

Intra-population variation in anadromy and reproductive life span in rainbow trout introduced in the Santa Cruz River, Argentina

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Scale patterns, maturational status and otolith microchemistry (strontium to calcium ratios) were analysed in sympatric anadromous and non-anadromous rainbow trout *Oncorhynchus mykiss* in the Santa Cruz River (Patagonia, Argentina) to investigate the life-history differences of anadromous and non-anadromous lifestyles and the association between maternal origin and progeny life history. The analyses revealed that both forms can give rise to one another, indicating a single population with alternative phenotypes. Anadromous fish smolted at ages 2 and 3 years, matured after 1 to 2 years in the ocean, and survived up to 11 years, spawning up to eight times. Non-anadromous fish survived up to 6 years, spawning up to three times. The extended reproductive life span associated with anadromy in this river suggests that increased energetic and physiological demands associated with ocean migration may not necessarily result in reduced postspawning survival, as has been suggested for salmonids in general. Alternatively, reduction in parity may be regarded as the evolutionary outcome of reproductive traits resulting from the adoption of anadromy (*i.e.* augmented reproductive investment) coupled with long-range migrations to and from the ocean. The life-history patterns of Santa Cruz River rainbow trout provide a natural experiment for investigating the evolutionary transition and maintenance of anadromy and non-anadromy within salmonid populations. © 2007 The Authors

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Key words: anadromy; iteroparity; *Oncorhynchus mykiss*; otolith microchemistry; reproductive life span; Sr:Ca.

INTRODUCTION

In Pacific salmon *Oncorhynchus* sp., the evolution of anadromy and parity modes (*i.e.* semelparity *v.* iteroparity) has been shaped by the energetic balance between the costs of lengthy migrations between fresh and salt water and high

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reproductive investment on one hand, and the benefits of exploiting the rich resources of the ocean environment on the other (Willson, 1997; McDowall, 2001; Crespi & Teo, 2002; Hendry *et al.*, 2004; Quinn & Myers, 2004). While the outcome of this balance resulted in the evolution of obligatory anadromy and semelparity in most *Oncorhynchus* species (Miller & Brannon, 1982; Crespi & Teo, 2002; Brannon *et al.*, 2004), although there are exceptions, *e.g.* chinook salmon *Oncorhynchus tshawytscha* (Walbaum) (Unwin *et al.*, 1999), the rainbow trout *Oncorhynchus mykiss* (Walbaum) evolved a great plasticity to express anadromy and non-anadromy and varying degrees of parity across populations, ranging from highly reduced iteroparity to high iteroparity (Shapovalov & Taft, 1954; Busby *et al.*, 1996; Willson, 1997).

In *O. mykiss*, the incidence of anadromy varies between populations and among individuals within populations across the native range. As such, *O. mykiss* populations can be monomorphic, composed exclusively of anadromous (referred to as steelhead) or non-anadromous individuals (referred to as resident rainbow trout) or, alternatively, polymorphic (also known as partially migratory), composed of both anadromous and non-anadromous individuals (Busby *et al.*, 1996; Zimmerman & Reeves, 2000; Narum *et al.*, 2004).

In general, when they co-occur in sympatry without physical barriers, steelhead and resident rainbow trout may interbreed and be genetically similar (Allendorf, 1975; Berg, 1987; Leider *et al.*, 1995; Busby *et al.*, 1996); individuals can sometimes shift from one form to another during their lifetime or across generations (Zimmerman & Reeves, 2000). Recently, the failure to find genetic differences in such anadromous–non-anadromous complexes and the capacity of the species to switch between forms has led many authors to propose that anadromous and non-anadromous life histories of *O. mykiss* represent alternative phenotypic options for the species rather than distinct evolutionary modes (McCusker *et al.*, 2000; McEwan, 2001; Brannon *et al.*, 2004).

The Santa Cruz River in Patagonia, Argentina, is the only drainage system in the world where introduced *O. mykiss* are known to have recreated this polymorphic behaviour, displaying both anadromous and non-anadromous lifestyles (Pascual *et al.*, 2001). Scale pattern analysis indicated that anadromy in Santa Cruz River fish not only results in much faster growth, but may also produce notable changes in the schedule of reproduction and post-reproductive survival, an observation that has not been validated by independent methods. Mitochondrial DNA and nuclear microsatellite analyses revealed that both forms shared a common genetic origin derived from anadromous and non-anadromous sources introduced from rivers in California, U.S.A. (Riva Rossi *et al.*, 2004), and that the two forms are now genetically indistinguishable (Pascual *et al.*, 2001). These findings suggested that anadromy and its related attributes may result as phenotypic responses of a single genotype to the local environment. Even limited gene flow between the sympatric forms that share a recent common origin, however, could prevent genetically based phenotypic differentiation, even for traits under directional selection. Hence, establishing the relatedness between anadromous and non-anadromous forms raises questions such as whether the expression of anadromy leads to reproductive isolation between types, whether a genetic predisposition exists for anadromy or non-anadromy, and whether these two factors combined can operate as agents

of population structuring, e.g. kokanee form of sockeye *Oncorhynchus nerka* (Walbaum) pairs (Wood, 1995); anadromous–non-anadromous brook charr *Salvelinus fontinalis* (Mitchill) (Boula *et al.*, 2002) and steelhead-resident rainbow trout (Pascual *et al.*, 2002; Kostow, 2003).

In this study, fishing and spawning surveys, scale pattern analysis, maturational status and the ratios of strontium to calcium (Sr:Ca) in otoliths were used to: 1) describe the anadromous behaviour in Santa Cruz River *O. mykiss*, 2) determine the maternal origin of anadromous and non-anadromous fish, testing for cross-ancestry, and 3) analyse the concomitant expression of longevity and iteroparity, two life-history traits typically associated to anadromy. The results of this study are discussed in terms of the underlying trade-offs between anadromy, growth and reproduction, and the prospects for the long-term coexistence of anadromous and non-anadromous forms in the Santa Cruz River.

MATERIALS AND METHODS

STUDY SYSTEM

The Santa Cruz River (50° S; 70° W, average flow 690 m³ s⁻¹, range 300–2000 m³ s⁻¹) flows for 382 km across the Patagonian plateau to drain into the Atlantic Ocean (Fig. 1). The upper basin is dominated by two large glacier-fed lakes, Viedma and Argentino, connected by the La Leona River, the second most important river of the drainage system after the Santa Cruz. There are no barriers to fish migrations. Average water temperature is 9° C with maxima registered in January (15° C) and minima in July (3° C).

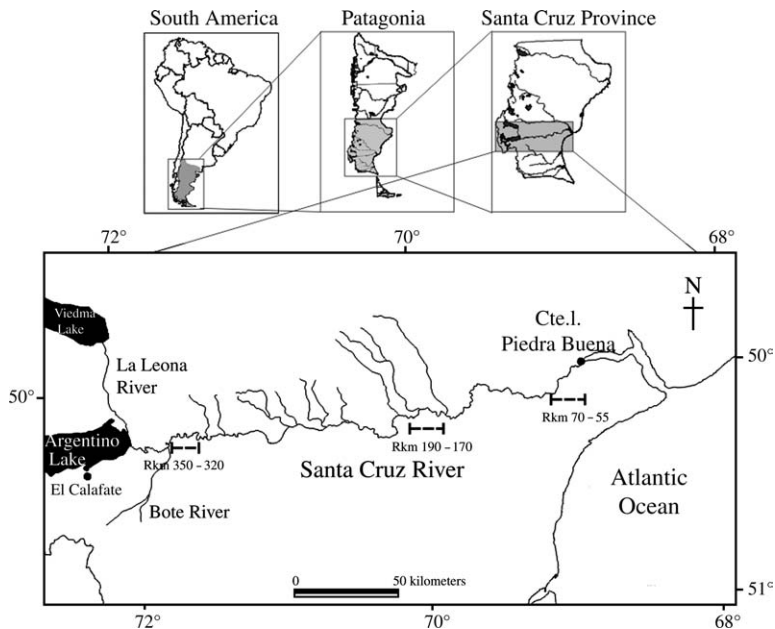


FIG. 1. Map of the Santa Cruz River, Argentina, showing the locations of the study river reaches.

FISH COLLECTION

A total of 467 fish (292 anadromous and 175 non-anadromous) were analysed in this study, collected at spawning and fishing areas that were identified through field and telemetry studies (Riva Rossi *et al.*, 2003): 61 non-anadromous and 90 anadromous fish from river reaches located in the upper portion (river km 350–320), 92 non-anadromous and 55 anadromous fish from the middle portion (river km 190–170), and 22 non-anadromous and 147 anadromous fish from the lower portion (river km 70–55), near the town of Piedra Buena (Fig. 1). Fish were measured (fork length, L_F , mm), weighed (g), and sex and spawning status were recorded on the basis of macroscopic appearance of the gonads. Scales and otoliths were collected for age and life-history determinations, and for microchemical analysis (otoliths, only).

SCALE ANALYSIS

Scales were analysed from 292 anadromous fish and 164 non-anadromous fish for age, evidence of sexual maturity and degree of iteroparity. Scales were taken from an area below the posterior margin of the dorsal fin and five scale rows above the lateral line. Three cleaned scales from each specimen were pressed on acetate sheets with heated plates; circuli impressions were highlighted by the application of ink and magnified on a microfiche reader.

For ageing, annuli were identified by the conventional technique of assigning crowding and narrowing of inter-circuli distances, typically associated to the cessation of growth during winter, to yearly events (Davis & Light, 1985; Pascual *et al.*, 2001). In anadromous fish, freshwater annuli are characterized by narrowly spaced circuli, clearly differentiated from ocean growth, where increased spacing and thickness of the circuli is evident (Pascual *et al.*, 2001). In mature anadromous fish, spawning takes place at the time of annulus formation and, therefore, the corresponding annuli are accompanied by or replaced by spawning checks, noticeable as erosion and scar-like rings caused by scale margin resorption. As in other summer steelheads, the amount of resorption in Santa Cruz River scales varies from moderate (loss of three to four circuli) to complete (loss of the previous winter annulus). Spawning checks were used to estimate age at first spawning and incidence of repeat spawning, by documenting the first appearance and repetition of checks on scales. Scales ages and spawning checks were examined twice by two experienced readers to ensure consistency and after a third joint reading identical fresh water and marine age assignments were obtained for >95% of the sampled fish.

The scale information corresponding to anadromous fish together with the age distribution, and maturational status of anadromous adults caught during the spawning run of 2000, 2001 and 2004 were evaluated for consistency and validation of ageing methods and interpretation of spawning events.

MATURATIONAL STATUS AND AGE DESIGNATION METHOD

On the basis of inspections of the gonads, four maturational categories were defined: immature fish were those with underdeveloped gonads and that were not ready to spawn that year; maturing fish were those with developing gonads (maturing), eggs or milt not produced when squeezed, and that were going to spawn later in the season; mature or imminent spawner fish were those in full spawning colours, had gonads at maximum size with eggs or milt easily extruded by a gentle pressure on the abdomen; spent or postspawned fish were those that still had spawning colours but eggs and sperm were totally discharged, and gonads empty except for a few remaining eggs or residual sperm. Based on these characteristics fish falling in the immature or maturing categories were considered as non-spawners, whereas mature or spent fish were considered as spawners (Kissner, 1973; Anon., 1997).

Taking into account the variability in smolting age, age at first maturity, longevity and the incidence of multiple spawning events in Santa Cruz River *O. mykiss*, the age of spawners was expressed as 'post-smolt age' defined as the number of years a fish survived to migrate to and from the ocean. In terms of the ageing system typically used for recording age in North American steelhead, post-smolt age would correspond to the sum of ocean age (the number of continuous years a fish spent in the ocean before entering fresh water to spawn) plus kelt age (the number of post-reproductive years a fish survive to repeat spawning) (Busby *et al.*, 1996; Whiteaker *et al.*, 2006). For example, a fish that returned to fresh water after 1 year at sea would be aged post-smolt age 1. A post-smolt that migrated to and from the ocean twice (spawning or not spawning after each trip) would be aged post-smolt age 2. A post-smolt that migrated to and from the ocean six times would be aged post-smolt age 6.

OTOLITH MICROCHEMICAL ANALYSIS

Microchemical analyses were conducted on the otoliths to measure the Sr:Ca ratio as a proxy for salinity. These Sr:Ca ratios can then be used as a tracer of residence in waters of different salinities to describe the environmental history of individuals across the oceanic-freshwater boundary (Kalish, 1990). One of the two sagittal otoliths from 20 fish were subjected to microchemical analysis: eight fish from the upper river, two fish from the mid portion and 10 fish from the lower river. The otoliths were embedded in epoxy resin and a transverse, dorso-ventral cross-section through the core of the otolith was made using a low-speed saw, exposing all annuli. The posterior halves of up to five otoliths were re-embedded in a standard 25.4 mm diameter acrylic, probe-mount. The exposed otolith surfaces were sequentially ground (wetted 30, 12 and 9 μm aluminum oxide lapping film), polished (dry 3.0 and 0.3 μm aluminum oxide lapping film), ultrasonically cleaned, photographed and finally carbon-coated to prevent charging during microprobe analysis.

The photograph of each otolith section was used to plot a linear path (transect) for the microprobe beam to cross all annuli from the nucleus running parallel to the sulcus to the outer edge of the otolith, corresponding to a life-history transect in time. A second transect was plotted across the nucleus of the otolith (nucleus transect) to determine the maternal origin of individual fish. The photographs were then used as references by the microprobe operator. The wavelength-dispersive electron microprobe used in this study was a Cameca SX-100, housed at the Department of Geological Sciences, University of Manitoba, Winnipeg, Manitoba, Canada. The electron beam was operated at an accelerating voltage of 15 kV and a current of 20 nA. The transects across the otoliths utilized a beam diameter of 5 μm and a centre-to-centre distance between sequential points of 7 μm . Strontium $L\alpha$ X-rays were counted at each point for 25 s. A small (ablation) pit was left on the otolith surface at every point. The 'life-history transect' typically consisted of 75 to 175 points, depending on the size of the otoliths. The nucleus transect to determine the maternal origin of the fish typically consisted of eight to 18 points. Sr:Ca was then plotted against distance (number of sample points) for each otolith.

After microprobe analysis was completed, otolith preparations were cleaned with ethanol to remove the carbon coat. For age estimation, otoliths were examined under a stereoscopic microscope, using reflected light against a black background to reveal the otolith banding patterns, annuli and marks left by the electron beam. Annuli were identified as bright opaque bands (regions of fast growth) separated by dark, translucent bands (regions of slow growth). Age was determined by counting the number of annuli while viewing the corresponding scales from the same fish, so otolith and scale ages are not independent. Sr:Ca plots (profiles) were superimposed on the digital optical image of the post-microprobed otolith section allowing Sr:Ca to be correlated with otolith annuli. In some cases the seasonal patterns in strontium uptake by the otoliths helped to resolve questionable annuli. Otolith regions were classified as nucleus, freshwater growth zone and seawater growth zone.

Maternal origin is indicated by otolith Sr:Ca as the water chemistries experienced by the female parent during vitellogenesis and yolk deposition affect Sr:Ca values in the otolith core regions of juvenile salmonids [Otolith core Sr:Ca values are higher for progeny from females maturing in sea water and lower for offspring of females maturing in fresh water (Kalish, 1990)]. In this study the relative value of Sr:Ca in the otolith nucleus and freshwater growth zones along the secondary transect was used to identify the progeny of anadromous *v.* non-anadromous fish. Individual fish were determined to be of anadromous maternal origin if the Sr:Ca in the nucleus was significantly higher than values in the freshwater growth region based on unpaired, one-tailed *t*-tests with $\alpha = 0.05$ (Zimmerman & Reeves, 2002).

To validate the interpretation from nucleus Sr:Ca, independent data obtained from the progeny of wild anadromous fish were used. In September 2001 wild, mature anadromous fish collected in the mainstem of the river (five females and two males) were spawned and their progeny reared in the Piedra Buena Municipal Hatchery (Fig. 1). In February 2003, sagittal otoliths from six juveniles (age 1+ years) from this cohort were collected and Sr:Ca were analysed for eight points sampled from the otolith nucleus of each fish.

RESULTS

SR:CA PROFILES: MARINE AND FRESHWATER RESIDENCE

The otoliths of 14 wild fish out of the 20 caught in the Santa Cruz River showed an oscillatory Sr:Ca profile beginning at age 2 to 3 years, indicative of anadromy [e.g. Fig. 2(a)–(e)]. The remaining six cases showed no oscillation in Sr:Ca, indicative of a freshwater resident lifestyle [e.g. Fig. 2(f)–(h)].

The anadromous otoliths showed a pattern of relatively low Sr:Ca in the freshwater growth zone during the first 2 to 3 years of life (average 0.0004 to 0.0020), followed by relatively high, seasonally fluctuating Sr:Ca in the saltwater growth zone. Within the saltwater growth zone, peaks of Sr:Ca increased to values of 0.0027 to 0.0080, with Sr:Ca values between peaks declining to levels of 0.0010 to 0.0025. Such a pattern is indicative of 2–3 years in fresh water as juveniles, and summer ocean migrations (peaks) followed by overwinter or spawning migrations into fresh water afterwards (between peaks) [Fig. 2(a)–(e)]. As documented in other studies of fish migrations based on otolith microchemistry, after the initial Sr:Ca peak, the otolith zones corresponding to life in fresh water usually show more elevated Sr:Ca levels [Fig. 2(b)] than in the initial freshwater growth zone (Radtke *et al.*, 1998).

Life-history reconstruction based on Sr:Ca profiles indicated that most fish spent 2 years in fresh water before migrating as smolts to the ocean [Fig. 2(a)–(d) and Table I], with only one fish remaining in the river for 3 years before migrating to the ocean [Fig. 2(e) and Table I]. All fish re-entered the river during their first year of ocean life, after only one summer at sea. Following this first river entry, Santa Cruz River fish continued migrating annually to and from the ocean [Fig. 2(b)–(e) and Table I]. Six of the fish sampled had successfully completed three migrations to and from the ocean and the maximum was eight upstream migrations for an age 10 year individual [Fig. 2(d) and Table I].

The remaining six otoliths analysed showed relatively constant and low Sr:Ca (0.0002 to 0.0022) from outside the nucleus area to the outer edge of the otolith, indicating that these fish spent their entire life in fresh water [e.g. Fig. 2(f), (g)]. Two of these fish showed Sr:Ca levels corresponding to areas

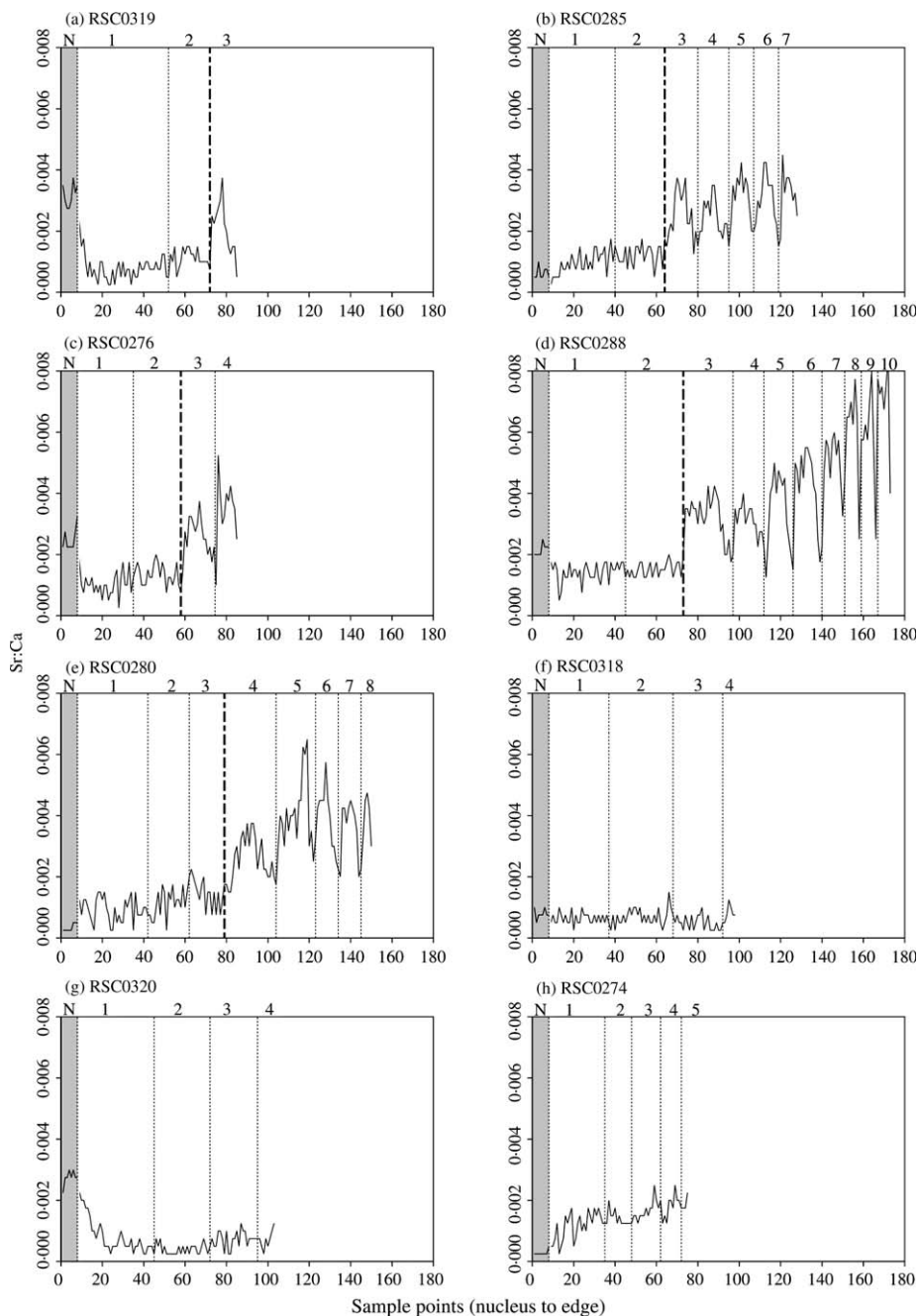


FIG. 2. Representative otolith Sr:Ca profiles from Santa Cruz River *Oncorhynchus mykiss*. (a)–(e) anadromous and (f)–(h) non-anadromous individuals. N, nucleus. Ages (years) are indicated in the upper x axis and approximate locations of annuli are delineated by dotted lines. Sr:Ca values for nuclei come from the nuclei transects and values for the remaining section come from the life-history transect. The broken line separates the freshwater growth zone (left) from the seawater and freshwater growth zone (right).

TABLE I. Capture, morphometric and life-history information of *Oncorhynchus mykiss* collected in the Santa Cruz River for otolith microchemistry analysis

Fish ID	Capture location	Capture date	L_F (mm)	Mass (g)	Sex	Fish ecotype	Maternal ecotype	Scale age (years)	Otolith age (years)	Age-at-first-seaward migration (years)	Post-smolt age-at-first-river return (years)	Number of years at sea	
												Scale annuli	Sr:Ca peaks
RSC0275	Lower portion	21 September 2001	565	2300	F	A	A	4	4	2	1	2	2
RSC0276	Upper portion	26 September 2001	569	2000	F	A	A	4	4	2	1	2	2
RSC0280	Upper portion	26 September 2001	805	7000	F	A	NA	8	8	3	1	5	5
RSC0285	Upper portion	29 September 2001	755	4800	F	A	NA	7	7	2	1	5	5
RSC0288	Upper portion	29 September 2001	800	5000	F	A	A	9	10	2	1	7	8
RSC0292	Upper portion	29 September 2001	555	1600	F	A	A	—	4	2	1	2	2
RSC0295	Upper portion	29 September 2001	520	1000	F	A	A	—	4	2	1	2	2
RSC0301	Upper portion	26 September 2001	670	3500	F	A	NA	—	7	2	1	5	5
RSC0306	Middle portion	4 October 2001	660	2600	M	A	A	7	7	2	1	5	5
RSC0307	Middle portion	4 October 2001	480	1250	M	A	A	3	3	2	1	1	1
RSC0315	Lower portion	26 March 2002	595	2800	M	A	A	4	4	2	1	2	2
RSC0316	Lower portion	30 March 2002	384	590	M	A	A	3	3	2	1	1	1

TABLE I. Continued

Fish ID	Capture location	Capture date	L_F (mm)	Mass (g)	Sex	Fish ecotype	Maternal ecotype	Scale age (years)	Otolith age (years)	Age-at-first-seaward migration (years)	Post-smolt age-at-first-river return (years)	Number of years at sea	
												Scale annuli	Sr:Ca peaks
RSC0319	Lower portion	1 April 2002	370	545	M	A	A	3	3	2	1	1	1
RSC0317	Lower portion	30 March 2002	442	860	M	A	NA	6	6	2	1	4	4
RSC0274	Lower portion	21 September 2001	335	390	M	NA	NA	5	5	—	—	—	—
RSC0318	Lower portion	30 March 2002	350	485	M	NA	NA	4	4	—	—	—	—
RSC0320	Lower portion	31 March 2002	360	510	F	NA	A	4	4	—	—	—	—
RSC0321	Lower portion	30 March 2002	426	695	F	NA	NA	4	5	—	—	—	—
RSC0322	Lower portion	30 March 2002	365	490	F	NA	A	4	4	—	—	—	—
LAR0004	Upper portion	26 March 1997	340	390	F	NA	NA	5	6	—	—	—	—

L_F , fork length; A, anadromous; NA, non-anadromous; F, female; M, male.

of slightly higher salinity, indicative perhaps of some movement between fresh water and the mouth of the river or the estuary [e.g. Fig. 2(h)]. Maximum age recorded for non-anadromous fish was 6 years.

SR:CA PROFILES: MATERNAL ORIGIN

The statistical comparison of Sr:Ca in nuclei and freshwater growth zones in otoliths for individual fish allowed identification of all four possible ecotype-maternal origin combinations (Fig. 3): 10 anadromous [e.g. Fig. 2(a), (c), (d)] and two non-anadromous fish [e.g. Fig. 2(g)] were determined to be the progeny of anadromous females because the mean Sr:Ca were significantly higher in their nucleus than in the freshwater growth region (unpaired one-tailed *t*-test for each fish; nine fish, $P < 0.001$; one fish, $P < 0.05$), whereas four anadromous [e.g. Fig. 2(b), (e)] and four non-anadromous fish [e.g. Fig. 2(f), (h)] were determined to be the progeny of non-anadromous females because the mean Sr:Ca were not significantly higher in their nucleus than in the freshwater growth region. As a general rule, all fish with a difference between nucleus and freshwater Sr:Ca ≥ 0.0004 were determined to be the progeny of anadromous females.

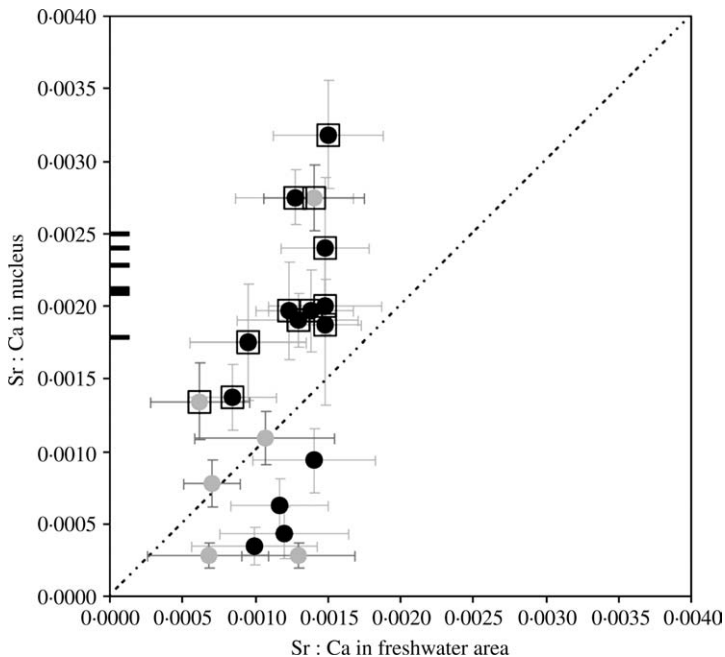


FIG. 3. Relationship between mean Sr:Ca in the otolith freshwater growth zone and in the otolith nucleus for Santa Cruz River anadromous (●) and non-anadromous (●) *Oncorhynchus mykiss*. Fish assigned as progeny of anadromous mothers by *t*-tests (□). Mean Sr:Ca in the otolith nucleus of six fish with known steelhead maternal origin are shown on the y-axis. Error bars are s.d.

In general, mean Sr:Ca in freshwater growth regions of the otoliths analysed ranged from 0.0006 to 0.0015 (mean 0.0012), while mean Sr:Ca in nuclei ranged from 0.0003 to 0.0032 (mean 0.0015; Fig. 3). Of those, the mean Sr:Ca in nuclei corresponding to the 12 fish assigned to anadromous mothers ranged from 0.0013 to 0.0032 (mean 0.0021). These values are consistent with those measured in nuclei of otoliths from the six hatchery reared fish of known steelhead maternal origin (Fig. 3). Although the range of values corresponding to the latter is somewhat narrower (0.0018–0.0025), an expected outcome given the small sample size and the reduced number of mothers used in the experiment, the distribution is centred at approximately the same mean value (0.0022 *v.* 0.0021). This correspondence between fish of putative anadromous maternal origin and an independent group of fish with known anadromous maternal origin provides a strong validation for the ecotype-progeny assignment.

VALIDATION OF THE INTERPRETATION OF PATTERNS IN SCALES

The number of annuli identified in the scales was compared with the number identified in otoliths. Overall, there was a 82.35% agreement between corresponding scale and otolith total ages from the fish with both structures sampled ($n = 17$). In this sample, maximum ages for scales and otoliths were 9 and 10 years, respectively. Scale age underestimated otolith age by 1 year in anadromous fish >9 years and non-anadromous fish >4 years (Table I).

The assignment of scale annuli to fresh water and ocean growth was validated by the ratios of Sr:Ca recorded in profiles along otoliths. There was complete agreement in the assignment of fish to anadromous and to non-anadromous ecotypes from scale pattern analysis and from enrichment patterns in Sr:Ca along otoliths, as in the assignment of individual annuli in anadromous fish to either fresh water or marine growth. The validation of proposed annuli and checks as yearly events in scales of anadromous fish was done by their correspondence with patterns of Sr:Ca ratios in otoliths. Radio-tracking experiments and run timing information (Pascual *et al.*, 2001; Riva Rossi *et al.*, 2003) clearly showed that migration to the ocean is a yearly event. There was a 91% agreement ($n = 11$) between the number of migration events, as indicated by the numbers of peaks in Sr:Ca ratio along otoliths transects, and the number of post-smolting annuli or checks identified in scales (Table I).

AGE AND MATURATION OF ANADROMOUS AND NON-ANADROMOUS FISH

The age structure of anadromous fish estimated from scale pattern analysis was markedly different from that of the non-anadromous component of the population (Fig. 4). Maximum ages for anadromous and non-anadromous fish were 11 and 7 years, respectively. The median age of both groups differed by a whole year (5 years for non-anadromous *v.* 6 years for anadromous), and the difference is more dramatic for extreme ages. Few non-anadromous fish >6 years were recorded but 20% of the anadromous fish caught were >7 years and as old as 11 years (one individual). The same difference was found in

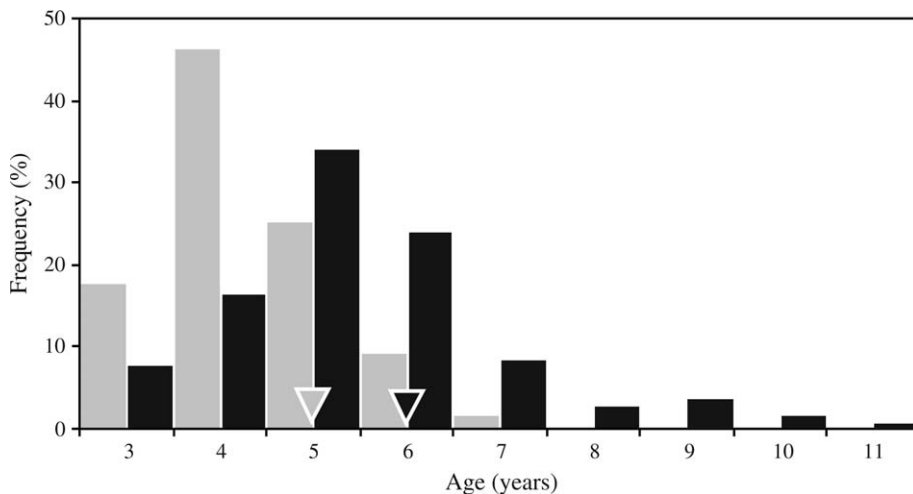


FIG. 4. Age distribution of Santa Cruz River anadromous (■) and non-anadromous (□) *Oncorhynchus mykiss* caught from 1996 to 2004. Median age for each ecotype (▽) are indicated.

the age structure of both groups when upper river samples taken in the spring (obtained by gillnetting during spawning season) were separated from lower river samples taken in the autumn (hook and line during the fishing season), indicating that it is unlikely that the observed pattern results from gear selectivity. The same pattern was found for the data disaggregated by sampling year.

The degree of parity of Santa Cruz River anadromous *O. mykiss* was then investigated to determine whether fish reached old ages by delaying maturation or, as suggested by the accumulation of checks in scales, by repeat spawning. Initially, the seasonal pattern of maturation and the ontogenesis of spawning checks in scales were investigated.

Fish caught between March to early May as they entered fresh water were primarily immature or maturing individuals ('non-spawners', $n = 152$), whereas those caught between July and late October in spawning grounds were either in a mature, spawning or postspawning condition ('spawners', $n = 140$). The edge of scales of 'non-spawners' showed from three to five narrowly spaced circuli, indicative of the beginning of annulus formation, with no evidence of erosion at the margin. On the other hand, the edge of scales of 'spawners' showed moderate to strong erosion with loss of the last three to four circuli deposited, indicative of the formation of the spawning check. The seasonal evolution of the scale edge provided conclusive evidence for the association between checks, annuli and spawning events.

The age structure of the spawning population was explored to verify and further characterize the degree of iteroparity in anadromous fish. The 140 spawners ranged from age 3 to 11 years, having spent from one to nine summers in the ocean (post-smolt age 1–9 years; Fig. 5). Scales of only 15% of all spawners of post-smolt age 2 years showed one previous spawning check, indicating

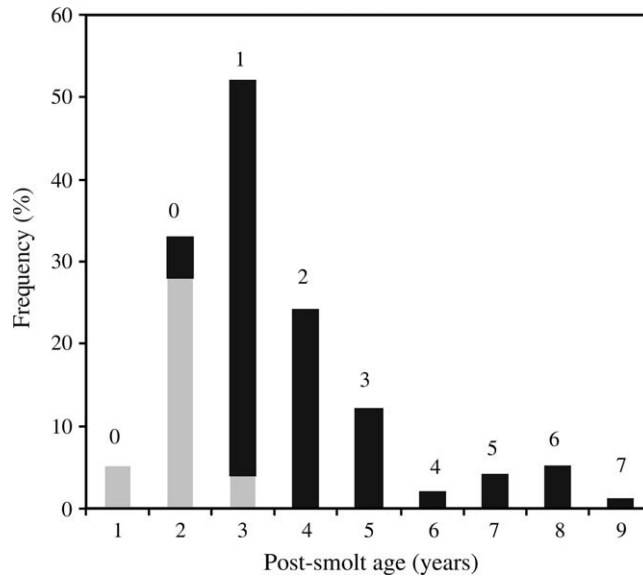


FIG. 5. Age distribution of anadromous spawning adults of Santa Cruz River *Oncorhynchus mykiss* caught during the spawning seasons of 2000, 2001 and 2004. Age expressed as years after the first ocean migration (post-smolt years). Bars show the composition of first time (□) and repeat (■) spawners at each age class. Numbers above each histogram column indicate the modal number of prior spawning events for each post-smolt age class.

that only a small fraction of fish spawn after only one summer in the ocean. In contrast, 94% of spawners of post-smolt age 3 years and 100% of post-smolt age 4 years had previous spawning checks, indicating that most fish mature at post-smolt age 2 years and all fish are reproductively mature afterwards. The number of spawning checks increased consistently with age (Fig. 5) and no evidence of annuli between spawning marks was detected; thus, indicating that once fish mature, they return yearly to the river to spawn, in some individuals as many as eight times. The association of spawning events to river entries is demonstrated by the correspondence of checks and profiles of Sr:Ca (Table I).

It is not clear why fish of post-smolt age 1 and 2 years are consistently less abundant than the next older age in both spawning ground and fishing season surveys (Figs 4 and 5). Being immature or first-time spawners, these fish might have different migratory patterns with respect to the mature portion of the population, somehow affecting the chances of catching them with the methods currently employed.

Meanwhile, the reproductive biology of non-anadromous rainbow trout remains poorly known. The lack of strong spawning checks in their scales did not allow for unambiguous inferences regarding past spawning events or maturation age, such as those produced for anadromous fish. Furthermore, non-anadromous fish were seldom caught during spawning surveys with gillnets, which have been largely dominated by anadromous fish. The possibility of some geographical segregation between types cannot be ruled out at this time.

Also, and judging from gonad inspection of a limited collection of resident fish, they appear to spawn later in the season compared to anadromous fish.

DISCUSSION

Anadromous salmonids provide good opportunities to study sympatric divergence in animal populations (Foote & Larkin, 1988; Skaala & Nævdal, 1989; Zimmerman & Reeves, 2000; Boula *et al.*, 2002; Saint-Laurent *et al.*, 2003; Narum *et al.*, 2004; Fraser & Bernatchez, 2005). While it is too early to claim that such a process is taking place within the Santa Cruz rainbow trout population, the exotic anadromous and non-anadromous complex in this river provides a new, unique setting to investigate the elements and processes that are key to ecological speciation (Schluter, 2001): phenotypic plasticity, the adaptive value of alternative phenotypes, together with their genetic bases, and their potential to produce reproductive isolation. This study substantiates the strong phenotypic component of anadromy in Santa Cruz River rainbow trout initially proposed by Pascual *et al.* (2001) and Riva Rossi *et al.* (2004) and documents variations in the expression of anadromy, as well as some unforeseen consequences on concomitant life-history traits.

Otolith-based Sr:Ca analysis of maternal origin clearly indicated that Santa Cruz River rainbow trout can express both anadromous and non-anadromous life-history types, suggesting that in this river they constitute phenotypic options of a sympatric breeding population, which can switch between lifestyles across generations.

The coexistence of distinctive life-history plans within a single population is paradoxical under certain theoretical life-history frameworks, *e.g.* optimality models (Roff, 1992; Stearns, 1992). Given that the Santa Cruz River population originated from common ancestors (*c.* 25 generations ago) that probably included *O. mykiss* from anadromous and non-anadromous sources (Riva Rossi *et al.*, 2004), it is plausible that alternative life histories are merely reflecting transitional states, where evolution has not had enough time to bring the population to an adaptive end-point (*e.g.* will one of the life-history options ultimately predominate, or will both coexist as alternatives within a common population), in response to the new selective landscape. Alternatively, the founder population could have found an environmental situation in the Santa Cruz River in which the ecological opportunities enabled the full expression of the anadromous and non-anadromous complex, a 'pre-adaptation' situation, where traits already present in the species become functional in new adaptive 'niches' of the receiving environment. In any event, the expression of alternative life-history plans in response to the conditions of the receiving environment could help illuminate some of the mechanisms underlying the phenotypic expression of critical life-history traits.

Ratios of Sr:Ca in otoliths provided a picture consistent with scale pattern analysis (Pascual *et al.*, 2001) in regards to river and ocean residency, longevity, iteroparity and post-reproductive behaviour. The most noteworthy feature of Santa Cruz anadromous life history is the high degree of iteroparity. Anadromous fish were as old as 11 years and spawned up to eight times. Such levels of post-reproductive survival and iteroparity are remarkable not only when

compared to North American steelhead (Busby *et al.*, 1996), but also compared to Santa Cruz River non-anadromous fish.

The extended reproductive life span associated with anadromy in Santa Cruz River *O. mykiss* may be regarded as a life-history paradox, because increased growth and fertility associated with ocean migration does not come at the expense of post-reproductive survival, as expected in principle from life-history theory, *e.g.* optimality models (Stearns, 1992; Schaffer, 2004). Anadromous fish seem able to amass energy stores during each ocean migration to allow them to promote not only growth and fertility, but also increasing post-reproductive survival, outliving non-anadromous fish.

Classical theoretical arguments have long postulated that reduced parity or even strict semelparity is linked to anadromy, because of the increased energy expenditure associated with the development of oceanic feeding migrations, the osmoregulatory stress of changing between fresh- and saltwater habitats, or the higher rates of mortality at sea than in fresh water that makes post-reproductive survival unlikely (Miller & Brannon, 1982; Fleming & Gross, 1989; Fleming, 1998; Quinn & Myers, 2004). The extraordinary reproductive life span of anadromous *O. mykiss* in the Santa Cruz River appears to be consistent with the view that reduced parity is in reality linked to traits that evolve following the adoption of anadromy to augment the effective fecundity of ocean-bound fish in situations of increasing inter- and intraspecific competition (*e.g.* increased somatic growth and egg size, strong sexual competition and elaborated secondary sexual traits) coupled with costly long-range migrations between feeding and breeding grounds (Willson, 1997; Crespi & Teo, 2002; Fleming & Reynolds, 2004).

Another ramification of these results is that the anadromous form in the Santa Cruz River appears to accumulate nothing but selective advantages, leaving the non-anadromous lifestyle with no apparent benefits. Factors that will shift the balance in favour of the persistence of the non-anadromous ecotype include: 1) a high pre-reproductive mortality associated with smolting and first-time ocean migration, 2) a weak genetic basis in characters mediating the expression of anadromy (*i.e.* phenotypic plasticity), 3) equalization in the fitness of alternative ecotypes by status-, density-, or frequency-dependent selection and 4) augmented value of partial-migration as a bet-hedging strategy in a variable environment.

Finally, the re-creation of polymorphic life histories in an introduced population highlights an issue of practical importance for the delineation of evolutionarily significant units (ESUs) of *O. mykiss* in its native range. The general consideration that there is not enough empirical information to assess the effective contribution of non-anadromous fishes to the viability of anadromous populations across different systems has precluded the inclusion of anadromous and non-anadromous population complexes under single ESU's (NMFS, 2003). In situations such as that presented by Santa Cruz *O. mykiss*, (*e.g.* alternating life histories sharing a unique gene pool) anadromous and non-anadromous forms clearly represent a single population unit that warrant integrated protection.

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