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Use of otolith microstructure to identify hatchery-reared and wild Pacific salmon

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Abstract

Daily growth increments in the otoliths of young chinook, coho, and chum salmon can be used to identify the rearing environment. For chinook salmon, daily growth increments that formed immediately after exogenous feeding were more uniform for hatchery-reared fish than for wild fish. A test of the accuracy of the determinations, using tagged hatchery fish, indicated that 89% were identified correctly. The otolith could also be marked by starving fry for 4 days.

Hatchery-reared coho smolts typically lacked an annulus, while wild smolts typically had an annulus in the otoliths. Hatchery-reared coho also had a more regular pattern of daily growth increments than wild coho, and produced increments throughout the winter, when increment formation may virtually cease or increments may be faint for wild coho. A test of the accuracy of the determinations for coho, using tagged hatchery fish, indicated 91% were identified correctly. Hatchery-reared coho also had a higher proportion of crystalline otoliths than wild coho. The percentage of these crystalline otoliths was significantly higher in the hatchery than in a sample of hatchery-tagged coho caught in the Strait of Georgia.

Hatchery-reared chum salmon were separated from wild chum salmon by the irregular pattern of the increments that formed in fresh water. Ninety-six percent of hatchery-reared chum salmon produced multiple checks shortly after exogenous feeding while only about 4% of wild chum salmon contained such checks in their

otoliths. It is proposed that the hatchery-reared chum produced more checks in fresh water than wild chum because hatchery-reared chum were prevented from starting their downstream migration.

Introduction

It has been known since 1971 (Pannella, 1971) that fish produce daily growth increments in their otoliths. An increment is composed of two zones, an incremental zone and a discontinuous zone. The incremental zone is mainly composed of calcium crystals and a small amount of protein and appears light under transmitted light. The discontinuous zone contains more protein than the incremental zone and appears dark under transmitted light (Zhang 1992, Zhang and Runham 1992). Otolith microstructure pattern is known to be closely related to environmental factors, such as temperature and amount of food (Volk et al. 1984; Campana and Neilson 1985; Wright et al. 1991). Because temperature and food affect the growth of daily increments, it is possible that daily growth increments will be more uniform in environments where temperature and feeding conditions are more regular. This would be particularly true for young fish when growth is relatively rapid.

For young salmon reared in hatcheries, the environment is carefully regulated compared to natural streams and rivers. Thus it is probable that the microstructure of otoliths of salmon reared in hatcheries could be distinguishable from salmon found in natural streams and rivers. If this is true, otolith microstructure could be used as a permanent natural tag to discriminate hatchery-reared and wild salmon. The ability to separate the early rearing environment in mixtures of hatchery-reared and wild salmon provides a method of studying the interactions of hatchery-reared and wild salmon in the ocean. Because the total marine mortality of salmon can exceed 90%, small changes in survival can result in large changes in the abundance of salmon returning to coastal areas. Thus it is important to know if the two rearing types grow and survive in the same way in the ocean. The identification of rearing types may also enable total population estimates of smolts to be made if the number of hatchery-reared fish is known and the behaviour of the smolts soon after they enter the ocean is also known.

In this study we identify the differences in the microstructure of hatchery-reared and wild chinook, *Oncorhynchus tshawytscha*, coho, *O. kisutch*, and chum, *O. keta* salmon. The method for separating chinook salmon has been described by Zhang et al. (1994) and the results are summarized in this report. We also briefly review the method of preparing otoliths and identify the difference in the microstructure of the otoliths from hatchery-reared and wild coho and chum salmon.

Materials and Method

Otolith Preparation

Fish were frozen prior to the removal of the otoliths (we used sagittae in this study). Extracted otoliths were cleaned of soft tissue. One otolith from each pair was mounted on a glass slide using thermosetting plastic resin. The otolith was then ground on a lapping film of 60 or 30 µm depending upon otolith size until the primordia were revealed. The resin was then melted on a hot plate and the otolith turned over. The other side of the otolith was ground in a similar manner until microstructure was clearly visible. The otolith was then polished on a lapping film of 0.3 µm to remove scratches on the otolith surface. If the otolith was damaged or the microstructure was not clearly revealed, the other otolith of the pair was used. Otolith were examined under a dissecting microscope using reflected light and under a compound light microscope using transmitted light.

Chinook Salmon

Hatchery-reared chinook smolts were collected from the Cowichan hatchery and the Cowichan river in 1991 and 1992 (Fig. 1). Those collected from the Cowichan river had been coded-wire tagged in the hatchery before release and thus were known to be hatchery origin. Wild fry were sampled in the Cowichan river in 1991 before the release of hatchery-reared fish into the river. In addition, samples of a mixture of hatchery-reared and wild chinook salmon smolts were collected using surface beam trawls (R. J. Beamish, Pacific Biological Station, Nanaimo B.C. unpubl. data) from the Strait of Georgia and the Fraser River plume in May, June and July 1993 (Fig. 1., Table 1). Some of these fish had been adipose fin-clipped and tagged at the hatchery using coded-wire tags, and thus were known to be hatchery-reared. Sixty-seven chinook salmon smolts with coded wire tags were obtained in the sample. From the coded-wire tag information these fish were known to have originated from at least 17 different hatcheries. The accuracy of classification of wild and hatchery-reared chinook salmon was assessed using the 67 coded-wire tagged fish. The rearing type of the total sample (*n* =

1,264) was classified using the otolith microstructure, without prior knowledge of which fish had coded wire tags, and the percentage of the known hatchery-reared fish that were correctly identified was determined.

The widths of daily growth increments in 54 randomly selected otoliths from Cowichan hatchery-reared chinook smolts and 49 randomly selected otoliths from wild chinook fry from the Cowichan river were measured using a Bio-Scan digitizing system. For each otolith, 25 to 60 daily growth increments that formed after the first feeding check were measured along the axis intercepting the daily growth increments at their widest portion in the dorso-posterior quadrant.

Starvation Marking

In 1993 a preliminary experiment was carried out in Robertson Creek hatchery (Fig. 1) to test the possibility of marking microstructure of the otoliths from chinook salmon by food-deprivation. Six hundred hatchery-reared chinook fry were moved from a raceway to a tank with flowing water in order to maintain a similar density of approximately 2.2 kg fry/m^3 as in the raceway, which was used as a control. The fish in the tank were deprived of food for 4 days and were then fed for 12 days. Twenty fish were sampled from the tank 4 and 12 days after resumption of feeding. Twenty fish were also sampled from the raceway 12 days after resumption of feeding of the fish in the tank.

Coho Salmon

Hatchery-reared coho were sampled before they were released into the wild in 1992 and 1993 from five hatcheries: the Big Qualicum, Capilano, Chilliwack, Inch and Puntledge (Fig. 1). Wild coho were collected before hatchery-reared coho were released from three rivers: Big Qualicum river, Black Creek and the Puntledge river (Fig. 1, Table 2). Both the hatchery-reared and wild coho were in fresh water for more than one year. Samples of a mixture of hatchery-reared and wild coho salmon smolts were also collected from the Strait of Georgia and the Fraser River plume. The rearing type of a sample containing 22 coded-wire tagged smolts obtained from samples of coho collected using beam trawls in the Strait of Georgia was classified using the otolith microstructure, without prior knowledge that the fish had coded-wire tags. The percentage of the known hatchery-reared fish that were correctly identified was determined.

Crystalline Otoliths

Some coho salmon contained crystalline (aberrant) otoliths (Mugiya 1972; Strong et al. 1986). The normal form of calcium crystal in the otolith is aragonite, while in the crystalline structure the calcium crystal form is calcite (Strong et al. 1986). Due to faint microstructure, crystalline otoliths were not used for studying the microstructure pattern. To determine the proportion of crystalline otoliths among the otoliths of hatchery-reared and wild coho salmon, 50 hatchery-reared coho were taken from a sample collected from the Big Qualicum hatchery in May 1993, and 50 wild coho were taken from a sample obtained from the Big Qualicum river and Black Creek in late March-May 1992 (Table. 2). In addition, 29 hatchery-reared, coded-wire tagged coho smolts were obtained from a sample of 437 coho collected from the Strait of Georgia in October 1993 (Beamish et al. 1994). Occurrence of crystalline otoliths were determined under a dissecting microscope.

Chum Salmon

In the spring of 1990, chum fry which had been fed for 42-49 days were released from Nitinat hatchery into Cook Creek (Fig. 1), a stream barren of salmon. Some of these fry were marked with ventral fin clips or with an adipose fin-clip and a coded wire tag. The sample of adults recovered from Cook Creek in 1992 contained some four and five year old chum in addition to the expected three years old, indicating that straying from other chum streams had occurred. To study the possibility of using otolith microstructure to separate hatchery-reared and wild chum, 62 ventral fin-clipped and 61 coded-wire tagged adults from the sample recovered in 1993 were selected for otolith examination. In 1993, a sample of 49 chum adults obtained from a neighbouring stream was collected. Chum in this stream were assumed to be wild, as no marked fish released from the Nitinat hatchery were found there. The otolith microstructure from these wild chum was compared with that from the hatchery-marked chum.

Results

Chinook Salmon

In most (92.7%) of the hatchery-reared and wild chinook otoliths, there was a prominent check associated with the transition from yolk absorption to exogenous feeding. We referred to it as the *first-feeding check*. The daily growth increments formed immediately after the first feeding check appeared different between hatchery-reared and wild chinook. In the otoliths of Cowichan hatchery-reared chinook salmon, daily growth increments formed in the hatchery after the first-feeding check were uniform in width and contrast. The average width was 3.97 µm in the dorso-posterior quadrant. Daily growth increments following the first-feeding check in the otoliths of most (92%) of the coded-wire tagged chinook, which were sampled from the Strait of Georgia and Fraser River Plum, were also uniform in width and contrast (Fig. 2). The average width was 2.89 µm in the dorso-posterior quadrant, slightly smaller than that in Cowichan hatchery-reared fish. This uniform or regular pattern in daily growth increments changed following the release of the fish from the hatchery into the wild. Daily growth increments that formed immediately after the release from the hatchery often appeared relatively irregular and narrow. The transition between these two different patterns of daily growth increments frequently corresponded with the formation of a check that we called a *releasing check*. In contrast, in the otoliths of wild fish, daily growth increments deposited following the first-feeding check varied a great deal in width and contrast (Fig. 3).

The average width of the increments was 2.37 μm in the dorso-posterior quadrant, thinner than in the hatchery-reared fish.

The difference in the uniformity of daily growth increments between hatchery-reared and wild chinook can be shown by comparing the coefficient of variation (standard deviation/mean) of daily growth increments of individual otoliths from the wild and Cowichan hatchery-reared chinook. The coefficient of variations for the two rearing types were ranged between 0.25 and 0.41 and between 0.06 and 0.17 respectively, and were, therefore, completely separated. The coefficient of variation for the wild fish was significantly higher than for the Cowichan hatchery-reared fish (t-test $P < 0.0005$).

Among the sample of 1,264 chinook salmon smolts collected from the Strait of Georgia in 1993, 633 were identified from otolith microstructure as wild fish, 620 as hatchery-reared, and 11 were unidentified because of crystalline otolith structure or poor otolith preparation. The sample contained 67 fish from various hatcheries identified by the coded wire tags. Approximately 10% of hatchery-reared chinook have coded wire tags. Thus, in the sample of 1,264 fish, the 67 tags would indicate that approximately 670 were of hatchery origin and the remaining 594 were wild. Our estimates of hatchery and wild chinook were within ± 5% of this expected number. Out of the 67 coded-wire tagged fish, 59 were correctly identified, 7 were misidentified as wild, and 1 was not identified because of crystalline otolith structure. Thus, the accuracy of identifying chinook salmon smolts known to have been released from various hatcheries was approximately 89%.

Starvation Marking

A hyaline zone consisting of 4 faint daily growth increments was formed in the otolith microstructure during the four day food deprivation period. The hyaline zone was distinguishable after 4 days of feeding, but more prominent after 12 days of feeding. Although the daily growth increments were visible in the hyaline zone, they appeared less evident than normal increments, probably because less protein was deposited. Daily growth increments formed after feeding for 12 days appeared normal, except for the few increments at the otolith margin. These marginal increments appeared faint because of the edge effect (Campana and Neilson 1985). In the fish sampled after 4 days of starvation, some marginal increments also appeared faint due to the edge effect. Daily growth increment formation was normal for all 20 fish in the control. No extra mortality was caused by food deprivation during this short experimental period.

Coho Salmon

In otoliths of both hatchery-reared and wild coho salmon, daily growth increments became narrower from summer to winter and wider from winter to spring and summer. Differences in regularity in daily growth increment thickness or contrast or both that were similar to the differences found for chinook salmon have not been identified for hatchery-reared and wild coho salmon. There is some difference in the uniformity of the increment formation between the two rearing types that facilitates separating the two rearing types but we have yet to quantify this difference. We did find that only 8.2% hatchery-reared coho smolts had a typical annulus, compared to 86.2% of wild coho smolts which had a typical annulus (Table 2). (An annulus is defined as a hyaline zone, which is formed in the slow-growing season, or in the winter, and preceded by increasingly narrow increments and followed by progressively wide increments.) In the annulus of wild coho increments were either absent or very faint. If a hyaline zone was present in otoliths of hatchery-reared coho, normal appearing daily growth increments were visible. Hatchery-reared coho differed from wild coho, therefore, in the overall regularity of the increments, the absence of a winter hyaline zone, and the presence of normal appearing increments in the winter.

The accuracy of the determination was assessed using a sample of 22 coded-wire tagged fish. Twenty (91%) were correctly identified as hatchery fish.

Crystalline Otoliths

All 129 otoliths started to grow normally with the aragonite form of calcium crystals. Some otoliths developed a crystalline structure after exogenous feeding. Of the 50 otoliths from Big Qualicum hatchery-reared coho salmon, 27 (54%) had one or more crystalline otoliths. This compares to only 2 (4%) for wild coho (Table 3). Of the 29 hatchery-reared, coded wire-tagged coho from the Strait of Georgia, 10 (34.5%) had one or more crystalline otoliths. The percentage of crystalline otoliths in the sample collected in the ocean was significantly lower than formed in the hatchery (chi-square test, $P < 0.001$). The high percentage of crystalline otoliths for hatchery-reared fish, therefore, is another difference for the two rearing types.

Chum Salmon

Daily growth increments in the central part of the chum salmon otolith appeared faint. Outside this area, daily growth increments were distinct. We know that daily growth increments formed before exogenous feeding in chinook salmon otoliths are not as distinct as those formed after exogenous feeding (Zhang et al. 1994), thus it is probable that the distinct increments formed after exogenous feeding.

The difference between hatchery-reared and wild chum salmon was found in the presence or absence of multiple checks produced shortly after exogenous feeding. A check was defined as a more intense dark zone, which is much darker and wider than the normal discontinuous zone. Of the 62 marked chum, 60 (96.8%) had multiple checks (between 4 and approximately 9 checks which were closely located to one another) and of the 61 marked chum, 58 (95.1%) had multiple checks. Of the 49 wild chum, 2 (4.1%) contained multiple checks.

Discussion

It has been verified that one increment was deposited daily in otoliths of juvenile chinook salmon (Neilson et al. 1985; Gauldie 1991). Although deposition of daily growth& increments has not been verified for coho and chum salmon, it is probable that growth increments also form daily (Campana 1989).

Chinook Salmon

Hatchery-reared chinook salmon in general are well fed and reared in a stable environment, while the wild fish experience both variation in food supply and the physical environment. Indeed, the daily growth increments deposited in the hatchery after exogenous feeding are not only more regular than those in the wild-fish otoliths, but also more regular than daily growth increments formed after the fish are released from the hatchery. The highly uniform and wide daily growth increments in the Cowichan hatchery-reared fish, as compared with other hatchery-reared fish, are possibly due to the constant and high temperature of the ground water and the abundant food supply.

The identification of the two rearing types of chinook salmon relies primarily on the difference in the daily growth increment pattern formed immediately after exogenous feeding. The existence of the releasing check and irregular daily growth increments deposited following release from the hatchery provide additional criteria to identify hatchery-reared fish from wild fish.

Our assumption that the technique can be used to separate chinook salmon of other hatcheries from wild chinook salmon is based on the observations from the hatchery-tagged fish caught in the Strait of Georgia in 1992 and 1993. In the sample of 67 coded-wire tagged smolts, only 7 were inaccurately identified. After being more carefully prepared, the other otolith of the pair from each of these 7 fish was studied. Six fish were incorrectly identified, because their otoliths did not contain a regular pattern of daily growth increments following the first-feeding check, as expected in the otoliths of hatchery-reared chinook. The other fish was initially erroneously identified as wild because the otolith was not well prepared and the otolith microstructure was not adequately revealed. A regular pattern of daily growth increments after the first-feeding check was observed in the other otolith of the pair. The high level of accuracy (89%) of identifying tagged fish among the sample collected in 1993 indicates that the determinations were reliable and that the method likely applies to other hatchery-reared chinook salmon. The ability to identify the rearing type from a large sample of chinook smolts within ±5% of the expected number indicates that the method probably applies to both wild and hatchery-reared chinook from other areas. More detailed studies from a selection of the other hatcheries and from more wild stocks must, however, be carried out for confirmation.

Starvation Marking

An otolith has two phases of growth; calcium crystal deposition and protein matrix formation. The calcium used for otoliths comes primarily from the surrounding water through the gills, while the protein originates from the diet. The matrix growth appears to be a biological process which is closely associated with somatic growth, while crystal growth appears to be a physicochemical process which seems to be less affected by biological events (Mugiya and Tanaka 1992). It is known that fish otoliths continue to grow during the initial cessation of somatic growth (Tzeng and Yu 1991; Wright et al. 1991), but this kind of otolith growth involves relatively less matrix growth and more crystal growth (Mugiya and Tanaka 1992).

The daily growth increments produced during the starvation period appeared relatively faint, probably due to lesser amounts of protein being incorporated into the otolith of the chinook salmon. Our food deprivation experiment on chinook salmon indicates that several cycles of food deprivation and food resumption would mark the otolith microstructure with a pattern of several consecutive hyaline zones. The 'starvation mark' would be most distinct after approximately one week of feeding because of edge effects. The 'starvation mark' is not as distinctive as a mark produced by the thermal marking technique, but starvation marking may be an inexpensive and convenient method of means marking large numbers of hatchery-reared salmon.

Coho Salmon

Coho salmon remain in fresh water for more than one year, consequently there are large numbers of daily growth increments relative to chinook salmon smolts. Also the otolith of chinook salmon is about twice as large as the coho salmon otolith. As a consequence of the difference in the otolith size and the duration of fresh water residence, daily growth increments in coho otoliths were narrower than in chinook otoliths and patterns were more difficult to observe. Nevertheless, the overall appearance of daily growth increments in hatchery-reared coho were more regular than in wild coho. Recognition of a regular appearance of the daily growth increments was the first indication that the fish was reared in a hatchery. The second and more diagnostic identification was the presence of an annulus or wide hyaline zone. Hatchery-reared fish continued to form increments throughout the winter, even though this was a period of slow growth. While some of these increments appeared hyaline, most of the increments that formed in the winter appeared normal. For wild coho, increments were not evident in the annulus. The annulus formation in coho otoliths appears to be related to food consumption. The wild coho salmon probably consume less food during the winter period than the hatchery-reared coho which were fed throughout the winter. Thus, even though it was more difficult to quantify the differences between hatchery-reared and wild coho salmon than for hatchery-reared and wild chinook salmon, we were able to separate the two rearing types using this technique.

Crystalline Otoliths

The percentage of crystalline otoliths is much higher for hatchery-reared coho than for wild coho. It is clear that the hatchery environment influenced the otolith development, but it was not determined why the crystalline structure was produced. The hatchery fish received an artificial diet, possibly indicating that occurrence of crystalline otolith structure is related to the diet. The impact of the aberrant otolith development on growth and survival is not known and it is not known if aberrant development occurs in other bones. The sample of hatchery-reared, coded-wired tagged coho collected in October 1993 had a significantly lower percentage of these crystalline otoliths, possibly indicating that crystalline development affected survival.

The presence of a crystalline otolith is an indication that the fish was probably reared in a hatchery. However until more is known about crystalline otolith development, it will be difficult to use the existence of crystalline otoliths as a method to separate rearing types.

Crystalline otoliths can present a problem when using daily growth increments to separate rearing types. When both otoliths are crystalline, it may be possible to see enough microstructure to make a determination. When this is not possible, we used the percentage of hatchery and wild fish with one crystalline otolith to represent the percentage of the rearing types in the sample with both otoliths crystalline.

Chum Salmon

Wild chum fry start their down-stream migration shortly after emergence. However, The Nitinat hatchery-reared chum fry were fed for about 45 days before release, in order to increase size at release and improve their survival rate. For the other two species, the hatchery environment reduced the variation in the factors affecting the formation of daily growth increments, resulting in a more regular pattern. For chum salmon, we believe the opposite is true. It is known that check production is often stress-related (Campana and Neilson 1985). The extended period of fresh-water residence may cause physiological stress to these hatchery-reared chum, because they were prevented from migrating to the sea immediately after the emergence, resulting in the formation of the multiple checks in their otoliths.

Our studies on chum salmon daily growth increment formation are preliminary and appear to be able to separate the two rearing types. However, because the samples were small and it was not absolutely certain that the wild chum used in the study were wild, more observations are required on the otoliths of hatchery-reared and known wild chum before we can be certain that otolith microstructure can be used to separate rearing types.

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Table 1. Samples of chinook salmon used in this study.

	Number	Year
Cowichan hatchery-sampled smolts	39	1991
Cowichan hatchery-reared smolts sampled from the Cowichan river	69	1991
Cowichan hatchery-reared smolts sampled from the Cowichan river	142	1992
Cowichan wild fry	118	1991
Mixture of hatchery-reared and wild smolts sampled from the Strait of Georgia and Fraser River plum	1,264	1993

Table 2. Location, date, and number of coho smolt samples and the rate of annulus occurrence in the otoliths of each sample group.

Location	Date	Number	Used Number*	Number of Fish with an annulus
Hatchery:				
Big Qualicum	Mar. 3rd 1992	45	38	4 (10.5%)
Big Qualicum	May 18th 1993	100	72	8 (11.1%)
Capilano	Late May 1993	43	35	2 (5.7%)
Chilliwack	May 11th 1993	50	30	2 (6.7%)
Inch	May 10th 1993	56	44	4 (9.1%)
Puntledge	Early May 1993	50	34	2 (5.9%)
Wild:				
Big Qualicum River	Late Mar. -- Mid-April 1992	31	29	25 (86.2%)
Black Creek	May 19th 1992	20	19	15 (78.9%)
Black Creek	April-May 1994	29	29	26 (89.7%)
Puntledge River	Mid-May -- Late June 1993	53	40	35 (87.5%)

* Due to occurrence of crystalline (aberrant) otoliths and a few poor otolith preparation, the actual number of otoliths used was smaller than the sample number in some instances.

Table 3. Proportion of crystalline otoliths in hatchery-reared and wild coho salmon smolts.

Crystalline otoliths	Wild coho (n = 50)	Big Qualicum hatchery-reared coho (n = 50)	Coded-wire tagged coho from the Strait of Georgia (n = 29)
Total	2 (4.0%)	27 (54.0%)	10 (34.5%)
Left otolith only	0 (0.0%)	10 (20.0%)	2 (6.9%)
Right otolith only	1 (2.0%)	10 (20.0%)	4 (13.8%)
Both otoliths	1 (2.0%)	7 (14.0%)	4 (13.8%)

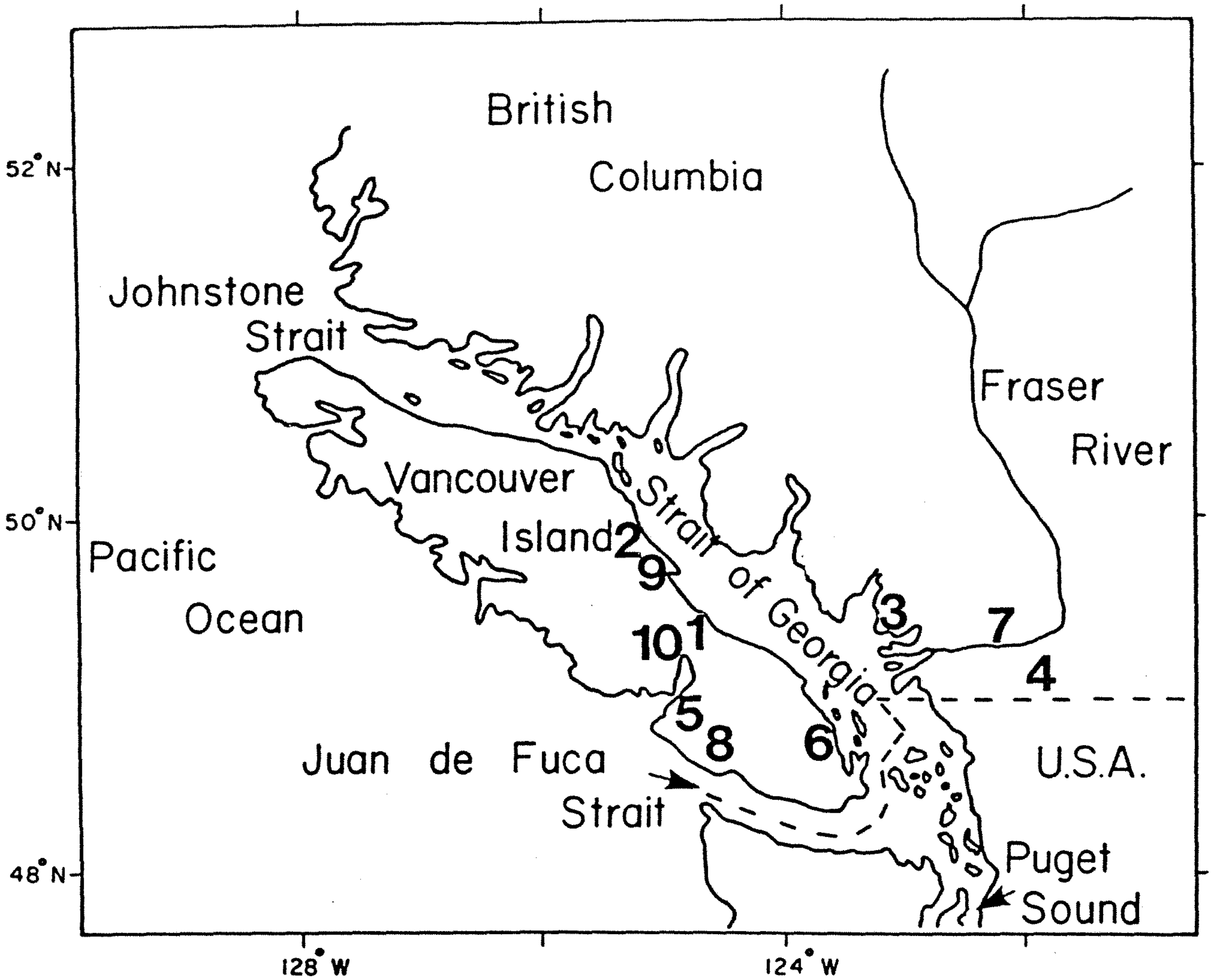

Fig. 1. Location of the hatcheries and rivers from which samples were collected.

1 -- Big Qualicum Hatchery and Big Qualicum River
2 -- Black Creek
3 -- Capilano Hatchery
4 -- Chilliwack Hatchery
5 -- Cook Creek
6 -- Cowichan Hatchery and Cowichan River
7 -- Inch Hatchery
8 -- Nitinat Hatchery
9 -- Puntledge Hatchery and Puntledge River
10 -- Robertson Creek Hatchery

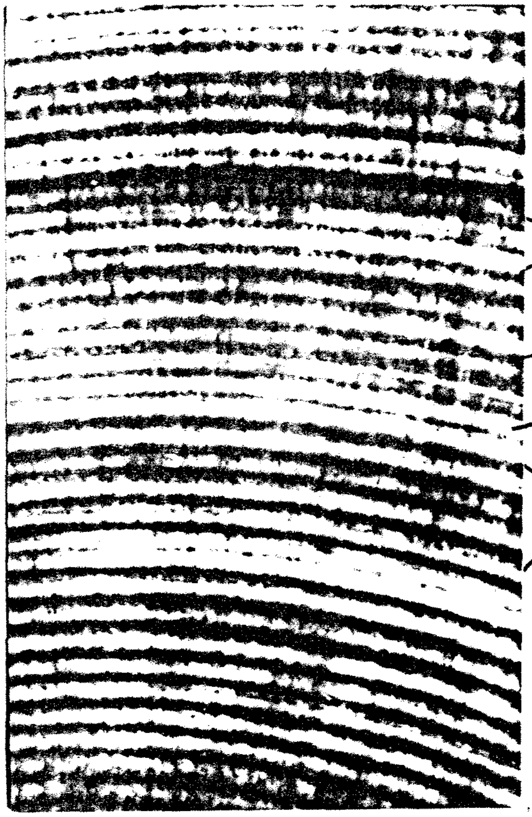

Fig. 2. Section of an otolith from a Cowichan hatchery-reared chinook salmon, showing the uniform daily growth increments. (x 600).

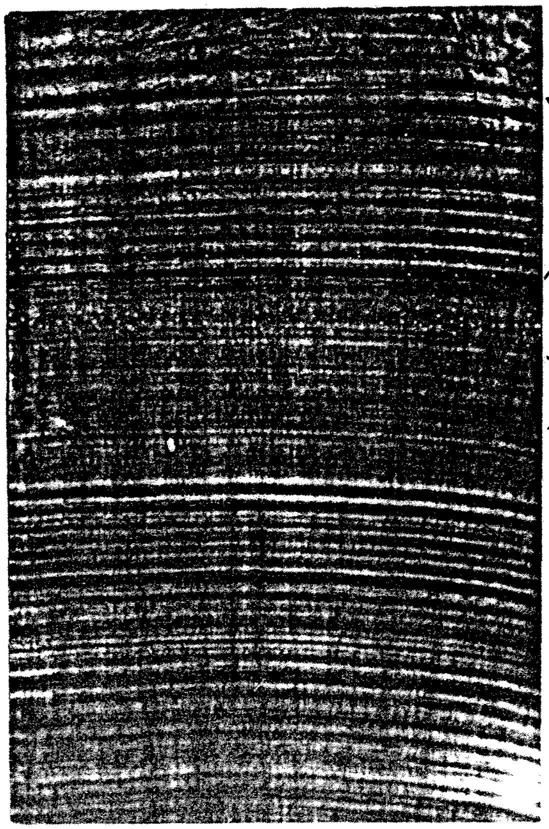

Fig. 3. Section of an otolith from a wild chinook fry from the Cowichan river, showing the irregular daily growth increments. (x 600).