DAILY RINGS IN OTOLITHS OF SOCKEYE SALMON (*Oncorhynchus nerka*) AND THEIR RELATIONSHIP TO GROWTH

by

Kenneth H. Wilson

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Department of Zoology

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

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ABSTRACT

This study reports the occurrence of daily rings in the otoliths of Oncorhynchus nerka fry and examines their relationship to growth. In experiment 1, sockeye salmon fry were collected from the Fulton River spawning channel at Babine Lake, British Columbia in May 1978. The fish were reared for 26 days in enclosures in the spawning channel and were sampled every seven to ten days. Sagittae were removed from 25 fish from each sample, and the growth rings in one otolith from each fish were counted. A regression of the number of rings on the number of days since capture showed that these rings are, on average, formed daily, beginning at the time of emergence. A number of possible technical and biological causes of variation in ring counts within and between samples are considered.

In Experiment 2, sockeye salmon fry were reared in the laboratory from fertilized eggs taken in the fall of 1978 at the Weaver Creek spawning channel near Mission, British Columbia. A random sample of 64 of these fry was marked to enable identification of individuals. Each individual was weighed initially on June 6 or 8, again on July 6, and surviving fish were weighed a third time on July 20. After a final weighing, sagittae were removed and a standard otolith radius was determined by counting back the appropriate number of daily rings which corresponded to each weight. The regression of ℓn otolith radius on ℓn fish weight was linear, and had an R^2 of 0.92, which demonstrates a relationship between the mean width of a daily ring in sockeye salmon fry sagittae, and a mean daily change in the weight of the fry. Using this regression line, we back-calculated the previous weight of the individual fish from

the corresponding otolith radius and a latter fish weight and otolith radius and found the errors to be relatively small -- in the order of 15 per cent.

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INTRODUCTION

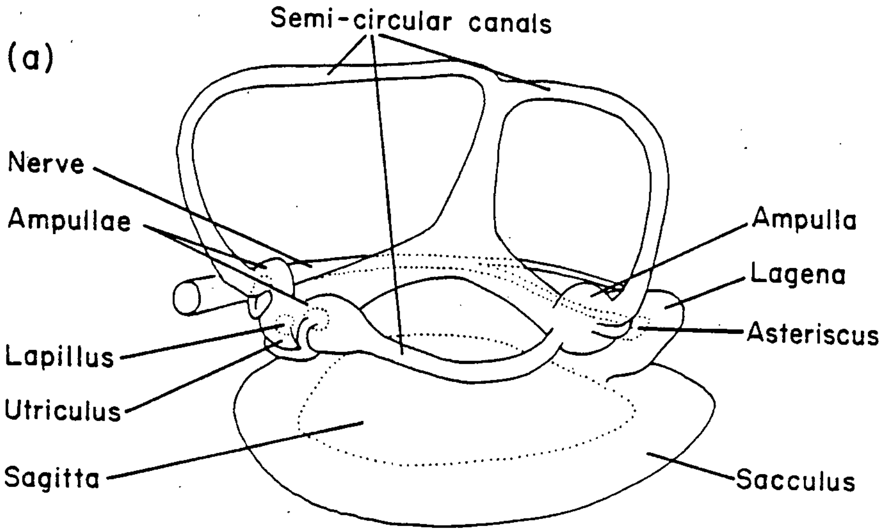
The otoliths of teleost fishes are parts of the labyrinth system. The typical teleost fish has six otoliths, three in each labyrinth; a sagitta in each sacculus, an asteriscus in each lagena, and a lapillus in each utriculus. The sagitta is almost invariably the largest otolith in non-osteriophysids. Figure 1 (after Blacker 1974) shows the labyrinth of a cod (Gadus morhua) and the associated otoliths. Figure 2 (after Taubert and Coble 1977) represents the sagitta of a sockeye salmon (Oncorhyncus nerka).

Each otolith is composed primarily (85-90 per cent) of argonite (calcium carbonate), and a protein related to collagen called otolin (Degens et al. 1969). Otolin constitutes between 0.25 and 10 per cent of the otolith. Small amounts of calcium phosphate and calcium sulphate as well as comparable sodium salts are also present (Morris and Kittleman 1967). An excellent review of the literature on otoliths and their growth is presented by R.W. Blacker (1974).

Daily growth rings were first reported to occur in the otoliths of Merluccius bilinearis, Utrophycis chuss and Gadus morhua by Pannella (1971). Subsequent research has confirmed their occurrence in at least seven species -- juvenile northern anchovy, Engraulis mordax (Brothers et al. 1976); juvenile and adult nehu, Stelaphorus purpurens (Strusaker and Uchiyama 1976); juveniles of the green sunfish, Lepomis cyanellus, pumpkinseed,

Figure 1. The labyrinth of the cod, *Gadus morhua* (L.) showing the relationship between the semicircular canals and the otolith chambers. (a) Left labyrinth viewed from the outer side and (b) dorsal view of the left labyrinth (after Blacker 1974).

(a)


Semi-circular canals
Nerve
Ampullae
Ampulla
Lagena
Asteriscus
Lapillus
Utriculus
Sagitta
Sacculus

(b)

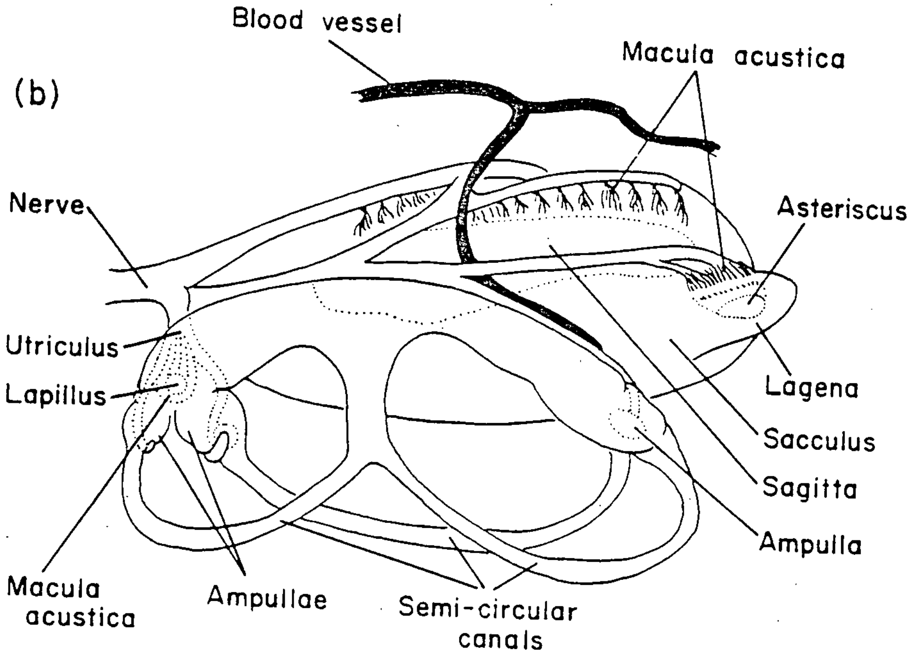
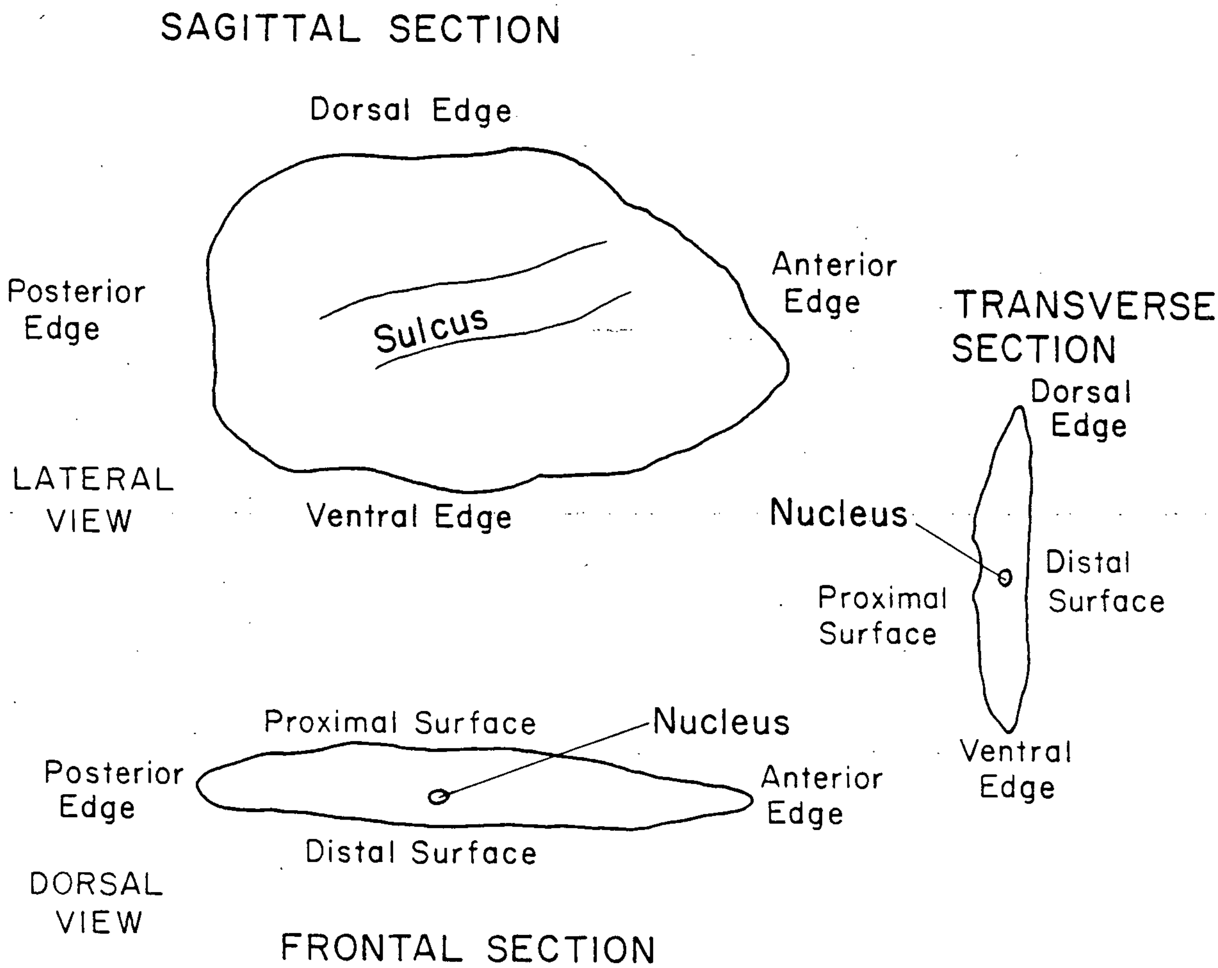

Blood vessel
Macula acustica
Nerve
Asteriscus
Utriculus
Lapillus
Lagena
Sacculus
Sagitta
Ampulla
Macula acustica
Ampullae
Semi-circular canals

Figure 2. Sagittae of O. nerka fry (after Taubert and Coble 1977)


SAGITTAL SECTION
Dorsal Edge
Posterior Edge
Sulcus
Anterior Edge
LATERAL VIEW
Ventral Edge
TRANSVERSE SECTION
Dorsal Edge
Nucleus
Distal Surface
Proximal Surface
Ventral Edge
Proximal Surface
Nucleus
Posterior Edge
Anterior Edge
Distal Surface
DORSAL VIEW
FRONTAL SECTION

L. Gibbosus, Mozambique mouthbrooder, Meidia menidia (Barkman 1978); and juvenile sockeye salmon, Oncorhynchus nerka (Wilson and Larkin 1980). Wilson and Larkin (1980) have also observed daily ring patterns in the sagittae of chinook salmon (Oncorhynchus tshawytscha), chum salmon (O. keta), pink salmon (O. gorbuscha), coho salmon (O. kisutch), rainbow trout (Salmo gairdneri), Pacific staghorn sculpin (Leptocottus armatus), starry flounder (Platichthys stellatus), Pacific halibut (Hippoglossus stenolepsis), and Olympic mud minnow (Novumbra hubbsi) and in the asteriscus of the northern squawfish (Ptychocheilus oregonensis).

Although daily growth rings were first recognized by Pannella, they were probably first described by Hickling (1931), who described the microstructures of the hake (Merluccius merluccius) otolith as concrete shells or lamellae of organic material spaced about two microns apart and connected by stout radial fibers. The inorganic component was described as interlocking needle-like crystals about 40 microns in maximum length and less than one micron wide, which are secreted among the radial fibers and pass through the lamellae. The concentric lamellae described by Hickling were examined by Pannella (1971). Pannella observed that the smallest bands in the otolith were made up of two distinct parts. Under "optical" examination the bands consist of a light zone of width 0.5 to 1 micron, and a dark zone 0.5 to 2.5 microns wide, with the dark zone containing a much denser mesh of organic fibers. Pannella states that the dark zone corresponds to periods of rapid protein deposition, and that "during fast deposition the production of organic fibers is high, but calcification is even higher, so that the ratio of organic to inorganic fibers is overwhelmingly in favour of the organic portion (over 90 per cent) (Sic). During slow deposition fewer organic fibers are produced, but the ratio is in favour of the organic portion, since calcification is almost nil." This statement may have resulted from the observation of both otoliths and acetate impressions of otoliths, and it is not clear whether the "optical" examination involved the use of transmitted or reflected light.

Another interpretation of these observations of otolith composition is possible. In the formation of the annulus, it is the fast growth zone which is relatively high in calcium content and which is opaque under observation with transmitted light (Blacker 1971). In temperate fishes, the fast growth zone usually forms in the summer. The hyaline zone usually forms in winter, is higher in protein content (Blacker 1971, Christensen 1964), and appears transparent under observation with transmitted light. Similarly, the hyaline portion of a daily ring (observed with transmitted light) is high in protein, analagous to Hickling's lamellae, is the thinnest of the two zones which comprise a daily ring, and is probably formed during the hours of slow fish and otolith growth. The thicker zone, which appears opaque under transmitted light, is composed primarily of calcium salts and is formed during the hours of more rapid growth. I speculate that the rate of calcium salt deposition is highest during period of most rapid otolith growth, but whether the protein lamellae are formed by a rapid increase in protein deposition, a decrease in calcium salt deposition, or a combination of both, cannot be determined by optical observation of preserved otoliths.

Pannella (1974) observed 4, 8, 14, and 28-day cycles in daily ring thickness in a variety of tropical fish otoliths, which he attributed to lunar and tidal effects. Brothers and McFarland (unpub ms.) observed a small number of French grunt (Haemulon flavolineatum) otoliths collected during different times of the day. Their observations suggest that the dark portion of the daily growth ring (using transmitted light) is formed during the night, and the light zone is formed during the day.

Taubert and Coble (1977) worked with three species of Lepomis and Tilapia mosambica, and attempted to differentiate between the effects of photoperiod and feeding regime in daily ring formation. One tank was kept in constant light and fed at three hour intervals. Another was also kept under constant light and fed at three hour intervals with a nine hour hiatus every 24 hours. A third was kept under a 24-hour to 12-hour light-dark cycle and fed every six hours, and a fourth was maintained under a 15-hour to 9-hour light-dark cycle and fed every six hours. Fish in groups one and two demonstrated no daily ring formation, but in some cases had as many as four times as many rings as the age in days. These presumably represent feeding rings. Group three did not produce a ring every 36 hours. Taubert and Coble concluded that a diurnal feeding regime was not sufficient to cause daily ring formation, and that a diurnal change in photoperiod was sufficient to produce daily rings. They further speculated that the fish under the 24-hour light to 12-hour dark photoperiod did not produce 36-hour rings because the light cycle was too abnormal to entrain a biological clock, and in fact had a damping effect. Taubert and Coble were also able to stop daily ring formation by reducing water temperature, producing an artificial annulus which resembled the annulus in wild fish otoliths. They concluded that daily growth ring formation ceases when fish growth ceases.

The relationship between daily growth ring width and fish growth is largely unexplored. Taubert and Coble found a linear relationship between sagitta radius and fish length (r=0.9936). Brothers and McFarland (unpub ms.) found a linear relationship between log otolith radius and log fish length for French grunts, and attempted to relate changes in daily growth ring width to life history transitions.

In summary, there is much speculation and little documented evidence regarding otolith growth in general, and daily growth ring formation in particular. It is commonly assumed that the finest visible rings in any fish otolith are formed daily, particularly if they occur in the fastest growth zone.

It was the purpose of this study:

1. to demonstrate the occurrence of daily growth rings in the sagittae of *O. nerka*.

2. to examine the relationship between fish growth and daily growth ring width.

3. to develop techniques for the observation and measurement of daily growth rings.

GENERAL METHODS

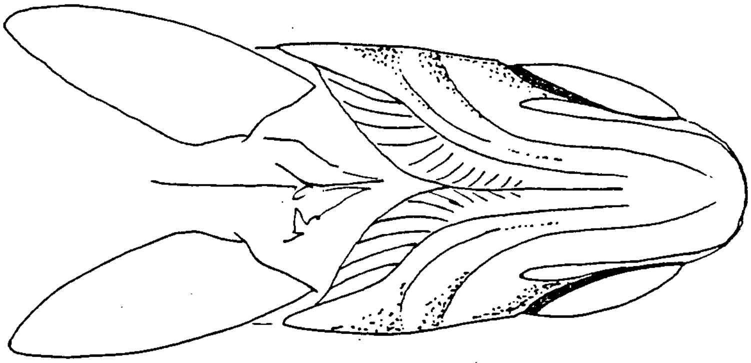
Fish Preservation

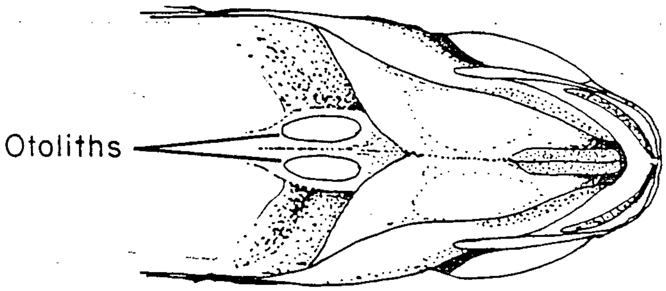
For this study, fry were preserved in 70 per cent ethanol. For long term storage (several weeks or more) care was taken to ensure that the ethanol had a pH of approximately 7, since acidic preservatives tend to decalcify otoliths.

Otolith Removal

Of the three pairs of otoliths, only the sagittae were used. The sagittae of large fish are usually removed by making a transverse cut three-quarters of the way through the head behind the eye orbits, or longitudinally through the center of the head between the orbits. For small fish these techniques are inefficient. We employed two techniques depending on the size of the fish. The otoliths of extremely small fish (1-2 cm.) were removed in a manner similar to that described by Taubert and Coble (1977). The head is placed or pressed on a piece of glass on the stage of a dissecting microscope. The otoliths appear as bright dots under side illumination. The head can then be teased gently apart using drawn glass probes and the otoliths picked up using probes or fine forceps. The otoliths of larger fry ($\geq$ 2cm) were removed by holding the fry on the stage of the dissecting microscope with the dorsal side down and removing the gills and opercular plates (Fig.3). The sagittae could then be seen under top illumination just posterior to the eye orbits, and

Figure 3. Appearance of O. nerka fry before and after removal of lower jaw, gill covers and gills to reveal sagittae.





lower jaw, gill covers, gills removed

were removed by teasing apart the cartilage.

Otolith storage

Otoliths were stored dry in the depressions of MicroTest 3034 tissue culture plates. Each of the sixty depressions in every plate was numbered and lettered for convenience, and the otoliths were held in the depressions with a sheet of 1/16th inch plexiglass cut to fit inside the plate. The plexiglass was held against the depressions by a piece of sponge between the lid and the plexiglass, and the entire unit was held together by several elastic bands.

Otolith Preparation and Observation

The otoliths of very small sockeye fry were examined whole. As the fry grow, the otoliths thicken and the internal structure becomes obscured. Thicker otoliths were ground into thin sections by attaching them to the center of a glass microscope slide using acrylic adhesive. Initially the otoliths were ground by hand, using Carborundum No. 50 aluminum oxide powder mixed with water on a sintered glass plate. Extreme care had to be taken to keep the slide parallel to the grinding surface, and thus avoid rounding the ground surface of the otolith. Subsequently, grinding methods developed by John Neilson (Neilson, In Press) were found to be superior and were used for latter work. Neilson's method employs a grinding jig which holds the slide parallel to the grinding surface to ensure a flat grind, and maintains constant and controllable pressure. Mylar grinding sheets (available from 3M in a variety of grit sizes) were used as the grinding surface. The finest grit, called lapping paper, had a particle size of 0.5 microns and was used to finish the otoliths. Rapid grinding was performed on 25 micron paper. After polishing one side of the otolith, the acrylic glue was softened by immersing the slide in acetone, and the ground surface of the otolith was reattached to the slide. The grinding process was then repeated. Neilson also employed heat sensitive resin as an adhesive instead of acrylic. We found that this method involved only slight heating to remove the otoliths and reattach them, and the grinding process was greatly simplified.

The grinding process was critical. Excessive grinding can remove rings from the otolith, while insufficient grinding can leave rings obscured by an overburden of calcium carbonate. The otolith must be observed numerous times during grinding, and a fairly high level of skill is required.

Several authors (Pannella 1971; Taubert and Coble 1977; Strusaker and Uchiyama 1976) have suggested etching the ground otoliths for several seconds in dilute HCl, either in preparation for taking an acetate impression, or to augment direct viewing. We did not use acetate impressions, and found considerable variation in etching rate within and between otolith sections. This variation resulted in occasional losses of material in both the center and edges of the otolith. Since there was only minor improvement in the visibility of the internal otolith structure, and there was a potential for introducing error, this technique was not used for routine work.

The prepared otolith was clarified by immersion in glycerine or immersion oil prior to viewing. The internal structure of the otolith becomes increasingly visible as the clarifying agent is absorbed, but excessive clarification obscures the daily growth rings by making the opaque portion of the ring transparent. For salmon fry otoliths, serial counts of daily rings during the process of clarification rapidly increased, then stabilized and then slowly decreased. The counts on very small otoliths or very thin sections of otoliths increased for up to six hours, remained stable for six to twelve hours, and then decreased for several days. It was accordingly made standard practice that for small otoliths counts were made after six to eight hours of clarification. Counts on thicker sections and larger otoliths increased for up to a day, remained stable for several days, and decreased for a week or more. Observations were made on these otoliths on the day following immersion. Overclarified otoliths were recovered by placing them in ethanol to remove the clarifying agent, and reclarified.

The clarified otoliths were photographed using an Olympus trinocular compound microscope and transmitted light, and fitted with a Wild 35mm automatic camera. Otoliths were photographed at 50X and 200X (based on the magnification factor for the film negative). A projector slide was prepared directly from the film negative and projected onto a white table top for counts and measurements.

A series of annotated photographs of sockeye otoliths has been prepared to assist others in interpreting daily growth patterns and understanding the methods used in interpreting the otoliths examined in this study. These photographs are presented in Appendix 1. The methods employed in counting, measuring and analysing the data are presented in the specific methods sections.

EXPERIMENT 1: DAILY GROWTH RINGS IN THE OTOLITHS OF JUVENILE SOCKEYE SALMON (*Oncorhynchus nerka*)

Introduction.

The early life history of salmon makes the interpretation of growth ring data difficult. Sockeye eggs hatch in the gravel of stream beds between December and January, but emergence may not occur until March, April or May. Fry generally move directly to a lake upon emergence, but short-term stream residency is not uncommon. Prior to emergence, the fry exist on yolk reserves (Dill 1968), and exogenous feeding begins as yolk reserves are exhausted. Taubert and Coble (1977) suggest that daily growth ring formation in the sagitta is prompted by fluctuations in light intensity and influenced by temperature. Salmon fry may occur at various depths in the gravel and may be exposed to diurnal changes in light intensity and temperature prior to emergence. Some sockeye salmon fry do not begin feeding even after completely absorbing their yolks. Such fry are called "pinheads" and are commonly observed in rearing facilities. These factors may all serve to increase the variation in the time at which an individual first produces growth rings and make suspect the application to wild populations of results obtained from laboratory studies. To make this study of otolith growth applicable to wild populations, we collected and reared fry in their natural environment.

Methods

Sockeye fry were collected from converging throat traps at the mouth of Fulton River spawning channel 2 at Babine Lake, British Columbia, in 1978. To determine the maximum time between emergence and capture at the channel mouth, 10,000 fry were dyed with neutral red and released at the upstream end of the channel. Experimental fry were captured in the late evening and early morning of May 12 and 13, and reared in enclosures in the channel. Samples were taken on May 13, 23, and 31, and June 9, and were preserved in 70 per cent ethanol. Twenty-five fish were examined from each sample. One otolith was used from each fish, and any otoliths which were crystalline or otherwise uncountable were rejected.

Sagittae were removed in the laboratory and affixed to glass microscope slides with the sulcus (*i.e.* proximal surface) upward. Thicker specimens (primarily those from the June 9 sample) were ground lightly on the proximal surface, using aluminium oxide on a sintered glass plate. All otoliths were clarified with glycerine and photographed at a magnification of 200X using transmitted light and a standard compound microscope with a 35 mm photographic attachment. A 35 mm projector slide was prepared directly using the developed film negative.

Ring counts were made on the projected images. A ring was defined as a pair of distinct bands, one light and one dark. All discernible rings were counted. Figure 4 shows a prepared otolith (from the June 9 sample) and the associated count.

Results

Of the 10,000 fry released at the upstream end of the channel, 340 were recaptured in sampling at the mouth; 339 were captured within 5 hours, and only one subsequently, during the second evening after release. With such a short passage of time through the channel, the date of capture of the fry from which otoliths were sampled may be taken as the date of emergence.

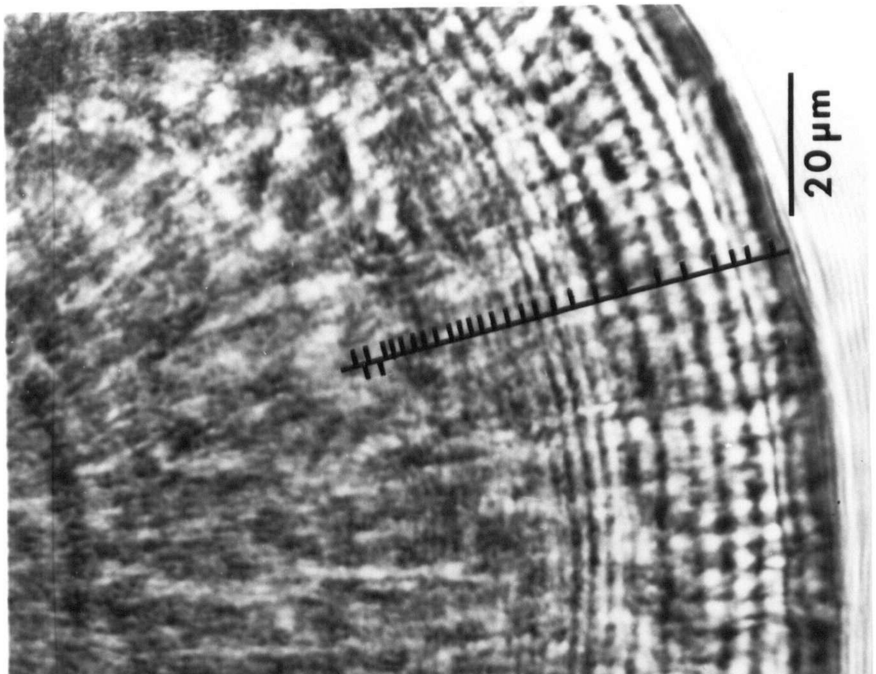
If rings are being formed daily in sockeye salmon sagittae, a least-squares linear regression of ring count against known age in days (after capture) should have a slope of 1. Further, if ring formation began on the average at the time of capture, the regression should still have a slope of 1 when constrained through the origin.

The mean and variance of daily ring counts in samples of 25 sockeye salmon from Fulton River spawning channel, Babine Lake, British Columbia, 1978 were as follows:

SAMPLE	1	2	3	4
DATE	May 13	May 23	May 31	June 9
Mean	3.9565	10.000	17.850	25.668
Variance	53.316	58.000	27.503	19.101

Bartlett's test of homogeneity of variance indicates the variances of the four samples are not significantly different. Samples 2, 3, and 4 are distributed around a mean which in each case is close to a line passing through the origin with a slope of 1.

Figure 4. Sagittae from an *O. nerka* fry removed from the enclosure on June 9.


20µm

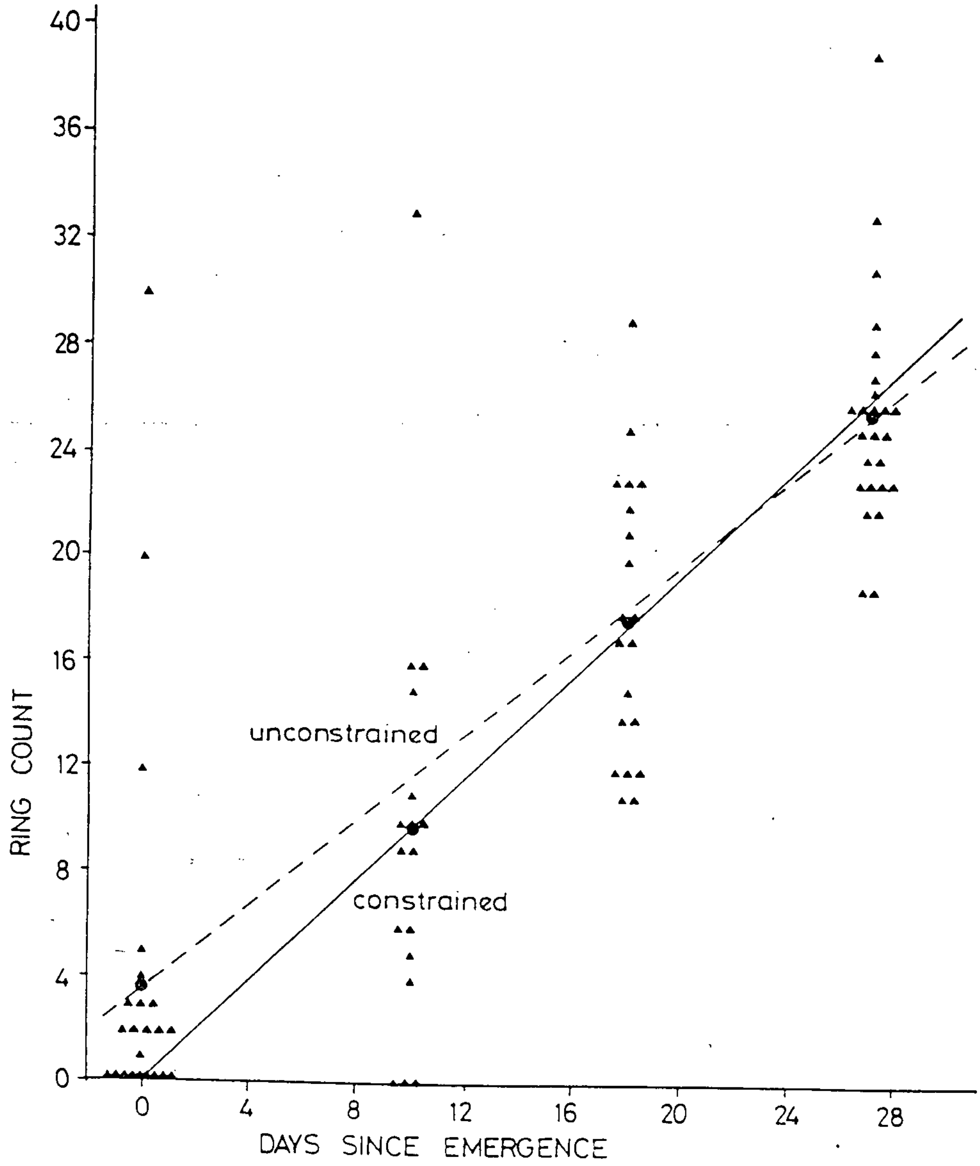
The counts below the mean in these samples must be represented in the first sample by zero counts because the variation below the mean is not due to counting error. Because negative counts are impossible, the first sample cannot have a mean of zero and was eliminated from the constrained regression. One regression line was fitted to all the data, and a second regression constrained through the origin was fitted to the data from the last three samples (Fig.5). The statistics of regression of daily ring count against time in days is as follows:

	Intercept	Slope	SD of slope
Standard regression (all data)	3.4869	0.81660	0.10020
Regression constrained through the origin (data for May 23, 31)	_	0.97584	0.033899

Discussion

The results suggest that growth rings in sockeye salmon sagittae are formed daily. Because the vast majority of fry migrated rapidly and hence were captured almost immediately upon emergence, the results further suggest that the average date of first discernible ring formation is the date of emergence. The apparent reduction in variance with age can be attributed both to the death of the fish which do not grow, and to the nature of pre-emergent growth rings which are formed while the fish are in

Figure 5. Graph of otolith ring count versus age in days since emergence.



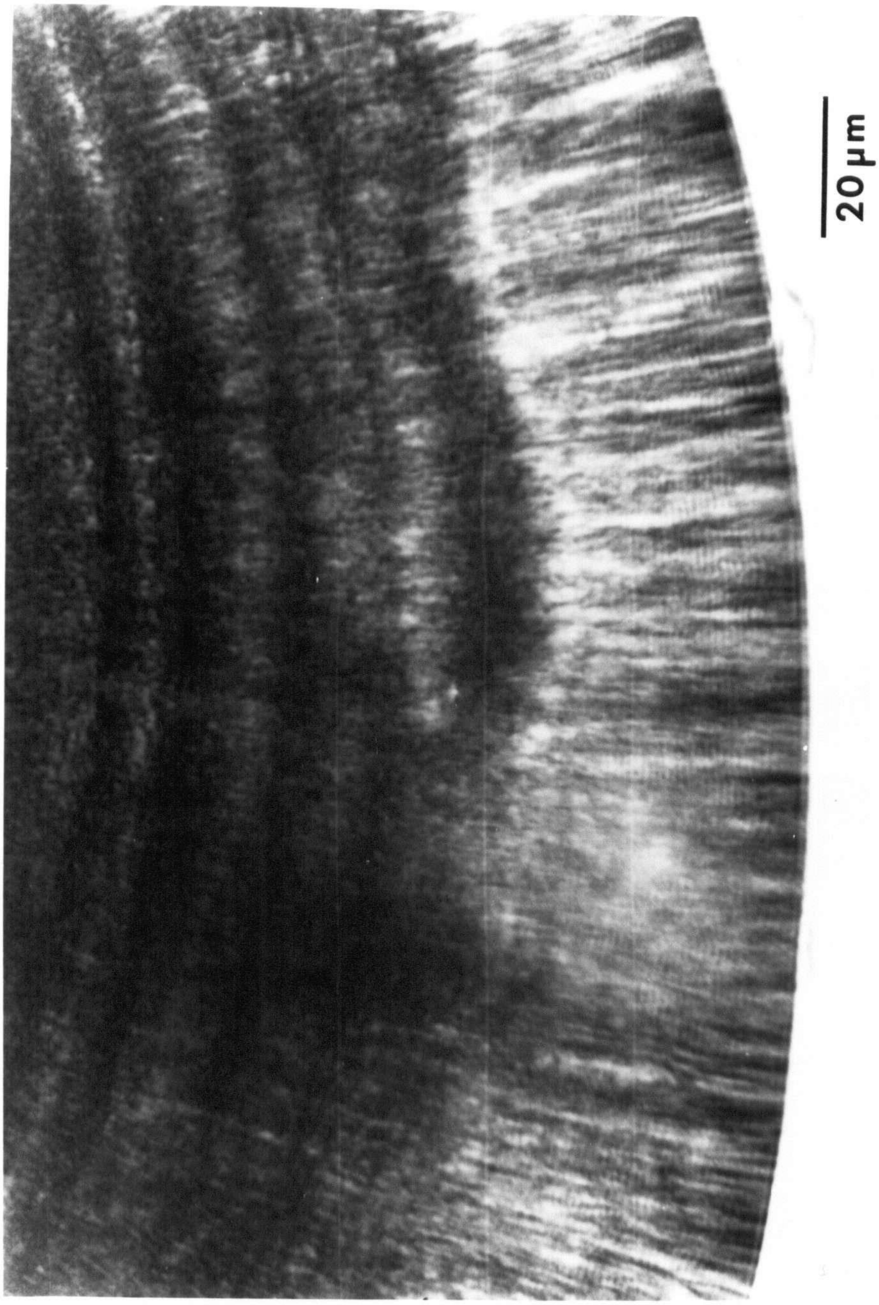
40
36
32
28
24
20
16
12
8
4
0
RING COUNT
unconstrained
constrained
0
4
8
12
16
20
24
28
DAYS SINCE EMERGENCE

the gravel of the channel. These rings have an extremely fine structure and might be obscured as the otoliths thicken (Fig.6).

Another possible explanation for the reduction of the variance of ring counts with age is the presence of subdaily rings at earlier ages. However, we have not observed subdaily rings, even in fish raised in the laboratory at growth rates substantially higher than those occurring in our experimental enclosures.

Finally, there is a possibility of faulty techniques as sources of error that account for the reduction of variance with age. Rings can be removed from the otoliths through excessive grinding, and can be obscured by the layer of calcium overburden where grinding is insufficient. Such errors are not considered to be serious for sockeye fry at early ages as here reported, though they may prove to be at later stages of the life history.

Figure 6. Sagittae of an *O. nerka* fry removed from the enclosure on May 13.


20 µm

EXPERIMENT 2: THE RELATIONSHIP BETWEEN WIDTH OF DAILY GROWTH RINGS IN SAGITTAE AND CHANGE IN BODY SIZE OF SOCKEYE SALMON (*Oncorhynchus nerka*) FRY

Introduction

Otoliths have been used as a method of aging fish since the turn of this century (Graham 1929). While growth studies utilizing otoliths are often confined to those species with unreadable scales, at least one recent study examined the relationship between fish growth and otolith size in salmonids (Jonsson and Stenseth 1977). Until recently, only techniques utilizing annular marks were available for age and growth studies. The occurrence of daily growth rings in teleost otoliths (Panella 1971) including those of sockeye salmon (Wilson and Larkin 1980) provides an important new methodology for age and growth studies.

The purpose of this experiment is to examine the relationship between fish weight and otolith size over short time intervals for sockeye salmon fry, utilizing daily rings instead of annuli as the time marker.

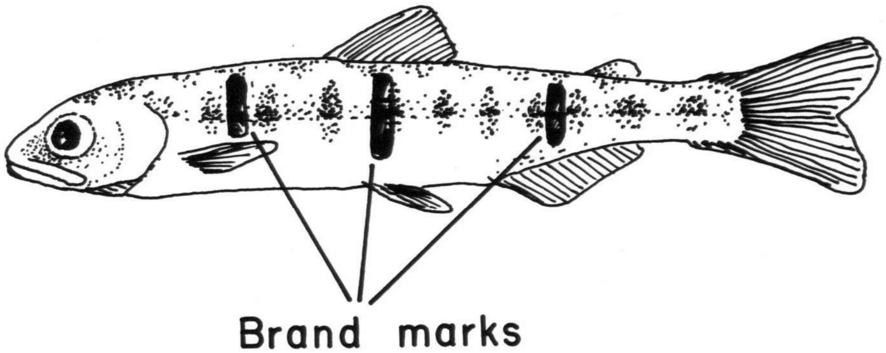
Methods

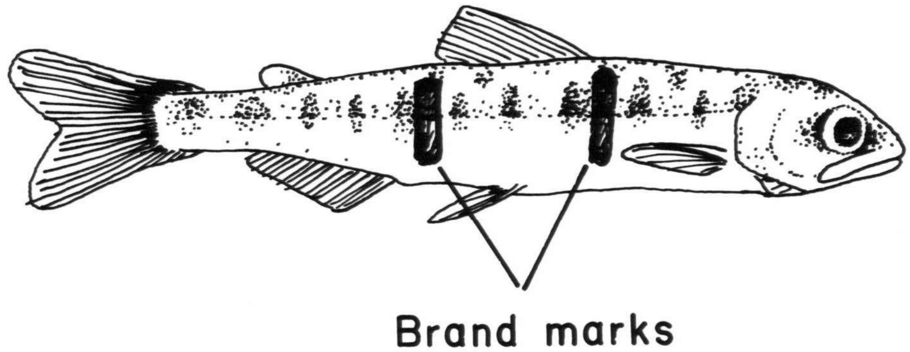
Sockeye salmon fry were reared in the laboratory from fertilized eggs taken in the fall of 1978 at the Weaver creek spawning channel near Mission, British Columbia. A random sample of 64 fry was branded to enable identification of individuals. The marks consisted of the presence or absence of a cold brand in any one of five distinct locations on the body. The fry were anaesthetized with MS222 and branded with a copper wire immersed in liquid nitrogen. The brand sites were: on the left side above the pectoral fin, below the dorsal fin, and above the anal fin, and on the right side above the pectoral fin and below the dorsal fin (see Fig.7).

These various patterns enabled 32 fry to be identified as individuals in each of two tanks. Each of the fry was weighed and branded before being placed in a tank (12in x 12in x 24in). One tank was stocked on June 6, 1979, and a second two days later. The fish were not fed for 24 hours prior to weighing in order to minimize variation in stomach contents; otherwise the fish were fed at one hour intervals eight times a day. The use of a Mettler automatic pan balance enabled the fish to be placed on the pan, a weight taken, the scale tared and a second weight taken by removing the fish from the pan. The two weights were compared to ensure agreement within 0.01 grams, and averaged. The fish were reweighed if necessary. The weighings were all carried out during the same period of the day, during the late afternoon.

Thirty days after the first tank was set up, the fish in both tanks were anaesthetized and reweighed. Fry surviving the second weighing were reared for an additional two weeks. Data

Figure 7. Cold branding sites on O. nerka fry.


Brand marks


Brand marks

were recorded for those fry which died immediately as a result of handling stress during the second weighing as well as for those still remaining at the end of the experiment. Those fish which died during the course of the experiment were discarded.

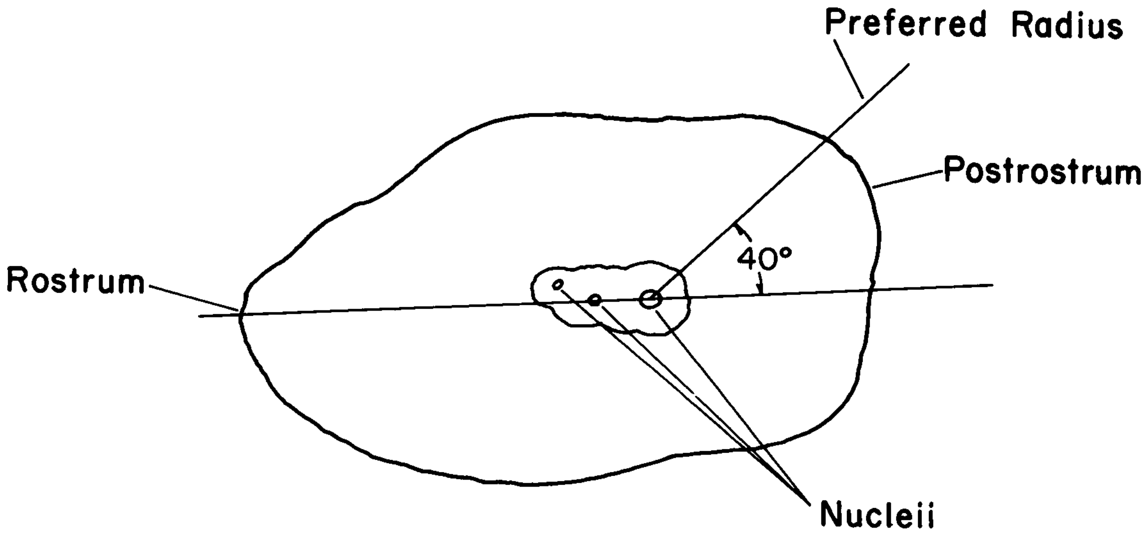
Sagittae were removed and ground on both sides (see General Methods). One otolith from each fish was photographed using Kodak panatomic X film in a 35mm camera mounted on a compound scope. Projector slides were prepared directly from film negatives. Slides were projected on a white table top using a projector held at a fixed height and angle, with a fixed focus, to achieve a magnification factor 382X. Measurements were taken directly from the projected image.

A brief description of the growth and morphology of sockeye salmon otoliths will facilitate a discussion of the measurement system used. Figure 8 represents a generalized sockeye otolith.

In sockeye salmon the sagitta typically has several foci, and changes shape as it grows. The sagitta of the yolk sac fry is oval in shape, but as the fry grows the sagitta becomes typically heart-shaped with the rostrum and postrostrum exhibiting the most rapid radial growth. These areas of greatest growth usually show the most distinct daily ring formations. The postrostrum in our samples was less prone to breakage during preparation, more uniformly shaped, and consistently had the most easily discernible daily ring patterns.

To ensure that measurements were taken on the same radius for each otolith, a line was drawn to the rostrum through the

Figure 8. Sagittae of an *O. nerka* fry showing rostrum, post-rostrum, nucleii and location of preferred radius.


Preferred Radius
Postrostrum
40°
Rostrum
Nucleii

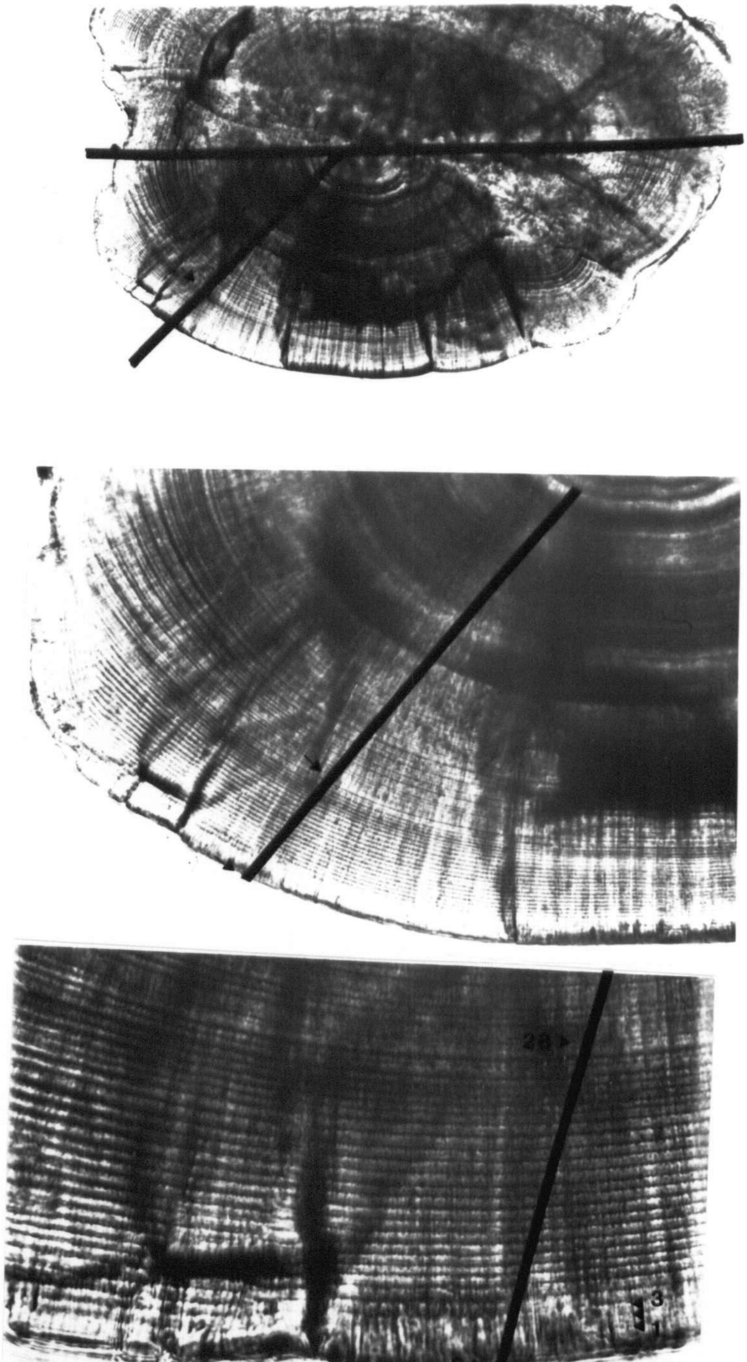
focus immediately adjacent to the post-rostrum. A radius was drawn in a straight line from this focus to the post-rostral bulge at a fixed angle of 40 degrees (Fig. 8).

For the fish from the first tank which survived to the end of the experiment, the radius was determined for three distances: (1) from the focus to the edge of the otolith; (2) from the focus to the growth ring 14 rings back from the edge; (3) from the focus to the growth ring 44 rings back from the edge. These radii corresponded to the final, middle, and initial weights respectively. Similarly, those fish which survived to the end of the experiment from the second tank (stocked two days after the first) were measured to the edge, and to the 14th and 42nd rings back from the edge. The otoliths of those fish from the first tank which died as a result of handling stress were measured to the edge and to the ring 30 rings back from the edge. Those from the second tank were measured to the edge and to the ring 28 rings back from the edge (Fig. 9). The branding and weighing process left a clear stress mark 28 or 30 rings back from the edge in every otolith (Fig. 9).

In each case the radius was measured three independent times and average values were calculated. Thus, for each fish there was determined a series of two or three weights and a standard otolith radius corresponding to each weight.

The relationship between otolith measurements and weights of fry was evidently curvilinear, as would be expected considering

Figure 9. Sagittae of *O. nerka* fry removed from Tank 2 on July 6.



that the relation is between linear and essentially volumetric measurements. Accordingly a standard regression line was fitted to the log of the weight and the log of the radius. Additionally, the data in raw form were fitted by orthogonal polynomials.

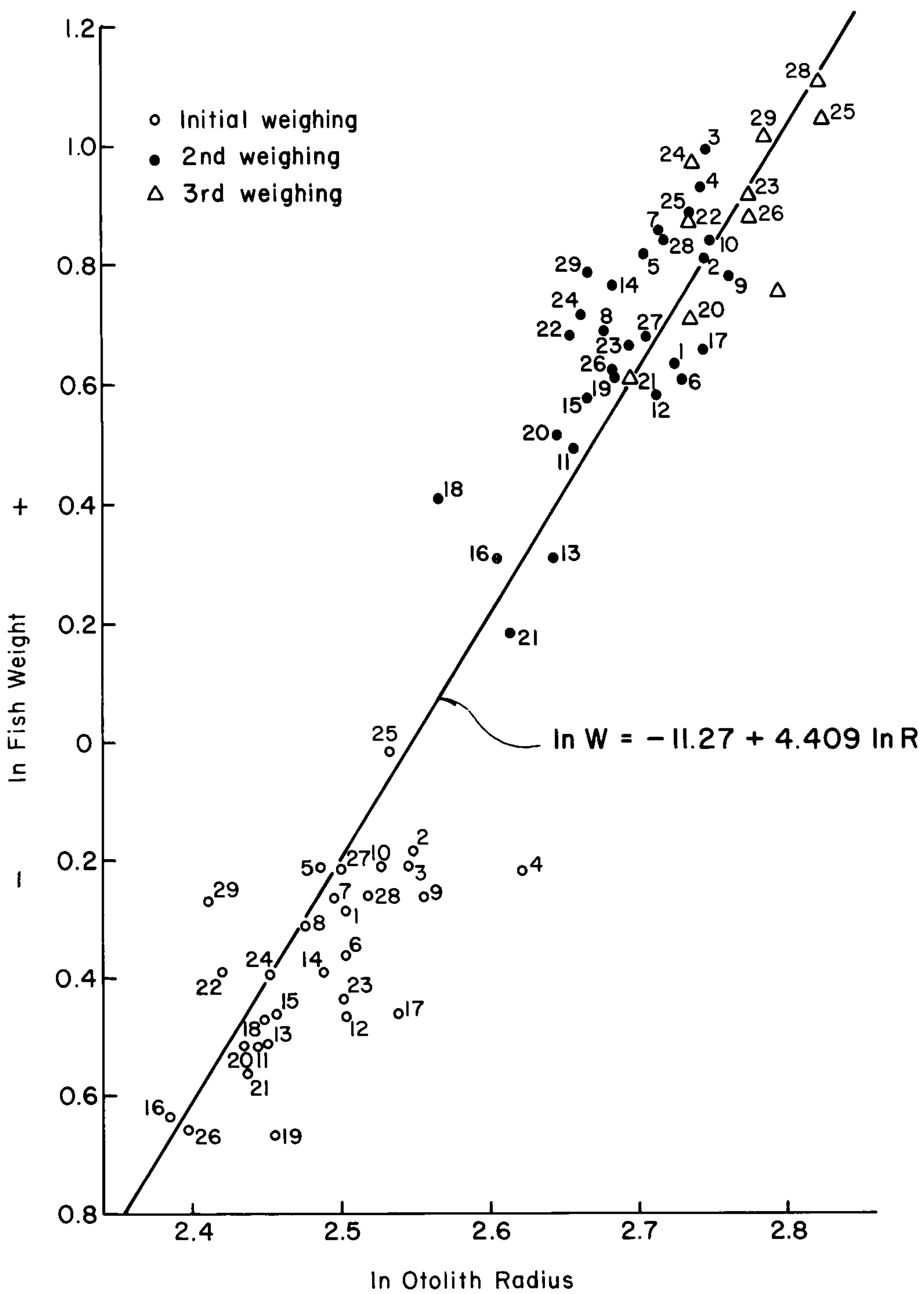
The log regression line was employed to back calculate the weight of each individual fish at the time of previous weighings, using a later weight and radius. The predicted weights were compared with those observed.

The relationship between otolith measurements and weights of fry was evidently curvilinear, as would be expected considering that the relation is between linear and essentially volumetric measurements. Accordingly a standard regression line was fitted to the log of the weight and the log of the radius. Additionally, the data in raw form were fitted by orthogonal polynomials.

Results

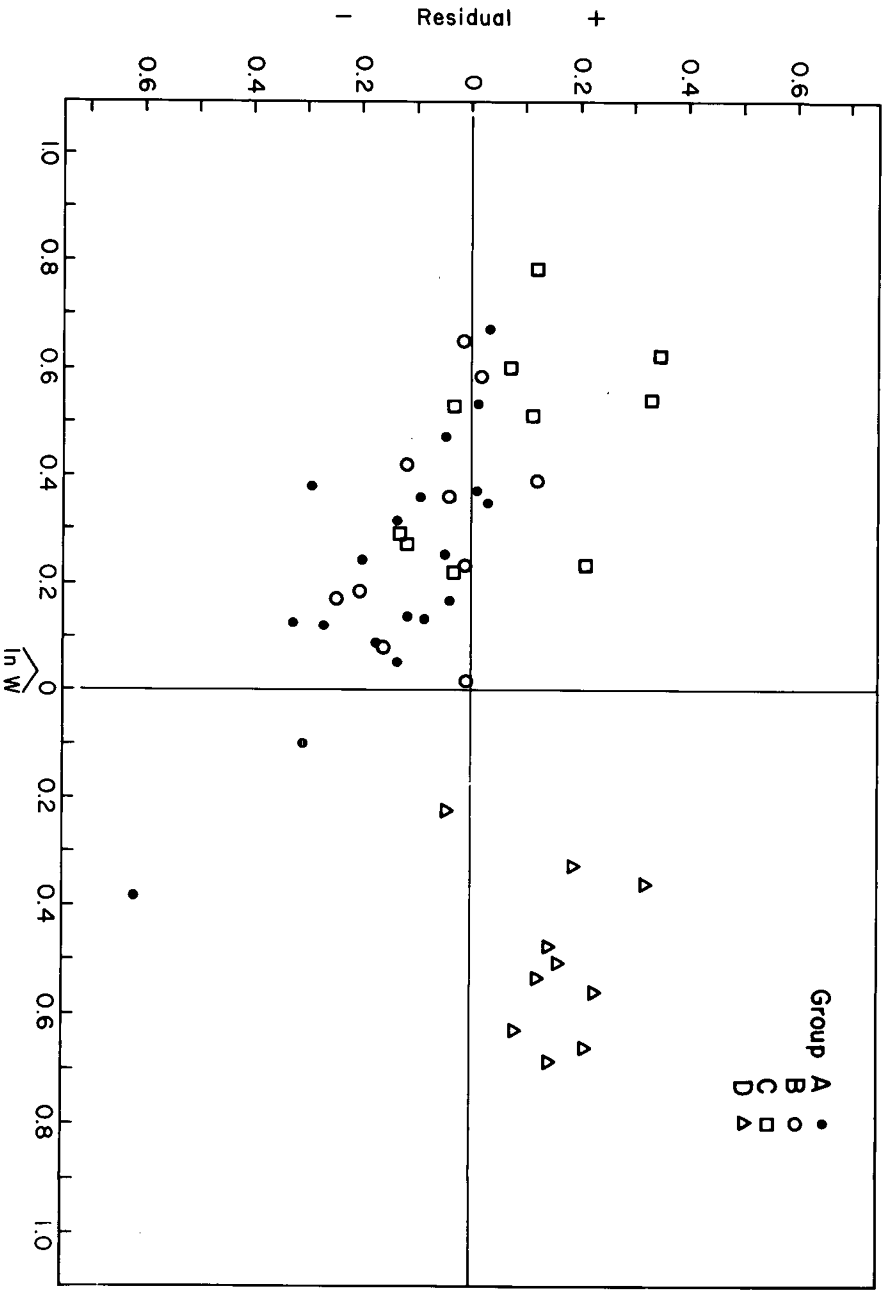
Fish weights and corresponding radii are given in Table I. The standard linear regression line fitted to the logged data had a line of best fit $\ell nW = -11.27 + 4.409^{*} \ell n^{R}$ and an r^2 of 0.9230 (see Fig.10). A visual inspection of the residuals (Fig.11) showed no marked non-random trends. Although the data points for each fish are linked, the conservative assumption was made that all data points were independent.

Figure 10. ℓn fish weight versus ℓn otolith radius for individual fish (No's 1-29) at the initial (June 6 or 8), second (July 6) and, where applicable, third (July 20) weighing.



Initial weighing
2nd weighing
3rd weighing
ln W = −11.27 + 4.409 ln R
ln Fish Weight
ln Otolith Radius
+
−
1.2
1.0
0.8
0.6
0.4
0.2
0
2.4
2.5
2.6
2.7
2.8

Figure 11. Plot of residuals from linear regression of ℓn weight on ℓn radius as shown in Figure 10.



Residual
−
+
0.6
0.4
0.2
0
0.2
0.4
0.6
1.0
0.8
0.6
0.4
0.2
0
0.2
0.4
0.6
0.8
1.0
ln W
Group
A •
B o
C □
D ▷

Table I ℓn otolith radius (ℓnR) and ℓn fish weight (ℓn) for experimental fry at the initial (June 6 or 8), second (July 6) and third (July 20) weighings.

	First Weighing		Second Weighing		Third Weighing	
Fish#	ℓnR	ℓnW	ℓnR	ℓnW	ℓnPl	ℓnW
1	2.5014	-0.31608	2.7246	0.63340		
2	2.5479	-0.18995	2.7428	0.80648		
3	2.5408	-0.20702	2.7447	0.99990		
4	2.6174	-0.22941	2.7402	0.92861		
5	2.4874	-0.21567	2.7020	0.81802		
6	2.5088	-0.36962	2.7324	0.60704		
7	2.4973	-0.27839	2.7147	0.86752		
8	2.4681	-0.30517	2.6797	0.68057		
9	2.5518	-0.25618	2.7606	0.78800		
10	2.5233	-0.20949	2.7498	0.83291		
11	2.4423	-0.51584	2.6589	0.49042		
12	2.5080	-0.45256	2.7114	0.58667		
13	2.4510	-0.52256	2.6426	0.30822		
14	2.4891	-0.39304	2.6831	0.73573		
15	2.4553	-0.45413	2.6672	0.57774		
16	2.3842	-0.63488	2.6064	0.30895		
17	2.5392	-0.45099	2.7441	0.65389		
18	2.4441	-0.47160	2.5665	0.40213		
19	2.4570	-0.67139	2.6844	0.61842		
20	2.4380	-0.52763	2.6469	0.51462	2.7350	0.70804
21	2.4380	-0.56212	2.6123	0.18482	2.6946	0.60103
22	2.4204	-0.38566	2.6525	0.67905	2.7344	0.87338
23	2.5031	-0.43232	2.6940	0.66783	2.7763	0.91228
24	2.4562	-0.39156	2.6617	0.72368	2.7376	0.97305
25	2.5337	-0.02020	2.7376	0.88830	2.8232	1.0435
26	2.3979	-0.66943	2.6851	0.61896	2.7763	0.87838
27	2.4998	-0.21691	2.7074	0.68914	2.7955	0.75565
28	2.5193	-0.25489	2.7279	0.84243	2.8214	1.1105
29	2.4105	-0.27181	2.6797	0.79706	2.7850	1.0282

The orthogonal polynomial regression yielded an r^2 of 0.9157, but the residuals showed a marked non-random trend in that the weights of the fish with the smallest radii were underestimated.

The weight of each individual at the time of previous weighings was back calculated using the formula:

$$\ell nW_1 = \ell nW_2 - b(\ell nR_1 - \ell nR_2)$$

where W1 is the weight to be estimated

W2 is some later measured weight

R1 is the radius corresponding to the weight to be estimated

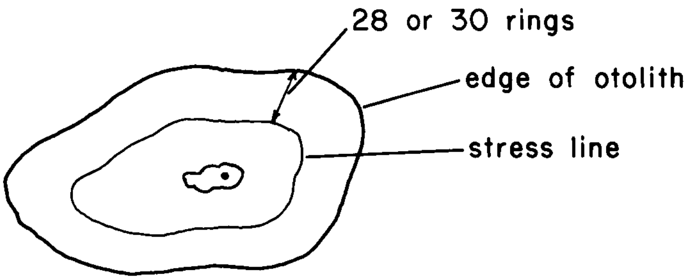
R2 is the radius corresponding to W2

A simple derivation for this procedure is given in Appendix 1.

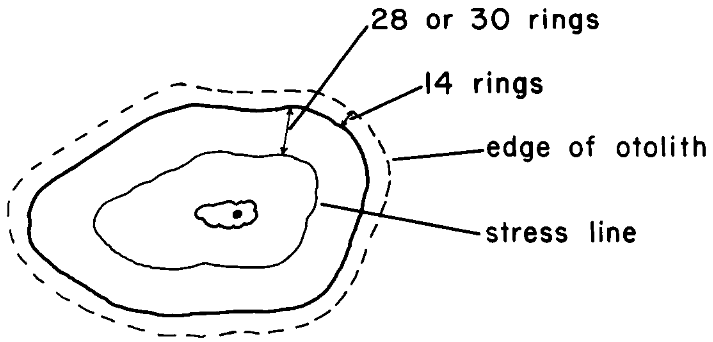
It should be noted that four different sets of back calculations are possible with these data. For those fish which died at the second weighing, only the initial weights can be estimated (group A, Fig. 12). For those fish which survived to the third weighing, the initial weight can be calculated from the weight and radius corresponding to either the middle or final weighings (groups B and C, Fig. 12), and the middle weight can be back calculated using the final weight and radius (group D, Fig. 12). It should be noted further that only the estimate of the initial weight based on the middle weight and radius (Group B) does not utilize a radius measured to the edge of the otolith and that back calculating the weight at the second weighing utilizes

Figure 12. Sagittae of *O. nerka* fry showing possible types of back calculations of previous fish weight using the corresponding previous otolith radius and a latter fish weight and otolith radius as described in the text.

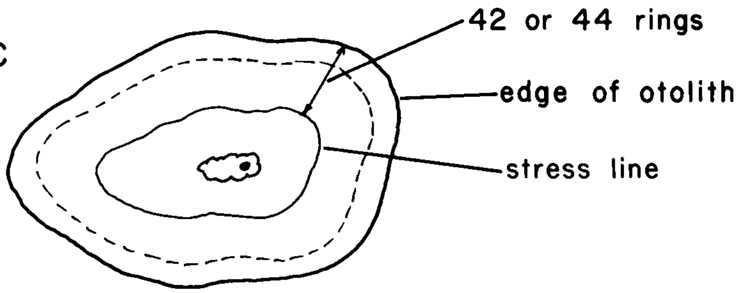
Group A



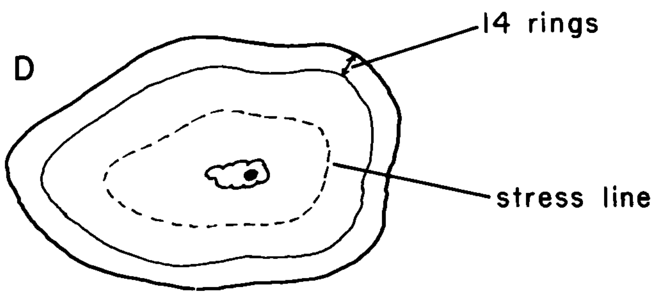
Group B



Group C



Group D



a change in radius for a period of only 14 days (compared with 28-30 and 42-44 days for the other estimates).

The difference between the weights estimated by back calculation and the weights observed, when plotted against the n of the predicted weight shows a non-random distribution (Fig.13). Groups A, B, and C, show a similar declining trend of differences from positive to negative as fish size increases. All but one of the differences in group D are positive.

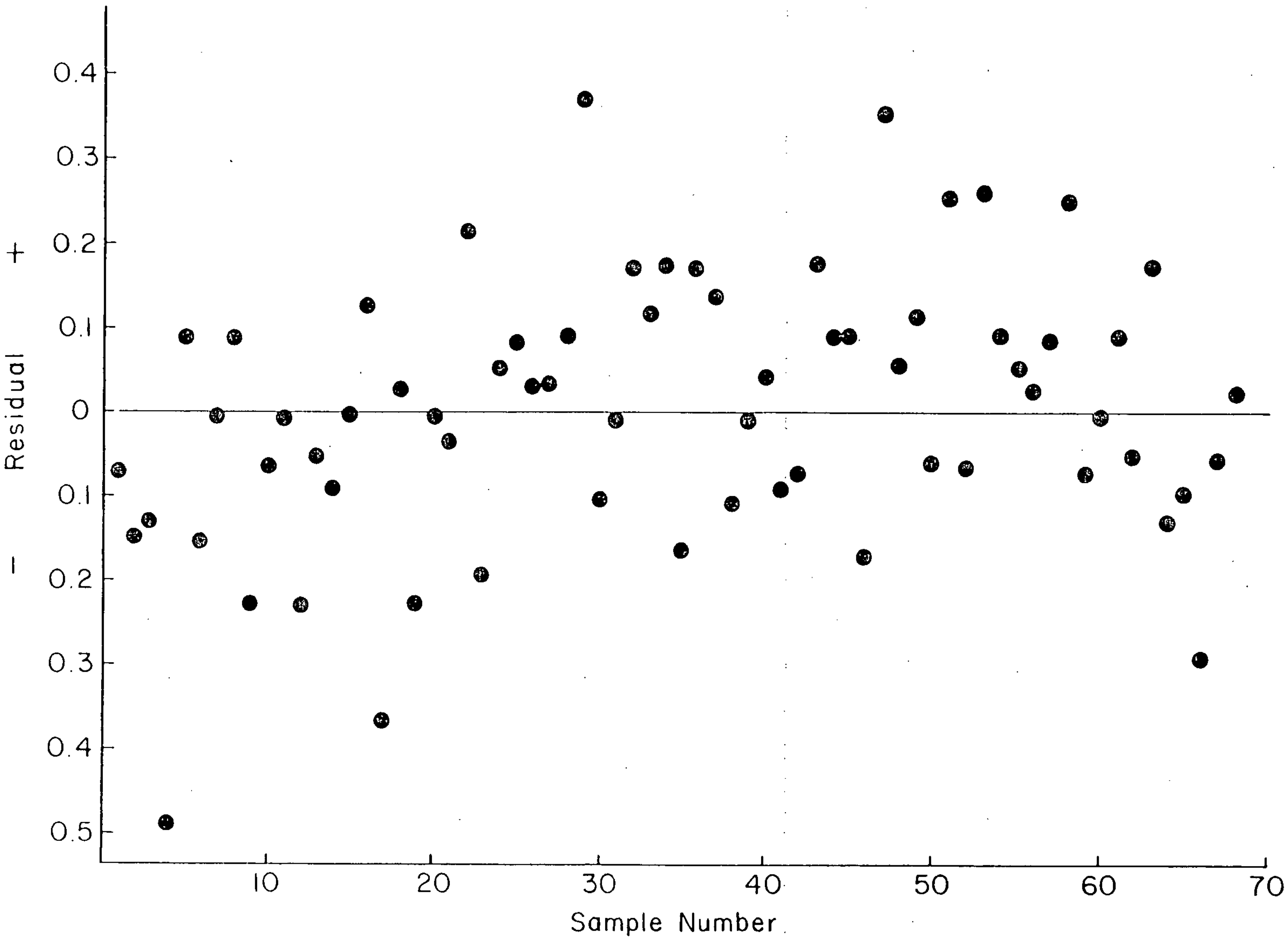
Discussion

Because the radius measurements were determined primarily on the basis of daily ring counts, the close relationship between otolith radius and weight as here described shows a relationship between the mean width of a daily ring in sockeye salmon fry sagittae, and a mean daily change in the weight of the fry. The r^2 value of 0.92 for the regression is identical to that determined by Jonssen and Stenseth (1978) for log fish length versus log otolith radius, even though a much smaller sample size and fish size range were used in our study.

The non-randomness of the difference between observed and back calculated weights of individual fish is presumably an expression of some inadequacy of the regression model as a reflection of the patterns of growth, a result of measurement error, or both.

Abrupt changes in the otolith growth pattern or the fishes weight may occur. Fish weight was used here as a measure of size

Figure 13. Residuals of back calculated weights (ℓn back calculated fish weight - ℓn observed fish weight) versus predicted ℓn weight.


Residual
+
−
0.4
0.3
0.2
0.1
0
0.1
0.2
0.3
0.4
0.5
10
20
30
40
50
60
70
Sample Number

because for very small fish, weight can be measured much more accurately than length. However, if the otolith is growing in direct proportion to other hard parts, abrupt changes in body form resulting from changes in growth or that might occur as a result of stress, could cause the weight to otolith radius relationship to deviate from a linear log-form. There is a possibility of time dependent changes in otolith radius. David Levy (pers. comm.) has noted that for samples of chinook salmon fry suffering from handling stress, weight decreased and length remained constant (over a two week period), but otolith growth rings were still formed daily.

Another possible cause of the pattern of departure of observed from predicted values could be selective mortality in the course of the experiment. If smaller fish tend to remain relatively smaller and larger fish tend to remain relatively larger, then size-selective mortality at the second weighing could have contributed not only to the relatively large value of the slope (4.4) but also to the pattern of departures of observed from back-calculated sizes. This does not appear to have been a factor in this analysis, because the mean initial weight of those fish which died at the second weighing (0.693 gms) was not significantly less than the mean initial weight of the fish which survived to the end of the experiment (0.700 gms).

Measurement error may be a factor because the statistical procedure of back calculation assumes a constant slope. If measurement error or other random effects are a significant component in the variation of weight and radius, this method of back calculation will always tend to overestimate the previous weight of fish with a fortuitously large weight to radius ratio, and underestimate those with a fortuitously small ratio. The back calculation formula assumes that the fish will maintain a constant rank order with respect to the weight and otolith radius relationship. Changes in rank order could thus generate the pattern of residuals seen for groups A, B, and C. By connecting the points representing the weights and corresponding radius measurements for the individual fish (as shown in Fig. 10), it is apparent that changes in rank order did occur.

Group D (Fig. 12 and Fig.13) represents the predicted weight of fish two weeks prior to the end of the experiment, as estimated from final weight and radius and the radius from the focus of the otolith to the growth ring 14 rings back from the edge. The precision of the weight estimates for an interval of two weeks are as good as the estimates for the 28-30 and 42-44 day intervals (groups A and B and group C respectively). The consistent underestimation of previous weight seen in group D has a number of possible causes. Stress related reductions in weight to otolith radius ratio at the final weighing (as previously discussed) or measurement errors associated with the edge of the otolith accentuated by the shorter time interval (and hence small change in otolith radius), could cause the observed deviation in the residuals.

Measurement error in weight would appear to be a minor contributor, because consecutive weights on each fish were seen to agree within 0.01 gms. Measurement error is, however, a major component of the variation in radius values. Errors of plus or minus 5 per cent over three measurements of a radius were not uncommon. Much of the variation resulted from difficulty determining the exact location of the appropriate focus. Other technical problems contributed to variation in the radius measurement; chipping or cracking of the otolith during grinding or preparation can obscure or shift the apparent location of the rostrum or post-rostrum, membranes adhering to the otolith can obscure the edge of the otolith, and variation in the amount of material removed during grinding can change apparent ring width.

Variation in the shape of the otolith with size, and between individuals of the same size also introduces variation in standard radius measurements, particularly when a relatively simple standard method is employed. For example, because the otolith changes shape as it grows, a straight line drawn from the focus to the outside edge of the otolith will not necessarily be perpendicular to any or all of the rings it intersects, nor will it necessarily intersect all of the rings at the same angle. The use of a curved radius, perpendicular to each ring it intersects, was considered. Unfortunately, the length of a curved line proved difficult to measure accurately and directly, and the resulting radius did not always leave the focus at the same angle to the longitudinal axis. Complex age, size and shape specific measurement systems would almost certainly reduce variation in radius measurement, as would improved techniques of otolith preparation. Appendix 2 gives a series of annotated photographs describing these and other technical problems associated with measuring and interpreting otoliths and daily growth rings in otoliths.

Despite these several considerations it is to be emphasized that the relationship between fish weight and otolith radius provides a satisfactory method for back calculation of weight at previous age. The errors that arise from the techniques used are relatively small (of the order of 15 per cent) and permissible in prediction even over as short a period as two weeks.

SUMMARY

Daily rings are found in the sagittae of sockeye salmon fry. Under the conditions of natural incubation observed, the formation of these rings commenced, on average, at the time of emergence. We have found similar ring formations in the otoliths of every teleost fish observed. The close relationship between the size of a fish, the size of its otoliths and the occurrence of daily rings, allows the back calculation of fish weight and, therefore, growth over intervals at least as short as two weeks. This shows a relationship between a fish's change in weight and the width of the corresponding daily growth ring.

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APPENDIX 1 THE BACK CALCULATION OF PREVIOUS FISH WEIGHT FROM CORRESPONDING PREVIOUS OTOLITH RADIUS AND A LATTER OTOLITH RADIUS AND FISH WEIGHT

When a straight line is fitted to the data for log fish weight and log otolith radius, a relationship is obtained of the form:

$$y = a+bx$$

which provides a prediction of the mean values of y for a given mean value of x.

To back calculate a value of y for some previous value of x from a given set of individual values of x and y requires an assumption about the constancy of either a or b.

If a is assumed to be an invariable quantity, the line of back calculation is given by the dashed line in Figure 1-1a. Algebraically, this is equivalent to establishing the proportion:

$$\frac{Y_2 - a}{Y_1 - a} = \frac{X_2}{X1}$$

The quantity b becomes different for each individual case. Alternatively, b may be considered as invariable, in which case

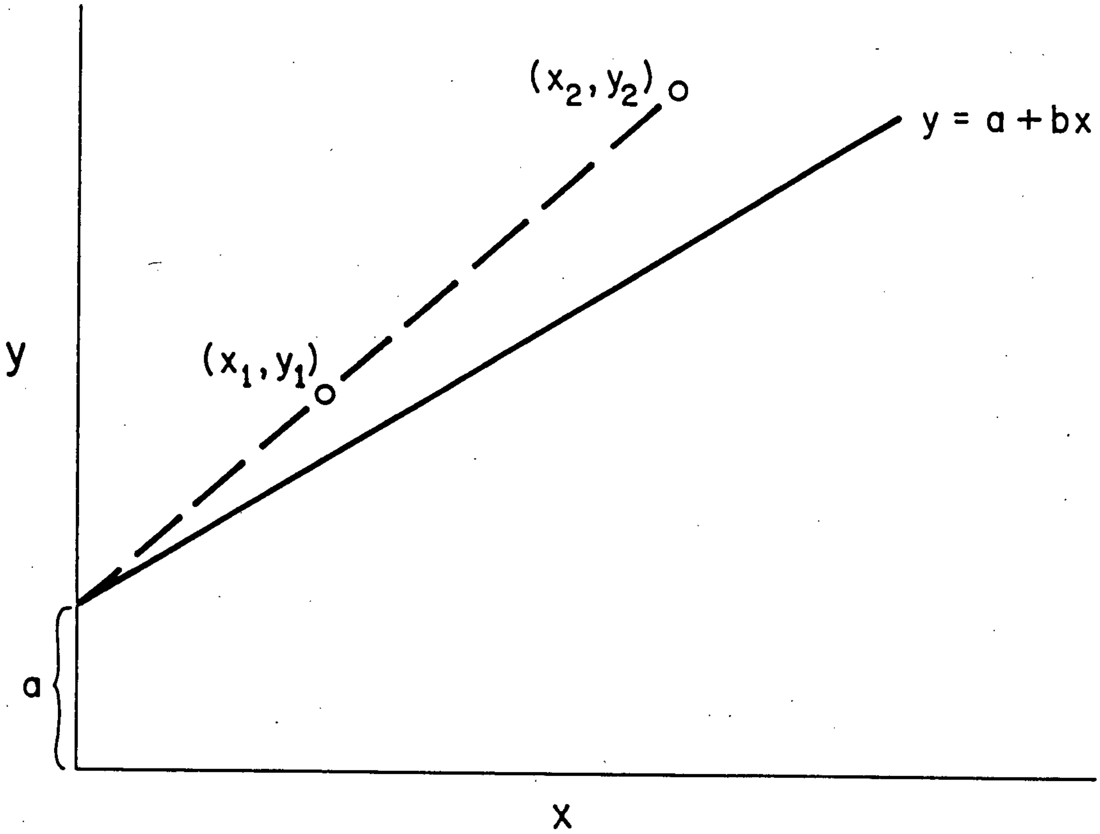


figure 1-1a

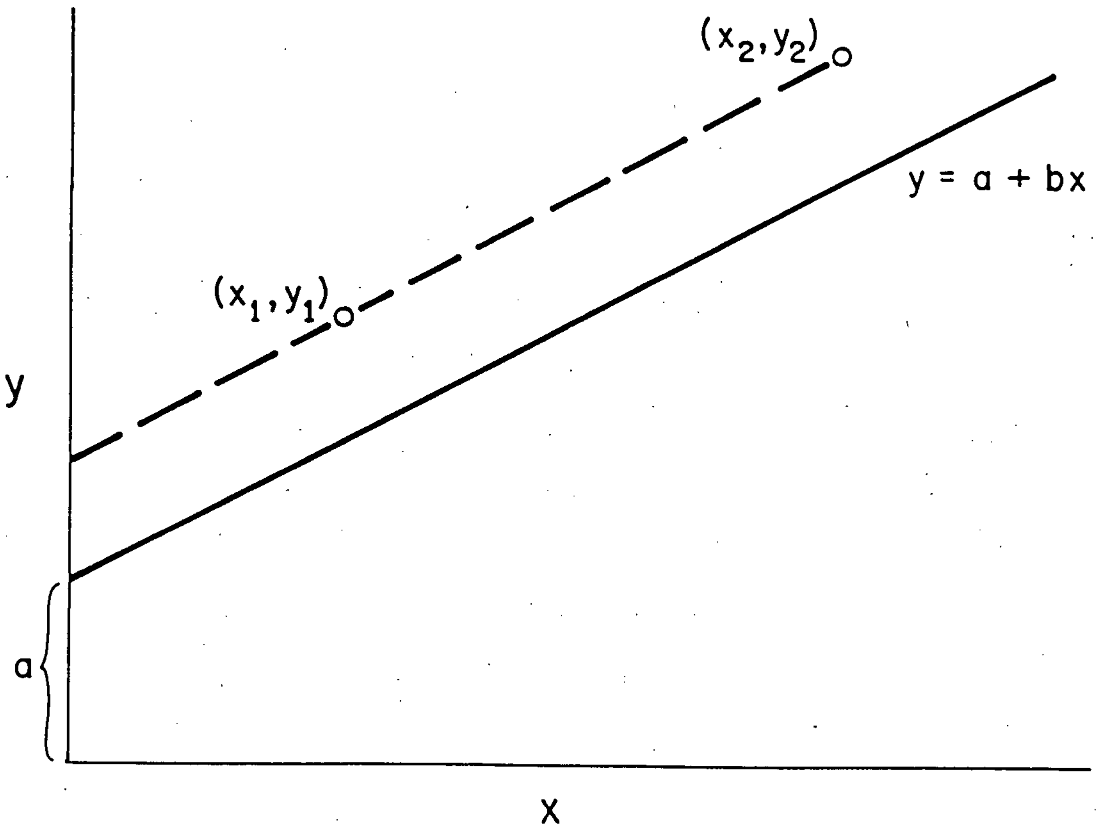


figure 1-1b

the dashed line in Figure 1-1b represents the line of back calculation and the proportionality is:

$$\frac{Y_2 - Y_1}{X_2 - X_1} = b$$

or $Y_2 - Y_1 = b(X_2 - X_1)$

or $Y_1 - Y_2 = b(X_1 - X_2)$

The value of a is different for each individual case.

The relation between fish weight (y) and otolith radius (x) was found to be of the form:

$$\ell n\ y = a + b\ \ell n x$$

In as much as there was no increase in variance of $\ell n\ y$ with increased $\ell n\ x$, it follows that the second procedure of assuming a constant slope is appropriate. Hence the formula for back calculation:

$$\ell n W_1 = \ell n W_2 - b\ (\ell n R_1 - \ell n R_2)$$

APPENDIX 2 ANNOTATED PHOTOGRAPHS OF ONCORHYNCHUS NERKA OTOLITHS AS AN AID TO AGE AND GROWTH ANALYSIS

Otolith

1 This fish was branded and weighed on June 6, weighed again 30 days later, and finally weighed and sacrificed 44 days after initial weighing.

The effects of stress of handling are evident at ring 44 and 14. The rings near the edge are slightly obscured. (This problem is accentuated by the difficulties of printing a high contrast negative.)

"A" shows an area where two rings come together along a fault in the otolith.

Finding the nucleus in an otolith such as this involves considerable uncertainty.

Prior to ring 44, this fish was held in a densely populated tank and fed less regularly; this is reflected in the growth rings.

2 Over-clarified and under-ground

3 Under-ground. Note the ring of glue seen through the otolith. This can be mistaken as a stress mark by inexperienced readers. Note also that the rostrum of both otoliths is crystaline.

4 Under-ground

5 "A" shows rings lost due to excessive clarification in glycerine.

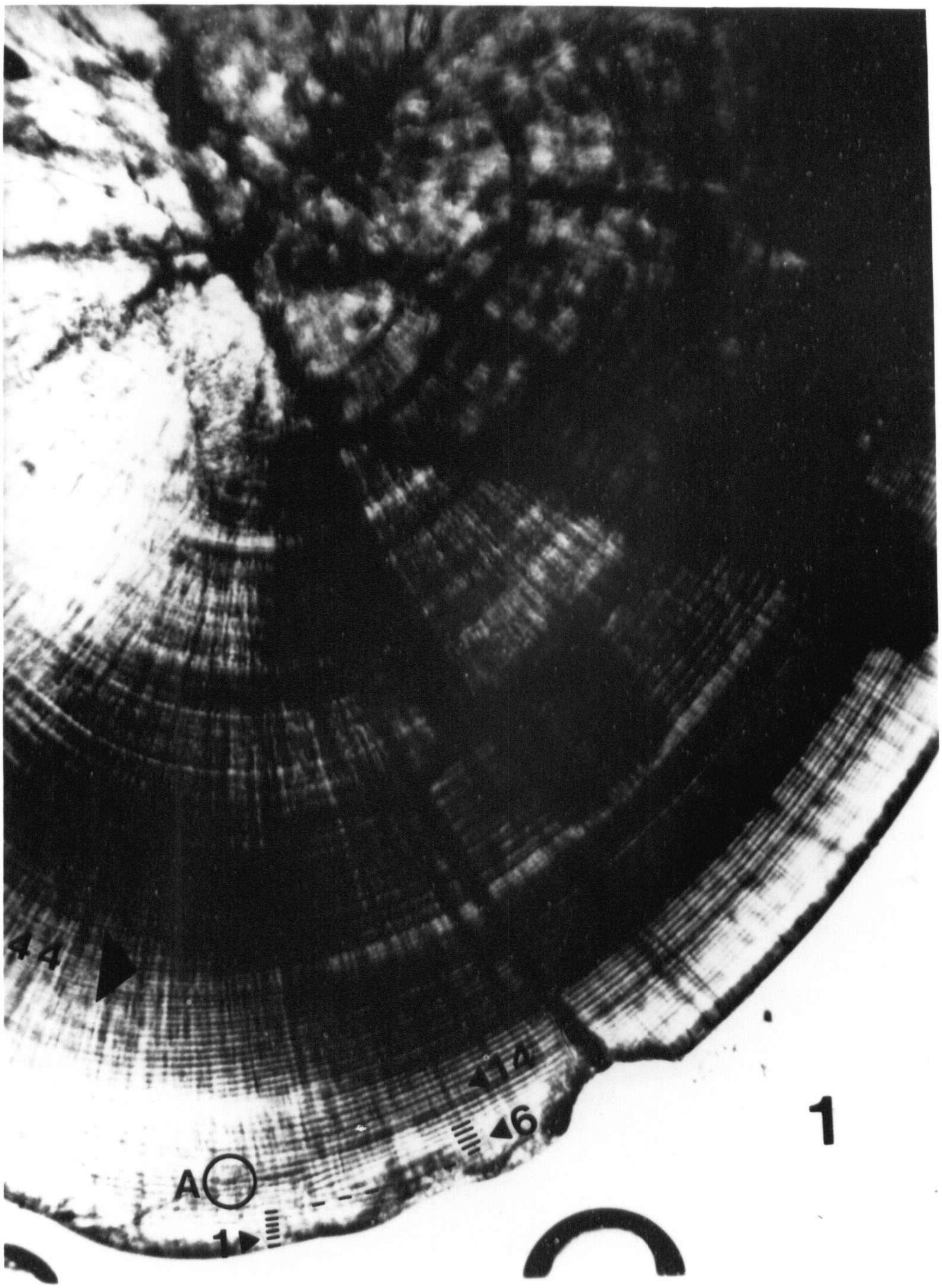
The rings at "B" are daily rings. None occur at "C". The first daily ring would likely be found near the arrow, although it is difficult to see it on this print.

Note the refraction lines near the edge.

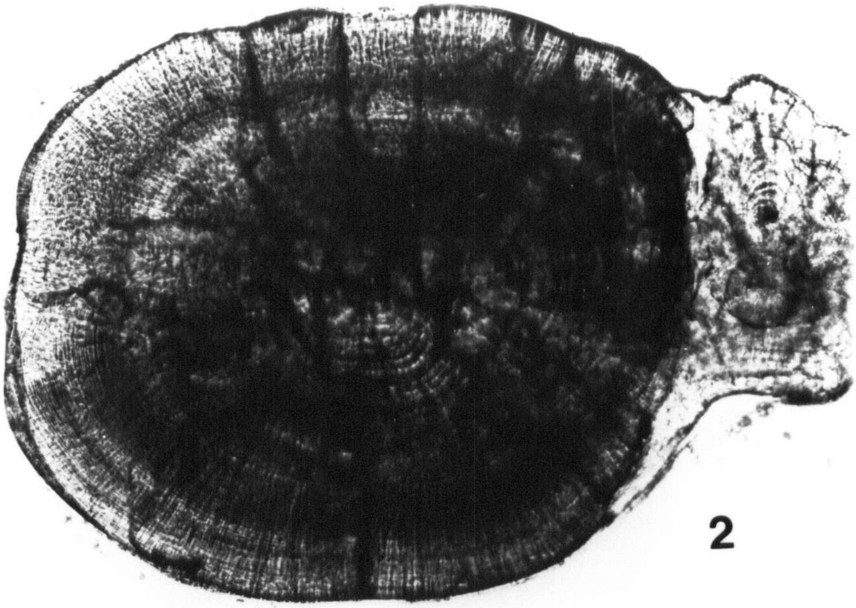
6 This is a fairly typical sockeye fry otolith properly ground and clarified.

The preferred radius would start at the nucleus "A".

Zone "B" shows rings typical of those found during periods of irregular growth; extreme care must be taken in counting rings such as these because each of the darker rings can contain two or more daily zones. Counts should be done at higher magnification than the one shown.



1



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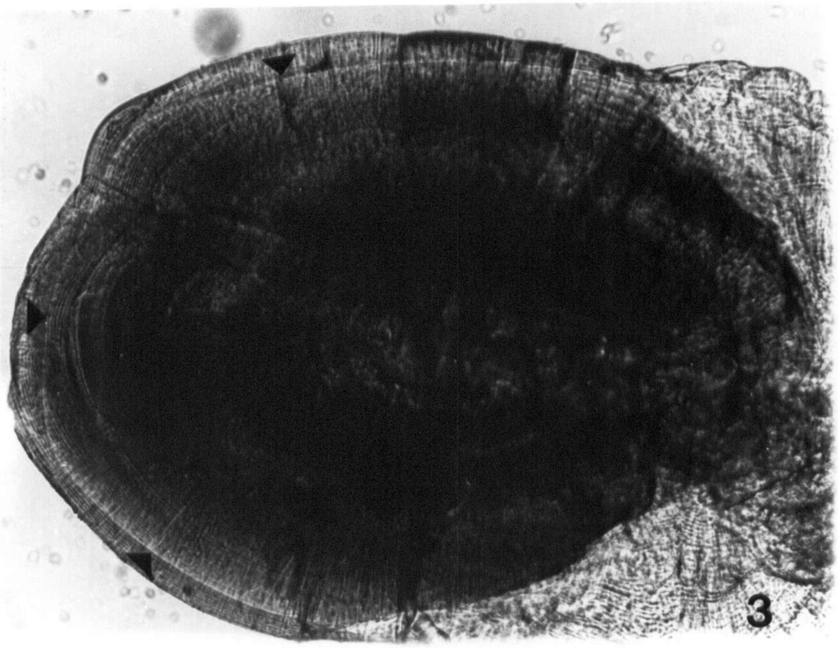

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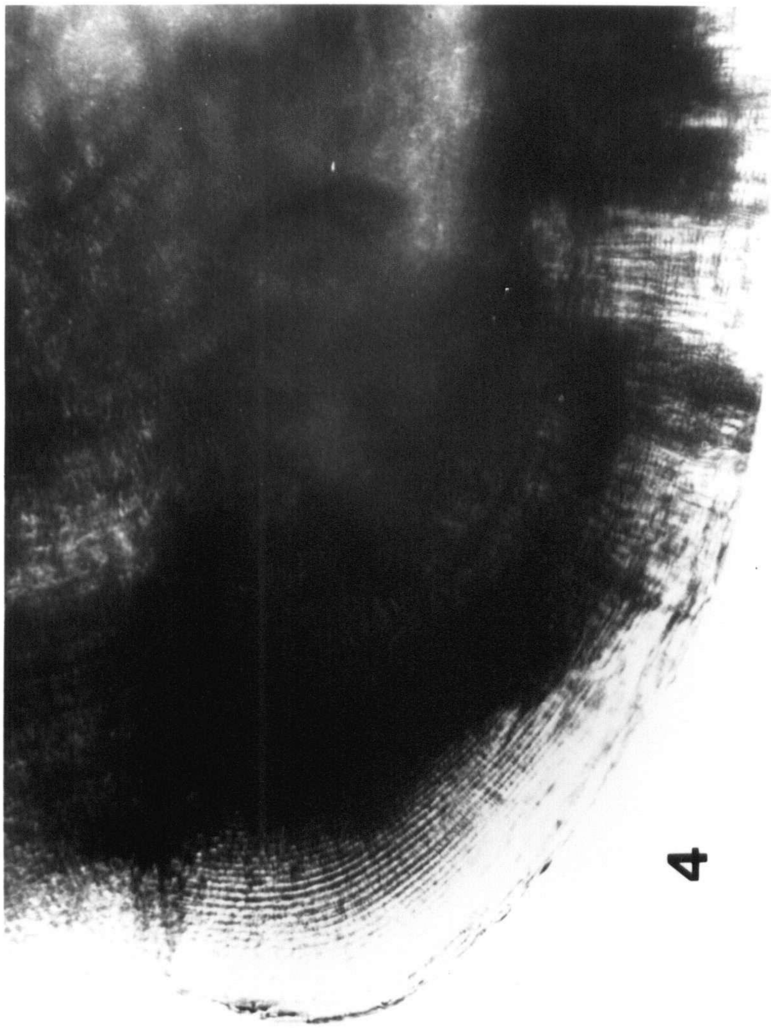
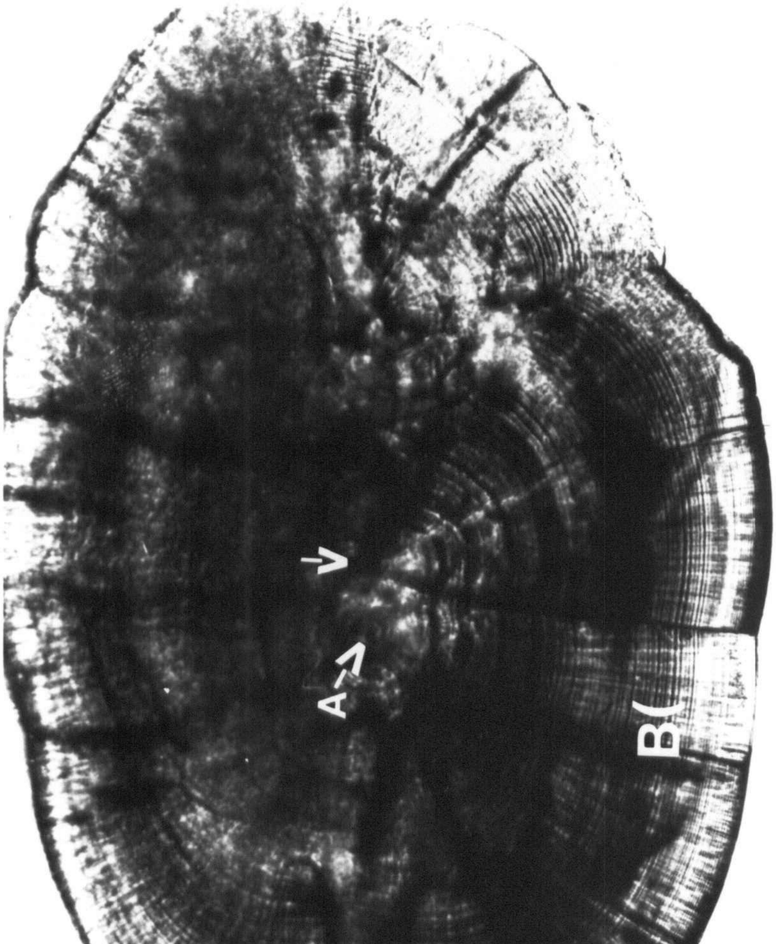


4


C
B
A
5


A
B