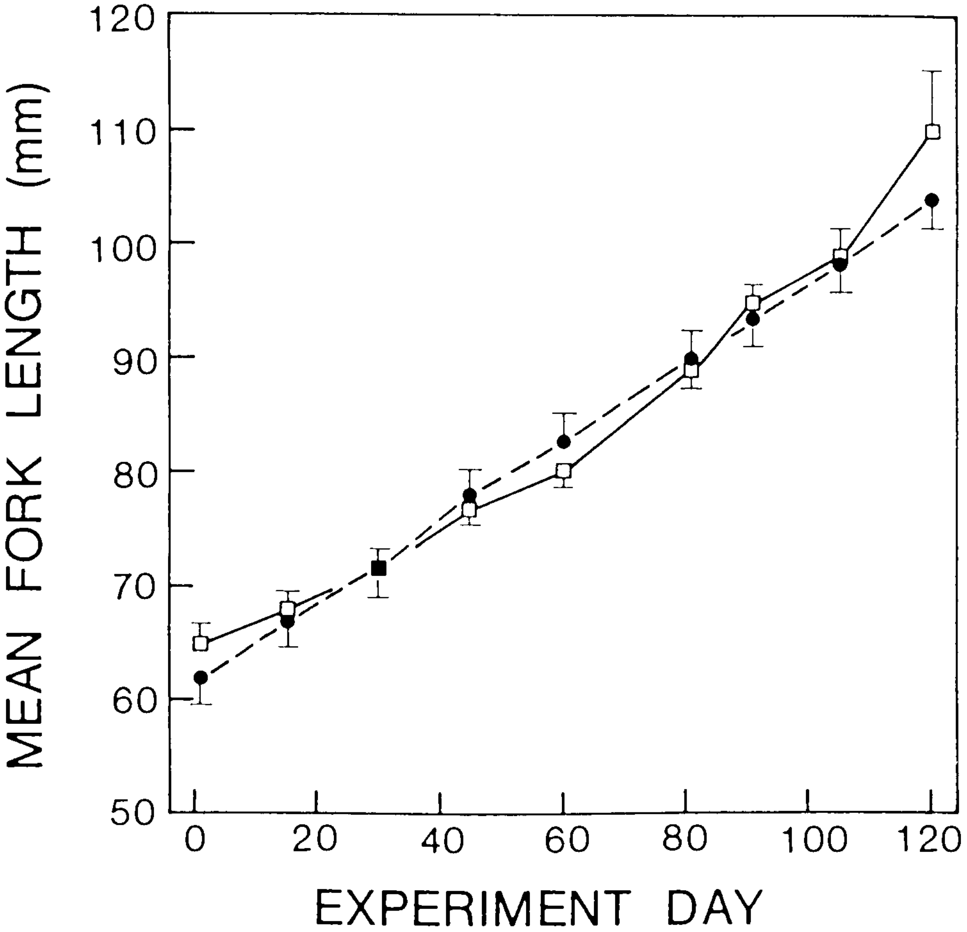
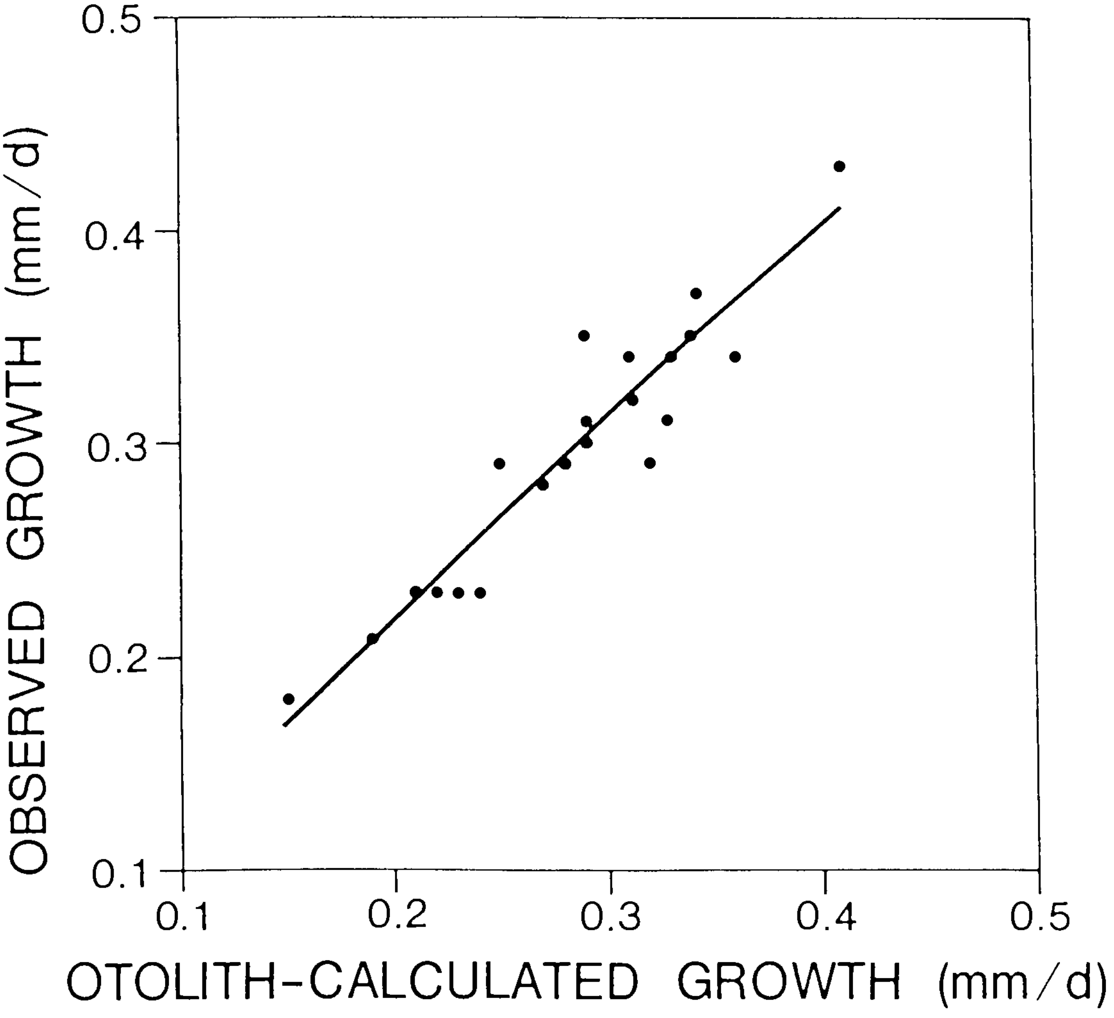

MEAN FORK LENGTH (mm)
50
60
70
80
90
100
110
120
0
20
40
60
80
100
120
EXPERIMENT DAY

Figure 1.4: Observed and back-calculated growth rates of individual chinook salmon in Treatment 2. Data are averaged over the last 51 days of the experiment. Equation of the line: $Y=0.97X-0.003$, $r=0.94$.


0.5
0.4
0.3
0.2
0.1
OBSERVED GROWTH (mm/d)
0.1
0.2
0.3
0.4
0.5
OTOLITH-CALCULATED GROWTH (mm/d)

variation: 16-20% vs. 29-55%), indicating that otolith growth was not as variable as body growth.

Discussion

Mean fish lengths back-calculated from otolith increment widths were generally accurate when compared to observed lengths. In Treatment 1, a significant difference occurred because otolith growth did not follow an unexplained increase in fish growth. Back-calculated mean fish lengths had 95% confidence limits 2-5% of mean length.

The 9-19% errors in the back-calculated lengths of individual fish were similar to the 15% error reported by Wilson and Larkin (1982) for back-calculations of juvenile sockeye salmon weight. The magnitude of these errors may limit the utility of size back-calculations of individual fish.

Otolith-derived growth rates did not exhibit the the same range of fluctuations as the observed growth rates. Although considerable variability exists between the widths of individual increments (e.g. Volk et al. 1984), growth rates derived from averaged increment widths were less variable than observed fish growth rates. Increases or decreases in fish growth rate were not necessarily coupled with corresponding changes in otolith growth. Thus size-at-time curves back-calculated from increment widths may not indicate the actual variation in fish growth.

Fish growth and otolith growth were not strongly correlated over the short sampling intervals of Treatment 2 (Table 1.3). Brothers (1981) suggested that the existence of an otolith size-fish size relationship is not sufficient to conclude that increment widths will closely reflect fish growth. The uncoupling of otolith and fish growth has been well documented for fish receiving little or no ration (Marshall and Parker 1982; Volk et al. 1984). In these cases otolith growth continued after fish growth halted. Even for the well-fed fish used in our experiments otolith growth and fish growth were not closely correlated for individual fish (Table 1.3). The scatter in the otolith radius-fish length relationship reflects the considerable variation that can occur in fish growth relative to otolith growth.

Over the long term, however, the relationship between otolith size and fish size implies that increment width must be related to fish growth. This was confirmed by the significant regression of actual fish growth on otolith-derived growth of individual fish for the final 51 days of Treatment 2. Significant correlations of fish growth rates and otolith increment widths have been found for groups of fish reared under different feeding regimes in the 40-60 day experiments of Volk et al. (1984) and Neilson and Geen (1985).

My results suggest that for individual chinook salmon increment widths will have to be averaged over at least 30-40 days for growth rate estimates to be accurate. When averaged over >50 days, it may be possible to resolve differences in growth between individuals of as little as 0.1 mm/day (Fig. 1.4).

The closeness of the coupling between fish and otolith growth will be species and fish-size specific. Smaller fish than those used in these experiments may have otoliths that respond more rapidly to changes in fish growth. In addition, the variability in the otolith size-fish size relationship is species specific (e.g. Fig. 1.2; Taubert and Coble 1977; Laroche et al. 1982) and would affect the reliability of growth estimates derived from increment widths.

Detailed examination of growth of juvenile fish based on individual otoliths or a short time-series of increments may have minimal value given the conservative nature of otolith growth in relation to fish growth. More accurate estimates of back-calculated size and growth rates may be possible over longer time intervals. The experiments reported here emphasize the need to carefully assess the reliability of fish size and growth estimates back-calculated from otolith increments in controlled experiments before field studies are undertaken with the object of reconstructing size and growth-rate histories.

CHAPTER 2: GROWTH ESTIMATES FROM OTOLITH INCREMENT WIDTHS OF FISH REARED UNDER VARIABLE ENVIRONMENTAL CONDITIONS

Introduction

In the experiments of Chapter 1 I found for fish reared under constant environmental conditions otolith growth tended to be insensitive to small short term fluctuations in fish growth. Over longer intervals, however, increment widths did provide an accurate record of growth.

If increment widths are to provide useful information on the growth of fish, otolith growth should vary in a consistent and predictable manner with fish growth. Increment width has been correlated with fish growth in experiments on juvenile salmon reared under constant environmental conditions for 30 or more days (Volk *et al*. 1984; Neilson and Geen 1985). Wild fish, however, live in an environment of changing temperature, salinity and food abundance, all of which may influence fish growth rate. It is not known if increment width can provide an accurate record of growth under natural conditions when fish growth is changing with environmental conditions.

In this chapter I examine the reliability of growth rates estimated from otolith increment widths of fish reared under conditions of changing ration and temperature. These two factors are probably the most important determinants of growth of juvenile salmonids (Brett 1979). These factors were varied within a range that wild chinook salmon fry might encounter, and observed growth rates in length and weight were compared to those obtained from increment widths.

Methods and Materials

Chinook salmon fry (mean fork length 65mm, mean weight 3.0g) from the 1983 Campbell R. brood were transported from the Department of Fisheries and Oceans (DFO) Quinsam hatchery to the DFO West Vancouver Lab in early March 1984. Four 200-L flow-through tanks supplied with 11°C well water were stocked with 115 fry each.

The experiment was originally planned to run for 60 days with a 20-day pre-treatment period (11°C water, 9% ration), a 20-day treatment period, as outlined below, and a 20-day post-treatment period (11°C water, 9% ration). A problem with the water supply between day 10 and 20 decreased water temperature to 7.5°C; the normal water supply (11°C) was restored on day 21. As a result the pre-treatment period was extended; the lengths of the 3 intervals were modified to 31,19 and 20 days. Ration was calculated as % of dry body weight and consisted of Oregon Moist Pellets, delivered once per day, between 1000-1200h.

The four treatments and their abbreviations used this experiment were:

a) constant water temperature (11°C), and a constant ration of 9%. This group served as the control treatment (CTCR).

b) constant temperature (11°C) and a reduced ration of 3% (CTRR).

c) warmed water (an average of 15.8°C, range 15.0-18.4°C) and a constant 9% ration (ITCR).

d) warm water as in c) and 14% ration (ITIR).

Random samples of 25 fish were sacrificed at day 0 and at the beginning and end of the treatment interval (days 31 and 50) from each tank. The remaining fish were sacrificed at the end of the experiment. Fork length to the nearest 1.0mm and wet body weight to the nearest 0.1g of each of the sacrificed fish was measured. Left sagittal otoliths were removed from sampled fish and prepared for examination as described by Neilson and Geen (1981). Total otolith radius was measured along the standard axis described in Chapter 1. Increment width measurements were taken from 22-24 otoliths from the final sample of each treatment using the techniques of Chapter 1.

I assumed, as in Chapter 1, that increments were formed daily, based on the results of laboratory experiments of Neilson and Geen (1982,1985) who found one increment formed per day for juvenile chinook salmon reared under similar conditions.

I used increment widths to derive both growth in length (mm/day) and instantaneous growth in weight (as % wet body weight/day) for comparison with observed rates. Fish growth in length was estimated from increments using the methods of Chapter 1. The observed instantaneous growth rate in weight was calculated from the fish weight data following Ricker (1975):

$$G=((\mathit{ln}W_2-\mathit{ln}W_1)/(t_2-t_1))100 \quad (2)$$

where W_1 and W_2 are wet weight measurements taken at t_1 and t_2.

Otolith radius measurements were converted to instantaneous growth rate (as %bw/day) by substituting the linear relation describing *ln* W as a function of *ln* R, otolith radius, for *ln* W in (2), yielding, after simplification:

$$G=\gamma(ln R_2-ln R_1)100$$

where γ is the slope of the *ln* fish weight-*ln* otolith length relation ($t_2-t_1=1$ when successive daily increments are used). Daily instantaneous growth was calculated for each day for each fish in the final sample and averaged over the 3 intervals for each of the 4 treatments.

Results

Lengths and weights of fish in the samples taken during the experiment are shown in Table 2.1. The group receiving reduced ration (CTRR) grew very little during the treatment period. Chinook salmon fry in the ITCR treatment were similar in size to the control group throughout the experiment, while fish reared in warmer water and receiving increased ration during the treatment period were slightly larger than the control group after the treatment period.

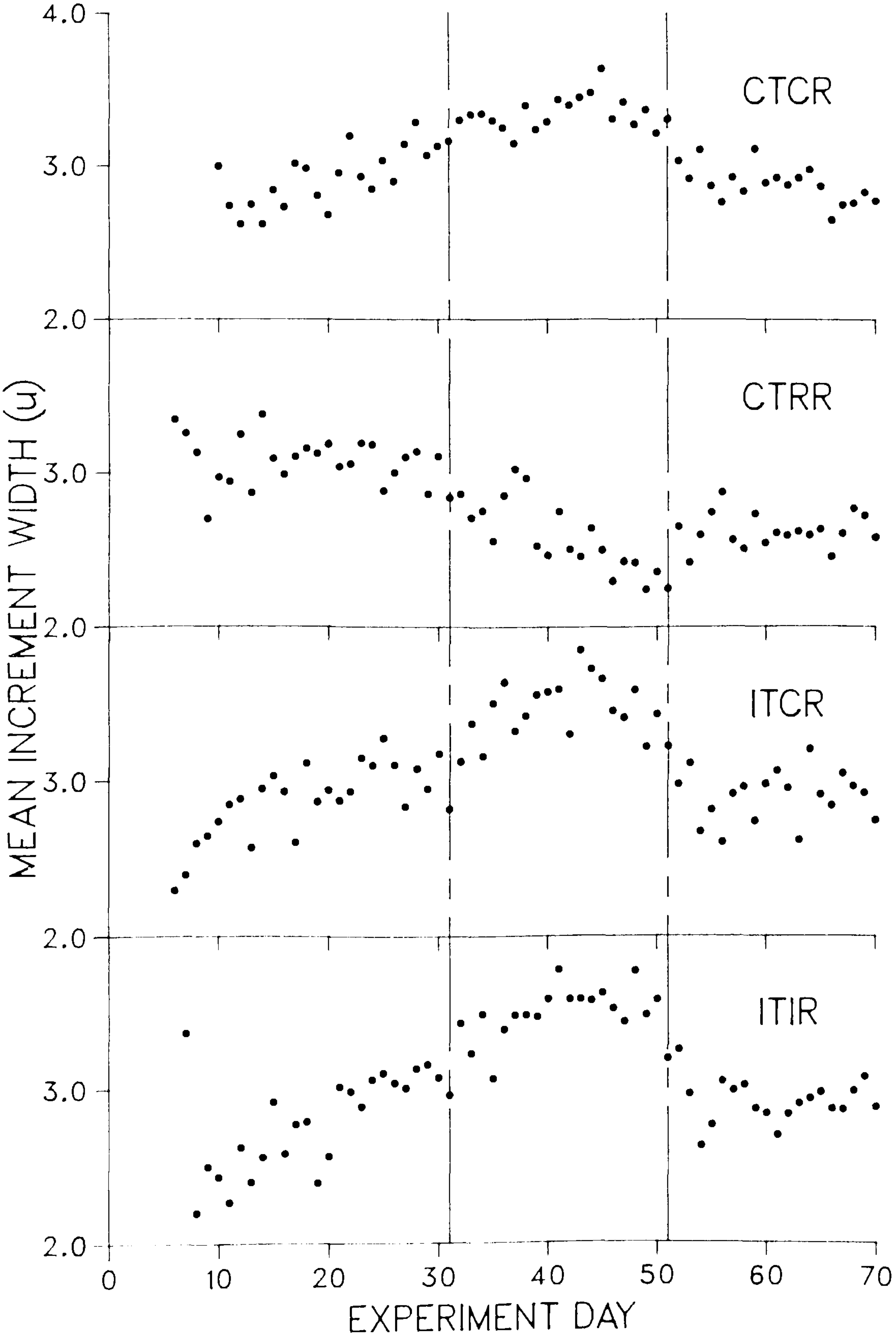
Linear relationships were found between fish length and otolith radius for all groups. There was no evidence of the curvilinearity in the data as observed in the Chapter 1 data. Slopes of the four groups were not significantly different from each other (P=0.103). Significant linear relationships were also found between fish weight and otolith radius when both variates were log-transformed. There was no difference between slopes of the regressions (P=0.2321). Regression slopes for each treatment were retained for the calculation of growth rates.

Mean increment width increased through the first half of the experiment and then declined over the last 30d in 3 of 4 treatments (Fig. 2.1). The increase in increment width during the pre-treatment interval may have been due to the the change in temperature due to the water supply failure. Mean increment width of the CTRR tank declined over the treatment period in response to the reduced ration; increment width increased

Table 2.1: Mean length and weight of juvenile chinook salmon in the 4 treatments at each measuring date (± standard error indicated). Days 32 and 51 denote the beginning and end of the treatment interval.

Tank	Day	N	Length(mm)	Weight(g)
CTCR	0	20	45.3±0.49	0.97±0.045
	32	22	53.5±0.59	1.83±0.070
	51	25	58.3±0.83	2.65±0.118
	71	53	65.2±0.65	3.29±0.107
CTRR	0	20	45.2±0.52	0.97±0.045
	32	21	53.5±0.59	1.83±0.082
	51	24	54.2±0.75	1.87±0.088
	71	55	61.8±0.68	2.77±0.095
ITCR	0	19	44.2±0.45	0.90±0.033
	32	21	52.6±0.74	1.70±0.085
	51	25	57.9±0.67	2.42±0.098
	71	55	66.8±0.61	3.25±0.092
ITIR	0	19	45.3±0.51	0.97±0.039
	32	21	53.3±0.66	1.69±0.057
	51	24	60.0±0.81	2.82±0.129
	71	56	67.8±0.91	3.63±0.156

Figure 2.1: Mean increment widths of otoliths of juvenile chinook salmon reared under various environmental conditions. Treatment abbreviations as in text; vertical lines separate pre-treatment (day 0-30), treatment (day 31-50), and post-treatment (day 51-70) intervals.


MEAN INCREMENT WIDTH (u)
4.0
3.0
2.0
3.0
2.0
3.0
2.0
3.0
2.0
CTCR
CTRR
ITCR
ITIR
0
10
20
30
40
50
60
70
EXPERIMENT DAY

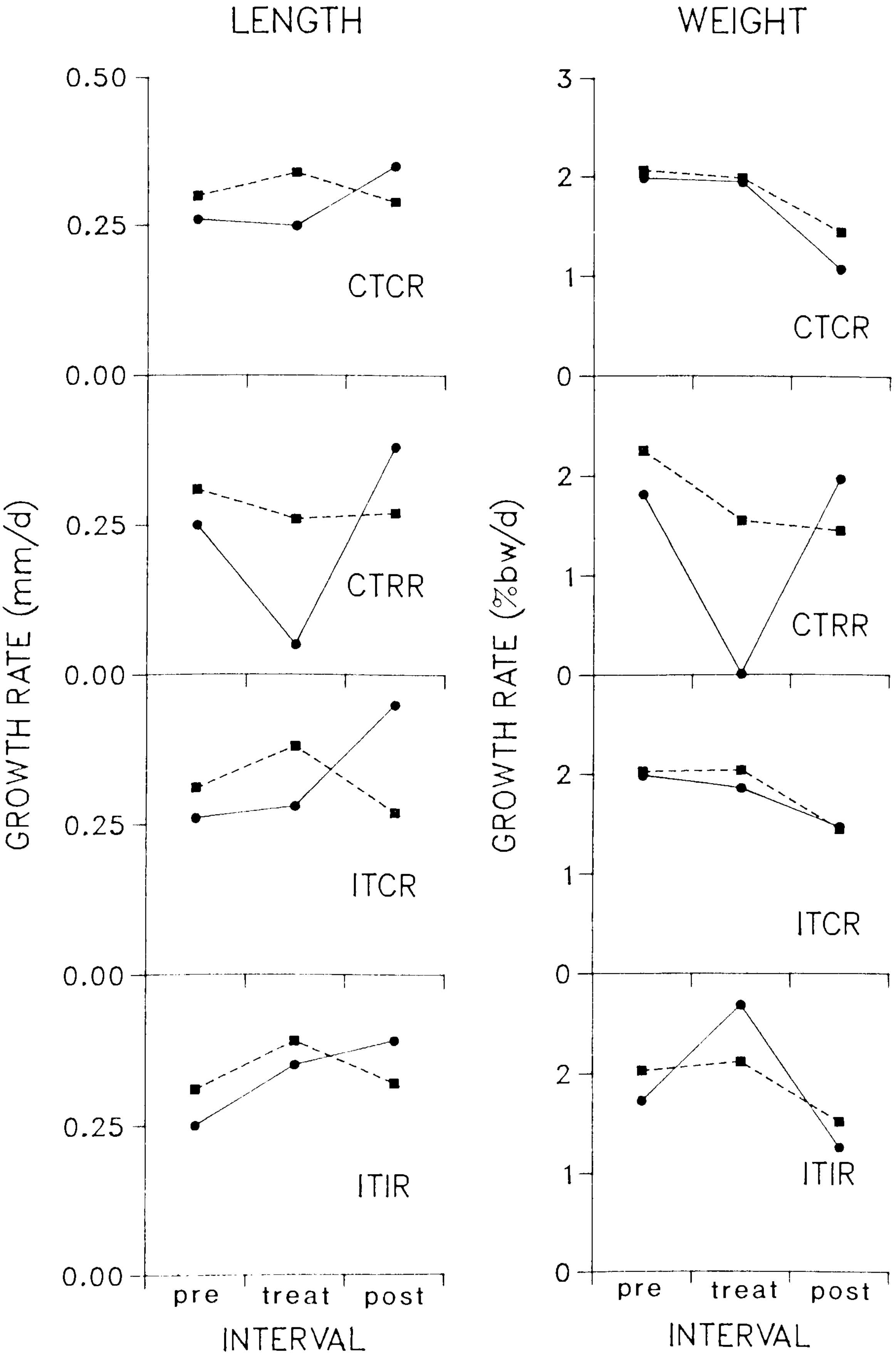
0.3-0.4μ 3-5 days after ration was restored (Fig. 2.1). Mean increment widths of the CTRR tank were significantly smaller than the control group in the post-treatment period (t-test, $p<0.0001$). Increment width patterns in fish from the two warmer water groups were very similar throughout the experiment. The average increment width of the ITCR and ITIR groups were both significantly greater than the control group for the treatment period (ANOVA, $p=0.0122$ and $p=0.0032$ for the ITCR and ITIR groups respectively), however they were not significantly different from each other.

Growth rates calculated from increment widths did not vary between intervals to the same extent as observed growth rates (Table 2.2, Fig. 2.2). With the exception of the CTRR group, during the post-treatment period growth in length increased while growth in weight declined. These trends in the observed growth rates were more accurately reflected in increment-derived growth in weight than growth in length (Fig. 2.2) The period of reduced growth in the CTRR treatment was not accurately reflected in either growth measure.

Table 2.2: Observed and otolith calculated growth rates of juvenile chinook salmon in the 4 treatments. Columns display actual growth in length (L), growth in length calculated from increment widths (L_0), observed growth in weight (G) and otolith-derived growth in weight (G_0).

Tank	Interval	L (mm/d)	L_0 (mm/d)	G (%bw/d)	G_0 (%bw/d)
CTCR	Pre	0.26	0.30	1.98	2.06
	Treat	0.25	0.34	1.95	1.99
	Post	0.35	0.29	1.08	1.45
CTRR	Pre	0.25	0.31	1.81	2.25
	Treat	0.05	0.26	0.01	1.55
	Post	0.38	0.27	1.97	1.40
ITCR	Pre	0.26	0.31	1.99	2.03
	Treat	0.28	0.38	1.86	2.04
	Post	0.45	0.27	1.47	1.45
ITIR	Pre	0.25	0.31	1.73	2.03
	Treat	0.35	0.39	2.69	2.12
	Post	0.39	0.32	1.26	1.52

Figure 2.2: Observed and increment width-calculated growth in length (left) and weight (right) of chinook salmon reared under various environmental conditions. Points represent mean growth rates during the pre-treatment, treatment, and post-treatment intervals. Solid line: observed growth, dashed line: otolith derived growth.


LENGTH
WEIGHT
0.50
0.25
0.00
0.25
0.00
0.25
0.00
0.25
0.00
3
2
1
0
2
1
0
2
1
0
2
1
0
CTCR
CTRR
ITCR
ITIR
CTCR
CTRR
ITCR
ITIR
GROWTH RATE (mm/d)
GROWTH RATE (%bw/d)
pre
treat
post
pre
treat
post
INTERVAL
INTERVAL

Discussion

Growth rates calculated from increment widths did not always vary in the same manner as observed rates to in relation to changes in environmental conditions (Fig. 2.2). The lack of variation in growth rates calculated from increment widths compared to the variation in actual fish growth rates supports my results of Chapter 1 that otolith growth is conservative and does not follow small or very rapid fluctuations in fish growth.

When fish growth was nearly halted by restricted ration otolith growth decreased gradually and growth rates were overestimated from increment widths (Figs. 2.1,2.2). Increment width increased only slightly during the post-treatment interval when ration was restored which resulted in growth rates that were underestimated by increment widths. Continued otolith growth in fish receiving no ration has been observed in a number of studies (Mugiya *et al*. 1981; Marshall and Parker 1982; Campana 1983; Neilson and Geen 1985) as well as for fish receiving less than maintenance ration (Volk *et al*. 1984).

Increment width has been shown to reflect fish growth only in long-term experiments where ration was held constant and was sufficient to result in positive growth (Volk *et al*. 1984; Neilson and Geen 1985; Chapter 1). My results indicate that the lag in response of the otolith to changes in ration level, and the small change in increment width that did occur even though growth was nearly halted, may make it difficult to detect changes in growth due to a variable food supply unless the change in ration is severe and prolonged.

Growth rates of chinook salmon fry reared in warmer water at constant ration were similar to the control group, yet their increments were wider during the treatment period (Fig. 2.1). In other studies wider increments have been found in fish reared at higher temperatures, although in all cases fish growth rate also increased as food was provided in excess (Marshall and Parker 1982; Neilson and Geen 1982; Radtke and Dean 1982). My results indicate that increment widths can increase in warmer water without an increase in growth rate, possibly due to an increase in metabolic rate. In order to maintain the proportionality of the otolith to other structures in the fish's inner ear, increment width would probably have declined eventually, had the treatment period continued longer. There was an indication of this occurring, as increment widths in the ITCR treatment were beginning to decline at the end of the treatment period compared to the ITIR group which received a larger ration during the treatment period (Fig. 2.1).

When temperature and ration were increased simultaneously, increment widths were similar to the ITCR treatment where only temperature was raised, suggesting that temperature had a stronger influence on otolith growth than ration (Fig. 2.1). Brothers (1981) states, without published evidence, that otolith growth and temperature may be coupled, so that increment width may provide an accurate record of water temperature in some species. Campana (1984) found a significant correlation between the widths of juvenile starry flounder (*Platichthys stellatus*) increments and air temperatures, although no data on fish growth rates were provided. Neilson and Geen (1985) also found that changes in temperature had a stronger influence on increment width than changes in ration. The effects of changes in ration on otolith growth will depend on the nutritional status of the fish and the rate of food metabolism, both of which may buffer the effect of the change in ration on otolith growth. If temperature influences otolith growth through metabolic rate (Campana and Neilson 1985), this more direct pathway may explain the increased sensitivity of otolith growth to temperature rather than to changes in ration level.

Trends in growth rates in all treatments except CTRR were more accurately recorded as otolith-calculated growth in weight than growth in length. (Fig. 2.2). The presence of protein matrix is required for the cryastallization of calcium carbonate (Dunkelberger *et al*. 1969); otolith growth may be limited by the deposition of protein. For this reason it is possible that otolith growth will be more closely coupled to the processes that control weight changes in the fish (i.e. protein metabolism) rather than growth in length alone.

The divergence between observed and calculated growth rates in the CTRR treament may be due to changes in metabolism that occur at low ration. While protein is the primary energy source for normally feeding carnivorous fish, starving fish utilize fat reserves for energy (Jobling 1980). In juvenile sockeye salmon (*O. nerka*) inorganic nitrogen excretion, an indicator of protein catabolism, did not increase during starvation (Brett and Zala 1975). The nitrogen pool is spared during periods of reduced energy intake, which may allow for continued deposition of protein matrix on the otolith. The use of well-fed hatchery fish which have a high fat content (Cowey and Sargeant 1972) in my experiments may have helped lessen the effects of food shortage on otolith growth.

The responsiveness of the otolith to changes in fish growth may be affected by the size, nutritional status and activity level of the fish. Smaller fish, such as larvae, and wild fish that live in a food-limited environment will have lower energy reserves that may tighten the coupling between otolith growth and fish growth compared to the juvenile salmon used in this study. The otoliths of more active species may show a more immediate response to changes in fish growth (Mugiya and Miromatsu 1982; Campana 1983).

My results demonstrate otolith growth can respond independently of fish growth to changes in environmental conditions. When environmental changes altered fish growth rate, otolith growth did not respond to the same extent, and gave a less variable record of growth. Rapid changes in temperature could result in a change in increment width without a corresponding change in fish growth. When both ration and temperature changes occur simultaneously, the temperature changes will have a stronger effect on the otolith. The effects of temperature on otolith growth and the lag in response of otolith growth to changes in ration are examples of the short-term uncoupling of fish and otolith growth which can result in inaccurate estimates of fish growth from increment width (Brothers 1981).

These experiments indicate that general trends in growth rates may be obtained from microstructure examination, however for more detailed investigations, factors such as ration and temperature should also be considered along with the increment width data.

CHAPTER 3: THE SIZE AND GROWTH OF JUVENILE CHINOOK SALMON REARING IN CAMPBELL R., B.C. ESTUARY

Introduction

The utilization of estuary habitats by a number of species of salmon (Healey 1982a) for varying time periods has led researchers to express concerns that anthropogenic degredation of estuary habitat may have negative impacts on salmonid populations (Dorcey *et al*. 1978). The key question is determining if estuary rearing confers a significant advantage to survival. The null hypothesis of Levings (1984) that juvenile salmonids are not dependent on estuaries provides a focus for research on alternative hypotheses on the mechanisms by which estuarine rearing enhances survival to maturity. Potential mechanisms include: a) increased growth rates due to high availability of food organisms and b) reduced mortality, either due to a lack of suitable predators, or increased water turbidity restricting visual predation (Simenstad *et al*. 1982). In addition, refuge from size-selective mortality in the estuary that has been observed in the ocean (e.g. Healey 1982b) may also increase overall stock survival rates.

Of the five species of salmonids the so-called "ocean type" chinook salmon are the heaviest users of estuary habitats (Healey 1982a,1983). These fish emerge from the spawning grounds and migrate immediately to the estuary, where they rear for up to 3 months before emigrating to sea. The other chinook salmon life history types spend longer rearing in the river. Though data are available that describe juvenile chinook salmon distribution, size and feeding habits in estuaries, attempts to test the above mentioned alternative hypotheses have been few. An exception is the work of Reimers (1973) on Sixes R., Oregon chinook salmon, who showed that the return rate of fish that spent 3 months or more in the estuary was greater than for other life history types, implying that estuarine rearing enhanced survival.

In this chapter I used otolith daily growth increments to examine the size and growth of juvenile chinook salmon rearing in a small British Columbia estuary. Neilson *et al.* (1985a) first used this technique to study the growth of chinook salmon in the Sixes R. estuary, and found that growth rates declined during mid-summer when peak water temperatures and fry abundances were measured. From indirect evidence they concluded that on average one increment was formed per day on the otoliths of estuary rearing fish. I used the methods developed in Chapter 1 to back-calculate fish size to look for evidence of size-selective mortality of estuary rearing chinook salmon. I compared increment widths to environmental conditions to determine the factors which may regulate seasonal fish growth patterns, and to look for evidence of density-dependent growth. The results of this chapter were compared to the results of the experiments in the first two chapters to assess the reliability of the inferences drawn from the otoliths of field collected fish.

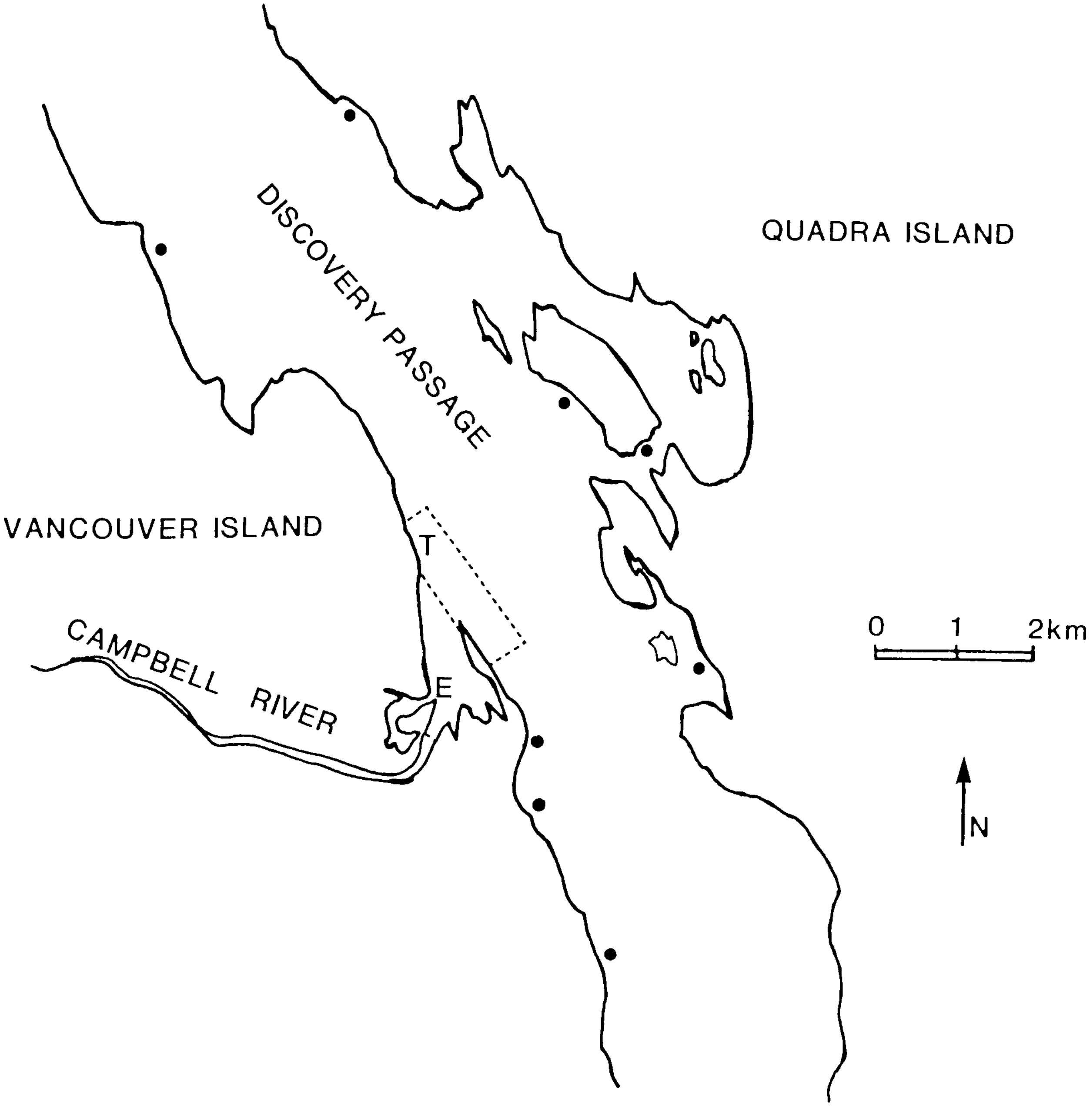
Study Site

Campbell River is located on the east side of central Vancouver Island and flows from a hydroelectric dam east 5 km through a small estuary into Discovery Passage (Fig. 3.1). A major tributary, the Quinsam R., flows into the Campbell R. below the dam.

The Campbell R. estuary and nearshore habitats have been divided into 3 zones by Levings *et al*. (1985). The estuary zone is approxiamtely 50 ha in area and extends from the upper penetration of seawater downstream to a spit which constricts the mouth of the estuary. This zone is characterized by generally low salinity surface water of river origin, underlain by seawater. The depth of the salt wedge depends on location, tidal height and river discharge. The area approximately 1-2 km north and south from the mouth of the estuary, including adjacent gravel bars and nearshore habitats has been termed the 'transition zone', because of the intermediate salinities caused by the mixing of river and ocean water. Finally, the 'marine zone' includes the waters of Discovery Passage extending 10-20km north and south of the Campbell estuary. This zone is characterized by strong tidal currents, and high (28-32ppt) salinities (Brown *et al*. 1984).

Chinook salmon escapement, as estimated by streamside counts, in the Campbell R. system has averaged 3500 fish between 1933 and 1973 (Goodman and Vroom 1974). In recent years

Figure 3.1: Map of the Campbell R. region showing the location of the estuary (E) and transition (T) zone sampling sites. Dashed lines separate estuary, transition and marine zones.


DISCOVERY PASSAGE
QUADRA ISLAND
VANCOUVER ISLAND
CAMPBELL RIVER
T
E
0
1
2km
N

escapement has dropped to about 2000 fish, of which an unknown number are the result of hatchery smolt production that began in 1974.

Emergent fry migrate downstream in April and May from the spawning grounds to the estuary. After rearing in the estuary for 1-2 months, chinook salmon fry move seaward through the transition and marine zones, in a pattern typical of ocean-type chinook salmon fry observed in other British Columbia estuaries (Dunford 1975; Healey 1980a; Levings *et al*. 1985).

Materials and Methods

Sampling for juvenile chinook salmon was conducted at approximately 2-week intervals throughout the spring and summer of 1982 and 1983. Seventeen estuary and 12 transition sites were sampled by beach seine one or more times per trip. Eleven marine stations were usually only sampled once per trip. Sampling sites were distributed throughout each zone although the exact locations were restricted by the type of sampling gear. At each site a boat-towed 14 X 3m beach seine was used to capture salmonids. Two sets were made at most stations; the catch was enumerated and surface water temperature and salinity were recorded. Detailed descriptions of the sampling sites and field methods are given in Brown *et al*. (1984) and Levings *et al*. (1985).

Large numbers of marked and unmarked hatchery fish were released into the estuary in late May and June of each year. Marked fish were identified by their lack of adipose fin. Early in the season the unmarked hatchery fish were readily separated from wild fry by their larger size. Later in the season length-frequency analysis or back-calculated size were used to separate naturally-spawned fry from hatchery-reared fish.

Catch per set of wild chinook salmon fry, an index of abundance, was calculated by Levings *et al*. (1985) for each zone and sampling trip using a weighting scheme that gave equal importance to each site within each zone regardless of the number of times it was occupied on each trip. This prevented inflated estimates of relative abundance from repeated sampling of sites that yielded large catches.

Subsamples of wild chinook salmon for otolith preparation were taken at roughly 3-4 week intervals from each zone. Samples from the estuary were taken from the Northern mouth of Baikies slough; the transition samples were from Painter's channel (marked by the E and T in Fig. 3.1). Samples from the marine zone were taken whenever a large enough (n>20) catch was made. After an initial (<1 day), preservation in formalin, samples were transferred to either ethanol or isopropanol prior to otolith dissection. Otolith preparation and data analysis followed the methods described in the first two chapters of this thesis.

Sampling for meiofaunal organisms by benthic sled was conducted in both years in the estuary, transition and marine zones. In 1983 only, zooplankton densities in the upper 1m of the water column were estimated by Miller plankton net tows in all three zones. Details of the methods and raw data are provided in Brown and Kask (1984), and Kask and Brown (1984,1985). I calculated a crude index of prey availability for juvenile chinook salmon by totalling the densities of all organisms caught in the samples, with the exception of eggs, in each of the three zones.

To examine the influence of environmental conditions on increment widths I calculated the correlation coefficient, r, between increment width and temperature or zooplankton abundance for the estuary and transition zone data. Because the environmental data were taken at 5-20 day intervals I averaged the daily increment widths for all available samples over these intervals such that the environmental observations formed the midpoint.

Mean fish lengths were back-calculated from each sample of fish at 10-15 day intervals using the methods of chapter 1 to look for evidence of size-selective mortality. I did not perform statistical tests on the differences between mean sizes of the different samples, but rather examined the data for trends in the size-at-time curves. An analysis of variance of mean lengths at one date would be statistically valid, but there would have to be an *a priori* reason for choosing a specific date for conducting the analysis. Without an *a priori* date for the test an elaborate statistical model is required to test for significant differences in mean size between all available samples at each of the dates when back-calculated sizes were estimated. This model would have to account for the chance occurrence of a few significant results given a large number of comparsons made, and the lack of independence of the data given that a number of back-calculations are made from a single sample of fish. In addition, the exact sampling distributions of back-calculated mean sizes are unknown because they are calculated from a number of random variables (i.e. mean otolith radius and the estimated coefficients of the fish size-otolith size relation). Because of these uncertainties, I felt an exact statistical approach was unwarranted.

Results

Relative abundances in the three zones peaked in a seaward progression from the estuary to the marine zone (Fig. 3.2). The estuary rearing period extended over 2 months, from mid-April to mid-June. Chinook salmon abundances were greater in all zones in 1982 than 1983 (Levings *et al*. 1985).

To back-calculate fish size from otolith measurements the relationship between fish size and otolith radius must be carefully described. In both years data were separated into 2 groups; estuary fish and 'outside' fish from the transition and marine zones. Insufficient data were available to form separate regressions for the transition and marine zones.

Linear regression models were used to describe the relationship between otolith radius and fish fork length. For 1982 the equations were:

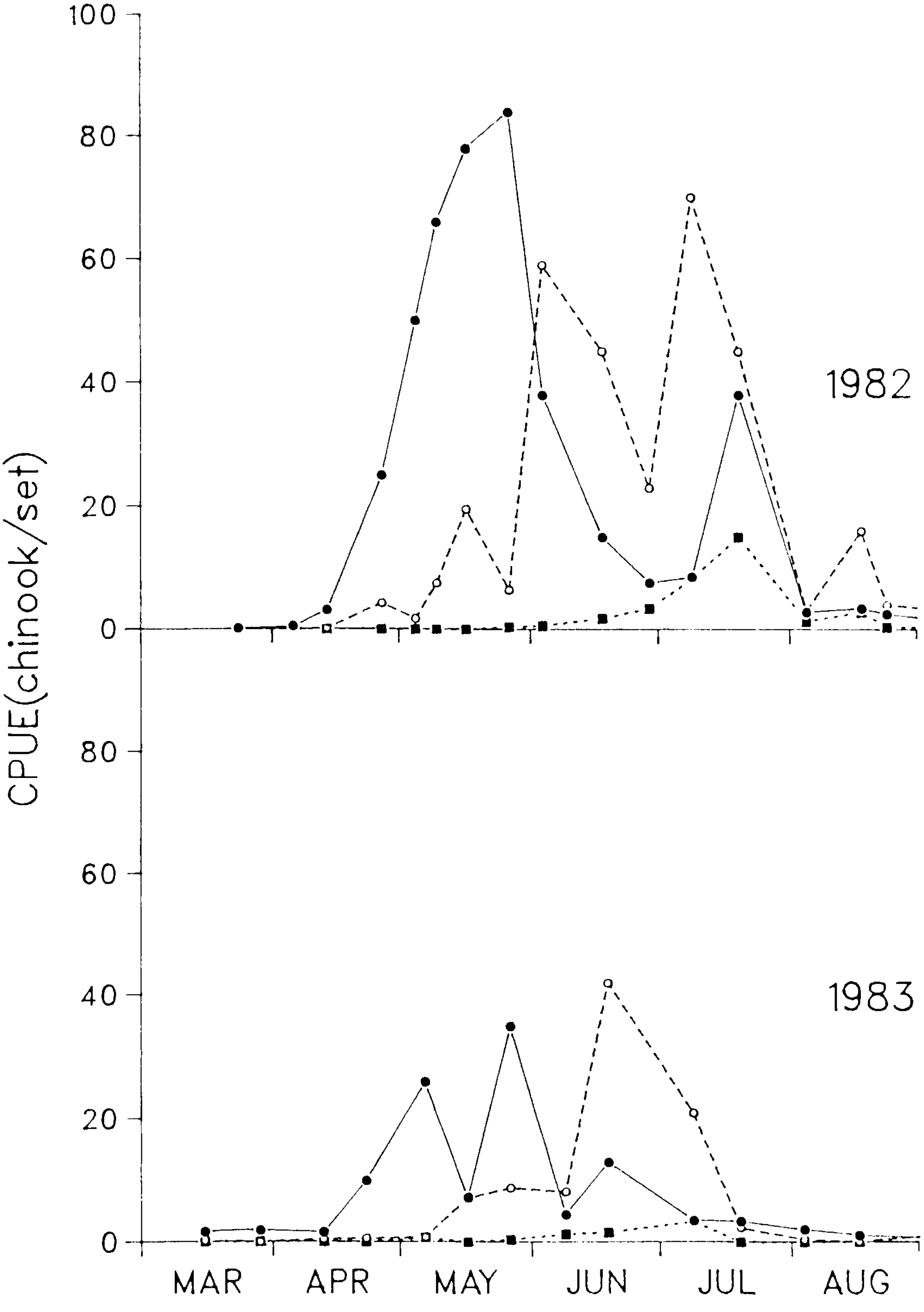
Estuary: $FL = 0.160R - 8.01$ (N=114, r=0.95)

Outside: $FL = 0.191R - 17.5$ (N=106, r=0.92)

Where FL is fork length (mm) and R is otolith radius (μ).

The scatter plot of 1983 estuary fish length vs. otolith radius revealed an inflection point at an otolith radius of about 350μ. This inflection was not evident in the 1982 samples, because fewer small (<40mm) fish were sampled that year. A two-piece regression was fit to the 1983 data and was found to

Figure 3.2: Mean catch per unit effort (fish/set) of naturally spawned chinook salmon beach seined in the Campbell R. region in 1982 and 1983. ● estuary, o transition, ■ marine zone. Redrawn from Levings et al. (1985). Means are weighted to give all sites in the zone equal weighting, regardless of sampling frequency.


CPUE(chinook/set)
100
80
60
40
20
0
1982
80
60
40
20
0
1983
MAR
APR
MAY
JUN
JUL
AUG

be a significant improvement over a simple straight line (F test, $P=0.0001$). A dummy variable (Z) was set to 0 for $R<350\mu$ and 1 for $R>350\mu$. A simple linear regression was used for the 1983 outside samples. The equations were:

Estuary: $FL = 0.055R + 0.118ZR + 25.18 - 38.36Z$ ($N=220$, $r=0.97$)

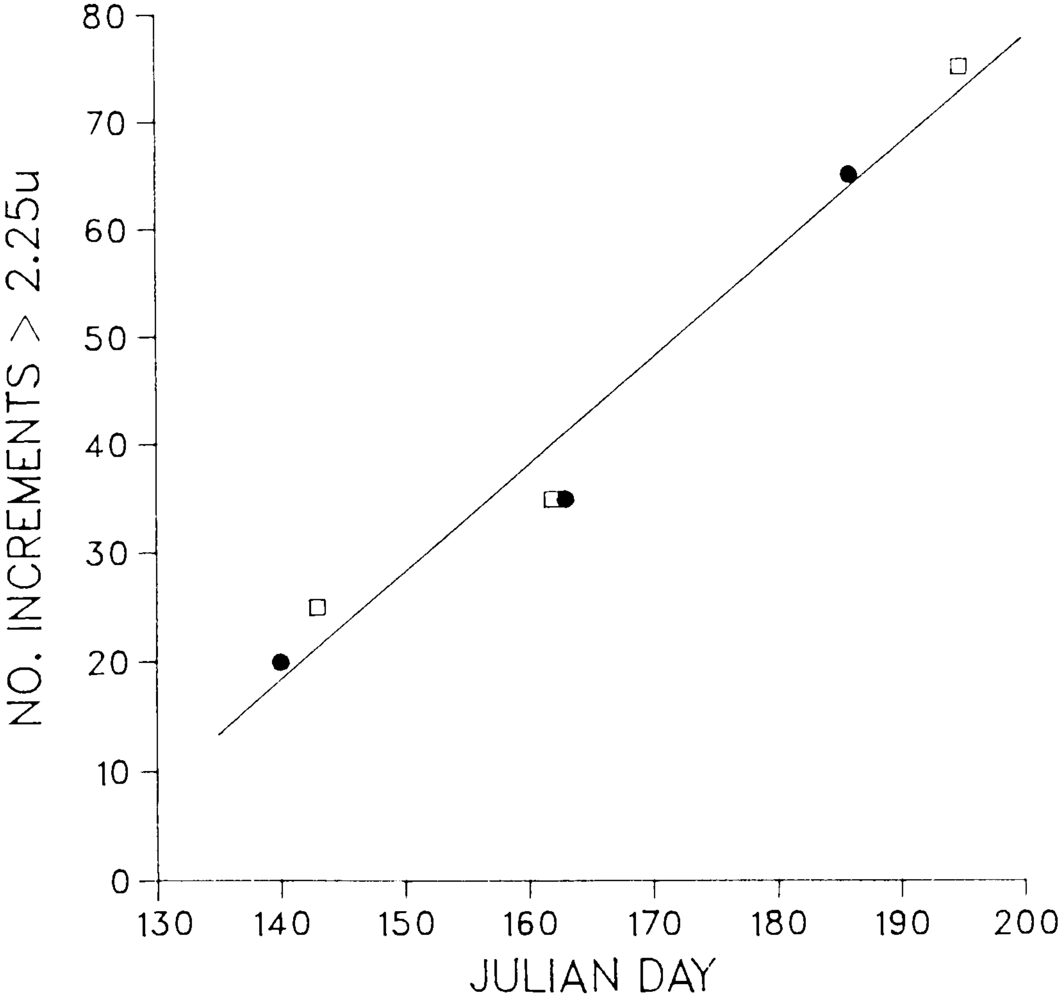
Outside: $FL = 0.167R - 7.79$ ($N=121$, $r=0.91$)

By using a separate regression for outside fish I am assuming that this relationship adequately describes otolith growth in relation to fish growth throughout their post-emergence rearing period. Since fish caught in the outside zones probably spent a month or more rearing in the estuary first, where the estuary regression may have been more appropriate, inaccurate back-calculations of size may have resulted because of the difference between the two regression lines. Within the size range of fish captured in the estuary the difference between lengths calculated from the two equations was 2-3 mm.

No transition or check was noted on the otoliths that indicated the migration of the the fish from river to estuary habitats as was found for Sixes R. chinook salmon by Neilson *et al*. (1985a). The transition in habitat type from the river to the inner estuary was apparently insufficient to provide a marked contrast on the otolith.

I could not directly determine whether increments were formed on a daily basis because there was no mark or check that could be used as a time marker on the otolith from which increment counts could be made. I also could not determine the total number of increments on the otolith as increments were very narrow and faint near the nucleus, probably due to cold winter water temperatures. I indirectly determined the frequency of increment formation by using the point on the otolith where increment widths exceeded an arbitrary width as a reference point, and counted the number of increments from this point to the edge of the otolith. This method is based on the assumption that increments of the threshold width are formed at approximately the same time for all fish captured in the estuary. I averaged increment widths in groups of 5 from each sample and determined the point on the otolith where the 5-increment average exceeded 2.25μ. I regressed the number of increments from this point to the edge of the otolith on the Julian date of capture of the sample (Fig. 3.3). If increments were formed daily, and if increments first became larger than 2.25μ at approximately the same time for all fish, then the slope of the regression should not be different from one. The regression was significant ($r=0.98$) with the slope not different from 1.0 ($P=0.901$), so I concluded that increments were formed daily.

Figure 3.3: Regression of the mean number of increments formed after the first 5-increment average exceeded 2.25µ on the Julian date of capture for samples captured in the estuary zone in 1982 (●) and 1983 (□).
Equation: Y = 0.98X - 120. Slope = 1 implies that increments are formed daily.


NO. INCREMENTS > 2.25u
80
70
60
50
40
30
20
10
0
130
140
150
160
170
180
190
200
JULIAN DAY

Back-calculated size

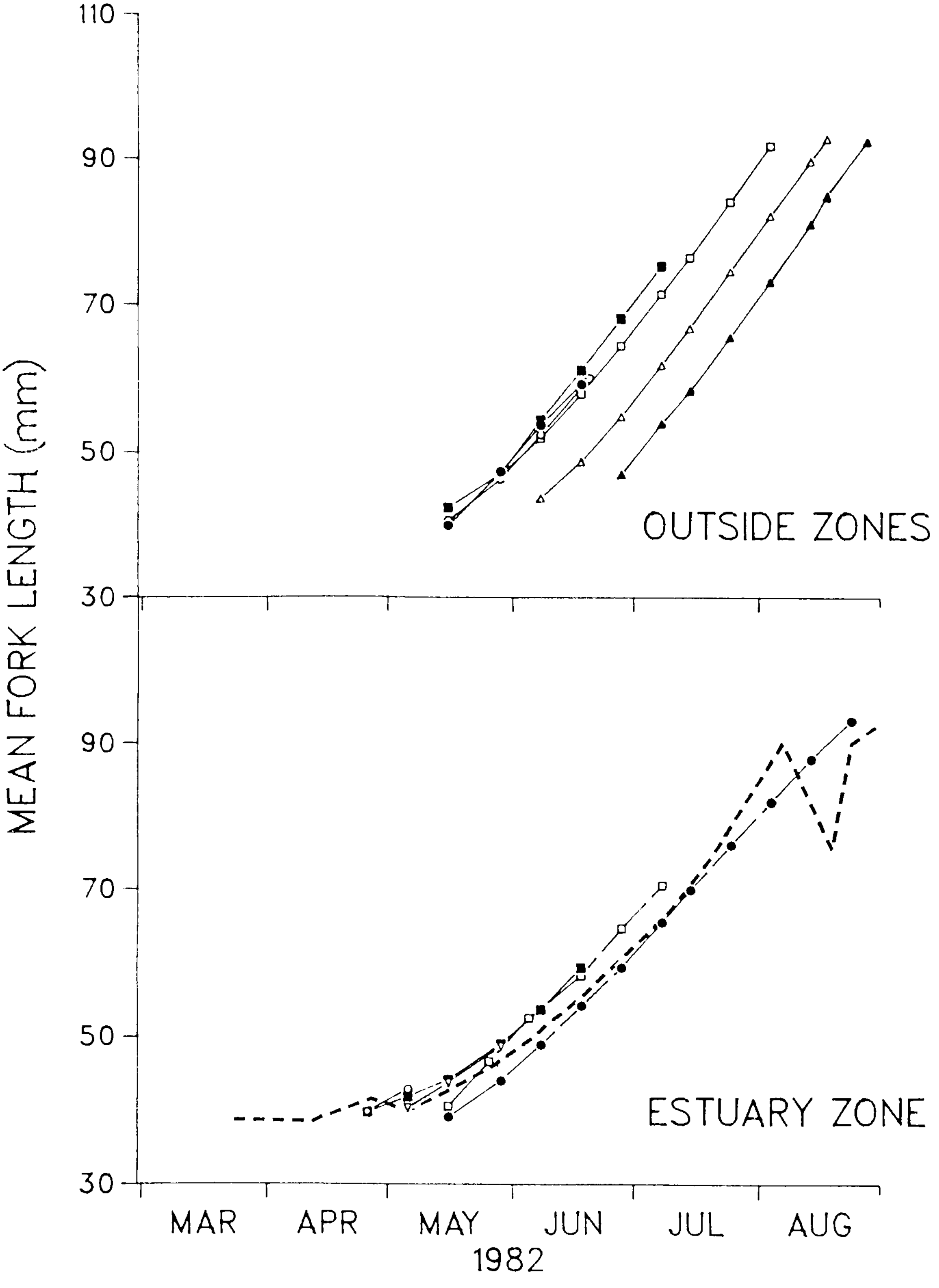
Mean lengths of juvenile chinook salmon were back-calculated at 10- to 15-day intervals back in time from the capture date of each sample. Back-calculations were not attempted when the number of otoliths in each sample fell below 3.

Back-calculated fork lengths of fish captured in the estuary were similar to the fish sizes observed by Levings *et al*. (1985) derived from field sampling (Fig. 3.4 and 3.5).

Mean lengths were back-calculated from samples taken at different times during the season to identify if size-selective processes were acting on the rearing chinook population. If samples caught later in the year had back-calculated sizes larger than the average back-calculated size found earlier in the season, size-selective mortality may be acting against smaller individuals so that only the larger fraction of the original population remained later in the season. Alternatively, if late residing fish had smaller back-calculated sizes than those caught earlier, the most likely explanation would be that the larger, fast growing fish were migrating out to the ocean. If both processes were operating simultaneously, they could cancel each other out.

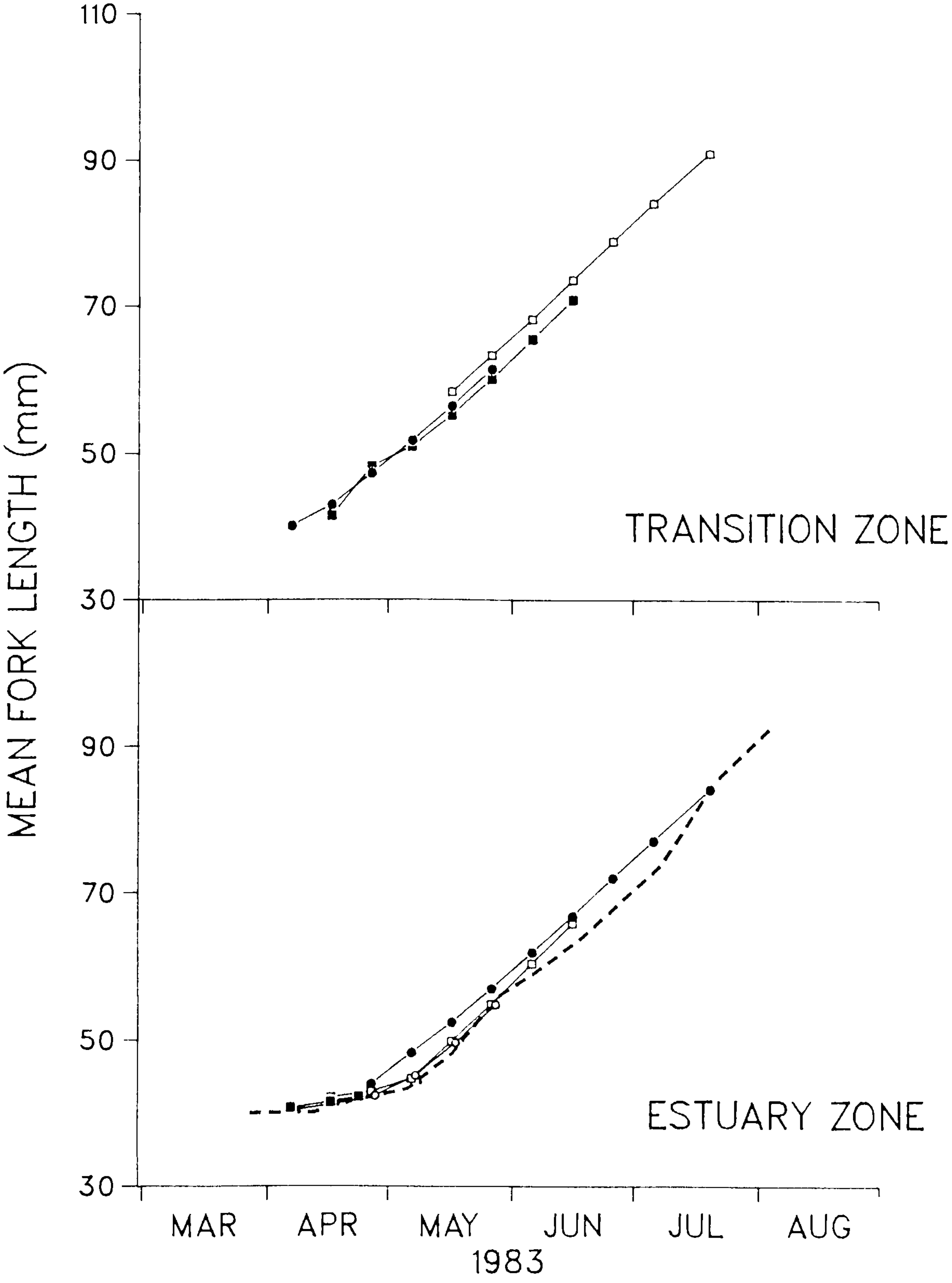
Size-at-time curves for all but one sample taken in the estuary in 1982 overlapped each other, suggesting that size-selective mortality was not acting on this population (lower panel of Fig. 3.4). Fish from the August 1982 sample were

Figure 3.4: Backcalculated fork lengths of juvenile chinook salmon sampled in estuary, transition and marine zones, 1982. Transition zone; by capture date (sample size): ● June 16 (12), ■ July 8 (16), Δ Aug 25 (9). Marine zone: o June 18 (8), ¤ Aug 4 (16), Δ Aug 18 (23).Lower panel: Estuary zone o May 5 (12), ∇ May 28 (25), ■ June 18 (23), ¤ July 8 (15), ● Aug 24 (11). Dashed line is mean length from field samples of Levings et al. (1985).



110
90
70
50
30
90
70
50
30
MEAN FORK LENGTH (mm)
OUTSIDE ZONES
ESTUARY ZONE
MAR
APR
MAY
JUN
JUL
AUG
1982

Figure 3.5: Backcalculated fork lengths of juvenile chinook salmon sampled in estuary and transition zones, 1983. Transition zone, by date of capture (sample sizes): • May 27 (27), ▪ June 16 (24), □ July 21 (6). Estuary zone: ▪ April 24 (13), □ May 27 (54), o June 16 (20), • July 21 (18). Dashed line is mean length from field samples of Levings et al. (1985).


MEAN FORK LENGTH (mm)
110
90
70
50
30
TRANSITION ZONE
90
70
50
30
ESTUARY ZONE
MAR
APR
MAY
JUN
JUL
AUG
1983

smaller than samples captured earlier in May and June. Since chinook salmon emerge from the spawning gravel at 35-40mm (Lister and Genoe 1970), this size-at-time curve implies that fish captured in the estuary late in the summer had emerged later in the spring. In 1983 the overlap of growth trajectories in the early spring indicates that size selective processes were also not acting on the estuary population in that year (Fig 3.5).

Samples taken in the marine and transition zones were also examined for evidence of size-selective mortality. The size-at-time curves of fish captured in the outside zones in June, July and early August 1982 overlapped, suggesting a lack of size-selective mortality (top panel of Fig. 3.4). As observed by Levings *et al*. (1985) transition zone fish were slightly larger than those captured in the estuary. Chinook salmon sampled in late August 1982 in the transition and marine zones had much smaller back-calculated sizes than fish caught earlier in the season. A number of factors might have been responsible for those differences. Beach-seined catches have been shown to be biased towards smaller fish in areas where deeper offshore water is available for larger fish to rear (Levings 1982). Second, fish remaining in the Discovery Passage region late in the season may be late recruits from Campbell R. with larger, earlier recruiting fish having left the area. Lastly, fish caught in the marine zone may not be from the Campbell system, but may originate from other Georgia Strait rivers where the growth pattern is somewhat different. Therefore the observed growth of these late-season samples are probably not representative of the bulk of the Campbell R. population.

In 1983 mean back-calculated sizes of samples taken in the transition zone were similar to each other, and as in 1982, were always slightly larger than estuary-sampled chinook salmon (Fig 3.5). Lower abundances in this zone and very small catches in the marine habitats did not yield sufficient data to analyze late summer growth.

Increment widths

The increment widths of otoliths of chinook salmon from estuary and outside zones are plotted as 5-day averages in Figs. 3.6 and 3.7. The linear relationships between fish fork length and otolith radius implies that increment widths are, on average, proportional to growth rates. Increment widths generally increased between April and June in both years (Figs. 3.6 and 3.7). These increment widths correspond to growth rates of 0.3 to 0.4 mm/d in April rising to a peak of 0.64 mm/d in 1982 and 0.52 mm/d in 1983.

The variation in increment widths of fish caught in the estuary was strongly correlated with changes in estuary surface water temperature in both years (1982: r=0.97, N=14; 1983: r=0.92, N=9). Water temperature increased sooner in April 1983, and temperatures were 2-4°C higher in the spring of 1983 compared to 1982 (Fig 3.8). Mean width of increments formed

Figure 3.6: Mean increment widths of juvenile chinook salmon in estuary and transition zones, 1982. Data plotted as 5-d averages, sample sizes as Fig. 3.3. Transition zone, by capture date: ● June 16, ■ July 8, Δ Aug 25. Marine zone: o June 18, ¤ Aug 4, Δ Aug 18. Estuary zone: o May 5, ∇ May 28, ■ June 18, ¤ July 8, ● Aug 24.

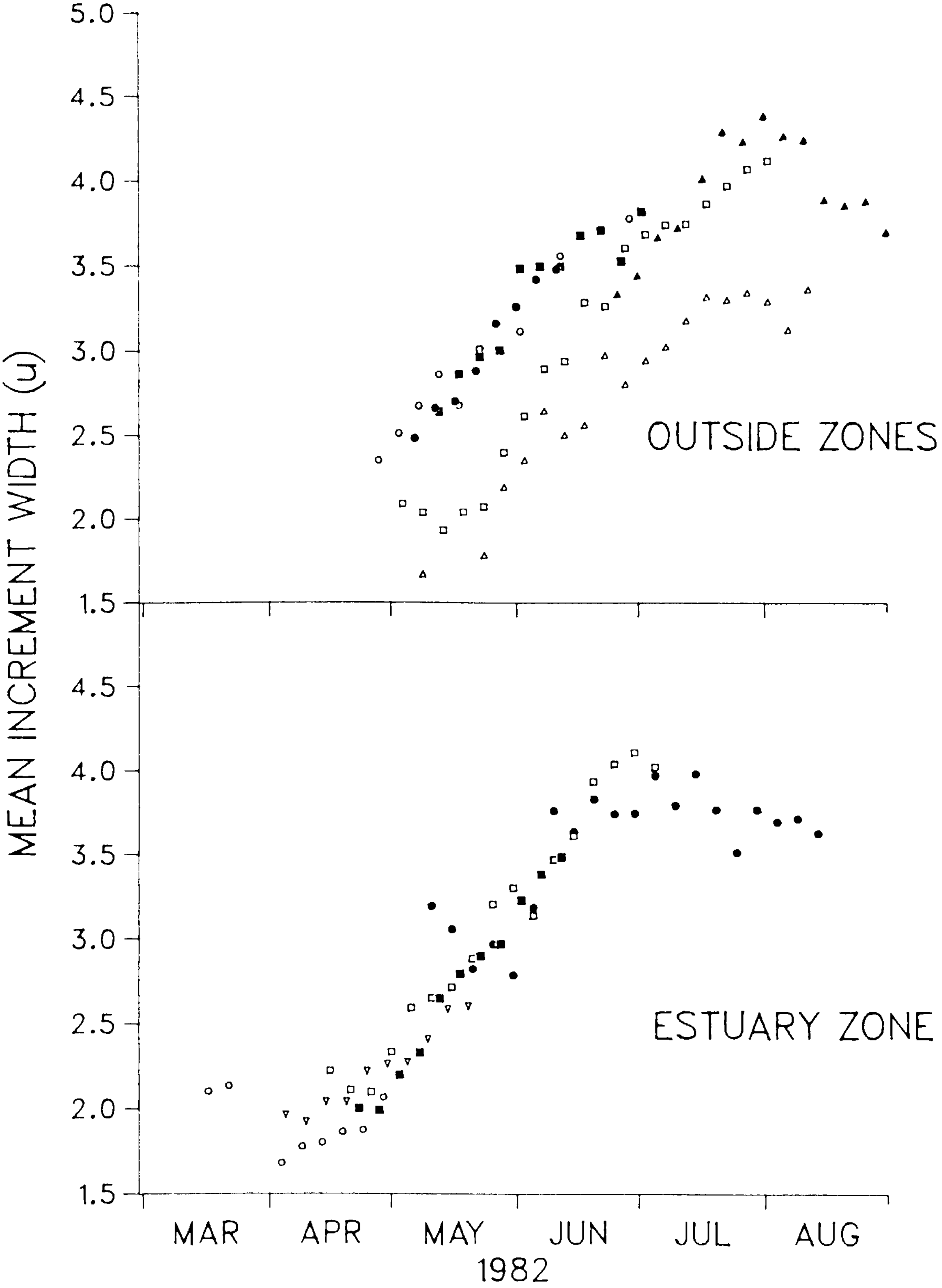

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
MEAN INCREMENT WIDTH (u)
OUTSIDE ZONES
ESTUARY ZONE
MAR
APR
MAY
JUN
JUL
AUG
1982

Figure 3.7: Mean increment widths of juvenile chinook salmon in estuary and transition zones, 1983. Data plotted as 5-d averages, sample sizes as Fig. 3.4. Transition zone, by date of capture: ● May 27, ■ June 16, □ July 21. Estuary zone: ■ April 24, □ May 27, o June 16, ● July 21.

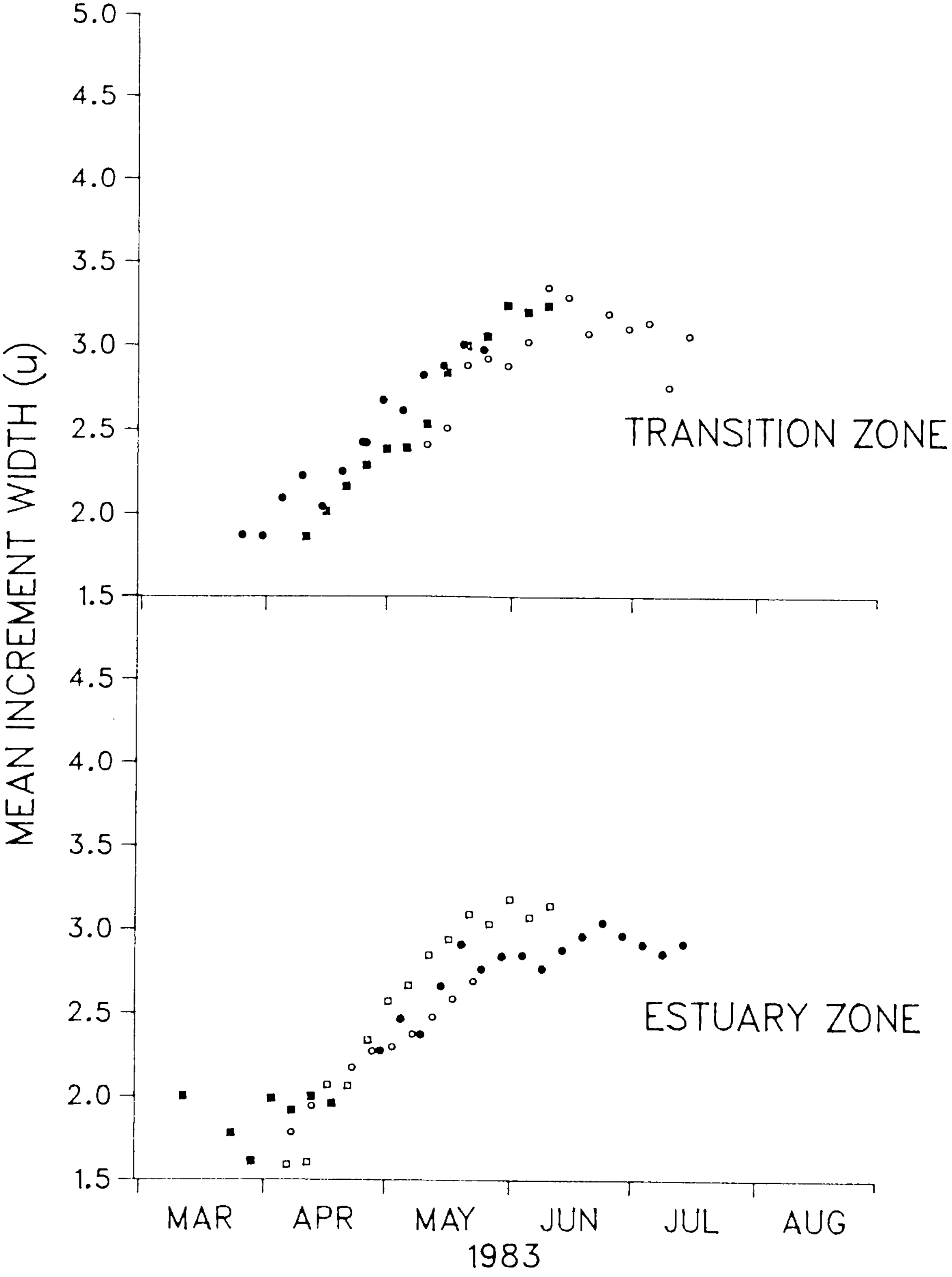
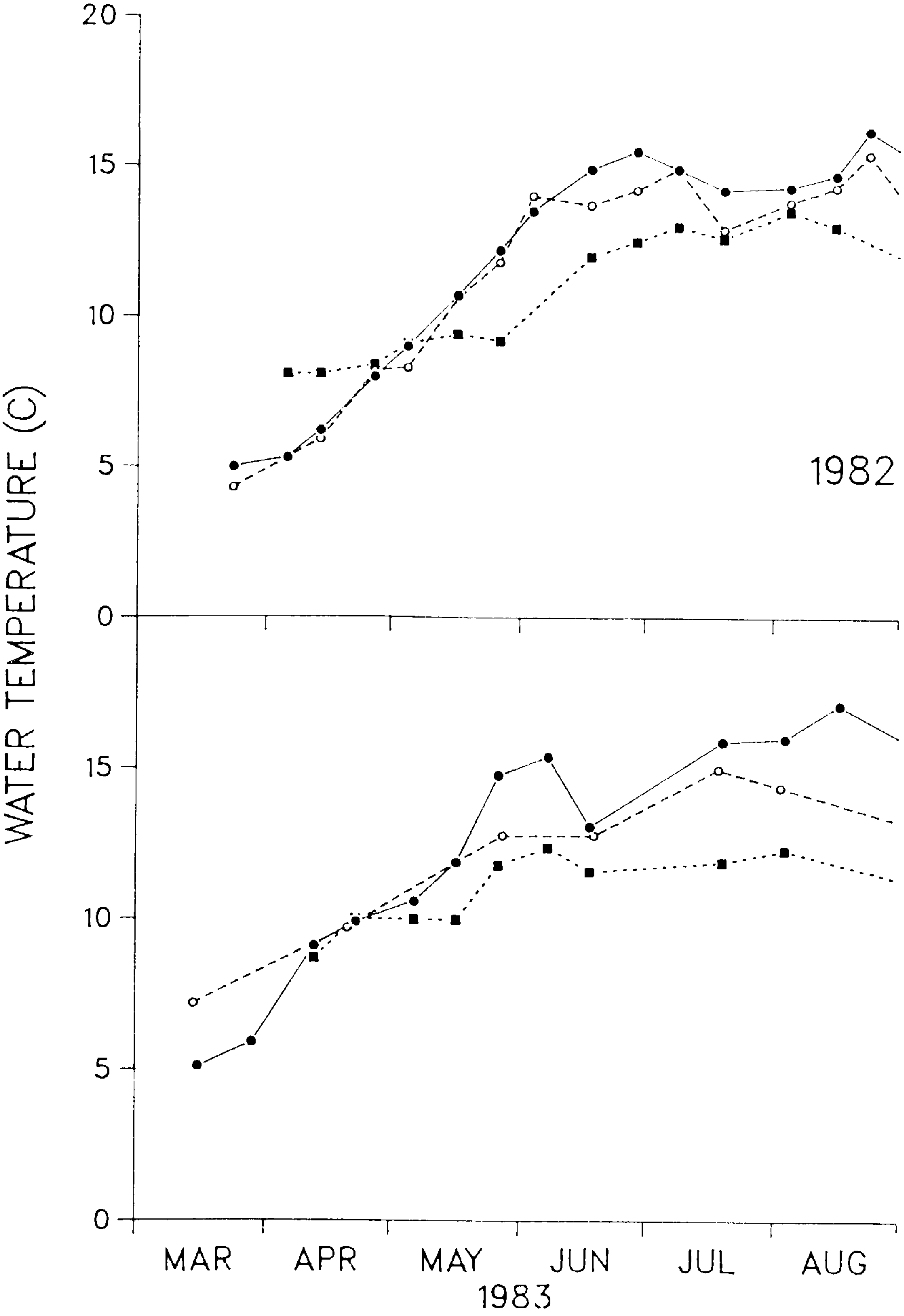

MEAN INCREMENT WIDTH (u)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
TRANSITION ZONE
ESTUARY ZONE
MAR
APR
MAY
JUN
JUL
AUG
1983

Figure 3.8: Surface water temperature for the three zones in 1982 and 1983. Points are the mean of all readings taken in each zone at random times on each 3-5 day sampling trip ● estuary zone; o transition zone; ■ marine zone. Redrawn from Levings et al. (1985).

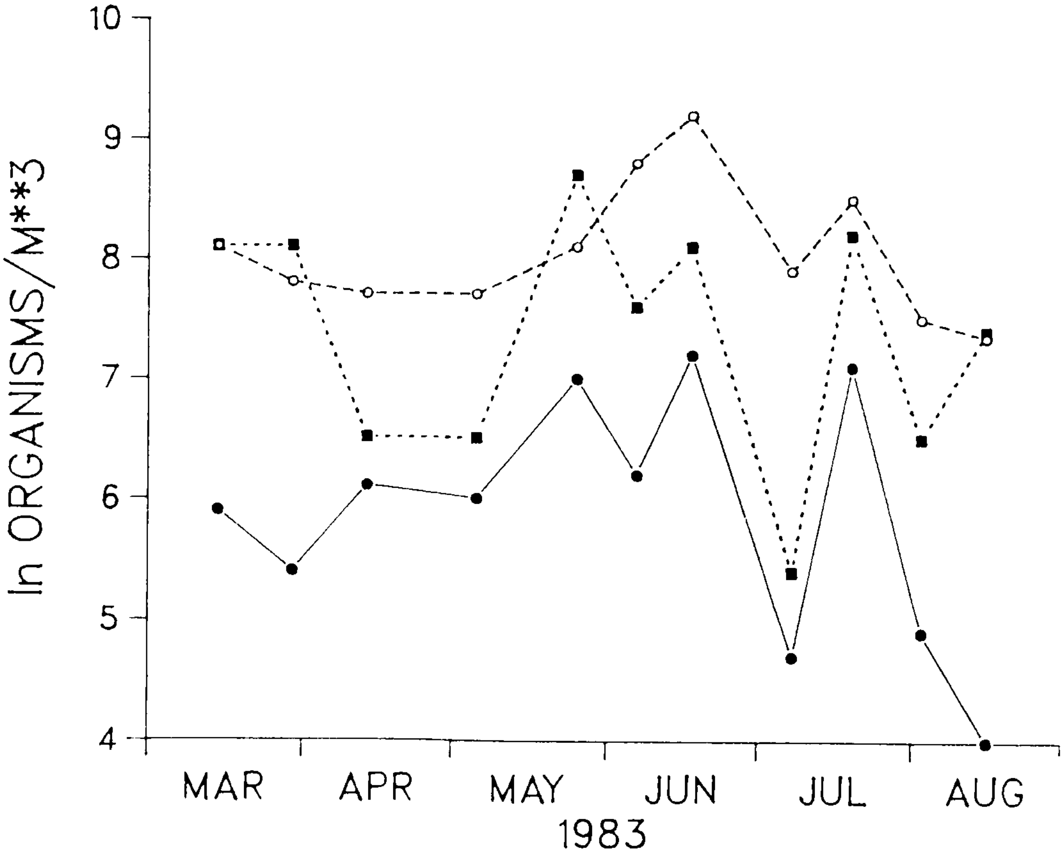

20
15
10
5
0
WATER TEMPERATURE (C)
1982
15
10
5
0
MAR
APR
MAY
JUN
JUL
AUG
1983

between April 15 and 30 were significantly larger in 1983 than in 1982 (ANCOVAR P=0.0025). Peak increment width in both years coincided with the end of the spring increase in temperature. Decreases in increment width after the peak match small mid-summer declines in temperature in both years.

I also compared an index of increment width patterns to the density of potential prey items. The diet of naturally spawned chinook salmon fry in the estuary consists largely of terrestrial insects and pelagic organisms, such calanoid copepods and cladocerans (MacDonald and Levings 1984); organisms which would be sampled by the Miller plankton net. Total organism densities from Miller net samples are available for 1983 only, and are shown in Fig. 3.9. The density of organisms increased through April and May, and was positively correlated with increment widths, although the correlation coefficients were lower than was found for water temperature (1983: r=0.41, N=9).

The peak increment width was larger in (4.0μ, Fig. 3.6) in 1982 than was observed in 1983 (3.0μ, Fig. 3.7). This difference could not be explained in terms of temperature as estuary temperatures similar during June in both years. (Fig. 3.8). Since zooplankton tow data were not available from both years, meicfauna abundances were compared using data in Kask and Brown (1984,1985). Total organism counts, except eggs, from samples taken at the estuary station marked in Fig. 3.1 were log-transformed and used in a two-way analysis of variance

Figure 3.9: Zooplankton densities (no./m^2) in Campbell R. estuary, transition and marine zones in 1983. Samples captured by 1.0μ Miller net in the upper 1m of the water column. ● estuary zone (n=4); o transition zone (n=1); ■ marine zone (n=3).



10
9
8
7
6
5
4
ln ORGANISMS/M**3
MAR
APR
MAY
JUN
JUL
AUG
1983

blocked by months (March-August) to test for difference in total organism density between years. There was no significant difference in invertebrate density between years (P=0.369). Thus total abundance of food organisms could not explain the differences in peak increment widths between years.

The seasonal patterns in increment width could not be attributed to density-dependent growth. Increments widths in both years increased throughout the buildup and decline of the estuary population and peaked after most of the fish had left the estuary zone. Nor was the difference in peak increment width between years related to fry abundance in the estuary, as both population size and peak increment width were greatest in the same year. There was no evidence that movement of fry from the estuary zone seaward was prompted by, or was coincident with, a reduction in growth as reflected as increment width. Most fry left the estuary before maximum growth rates were attained (Fig. 3.2)

Mean increment width of fish caught in the transition and marine zones in 1982 were more variable than the estuary samples, probably because some of the samples were not typical of the Campbell R. population. Increment widths of the transition zone samples were also correlated with water temperatures (1982: r=0.90, N=11; 1983: r=0.91, N=6). The correlation between 1983 zooplankton density and increment width in the transition zone was weaker (r=0.73, N=8).

Discussion

The high productivity of estuaries has led researchers to hypothesize that estuary rearing enhances growth and therefore subsequent survival of chinook salmon (Simenstad and Wissmar 1984). Levy and Northcote (1982) further speculate that chinook salmon stocks originating from rivers with estuaries will be more productive than those from rivers without estuaries. Evidence from Oregon suggests that the productivity of chinook salmon stocks may be limited by the rearing capacity of the estuary (Reimers *et al*. 1979).

Water temperature and food abundance appeared to have the greatest affect on the seasonal pattern of growth of chinook salmon fry in the Campbell estuary. Temperature has been termed a 'Controlling Factor' by Brett (1979), because it controls the rate of metabolism and paces the activity and food demands of the fish. The optimum temperature for growth for nearby Qualicum R. chinook fry was found to be 20°C (Brett *et al*. 1982) slightly above the maximum temperature reached in the Campbell estuary in mid-summer. If this relation is applicable to the Campbell R. stock, increases in water temperature up to 20°C should result in increased growth, if sufficient food resources are readily available. While some growth occurred at the winter water temperatures of 4-5C, not until temperature began to rise in the spring did growth rates increase (Fig. 3.6,3.7 and 3.8). I concur with Levings *et al*. (1985) who noted that the observed size advantage of the 1983 fry was probably due to the slightly earlier rise in water temperature compared to 1982. The results of the benthic sampling suggest that the differences in growth, as reflected by increment widths, between years were not related to invertebrate densities. However, with the very crude index of prey availability that I used it is difficult to draw detailed inferences about ration levels and growth.

I could not find evidence that within-season changes in fish density affected growth. Levings *et al*. (1985) also concluded that growth was not affected by density based on the similarity of growth rates between years despite the differing fish abundances. Within-season growth depressions have been shown to occur occasionally in the Sixes R. estuary due to a combination of above optimum temperatures and competition for scarce food organisms (Reimers and Downey 1982; Neilson *et al*. 1985a). Healey (1980a) was unable to find any indication of density-dependent foraging success in three years of data on chinook salmon fry rearing in the Nanaimo R. estuary. He concluded that there was little convincing evidence for density-dependent growth of rearing chinook salmon fry in the British Columbia estuaries studied to date (Healey 1980b), however within-season density-dependent growth may only occur occasionally in years when both fish density is high and secondary productivity is low.

Historically, density-dependent growth may have been much more common than is currently observed. Escapements that gave rise to the fry densities observed in this study are about half of those found during the early part of the century and are probably 25-50% of the unfished population levels. The historical escapements would have increased fry densities considerably over the observed abundances.

I was unable to detect differences in growth, as reflected by increment widths, between fish caught in the estuary and transition zones. Transition zone fish would have spent some time in the estuary before moving seaward, so that the early part of their otolith record would reflect estuary growth. The estuary and transition zone water temperatures were in the range of temperatures that were shown by Brett *et al*. (1982) to be on the dome of the temperature-growth relation for chinook salmon fry and the differences in temperature may not have been enough to cause detectable differences in growth rate. Also, increases in growth due to the higher densities of zooplankton in the transition zone (Fig. 3.9) may not have been recorded on the microstructure (Chapter 2). Little can be said about growth in the marine zone, as samples obtained from that zone did not appear to be typical of the population.

The use of otolith microstructure to estimate growth is based on the assumption that increment width is proportional to fish growth rate. This assumption is not always valid, as uncouplings of otolith and fish growth can occur (Campana and Neilson 1985; this thesis). In Chapter 1 I found that short-term changes in fish growth were not recorded on the otolith. Otolith growth also responded independently of fish growth to changes in water temperature (Chapter 2). The gradual change in water temperature in the Campbell estuary (Fig. 3.8) should have reduced the uncoupling between fish and otolith growth, increasing confidence in growth estimates from increment widths. That increment widths were more strongly correlated with temperature than estuary invertebrate abundance agrees with the observation that otolith growth is less responsive to changes in ration than temperature (English 1983; Neilson and Geen 1985; Chapter 2). Because otolith growth and fish growth can respond differently to environmental changes, from microstructure examination alone I cannot quantify the relative importance of temperature or ration in contolling the growth rate of fry. Determining if either factor limits growth would be useful in assessing the likelihood of density-dependent growth of estuary rearing fry.

Although variations in increment width patterns were similar to the variations in environmental variables considered, there were unexplained differences in the maximum increment widths between years. These differences may have been related to growth conditions that I have not considered, such as the composition and nutritional value of available prey items. Neilson *et al.* (1985a) also could not relate the changes in increment widths of estuary rearing chinook salmon fry to changes in estuary water temperatures or prey abundances. These unexplained patterns highlight our lack of knowledge of the processes regulating otolith growth, and reemphasize the need to interpret the results of microstructure examination with caution.

In Chapter 1 I found that back-calculated size-at-time curves were less variable than was observed because otolith growth was not closely coupled to small changes in fish growth. The relatively smooth back-calculated size-at-time curves of the Campbell River fry are similar to those found in Chapter 1. The actual size-at-time curves probably vary around the back-calculated curves. Thus, the back-calculated size-at-time curves will not indicate any small changes in growth that may have been due to, for example, competition for food.

The similarity of the size-at-time curve of Levings *et al*. (1985) and my back-calculated sizes (Figs. 3.4 and 3.5) indicate that seasonal growth estimates calculated from field samples are accurate. Concerns expressed by those authors that recruitment and emigration may affect growth rate estimates may not be applicable in this population. In other systems, such as the Nanaimo R. where strong size-dependent emigration occurs, growth rates determined from field sampling were found to be lower than rates estimated from other methods (Healey 1980a).

From the back-calculations of size there was no measurable size-selective mortality occurring in the estuary or transitional zones (Fig 3.4 and 3.5). In contrast, size-selective mortality on small fish has been observed on lake-rearing sockeye salmon fry (West 1983), river migrating chinook salmon smolts (Patten 1971), in the marine environment for hatchery-reared coho salmon (Bilton *et al*. 1982), wild chum (Healy 1982b), and for pink salmon in experimental predation experiments (Parker 1971). The difference in size between estuary and transition zone fish is probably due to the seaward migration of larger fish from the inner estuary. Size-selective mortality acting on small fish in the estuary would have to be intense to be detected if size-related emigration from the estuary was occurring. Neilson (1984), using otolith microstructure, also did not find any evidence of size-selective mortality in juvenile chinook salmon rearing in the Sixes R. estuary. Predation rates in the inner estuary may be low; coho smolts and sculpins do not prey on chinook fry, and the piscivorous cutthroat trout are present in very low numbers (C.D. Levings, pers. comm.). The importance of bird predation is unknown. Size-selective mortality may be occurring but it may be at a rate too low to be detected. The rate of predation is probably much higher in the marine zone, because of the wider variety of potential predators. I could not, however, obtain enough samples to look for size-dependent mortality in this zone.

Available evidence, although indirect, indicated that one increment was formed per day (Fig 3.3). This conclusion was based on the assumption that the spring increase in increment width occurred simultaneously in all fish, perhaps caused by the increasing water temperatures. If increments were not formed daily, then the timing of the increase in increment width would have had to exactly offset the rate of increment formation in order for the slope of the regression in Fig. 3.2 to be close to one. This seems unlikely. Neilson *et al*. (1985a) also concluded that increments were formed daily on the otoliths of Sixes R. chinook salmon. From laboratory experiments with juvenile chinook salmon temperature, feeding, or activity cycles with a period less than 24 hours can result in higher than daily increment formation rates (Neilson and Geen 1982,1985). In the estuary it is conceivable that the twice daily tidal cycle could generate a higher rate of increment formation. However, other environmental stimuli, such as the daily cycle in river temperature or diurnal feeding activities may have been stronger influences on increment formation, with the result that one increment appeared to be formed per day.

The evolution of estuary rearing life history strategies implies that no other strategy can bring greater fitness to the individual. Increased fitness can result from greater survival or through increased growth, which may increase subsequent survival.

While little can be said about estuary mortality rates in comparison to alternate habitats, my results and those of Neilson (1984) suggest that the estuary may provide smaller individuals with protection from size-selective mortality in the ocean.

The estuary does not seem to provide growth opportunities that are not available elsewhere. Chinook salmon fry left the Campbell estuary before peak growth rates were reached. In addition, invertebrate densities were higher in the marine environment compared to the estuary. The observation made by Healey (1980a) that river-rearing fry migrants are similarly sized as conspecifics rearing in the estuary suggests that in the Nanaimo system, estuary rearing may not result in greater growth compared to fish rearing in the river. A comparative study of the growth of estuary and stream dwelling fish could establish whether estuary rearing confers a growth advantage over river rearing.

The estuary rearing behavior of juvenile chinook salmon may be the result of a physiological constraint to seawater entry rather than a trait selected for because of increased growth or survival. Chinook salmon fry cannot tolerate full-strength seawater until they reach 60-80mm in length (Wagner et al. 1969). Fry remain long enough in the estuary to attain sufficient size to be able to physiologically tolerate seawater, and then move directly to the ocean. This view is supported by the observation that outmigration by wild fish from the estuary tends to be size-dependent (Healey 1980a) and larger hatchery-reared fry spend less time in the estuary (Levings *et al*. 1985). Thus we may be viewing chinook salmon in a transitional stage as they evolve towards a life history strategy similar to that of chum and pink salmon.

GENERAL DISCUSSION

The purpose of this thesis was to determine the utility of otolith increment widths in estimating growth rates and size-at-time of juvenile salmonids. Although the technique has, in theory, great potential recent concerns have been expressed about the closeness of the relationship between otolith and fish growth (Brothers 1981; Campana and Neilson 1985). Uncouplings of fish and otolith growth may result in inaccurate estimates of size and growth.

The potential problems in estimating the growth of fish from daily increments are somewhat different than growth estimates made from annular structures. Some of these differences are related to the relationship between the periodicity of the formation of the aging structure and the duration over which environmental factors affect fish growth rates. Seasonal environmental factors have been found to influence the relationship between fish growth and the growth of the hard part used for aging. Reay (1972) has shown that the relationship between otolith size and fish size changes throughout the growing season, however, by the time of annulus formation, the width of the zone between annuli was proportional to the growth that occurred over the whole year. In contrast, the width of daily growth increments does not seem to reflect daily fish growth. Volk *et al*. (1984) note that the day-to-day variation in the increment widths of individual chum salmon implies an unlikely 14-fold change in fish growth between successive days. Only when increment widths are averaged over time will they reflect fish growth (Chapter 1). In addition, the lag in the response of the otolith to changes in fish growth that is sometimes observed (e.g. Marshall and Parker 1982; Chapter 2) implies that increment widths can reflect both past and present growth conditions.

This thesis raises concerns about the extent to which increment width reflects fish growth. Although the otolith grows allometrically with respect to fish growth, I have documented that otolith growth can respond independently of fish growth to short term change in environmental factors (Chapter 2). Thus, increment widths will reflect a combination of environmental conditions and fish growth. Based on the increment record alone, it will generally not be possible to separate these effects. For this reason increment width data should be interpreted with some knowledge of environmental conditions that prevailed when the increments were formed. Abrupt environmental change, such as the estuarine entry observed by Neilson *et al*. (1985a) can provide a marker on the otolith for increment counts and size back-calculations. However, inaccurate growth estimates may result if fish and otolith growth become uncoupled. Gradual environmental change may help to maintain the allometry between fish size and otolith size, and may render growth estimates more accurate.

My comments are not intended to imply that increment widths are of limited use in age and growth studies of juvenile salmonids. The lack of correspondence between increment width and growth of individuals over the short term indicates that inferences about individuals or very small samples should not be made. Variability in the otolith radius-fish size relationship demands that it be evaluated carefully for each study population because its form will affect growth estimates made from increment measurements. Although increment width-derived growth estimates may be free of some of the sampling biases of length-frequency estimates (Volk *et al*. 1984), the extensive labor demands of otolith examination should be weighed against the potential gains in information. The results of this thesis should help to define the types of problems that could be addressed fruitfully with microstructure examination.

REFERENCES

Beamish, R.J. and G.A. McFarlane. 1983. The forgotten requirement for age validation in fisheries biology. Trans. Am. Fish. Soc. 112:735-743.

Bilton, H.T., D.F. Alderdice and J.T. Schnute. 1982. The influence of size and time at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. Can. J. Fish. Aquat. Sci. 39:426-447.

Brett, J.R. 1979. Environmental factors and growth. *In* Fish Physiology. Hoar, W.S., D.J. Randall and J.R. Brett (eds.). Academic Press. New York. p. 599-675.

Brett, J.R., W.C. Clarke, and J.E. Shelbourn. 1982. Experiments on the thermal requirements for growth and food conversion efficiency of juvenile chinook salmon, *Oncorhynchus tshawytscha*. Can. Tech. Rep. Fish. Aquat. Sci. 1127: 27p.

Brett, J.R. and C.A. Zala. 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. J. Fish. Res. Bd. Can. 32:2479-2486.

Brothers, E.B. 1981. What can otolith microstructure tell us about daily and subdaily events in the early life history of fish? Rapp. P.-v. Reun. Cons. Int. Explor. Mer 178: 393-394.

Brown, T.J., C.D. McAllister, C.D. Levings, M.S. Kotyk, B.D. Chang and J.S. MacDonald. 1984. Salmonid catch-data from Campbell River and Discovery Passage, 1983. Can. Data Rep. Fish. Aquat. Sci. No. 444:97 p.

Brown, T.J. and B.A. Kask. 1984. 1983 Campbell River Miller net samples. Can. Data Rep. Fish. Aquat. Sci. 471: 73pp.

Campana, S.E. 1983. Feeding periodicity and the production of daily growth increments in otoliths of steelhead trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). Can. J. Zool. 61:1591-1597.

Campana, S.E. 1984. Lunar cycles of otolith growth in the juvenile starry flounder *Platichthys stellatus* . Mar. Biol. 80: 239-246.

Campana, S.E. and J.D. Neilson. 1985. The incremental growth of fish otoliths. Can J. Fish. Aquat. Sci. 42:1014-1032.

Cowey, C.B. and J.R. Sargent. 1972. Fish nutrition, p. 382-492 *in* F.S. Russel and M. Young (ed.) Adv. Mar. Biol., Vol 10. Academic Press, New York, NY.

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

Dorcey, A.H.J., T.G. Northcote and D.V. Ward. 1978. "Are the Fraser marshes essential to salmon". Westwater Lectures No. 1. Westwater Inst., Univ. British Columbia. 29pp.

Dunford, W.E. 1975. Space and food utilization by salmonids in marsh habitats of the Fraser River estuary. M.Sc. thesis Univ. British Columbia, Vancouver, B.C. 81 p.

Dunkelberger, D.G., J.M. Dean and N. Watabe. 1980. The ultrastructure of the otolithic membrane and otolith in the juvenile mummichog *Fundulus heteroclitus*. J. Morph. 163:367-377.

English, K.K. 1981. Growth and feeding of juvenile chinook salmon, *Oncorhynchus tshawytscha*, in "in situ" enclosures. M.Sc. thesis, Univ. British Columbia Vancouver, B.C. 80pp.

Goodman, D. and P.R. Vroom. 1974. An assessment of the impact of proposed marina developement on the fisheries resources of the Campbell River estuary. Environment Canada Tech. Rep. No. PAC/T-74-13: 93 p.

Healey, M.C. 1980a. Utilization of the Nanaimo River estuary by juvenile chinook salmon (*Oncorhynchus tshawytscha*). Fish. Bull. 77:653-668.

Healey, M.C. 1980b. The ecology of juvenile pacific salmon in Georgia Strait, British Columbia. pp. 203-229. *In* Salmonid ecosystems of the North Pacific. (eds.) W.J. McNeil and D.C. Himsworth. Oregon State Univ. Press, Corvallis Ore.

Healey, M.C. 1982a. Juvenile Pacific salmon in estuaries: the life support system. p. 315-341. *In* V.S. Kennedy (ed.) Estuarine comparisons. Academic Press, New York, NY. 709 p.

Healey, M.C. 1982b. Timing and relative intensity of size-selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. Can J. Fish. Aquat. Sci. 39:952-957.

Healey, M.C. 1983. Coastwide distribution and oceanic migration patterns of stream- and ocean-type chinook (*Oncorhynchus tshawytscha*). Can. Field-Nat. 97:427-433.

Jobling, M. 1980. Effects of starvation on proximate chemical composition and energy utilization of plaice (*Pleuronectes platessa*). J. Fish. Biol. 17: 325-334.

Kask, B.A. and T.J. Brown. 1984. Meiofauna sled samples from Campbell River estuary and Discovery Passage 1982. Can. Data Rep. Fish. and Aquat. Sci. 476: 157pp.

Kask, B.A. and T.J. Brown. 1985. Meiofauna sled samples from Campbell River estuary and Discovery Passage 1983. Can. Data Rep. Fish. and Aquat. Sci. 499: 207pp.

Laroche, W.A., S.L. Richardson, and A.A. Rosenberg. 1982. Age and growth of a pleuronectid, *Parophrys vetulus* during the pelagic larval period in Oregon coastal waters. Fish. Bull. 80: 93-104.

Levings, C.D. 1982. Short term use of a low tide refuge in a sandflat by juvenile chinook salmon (*Oncorhynchus tshawytscha*), Fraser River estuary. Can. Tech. Rep. Fish. Aquat. Sci. No. 1111: 33 p.

Levings, C.D. 1984. Commentary: Progress in attempts to test the null hypothesis that juvenile salmonids aren't dependent on estuaries. p. 287-296. *In* W.G. Pearcy (ed.) The Influence of Ocean Conditions on the Production of Salmonids in the North Pacific. Oregon St. Univ. Corvallis, OR.

Levings, C.D., C.D. McAllister, and B.D. Chang. 1985. Use of the Campbell River estuary, British Columbia, by wild and hatchery-reared juvenile chinook salmon (*Oncorhynchus tshawytscha*). Manus. in prep.

Levy, D.A. and T.G. Northcote. 1982. Juvenile salmon residency in a marsh area of the Fraser River estuary. Can. J. Fish. Aquat. Sci. 39:270-276.

Lister, D.B. and H.S. Genoe. 1970. Stream habitat utilization by cohabiting underyearlings of chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon in the Big Qualicum River, British Columbia. J. Fish. Res. Board Can. 27:1215-1224.

Lough, R.G., M. Pennington, G.R. Bolz and A.A. Rosenberg. 1982. Age and growth of larval Atlantic herring, *Clupea harengus* in the Gulf of Maine-Georges Bank region based on otolith growth increments. Fish. Bull. 80: 187-199.

MacDonald, J.S. and C.D. Levings. 1984. Feeding of juvenile salmonids in a newly restored estuarine habitat. Trans. Am. Fish. Soc. 00:0000

Marshall, S.L. and S.S. Parker. 1982. Pattern identification in the microstucture of sockeye salmon (*Oncorhynchus nerka*) otoliths. Can. J. Fish. Aquat. Sci. 39:542-547.

Mugiya, Y. and J. Muramatsu. 1982. Time marking methods for scanning electron microscopy in goldfish otoliths. Bull. Jpn. Soc. Sci. Fish. 48: 1225-1232.

Mugiya, Y., N. Watabe, J. Yamada, J.M. Dean, D.G. Dunkelberger and M. Shimizu. 1981. Diurnal rhythm in otolith formation in the goldfish, *Carassius auratus*. Comp. Biochem. Physiol. 68A:659-662.

Neilson, J.D. 1984. Formation of otolith growth increments and their potential for assessing the early life history of chinook salmon (*Oncorhynchus tshawytscha*) Ph.D. Thesis. Simon Fraser Univ. 167pp.

Neilson, J.D. and G.H. Geen. 1981. Method for preparing otoliths for microstructure examination. Prog. Fish-Cult. 43: 90-91.

Neilson, J.D. and G.H. Geen. 1982. Otoliths of chinook salmon (*Oncorhynchus tshawytscha*): daily growth increments and factors influencing their production. Can. J. Fish. Aquat. Sci. 39: 1340-1347.

Neilson, J.D. and G.H. Geen. 1985. Effects of feeding and diel temperature cycles on otolith increment formation in juvenile chinook salmon (*Oncorhynchus tshawytscha*). Fish. Bull., in press.

Neilson, J.D., G.H. Geen and D. Bottom. 1985a. Estuarine growth of juvenile chinook salmon (*Oncorhynchus tshawytscha*) as inferred from otolith microstructure.

Neilson, J.D., G.H. Geen and B. Chan. 1985b. Variability in dimensions of salmonid otolith nuclei: implications for stock identification and microstructure interpretation. Fish. Bull. 82 (in press).

Pannella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science 173: 1124-1126.

Pannella, G. 1980. Growth patterns in fish sagittae. Pages 519-560 *in* D.C. Rhoades and R.A. Lutz, editors. Skeletal growth of aquatic organisms: Biological records of environmental change. Plenum Press, New York, USA.

Parker, R.R. 1968. Marine mortality schedules of pink salmon of the Bella Coola River, central British Columbia. J. Fish. Res. Bd. Can. 25: 757-794.

Parker, R.R. 1971. Size-selective predation among juvenile salmonid fishes in a British Columbia inlet. J. Fish. Res. Bd. Can. 28:1503-1510.

Patten, B.G. 1971. Predation by sculpins on fall chinook salmon, *Oncorhynchus tschawytscha*, fry of hatchery origin. U.S. National Marine Fisheries Service Spec. Sci. Rept. Fisheries No. 621. 14 p.

Radtke, R.L. and J.M. Dean. 1982. Increment formation in the otoliths of embryos, larvae, and juveniles of the mummichog, *Fundulus heteroclitus*. Fish. Bull. 80:201-215.

Reay, P.J. 1972. Seasonal pattern of otolith growth and its application to back-calculation studies in *Ammodytes tobianus*. J. Cons. int. Explor. Mer 34: 485-504

Reimers, P.E. 1973. The length of residence of juvenile fall chinook salmon in Sixes River, Oregon. Fish. Comm. Oreg. Res. Rept. 4: 43pp.

Reimers, P.E. and T.W. Downey. 1982. Population dynamics of fall chinook salmon in Sixes River. Ann. Prog. Rept., Fish. Div., Oreg. Dept. Fish Wildl., Portland, OR. 27 p.

Reimers, P.E., J.W. Nicholas, D.L. Bottom, T.W. Downey K.M. Maciolek, J.D. Rogers, and B.A. Miller. 1979. Coastal salmon ecology project. Ann. Prog. Rept. Fish. Res. Proj. Oregon Dept. Fish Widl. 43 pp.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Bd. Can. 191:382 p.

Simenstad, C.A. and R. Wissmar. 1984. Variability of estuarine food webs and production may limit our ability to enhance Pacific salmon (*Oncorhynchus* spp.). p. 273-286 *In* W.G. Pearcy (ed.) The Influence of Ocean Conditions on the production of Salmonids in the North Pacific. Oregon St. Univ. Corvallis, OR.

Simenstad, C.A., K.L. Fresh, and E.O. Salo. 1982. The role of Puget Sound and Washington coastal estuaries in the life history of Pacific salmon: an unappreciated function, p. 343-364. *In* J.S. Kenney (ed.) Estuarine comparisons. Academic Press, New York, NY. 709 p.

Taubert, B.D. and D.W. Coble. 1977. Daily growth rings of three species of *Lepomis* and *Tilapia mossambica*. J. Fish. Res. Bd. Can. 34: 332-340.

Volk, E.C., R.C. Wissmar, C.A. Simenstad and D.M. Eggers. 1984. Relationship between otolith microstructure and the growth of juvenile chum salmon (*Oncorhynchus keta*) under different prey rations. Can. J. Fish. Aquat. Sci. 41:126-133.

Wagner, H.H., F.P. Conte, and J.L. Fessler. 1969. Developement of osmotic and ionic regulation in two races of chinook salmon *Oncorhynchus tshawytscha*. Comp. Biochem. Physiol. 29:325-341.

West, C.J. 1983. Selective mortality of juvenile sockeye (*Oncorhynchus nerka*) in Babine lake determined from body-otolith relationships. MSc. thesis. Univ. of British Columbia. 62pp.

Wilson, K.H. and P.A. Larkin. 1982. Relationship between thickness of daily growth increments in sagittae and change in body weight of sockeye salmon (*Oncorhynchus nerka*) fry. Can. J. Fish. Aquat. Sci. 39: 1335-1339.