Natural otolith microstructure patterns reveal precise homing to natal incubation sites by sockeye salmon (*Oncorhynchus nerka*)

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Abstract: Experimental results suggest that anadromous salmon and trout learn (imprint) the odors of their natal site just prior to or during seaward migration. In contrast, information on the life histories of several species and the genetic structure of populations indicate that they must imprint earlier in life, probably during incubation in the gravel or when they emerge as free-swimming fry. To test the hypothesis that sockeye salmon (*Oncorhynchus nerka*) home to their incubation–emergence sites (rather than just to the lake where they reared before migrating to sea), we took advantage of the natural variation in otolith microstructure caused by differences in thermal regimes during incubation. We collected otoliths from adult sockeye salmon that returned to discrete spawning areas in Iliamna Lake, Alaska, and Lake Washington, Washington, and, in blind trials, these were classified based on comparison with otoliths from juveniles from the same sites and using information on site-specific thermal regimes. Our analysis showed that the salmon were much more likely to return to their natal incubation site than would have occurred by chance. Estimated straying rates were about 0.1% from the Woody Island population to the Pedro Pond population in Iliamna Lake and about 1% from the Cedar River population to the Pleasure Point population in Lake Washington. The results were consistent with genetic evidence for fine-scale structure of salmon populations and with conservation based on spatial scales appropriate for the early life history of the fish.

Résumé : Les résultats d'expériences ont démontré que les saumons et les truites anadromes font l'apprentissage (empreinte) des odeurs de leur lieu de naissance juste avant ou durant leur migration vers la mer. Par ailleurs, nos connaissances sur la dynamique de population de plusieurs espèces et la structure génétique des populations indiquent que cet apprentissage se fait plus tôt, probablement pendant l'incubation dans le gravier ou alors au moment où les poissons se libèrent du nid sous forme d'alevins capables de nager. Dans le but d'éprouver l'hypothèse selon laquelle les Saumons rouges (*Oncorhynchus nerka*) regagnent leur site d'incubation–émergence (plutôt que seulement le lac où ils ont été élevés avant de migrer vers la mer), nous avons profité d'une variation naturelle de la microstructure des otolithes causée par exposition à de régimes thermiques différents pendant l'incubation. Nous avons prélevé les otolithes de saumons adultes migrant vers des frayères différentes au lac Iliamma, Alaska, et au lac Washington, Washington, et les avons classifiés au cours de tests aveugles par comparaison aux otolithes des juvéniles des mêmes sites et en tenant compte des informations sur les régimes thermiques spécifiques à ces sites. Notre analyse a démontré que les saumons étaient beaucoup plus susceptibles de regagner leur site d'origine que si leur destination était aléatoire. Les taux estimés d'errance étaient d'environ 0,1% chez la population de la zone allant de Woody Island à Pedro Pond dans le lac Iliamna et d'environ 1% chez la population de la zone allant de Cedar River à Pleasure Point dans le lac Washington. Ces résultats corroborent les données génétiques qui mettent en lumière la structure fine des populations de saumons et appuient les stratégies de conservation qui tiennent compte des échelles spatiales appropriées à la dynamique des poissons au début de leur vie.

[Traduit par la Rédaction]

Introduction

The tendency to return to natal sites for breeding, often referred to as philopatry or homing, is displayed by a wide variety of organisms and has been the focus of extensive research (see reviews by Papi 1992; Dingle 1996; Wehner et al. 1996). Homing depends on a combination of innate responses and the animal's ability to learn site-specific characteristics that can guide its return migration at maturity. Such homing restricts gene flow among breeding groups and the resultant reproductive isolation is a prerequisite for the evolution of population-specific traits (Futuyma 1986). Thus the

population structure that is so critical to the conservation and management of animal populations (Nielsen 1995) depends on homing and the size of the spatial unit defined as home.

Although many fishes home (Quinn and Dittman 1992), the phenomenon has been most extensively studied in the family Salmonidae (Hasler and Scholz 1983; Dittman and Quinn 1996). Experiments have indicated that salmon and trout exposed to natural or artificial odors during their transformation from freshwater resident parr to seaward migrating smolts will learn ("imprint") the odors and home to them at maturity (e.g., Scholz et al. 1976; Dittman et al. 1996). Salmon incubated and reared at one site but transferred to another location just prior to release as smolts tend to return to the site where they were released, not to the one where they were incubated and first reared (reviewed by Quinn 1993). Although such experiments seem to indicate that salmon home only to odors learned as smolts, the movements of wild salmon are inconsistent with imprinting exclusively at this stage (Quinn 1990). Juveniles of several species may move away from their incubation sites weeks or months prior to seaward migration (e.g., coho salmon, *Oncorhynchus kisutch*, Peterson 1982; chinook salmon, *Oncorhynchus tshawytscha*, Murray and Rosenau 1989; Scrivener et al. 1994; sockeye salmon, *Oncorhynchus nerka*, Burgner 1991). Notably, juvenile sockeye salmon commonly emerge from gravel nests in streams and migrate downstream to a lake shortly thereafter. They then rear in the lake for 1 or 2 years and leave as smolts from the outlet of the lake, which may be distant from the spawning stream (Burgner 1991). Nevertheless, sockeye salmon spawning at discrete sites within lake systems often differ in allozyme allelic frequencies (Varnavskaya et al. 1994; Wood 1995), as well as in morphology, age, length, egg size, fecundity, and timing of reproduction (Gard et al. 1987; Rogers 1987; Blair et al. 1993; Quinn et al. 1995). Such variation indicates that salmon home to spawning and incubation sites and that these are the fundamental units of the populations, rather than the lake system as a whole.

The movement patterns of wild salmonids suggest that they imprint as developing embryos, yolk-bearing larvae ("alevins"), or newly emerged fry, but there have been no marking programs to document homing at this spatial scale in natural populations. Fry are small and fragile and can be extremely numerous (10^5–10^7 in most populations), making it difficult or impossible to mark all or most of a natural population. Therefore, the most practical approach to studying homing may be to employ natural marks acquired during incubation to distinguish among populations.

The otoliths of fishes are composed primarily (~95%) of calcium carbonate in the aragonite mineral form. The other major component, aside from an array of trace elements, is a collagen-like organic fraction that functions as a template for the deposition of calcium carbonate (Degens et al. 1969; Dunkelberger et al. 1980). Although an organic matrix is distributed throughout the growing otolith, the characteristic dark and light bands observed in sectioned otoliths reflect the bipartite nature of otolith increments, each of which consists of a calcium-rich component (translucent when viewed under transmitted light) and an organically rich component (optically dense under transmitted light). Life-history transitions that reflect changes in environment and growing conditions, such as hatching and migration, may be recorded as changes in the appearance of the otoliths (Marshall and Parker 1982; Campana and Neilson 1985; Volk et al. 1990, 1996). Some of the most dramatic and easily observed variations in otolith microstructure result from temperature changes (e.g., Neilson and Green 1985), which alter the deposition of organic and calcified material in the growing otolith. Sudden temperature drops enhance the density of the organic fraction, and a series of such events creates a corresponding pattern of very dark increments. It is possible to artificially mark embryos by systematically manipulating their incubation temperature (Mosegaard et al. 1987; Brothers 1990; Volk et al. 1990, 1994). Variation in temperature and growth can also produce otolith increment patterns that are population-specific (juvenile rainbow trout, *Oncorhynchus mykiss*, Hayes 1995; chinook salmon, Zhang et al. 1995; Volk et al. 1996; sockeye salmon fry, Finn et al. 1997). Thus, the otolith characteristics of a fish might reveal its origin, provided the fish incubated under a sufficiently distinctive thermal regime.

The first objective of the present study was to use natural variation in otolith patterns to determine whether individual adult sockeye salmon homed to (or strayed between) discrete nearby spawning areas within each of two lakes. We then combined these data with estimates of salmon abundance, in order to estimate levels of straying between the populations within each lake. We selected the spawning sites for their different temperature regimes, hypothesizing that these regimes would result in different banding patterns in the central region of the otoliths produced during intragravel development of embryos and alevins. If the adult salmon homed to their incubation sites, then otolith samples from adults would differ among sites in the increment patterns in this central region. An inability to distinguish adult otoliths from different sites could result from insufficient differences between the incubation temperature regimes or from widespread straying. However, consistent differences in the otolith patterns of adults between sites would be strong evidence for homing to the natal incubation site, and would permit estimates of straying between sites.

Methods

Study sites

The study populations were located in the Iliamna Lake system in southwestern Alaska and the Lake Washington system in western Washington. Sockeye salmon spawn in August at numerous sites within the Iliamna Lake system, including beaches on low islands in the eastern end of the lake, especially those of the Woody Island and Porcupine Island groups (Fig. 1). Our primary "beach type" sampling site was a location where sockeye salmon spawn at high densities (Northwest Woody Island No. 1 of Demory et al. (1964), referred to hereinafter as Woody Island). We also collected a smaller number of samples from another beach site, Fuel Dump Island in the Porcupine Island group. These island beaches are bathed in lake water that is circulated by wind and are about 10–12°C at the time of spawning (Hendry et al. 1995; Leonetti 1997). Water temperatures fluctuate with patterns of calm sunny weather and stormy conditions (Leonetti 1997), but decrease to near freezing just below the ice in winter before warming in the spring (Fig. 2). Sockeye salmon also spawn in spring-fed ponds, including

a series of ponds near Pedro Bay (Fig. 1). These ponds are much colder than the beaches at the time of spawning (about 4°C in August) but are warmer in the winter (Fig. 2). We selected the population of one pond, which we refer to as Pedro Pond, for comparison with the Woody Island population, anticipating that the different thermal regimes would produce distinguishable otolith patterns.

To investigate homing on a finer scale, we compared Pedro Pond otoliths with those collected from salmon in a nearby pond, which we refer to as Grass Pond. The two ponds are similar in substrate (fine granite sand) and size (Grass Pond is 59 m long and 20 cm deep and Pedro Pond is 46 m long and 26 cm deep). Less than 1 km downstream, the outlets of the ponds join in a slow-moving creek that flows into Pedro Bay. Grass Pond was slightly warmer than Pedro Pond at the time of spawning but exhibited similar seasonal patterns (Fig. 2), suggesting that any differences in otolith patterns between the ponds would be much more subtle than those between the islands and the ponds.

The two comparisons from the Iliamna Lake system described above represent extremes in the range of the potential utility of otolith microstructure for population discrimination. Pedro Pond and Woody Island are very different habitats and, if sockeye salmon home to their incubation site, there should be few strays between them. The very different thermal regimes also made it likely that natural variation between sites in otolith increments would be detectable. Pedro and Grass ponds, on the other hand, are close to each other, physically similar, and probably more difficult for the salmon to distinguish between than the island-beach and pond systems. In addition, the similarity in thermal regimes between the ponds would make discriminating between their otolith patterns more daunting. After evaluating our success in discriminating between these two pairs of incubation sites (i.e., very different and very similar), we applied our approach to an intermediate situation found within another lake system. This latter case provided a practical test of our technique, with considerable implication for salmon conservation and management within that system.

In the Lake Washington system, most sockeye salmon spawn (primarily from October to December) in the Cedar River, which flows into the south end of the lake (Fig. 3). This river has a varying temperature regime (Fig. 4), but the size of the river and the diversity of spawning habitats (main channel, side channels, and ponds) suggested that all embryos might not display similar otolith banding patterns. Seven kilometres north of the Cedar River, a much smaller population of sockeye salmon spawns on beaches in southern Lake Washington (primarily in November), particularly at a site known as Pleasure Point (Fig. 3). In contrast with the Cedar River (and with the beach spawning environment of Woody Island), Pleasure Point is fed by ground water with a remarkably stable temperature profile (Woodey 1966; Fig. 4). Habitat features, such as flow and substrate, also differ dramatically between the Cedar River and Pleasure Point environments (Hendry and Quinn 1997).

Specimen collection, preparation, and analysis

Otoliths were collected from adult sockeye salmon at the Woody Island and Fuel Dump Island spawning sites in 1994 and 1995, at the Pedro Pond and Grass Pond sites in 1995, and at the Cedar River and Pleasure Point sites in 1992 and 1993. We also collected sockeye salmon fry from Pedro and Grass ponds in August 1996 and from the Cedar River in February 1996, to test our assumption that otolith increment patterns from these sites reflected relatively constant and variable thermal incubation regimes, respectively. Logistic constraints prevented us from collecting fry from the beach-spawning populations.

All adult otoliths used in this study were prepared at the Washington Department of Fish and Wildlife otolith laboratory (Volk et

Fig. 1. (A) Map of Alaska showing the location of Iliamna Lake. (B) Detail of the eastern end of Iliamna Lake and (C) detail of the Pedro Bay ponds, showing the locations at which sockeye salmon were collected for otolith analysis.
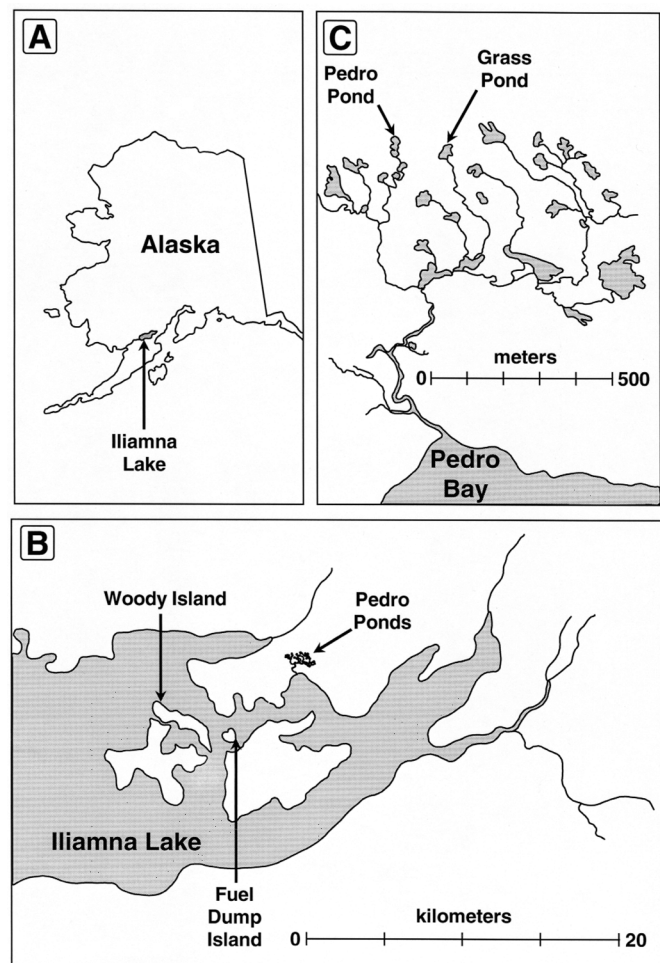

al. 1990, 1994, 1996). Otoliths were removed from their storage vials, placed medial side down on a glass plate, and then cast into a solid resin block. Their medial surface was lapped on a rotating wheel with a 500-grit abrasive pad until the core region of the otolith was exposed. The surface was polished with a 3-µm aluminum oxide abrasive and then fixed to a glass slide with quick-bonding cement. The portion of the resin block lying above the otolith was removed with a cut-off saw and the the lateral surface was then ground and polished in the same manner as the medial, leaving a thin section approximately 200 µm thick through the core of the otolith. Juvenile otoliths were cast into a solid resin block and then lapped until the primordial region of the otolith core was exposed; this was sufficient to observe pre- and post-hatch increment patterns.

Otoliths collected from either Woody Island or Pedro Pond and either Pleasure Point or the Cedar River were classified in blind trials, based on increment patterns recorded in the region between the otolith's core and the onset of regular increment production following emergence. Many salmonid otoliths display a "check" in the form of a very dark band or structural discontinuity that corresponds to the time of hatching. At emergence, a transition in increment appearance often occurs, in which broad or indistinct increments characteristic of the post-hatch alevin give way to the well-defined daily increments of the emergent fry (Volk et al. 1995). Typically,

Fig. 2. Continuous temperature records for sockeye salmon spawning and incubation sites in Iliamna Lake, recorded from 1990 to 1992 at Woody Island (recorded about 3 m below the surface of the lake and 1 m above the gravel) and from 1995 to 1997 at Pedro Pond and Grass Pond (recorded about 15 cm below the surface of the gravel). Bars at the bottom indicate the periods during each year when sockeye salmon embryos would have been incubating at each site, if they were spawned between 14 and 31 August and incubated at site-specific rates as estimated from Jensen (1988).
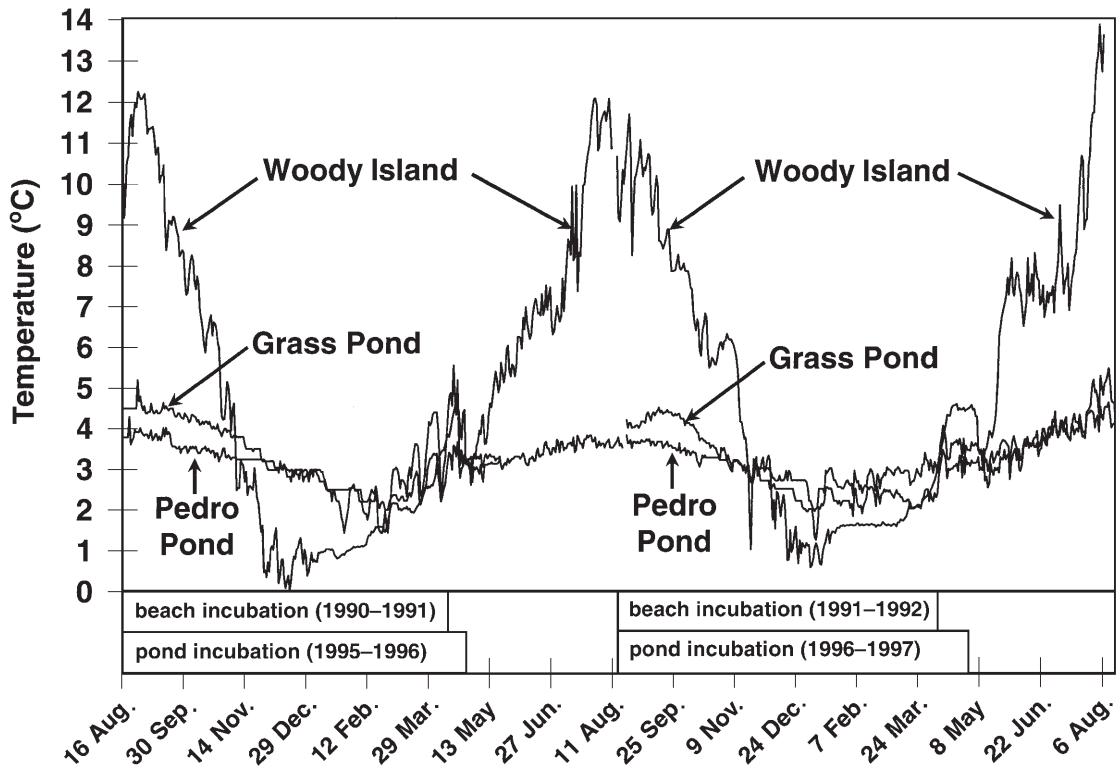
in juvenile otoliths, these features were discernible, allowing us to estimate the radial distance from the core to the feature. In otoliths from adults, where exact locations of hatching checks and emergence transitions were not always as obvious, the measurements from juveniles allowed us to approximate those regions of the otoliths that corresponded to pre-emergence life. The structural features used to classify adult otoliths were well within this portion of the otolith, so the precise determination of emergence was not needed for our purposes.

We examined and classified samples visually rather than by using discriminant function analysis (e.g., Hayes 1995; Finn et al. 1997), because preliminary examination of otoliths (see details below) revealed obvious differences in the banding patterns that were consistent with the evidence that the Pedro Pond and Pleasure Point fish incubated under less variable thermal regimes than the Woody Island, Fuel Dump Island, and Cedar River fish. Classification was based on differences in otolith increment patterns produced under more variable versus more constant incubation thermal regimes. Each specimen, identified only by code, was examined on three separate occasions (at least 1 week apart), without knowledge of prior scores. When the three trials did not yield a unanimous classification, the consensus was used for analytical purposes.

For comparisons between fish from Pedro Pond and Grass Pond, we first examined juvenile specimens from each site to determine what differences, if any, were noticeable in the otoliths of fish from these ponds, whose thermal regimes were much more similar than those of Pedro Pond and Woody Island. Based on these fry samples, the adult samples were examined on three separate occasions to assign probable origins to them. Although the primary purpose for examining the Grass Pond samples was to compare them with

the Pedro Pond samples, 50 of them were also scored subsequently for the presence of otoliths characteristic of island-beach spawners. Finally, otoliths from 10 adults collected at Fuel Dump Island were examined to test our assumption that the Woody Island samples were characteristic of the island beach spawning group and not unique to one site.

Estimates of homing and straying

We obtained estimates of the numbers of adult salmon returning to the spawning sites in Iliamna Lake and Lake Washington from natural resource agencies (the Alaska Department of Fish and Game and the Washington Department of Fish and Wildlife, respectively). In Iliamna Lake, aerial surveys indicated that the average estimates of sockeye salmon in the Woody Island and Porcupine Island groups were 35 831 and 12 065, respectively, from 1979 to 1996 (Regnart 1996). The Pedro Bay ponds had an average of 2574 spawning sockeye salmon per year from 1979 to 1996 (Regnart 1996). We combined the results of the 1995 surveys with the results of the otolith examination to estimate the straying between sites. For example, if 1% of the otoliths collected from adults in Pedro Pond were similar to the island-beach fish, then we would estimate a straying rate of 1% *into the pond*. If the beach population was 10 times larger than the pond population, then we would estimate a straying rate of 0.1% *from the beach*. In addition, our own foot surveys of the Pedro Bay ponds (Quinn and Kinnison, 1999) provided data on the relative abundance of salmon in Pedro and Grass ponds in 1995 for comparison with straying estimates between ponds.

In the Lake Washington system, most sockeye salmon spawn in the Cedar River (annual average = 190 009 from 1979 to 1993). The Pleasure Point beach spawning population is much less numer-

ous than the Cedar River population, having averaged only 3505 sockeye salmon from 1982 to 1996 (Washington Department of Fish and Wildlife, unpublished data). These abundance estimates were combined with the results of otolith examination to generate straying estimates in the same manner as for the Iliamna Lake populations.

Results

Otolith classification

Otoliths from juveniles collected in Pedro Pond showed pre- and post-hatch regions with little contrast and poorly defined increments, which is characteristic of relatively constant thermal regimes. Otoliths from all Woody Island adults showed highly contrasting otolith increments or increment groups, which is typical of fluctuating thermal regimes (Fig. 5). Even where preparation quality was imperfect, the groups of very dark and sharply contrasting increments recorded in the pre-emergence area of these otoliths suggested significant temperature changes. The 10 Fuel Dump Island samples resembled those from Woody Island, indicating that otoliths with contrasted increments were typical of that island beach spawning population as well.

Blind classification of Woody Island and Pedro Pond samples based on the correspondence between thermal regime and otolith increment appearance was consistent (97% of classifications were unanimous across the three blind trials). The classification of beach and pond fish was much more accurate than would have occurred by chance; all adult otoliths classified as Pedro Pond were collected there and all but four adult otoliths classified as Woody Island were collected there (Table 1; $\chi^2 = 85.28$, $P < 0.001$). Each of the four otoliths collected from Pedro Pond adults that were classified as coming from Woody Island fish received the same score on all three readings, and their increment patterns were indistinguishable from the specimens collected from Woody Island, even when reexamined after their origin was known.

The otoliths of juvenile sockeye salmon from Grass and Pedro ponds differed little in increment characteristics. There were often one or two groups of increments in the post-hatch region of the Grass Pond otoliths that were darker than the rest and differed somewhat from the very even increment contrast seen in Pedro Pond otoliths. Based upon this very subtle difference, we examined a mixed sample of 95 adult otoliths. These otoliths were classified less consistently than those in the Pedro Pond – Woody Island comparison, but 83.2% were classified similarly in all three readings. The proportion of the specimens correctly classified to their origin (57 of the 95 specimens or 60%) was better than would have occurred by chance ($\chi^2 = 4.14$, $P < 0.05$). Twenty-five fish collected from Grass Pond were assigned to Pedro Pond and 13 fish collected from Pedro Pond were assigned to Grass Pond (Table 2). Otoliths for which readings were inconsistent (i.e., not classified the same in all three readings) were equally numerous among those correctly and incorrectly classified (82–85% in all four combinations of origins and classifications). Subsequent examination of 48 samples from Grass Pond revealed 2 with the increment pattern characteristic of island-beach otoliths.

Fig. 3. (A) Map of northwestern Washington showing the location of Lake Washington. (B) Detail showing the locations where sockeye salmon were collected for otolith analysis.
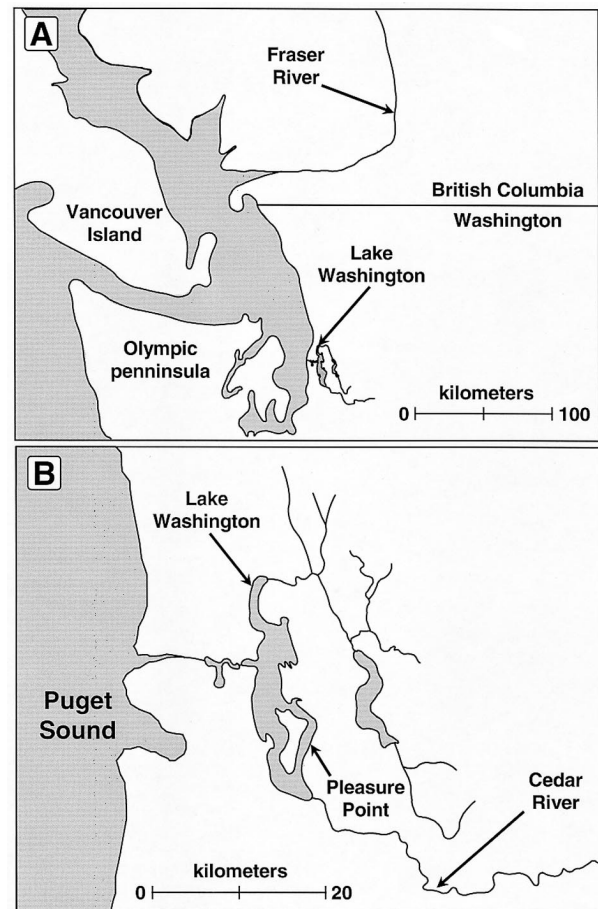

Initial examination of otoliths from 100 fry from the Cedar River revealed a range of patterns; most of these indicated incubation under fluctuating thermal regimes but some showed patterns characteristic of more stable regimes. As with the Iliamna Lake samples, blind classification of the Lake Washington adult samples was consistent: 89% of the specimens were classified the same in all three readings. The overall classification was much better than would have been expected by chance ($\chi^2 = 44.41$, $P < 0.001$), but 35 of the 139 specimens (25%) were misclassified. The classification success with the 1992 samples (77.6%) was similar to that with the 1993 samples (72.2%). The misclassifications were not randomly distributed; otoliths from 26 of the 66 fish (39.4%) collected from the beach had increments consistent with variable incubation temperatures, but otoliths from only 9 of the 73 fish (12.3%) collected from the river suggested more constant temperatures.

Estimates of homing and straying

In 1995, an estimated 132 750 sockeye salmon spawned on the Woody Island beaches (including our collection site), another 38 400 spawned on beaches in the Porcupine Island group, and 3000 spawned in the Pedro Bay ponds (Regnart 1996). If the six salmon with beach-type otoliths collected

Fig. 4. Temperature profiles for Pleasure Point (the upper dark line is the mean of two data loggers during 1995–1996) and the Cedar River (lower dark line and shaded area). Cedar River data are from a gauging station at river kilometre 2.7, and show the daily average from 1980 to 1996 (line) and the 10th–90th percentiles (shaded area) (see Hendry et al. 1998 for details of temperature records). Bars at the bottom indicate the periods when sockeye salmon embryos would have been incubating at each site, based on average dates of spawning at each site (Washington Department of Fish and Wildlife, unpublished data) and site-specific incubation rates as estimated from Jensen (1988).
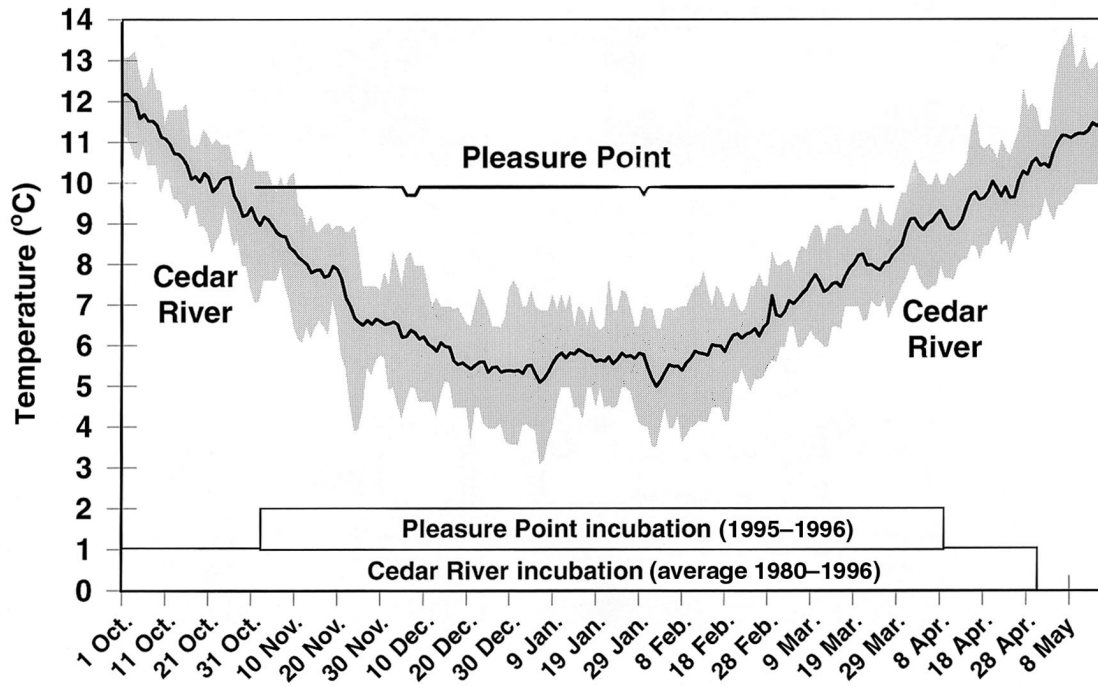
Table 1. Number of otoliths collected from adult sockeye salmon at three spawning sites in the Iliamna Lake system and their classification in two sets of blind trials based on knowledge of their incubation temperature regimes and examination of reference specimens.

Classification	Collection site			
	Pedro Pond	Woody Island	Pedro Pond	Grass Pond
Pedro Pond	46	0	—	—
Woody Island	4	50	—	—
Pedro Pond	—	—	34	25
Grass Pond	—	—	13	23

Table 2. Number of otoliths collected from adult sockeye salmon at two sites in the Lake Washington system in 1992 and 1993 and their classification in blind trials based on knowledge of their incubation temperature regimes and examination of reference specimens.

Year	Classification	Collection site	
		Pleasure Point	Cedar River
1992	Pleasure Point	18	1
	Cedar River	14	34
1993	Pleasure Point	22	8
	Cedar River	12	30
Total	Pleasure Point	40	9
	Cedar River	26	64

from the ponds (4 from Pedro Pond and 2 from Grass Pond out of 98 examined) were strays, then an estimated 6.12% of the 3000 sockeye salmon spawning in the ponds might have

been strays from beach populations. These strays, estimated to number 184 beach fish, would correspond to about 0.11% straying from the beach-spawning populations (171 150 adult salmon) into the ponds. If the 174 150 fish returning to the beaches and ponds in 1995 had distributed themselves randomly with respect to their origin, only 1.7% of the 3000 fish in the ponds would have had pond-type otoliths rather than the 94% that we observed.

Twenty-five of the 48 otoliths collected from Grass Pond adults were classified to Pedro Pond, roughly twice the proportion of Pedro Pond otoliths classified to Grass Pond (13 of 47). Surveys of the ponds revealed that Pedro Pond had roughly twice the abundance of sockeye salmon as Grass Pond in 1995. Total counts of adults (live and dead) were 1290 and 559, respectively, on 29 August, near the end of the spawning period for these populations.

In 1992 and 1993, the ratios of Cedar River to beach fish were 72:1 and 28:1, respectively (87 000 vs. 1210 and

Fig. 5. Photographs of representative otoliths from adult sockeye salmon collected at Woody Island (top) and Pedro Pond (bottom), Iliamna Lake, Alaska. Note the pronounced striations near the core of the Woody Island otolith and the more uniform appearance of the Pedro Pond otolith.
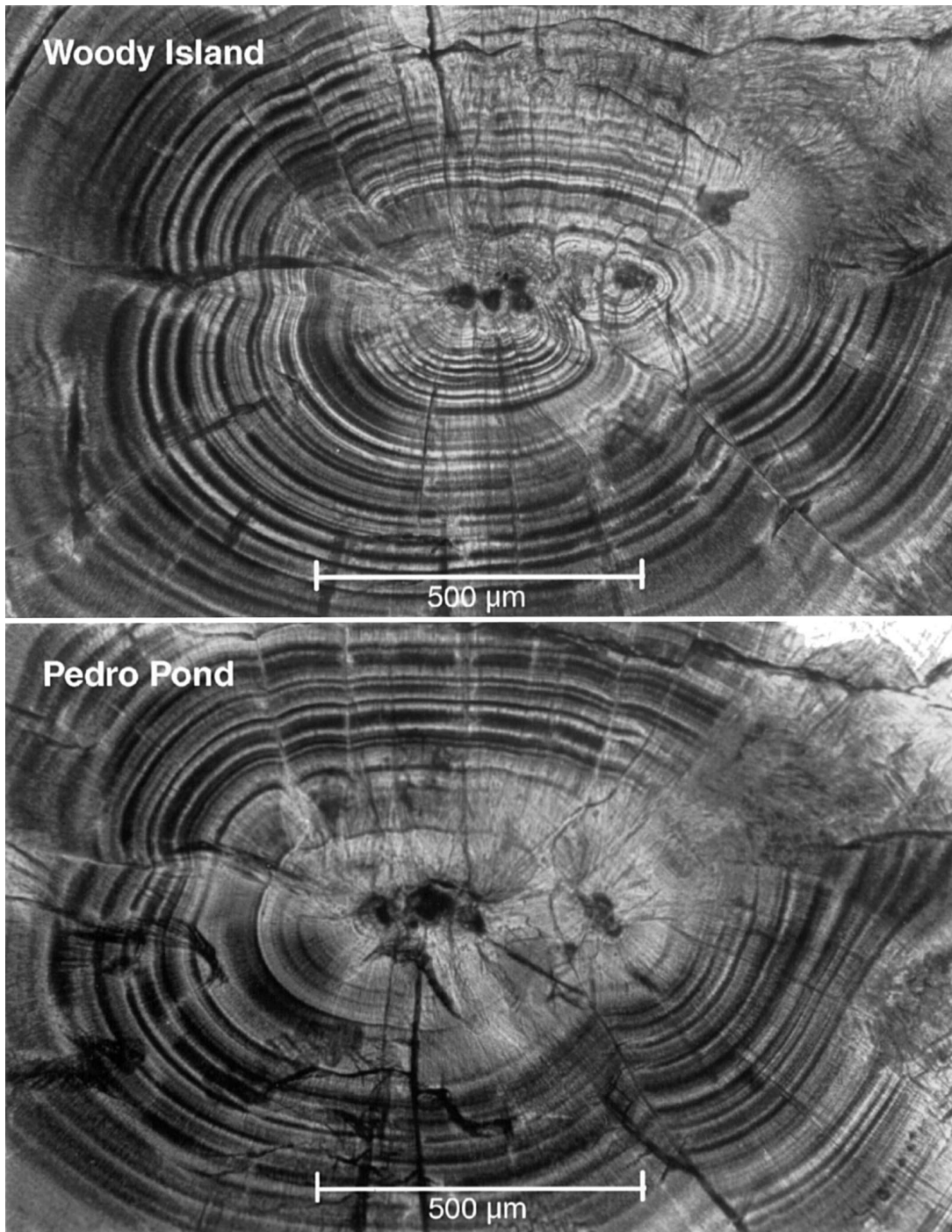
76 000 vs. 2720; Washington Department of Fish and Wildlife, unpublished data). If the 14 fish collected from Pleasure Point in 1992 with river-type otoliths were strays from the Cedar River (out of 32 specimens in total), then we would estimate 529 strays into a beach population of 1208 (44%). In 1993, the 12 fish with river-type otoliths (classified as Cedar River fish) in the sample of 34 from the beach gives an estimate of 35% straying into the beach population from

the Cedar River population. Although these are high levels of straying relative to the beach population, they are very small percentages relative to the Cedar River population (0.6 and 1.3% in 1992 and 1993, respectively). If Cedar River and Pleasure Point sockeye salmon distributed themselves randomly between the spawning areas, only 3% (1992) and 1% (1993) of the Pleasure Point fish would have had beach-type otoliths, rather than the 56 and 65% that we observed.

Discussion

The cornerstone of this study was the well-established effect of temperature on otolith increment patterns (Mosegaard et al. 1987; Brothers 1990; Volk et al. 1990, 1994). We viewed the generalized pattern of increment densities recorded over an easily defined region of the growing otolith formed prior to emergence, which insured that our classification was based on features representing natural variation in only the incubation environment. Examination of the Iliamna Lake samples indicated that all sockeye salmon collected at Woody Island had incubated under similar variable thermal regimes. However, only 4 of 50 adults collected from Pedro Pond and 2 of 48 from Grass Pond had incubated under such conditions. We infer that no sockeye salmon that had incubated in the pond or a similar environment were collected at the beach, and that 6% of the fish collected at the ponds were strays from the beaches. This does not mean that all the beach fish were spawned at Woody Island, nor does it mean that the apparent strays to Pedro Pond came exclusively from Woody Island. Otoliths from Fuel Dump Island sockeye salmon were similar to those from Woody Island sockeye salmon and all the nearby beaches seem to have similar temperature profiles (Olsen 1964; Hendry et al. 1995; Leonetti 1997), which would induce similar patterns on the otoliths. It is also possible that some fish strayed from other populations in the lake, but the high degree of classification success suggests that this was rare, because strays from other populations would only have obscured our ability to classify samples.

These results, implying an ability to home to distinct incubation habitats, were consistent with displacement experiments on adult male sockeye salmon in Iliamna Lake (Blair and Quinn 1991) and with differences between the island and pond populations in egg size (Quinn et al. 1995), morphology, and length at age (Quinn and Kinnison 1999). No pond fish were detected in the beach samples but, given the great disparity of abundance, only a very high rate of straying from the ponds would have been detected in our sample from Woody Island. For example, if 6000 sockeye salmon originated in the ponds and 50% of them strayed (i.e., 3000 strays plus the 3000 estimated in the ponds) to the beaches, they would have constituted only 1.75% of the beach fish or fewer than one in 50, the sample size we used. Thus the absence of pond-type otoliths in the sample from the beach does not mean that such straying does not occur.

Pedro and Grass ponds had similar temperature regimes and it was much more difficult to distinguish between the otolith increment patterns of known-origin juveniles. Given the similarity between ponds, even the modest success in classifying adults in blind trials (60%) indicates some level of homing to these two sites. Interestingly, there were about twice as many Grass Pond fish classified to Pedro Pond as vice versa. Given the fact that there were twice as many adults in Pedro Pond as in Grass Pond in 1995, this would be consistent with roughly equal rates of straying from each pond into the other. The sites not only have similar (though not identical) thermal regimes, but they are near each other, are both fed by ground water, and have similar soil and vegetation in the surrounding areas. Thus, distinguishing between them based on odors must pose a considerable challenge for adult salmon. Regardless, the evidence that the salmon in the two ponds are apparently not freely mixing between generations indicates population structuring on a very small spatial scale. This fine structure is also consistent with evidence of limited exchange of spawners between adjacent breeding areas within seasons (Hendry et al. 1995).

Both the Cedar River and Pleasure Point adult samples and the Cedar River fry samples included specimens that had apparently incubated under very different thermal regimes (some variable and some constant), despite the fact that our records indicated a virtually isothermal regime at Pleasure Point and a fluctuating one in the Cedar River. There are two possible explanations for this mixture of otolith types: within-site variation in thermal regimes and between-site straying. It is unlikely that the ground water at Pleasure Point varies as much as the Cedar River, though not all locations within the beach may be as isothermal as the two at which our temperature probes were placed. However, the Cedar River is a diverse habitat and includes several side channels and ponds that may have more stable temperatures than the main river. Consistent with this heterogeneity in incubation habitat, the otolith patterns of Cedar River fry varied considerably.

The "beach-type" fish from the Cedar River collections were probably not strays, because the Pleasure Point population is so small relative to the Cedar River population that the chances of our collecting beach-origin fish in the Cedar River would be minute, even if most of the beach population strayed to the Cedar River. However, it is likely that Pleasure Point adults with river-type otoliths were strays from the Cedar River. Given the relative abundance of the populations and their proximity, some straying is not surprising. The estimated straying levels (44 and 35% in the 2 years) are high relative to the beach population but they are only about 1% of the Cedar River population. Cedar River sockeye salmon thus show strong fidelity to their natal site, despite the proximity of the beach. The large number of apparent strays at the beach results simply from the overwhelming abundance of the Cedar River population. We cannot rule out straying from other sockeye salmon populations within the Lake Washington system, but these populations are much smaller and more distant than the Cedar River population, so straying from them is less likely.

If the otoliths with "river-type" patterns collected from Pleasure Point represented strays from the Cedar River, this straying rate would be sufficient to prevent any genetic differentiation between the populations, if the reproductive success of the strays was equal to that of the local fish. However, these two populations differed in morphology and life history (Hendry and Quinn 1997), developmental biology (Hendry et al. 1998), and gene frequencies (J. Wenburg and A.P. Hendry, unpublished data), implying a much lower reproductive success in strays.

Conclusions

From the standpoint of homing, our results support the hypothesis that sockeye salmon return to their incubation site, not just to the site where they underwent smolt transformation (i.e., the outlets of their respective lakes). We do not

contest the ample evidence that salmon imprint at the smolt stage (Hasler and Scholz 1983; Dittman et al. 1996). However, salmon also apparently imprint or learn odors earlier in life. The experimental studies conducted to date have failed to demonstrate the imprinting by embryos or emerging fry that must occur routinely in natural populations.

Our results are consistent with studies of gene frequencies indicating that sockeye salmon populations are structured at the level of spawning sites as well as that of nursery lakes, including Iliamna Lake (Varnavskaya et al. 1994) and Lake Washington (Hendry et al. 1996). However, while gene frequency data can indicate that the populations in question are not freely interbreeding, such data may not be able to identify specific individuals as strays. If the patterns of otolith microstructure are sufficiently distinct, individuals can be identified as strays independent of any genetic analysis. In the future it would be valuable to conduct genetic analyses of salmon populations that might also be distinguishable by otolith patterns. Individuals identified as possible strays at one spawning site might display genotypes more closely resembling those at the other spawning site, allowing for more accurate characterization of the genotypes.

Acknowledgements

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