

Selection on breeding date and body size in colonizing coho salmon, *Oncorhynchus kisutch*

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Abstract

Selection during the colonization of new habitat is critical to the process of local adaptation, but has rarely been studied. We measured the form, direction, and strength of selection on body size and date of arrival to the breeding grounds over the first three cohorts (2003–2005) of a coho salmon (*Oncorhynchus kisutch*) population colonizing 33 km of habitat made accessible by modification of Landsburg Diversion Dam, on the Cedar River, Washington, USA. Salmon were sampled as they bypassed the dam, parentage was assigned based on genotypes from 10 microsatellite loci, and standardized selection gradients were calculated using the number of returning adult offspring as the fitness metric. Larger fish in both sexes produced more adult offspring, and the magnitude of the effect increased in subsequent years for males, suggesting that low densities attenuated traditional size-biased intrasexual competition. For both sexes, directional selection favoured early breeders in 2003, but stabilizing selection on breeding date was observed in 2004 and 2005. Adults that arrived, and presumably bred, early produced stream-rearing juvenile offspring that were larger at a common date than offspring from later parents, providing a possible mechanism linking breeding date to offspring viability. Comparison to studies employing similar methodology indicated selection during colonization was strong, particularly with respect to reproductive timing. Finally, female mean reproductive success exceeded that needed for replacement in all years so the population expanded in the first generation, demonstrating that salmon can proficiently exploit vacant habitat.

Keywords: behaviour, conservation, dams, natural selection, reintroduction, sexual selection

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Introduction

Sexual selection tends to favour traits in males that increase their access to receptive mates and traits in females that aid in competition for breeding resources required for nest sites or parental care (Andersson 1994; Clutton-Brock 2009). However, reproductive success is determined not only by the number of offspring that an individual produces but also how many of them survive to reproductive maturity (Clutton-Brock 1988). Body size and breeding date are among the most

important traits influencing production and survival of offspring.

Large individuals of both sexes enjoy reproductive advantages. Large males can dominate competitors in contests for mates or breeding territories, and females may prefer large males (Andersson 1994). However, small males can sometimes be successful, especially if they gain fertilizations via sneaking or other alternative behaviours (Taborsky 1994). Large females can produce more numerous and larger offspring (Stearns 1992; Eium *et al.* 2004). In addition, large size may provide advantages in competition for nest sites (van den Bergh & Gross 1989) or other resources necessary for parental care provided by females (Andersson 1994).

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Breeding date is also linked to sexual selection and offspring survival. Breeders arriving early may have a prior residence advantage in competition for mates or reproductive territories (Foote 1990; Haley 1994). In birds, both clutch size and offspring survival often decline over the course of the breeding season (Perrins 1970; Svensson 1997). Early emergence of young provides increased growth opportunities, resulting in higher survival rates when mortality is size selective (Landa 1992; Enum & Fleming 2000; Feder *et al.* 2008). However, juveniles hatching too early may face increased predation risk (Brännäs 1995; O'Donoghue & Boutin 1995) or unfavourable habitat conditions and food availability (Crecco & Savoy 1985).

Coho salmon (*Oncorhynchus kisutch*) are ideal subjects for studies of competition in sexual and natural selection. Females compete for, construct, and guard nest sites, and the largest individuals were estimated to enjoy a 23-fold advantage over the smallest due to egg production, territory quality, and nest defence (van den Bergh & Gross 1989). Males compete for access to receptive females and large males typically win (Fleming & Gross 1994) but small males maturing at a younger age ('jacks') can sneak fertilizations and may represent an evolutionarily stable strategy (Gross 1985). Other than a brief (*c.* 1–2 weeks) period of nest defence by the female following egg deposition, coho salmon provide no parental care and invariably die after breeding (Sandercock 1991). Thus females determine the offspring's environment almost exclusively by spawning site and date, and parents cannot compensate for adverse conditions by feeding or protecting their offspring from predators. Adults breed in late fall or winter, embryos incubate in streambed gravels for several months before emerging in the spring, and juveniles commonly grow in freshwater for 1 year before migrating to sea (Sandercock 1991).

Pacific salmon have repeatedly colonized new habitats throughout their range, and such episodes provide special opportunities for phenotypic evolution and local adaptation. Much of the native habitat currently occupied by Pacific salmon was colonized following glacial recession (*c.* 10 000–15 000 years, McPhail & Lindsey 1986), and indeed this is an ongoing process (e.g. Glacier Bay, Alaska, Milner *et al.* 2000). In general, introduced or invading populations often experience rapid rates of adaptive evolution due to the opportunity for population growth (Reznick & Ghalambor 2001), and selection may be primarily responsible for phenotypic evolution during population expansion (Clegg *et al.* 2002; Koskinen *et al.* 2002; Yeh 2004). Although rapid evolution would imply strong selection during colonization, low densities typical of colonization might attenuate sexual selection (Kokko & Rankin 2006), reducing

selection on traits such as body size. Thus there are conflicting predictions regarding the strength of selection during colonization, but its role in adaptive evolution has most often been inferred well after population establishment and not directly measured in the very first generations.

Here we present the results of a study using DNA-based parent–offspring assignments to characterize selection on breeding date and body size in a population of coho salmon expanding above a former migration barrier. Our primary goal was to quantify the form, direction and magnitude of selection on these traits. To identify a potential mechanism for selection on breeding date, we measured the body size of age-0 juvenile coho salmon sampled from the stream and predicted that early breeding parents would produce offspring that were larger at the end of the summer than those produced by later breeding parents. We also compared selection on body size with a simple length-based egg production model to evaluate the importance of breeding competition amongst females. Finally, to test the hypothesis that selection is strong during colonization, we compared our selection gradients with others in a comprehensive review of selection in natural populations (Kingsolver *et al.* 2001) and similar salmonid studies (Seamons *et al.* 2007; Ford *et al.* 2008).

Methods

Sampling

This study was conducted in the Cedar River, Washington, USA, where Landsburg Diversion Dam blocked fish migration from 1901 to 2003. During the past century, coho salmon persisted in the Cedar River and its tributaries below the dam (located at river 35.1 km), although precise abundance estimates were unavailable. In fall 2003, the dam was modified with fish passage structures, and management adopted a policy of natural recolonization rather than translocation. Salmon accessed the new habitat on their own volition and spawned primarily in the lower reaches of the Cedar River immediately above the dam (Anderson & Quinn 2007). Thus, expansion of an existing population into newly accessible habitat, as opposed to the founding of a population in an isolated new area, best describes the colonization demographics in this case.

Adult salmon were sampled as they ascended the fish ladder, including a measurement of body length (tip of snout to fork of tail), determination of sex based on external examination, and excision of a small piece of tissue for subsequent DNA analysis. The ladder was operated such that all salmon accessing the new habitat were handled from well before the first coho salmon

arrived (approximately 1 month) until the run dwindled to 1–2 salmon per week (late January in most years). A few un-sampled salmon ascended the ladder after this point but were counted by an underwater camera (e.g. Shardlow & Hyatt 2004) at the ladder's exit. In 2008, high flow intermittently compromised the ladder's ability to prevent fish from moving upstream for 5 days in mid-November when the camera was not operational and approximately 50 salmon probably ascended un-sampled. Estimates of the proportion of adult coho salmon sampled were: 2003 = 100%, 2004 = 100%, 2005 = 96%, 2006 = 92%, 2007 = 95%, and 2008 = 85%.

We could not precisely determine the dates when individual salmon bred, and breeding occurs over several days (especially in males). Rather, we used the date of dam passage to characterize the timing of breeding for each salmon. Two lines of evidence suggest that salmon initiated breeding activity shortly after bypassing the dam, relative to the protracted (3–4 months) spawning period. First, expression of secondary sexual traits (e.g. coloration, hooked snout) of salmon sampled at the dam consistently indicated sexual maturity. Second, a portion of the salmon bypassing the dam in 2003 and 2004 was given radio transmitters and displayed movements consistent with breeding behaviour (Anderson & Quinn 2007). Males ($n = 66$) searched extensively for mates, including repeated movements both up- and down-river, and were active for approximately 2 weeks (mean \pm SD = 13.4 ± 8.7 days, based on date of final upriver movement). Females ($n = 18$) tended to move upriver deliberately (i.e. rarely changing directions) and settle in spawning habitats shortly after dam passage (mean \pm sd = 10.5 ± 10.0 days).

Juvenile coho salmon were collected above Landsburg Dam in the Cedar River and its main tributary, Rock Creek. Locations were targeted for sampling juveniles based on adult spawning (Anderson & Quinn 2007) and summer snorkel surveys (Anderson *et al.* 2008). Most samples were collected from the Cedar River in the summer [brood year (BY) 2003: $n = 280$, Aug 18–Sep 15 2004; BY 2004: $n = 432$, Jul 25–Aug 9 2005], with a few collected in Rock Creek over a wider range of dates (BY 2003: $n = 49$, Aug 13 2004–Mar 11 2005; BY 2004: $n = 139$, Aug 9 2005–Feb 21 2006). For BY 2005, juvenile coho were only collected from Rock Creek ($n = 194$) over a narrow range of dates (Sept 25–28 2006). In this portion of their geographic range, coho salmon juvenile typically spend 1-year rearing in freshwater before seaward migration, and thus age was assigned unambiguously from size data. The unimodal size distribution in summer represented age-0 fish, whereas spring samples contained two nonoverlapping size classes representing newly emerged age-0 fish, and

age-1 fish about to migrate downstream. Juveniles were collected over a range of dates so we adjusted observed length to length on a common date at the end of the growing season (Sept 15) using the von Bertalanffy growth curve; see Anderson *et al.* (2008) for details.

Genotyping and parentage

Tissue samples were genotyped at a suite of 10 microsatellite DNA markers (Table S1, Supporting Information). DNA was extracted using DNEasy kits (Qiagen, Valencia, CA, USA), and amplified via Polymerase Chain Reaction (PCR). Approximately 10 μ L PCRs included: 2 μ L genomic DNA from extraction, 10 \times PCR buffer, 1.0 mM MgCl₂ (except Omm1295 which was 1.5 mM), 0.2 μ M each of two primers, 0.1 mM each dNTP, and 0.5 units *Taq* DNA polymerase. Two brands of *Taq* polymerase and associated 10 \times PCR buffer (GeneChoice, Frederick, MD and Bionline, Taunton, MA, USA) were used but positive controls (discussed below) confirmed that there was no difference in allele scoring between the two manufacturers. In the thermocycler (MJ Research, Watertown, MA, USA), each reaction had an initial denature step at 95°C (5 min), followed by 30 cycles of 95°C (30 s) + X°C (30 s) + 72°C (1 min), followed by a final extension at 72°C (45 min) where X is an annealing temperature ranging from 54–60°C (Table S1, Supporting Information). There were two exceptions to this general protocol: Omm1300 and p53 cycled 28 rather than 30 times, and Omm1189 followed a different two step thermocycler profile in which extension step 72°C was eliminated both from the cycle and the final step. Microsatellite PCR products were visualized on a MegaBACE 1000 automatic genotyper (GE Healthcare, Piscataway, NJ, USA), as one primer from each pair was fluorescent labelled (FAM, HEX or NED), and a ROX labelled 550-bp DNA ladder provided the size standard. Genotypes were assigned using Genetic Profiler version 2.2.

All samples (both adults and juveniles) genotyped at seven or more loci were included in the analysis; 90.1% of these samples were genotyped at nine or ten loci. Nearly all adults sampled in 2003–2008 were included in the analysis, only 10 (three of which were potential parents) were excluded because they had less than seven markers genotyped. To quantify our genotyping error rate, we re-extracted $n = 120$ individual samples (5.7%) and genotyped them as a positive control, including one individual on each 96-well plate of genomic DNA used in the study. These samples provided 2580 single locus genotypes which were independently amplified and scored from at least two different sources of genomic DNA. Only 0.66% of these genotypes conflicted with the consensus genotype, and we consider

this to be our error rate due to mishandling of samples, scoring mistakes or other human errors.

We calculated Weir and Cockerham's F_{IS} and performed Hardy-Weinberg exact tests in GenePop version 4.0.10 (Raymond & Rousset 1995). Separate tests for each locus and adult return year or juvenile brood year were performed to determine if systematic allele scoring errors were present in the data. Null alleles or upper allele dropout would show a consistent excess of homozygotes, with a large deviation from expected Hardy-Weinberg proportions (i.e. large F_{IS} value).

We used Cervus version 3.0.3 (Marshall *et al.* 1998; Kalinowski *et al.* 2007) for all parentage assignments. Cervus assigns parentage when the likelihood ratio, or LOD score, of a given parent-parent-offspring or parent-offspring combination exceeds a threshold. We conservatively used the 99% confidence LOD threshold and excluded assignments with two or more mismatching loci. Furthermore, we assessed assignment error rate by deliberately ignoring critical phenotype information in determining the pool of potential parents for each offspring cohort. Thus, the initial assignments permitted putative assignments matching two parents from different years or two parents of the same sex. We reasoned that if the assignments were accurate, parent combinations with incompatible phenotypes (i.e. false positives) should be infrequent. Thus all offspring had a single pool of potential parents including males and females from two parental cohorts. For adults returning in 2005 adults, the pool of potential parents included all adults from 2003 and 2004; for adults returning 2006–2008, the pool of potential parents included all adults sampled both 2 and 3 years prior. Juvenile offspring of known age (discussed above) was compared with all adults in the correct cohort as well as all adults in the following cohort. These juveniles sampled above the dam additionally allowed us to assess the rate of failure to assign parentage because all were produced by colonists that ascended the fish ladder, whereas adults considered as offspring might have been produced in the lower river or elsewhere.

Data analysis

We used reproductive success (RS), defined as the total number of returning adult offspring produced by each breeder, as the fitness metric for selection analysis. Juvenile offspring samples were not used for reproductive success, but only to compare juvenile body size between breeding dates. To analyse the variance in RS, we calculated the opportunity for selection, $I = \frac{\text{var}(\text{RS})}{\text{mean}(\text{RS})^2}$, which is the upper limit on the strength of selection (Arnold & Wade 1984; Brodie *et al.* 1995).

Female reproductive success may vary with body size through a positive correlation between length and egg production. We did not sacrifice any female salmon in this population because of conservation concerns. Therefore, we used data from nearby hatcheries to estimate fecundity using a regression with body length as a predictor, reported by Quinn *et al.* (2004) for the University of Washington's hatchery and similar data from the nearby Issaquah Creek and Soos Creek hatcheries, run by the Washington Department of Fish and Wildlife (Quinn, unpublished data). A linear ordinary least squares regression of the form $\log_e(\text{fecundity}) = 2.14 \cdot \log_e(\text{length}) - 5.79$ was used to predict the fecundity of each female ascending the fish ladder ($F_{1,95} = 163.8$, $P < 0.0001$, $r^2 = 0.63$).

Selection gradients were calculated using methods developed by Lande & Arnold (1983) and reviewed by Brodie *et al.* (1995). Briefly, both body size and breeding date were standardized to a mean of zero and a SD of one; we denoted these standardized trait values as z_s (body size) and z_d (breeding date). Relative fitness, denoted as w , was each individual's RS divided by the within-season and within-sex mean. Linear directional selection gradients (β) were estimated via least squares regression as the partial coefficients from a multiple regression with w as the response variable and two predictors: z_s and z_d . Similarly, nonlinear selection gradients (γ) were estimated from a different multiple regression with w as the response variable and five predictors: z_s^2 , z_s , z_d^2 , z_d and $z_s z_d$. Stabilizing or disruptive selection gradients (γ) were estimated as two times the partial regression coefficients of z_s^2 and z_d^2 . A positive value of γ indicates disruptive selection, whereas a negative value indicates stabilizing selection. Bivariate selection gradients were the partial regression coefficients of $z_s z_d$.

We performed univariate cubic splines (Schluter 1988) to visualize the relationship between RS and both breeding date and body size. The plots were created with the generalized additive model ('GAM') function in R (R Development Core Team 2009) using negative binomial error structure designed for overdispersed count data, with the dispersion parameter θ estimated as $\frac{\text{mean}(\text{RS})^2}{\text{var}(\text{RS}) - \text{mean}(\text{RS})}$ (Hilborn & Mangel 1997). The smoothing factor λ was determined separately for each curve by generalized cross-validation. For females, we estimated an additional fitness metric by multiplying each female's estimated fecundity by the within-year ratio $\frac{\text{mean}(\text{RS})}{\text{mean}(\text{fecundity})}$ representing the average egg to adult survival, and rounding to the nearest integer. This provided an expected number of offspring if fecundity alone determined female fitness, and was plotted via cubic splines with Poisson errors.

Table 1 Body size, date of arrival to the breeding grounds, and reproductive success (RS) of colonizing coho salmon; reproductive success is defined as the number of returning adult offspring. *N* does not include fish that were sampled but not genotyped ($n=1$ male and $n=1$ female in 2003; $n=1$ male in 2005) and *I*=opportunity for selection

Year	Sex	<i>N</i>	Mean length \pm SD (mm)	Mean date \pm SD (days)	Mean RS	Variance RS	<i>I</i>
2003	Male	25	630.9 \pm 65.7	Nov. 20 \pm 20.4	4.12	71.3	4.20
	Female	20	622.4 \pm 79.2	Nov. 16 \pm 25.4	5.20	51.7	1.91
2004	Male	65	699.5 \pm 65.9	Dec. 14 \pm 25.8	1.43	5.06	2.47
	Female	34	662.9 \pm 72.2	Dec. 14 \pm 27.2	2.74	10.4	1.39
2005	Male	103	574.3 \pm 115.7	Nov. 30 \pm 32.4	2.53	15.8	2.45
	Female	66	601.9 \pm 83.3	Dec. 5 \pm 30.6	3.67	18.9	1.41

Results

The number of adult coho salmon ascending the fish ladder increased over the 3 years of the study (Table 1) and in each year, the male:female ratio exceeded 1 (2003: 1.24; 2004: 1.91; 2005: 1.58). A two-way ANOVA detected no difference in the length of males and females ($F_{1,307} = 0.066$, $P > 0.10$, Table 1) but size differed between years ($F_{2,307} = 40.0$, $P < 0.0001$) and there was an interaction between sex and year ($F_{2,307} = 3.74$, $P = 0.025$). Notably, in 2005 a size class typical of age-2 males appeared, reducing mean length and increasing the variance (Table 1). The date coho salmon ascended the fish ladder differed between years (two-way ANOVA, $F_{2,307} = 13.1$, $P < 0.0001$, Table 1), but males and females were similar ($F_{1,307} = 0.559$, $P > 0.10$), and there was no interaction between sex and year ($F_{2,307} = 0.628$, $P > 0.10$).

Within the 90 locus-cohort combinations (10 markers in each of six adult cohorts and three juvenile cohorts), observed heterozygosities (H_O) ranged from 0.67 to 0.98, with a median of 0.87. There was strong evidence for deviation from Hardy–Weinberg equilibrium, as 71 of the 90 exact tests were significant. The pattern of homozygote excess vs. deficit lacked consistency, as all markers deviated in both directions (Tables S2 and S3, Supporting Information). More of the significant tests showed a deficit of homozygotes ($n = 41$) than an excess ($n = 30$), and F_{IS} values tended to be small (average absolute value = 0.035, Tables S2 and S3, Supporting Information). Furthermore, the frequency of null alleles estimated by the parentage software was low (<0.02) for all loci. Thus, there was no evidence for major systematic genotypic scoring errors. The microsatellite dataset provided sufficient genetic variation for parentage analysis, as the global exclusion probability for two-parent and single-parent assignments were both >0.9999 .

The vast majority of parentage assignments matched parents and offspring of compatible phenotypes (e.g. sex and year). Of the 1954 offspring, 1406 had distinct

best parent pairs with LOD scores exceeding the 99% confidence threshold (LOD = 12.44) and ≤ 1 mismatching locus. The rate of erroneous assignments was low: only 32 assignments (2.3%) matched two parents of the same sex or two parents of different years, and all were discarded. For another 65 offspring, there were multiple parent pairs with the same number of mismatching loci and similar LOD scores (range in LOD difference between top two pairs: 0–2.8) above the threshold. Of these, 32 were assigned unambiguously based on parent phenotypes (matching parents of opposite sex from the same year); the other 33 were discarded because multiple or no parent pairs were compatible. We then attempted to assign single parents to the 548 offspring that were not assigned two parents simultaneously, including offspring that were excluded because of incompatible parental pair phenotypes. Similar to the procedures for parent pairs, 127 offspring were unambiguously assigned to a single parent with an LOD greater than the 99% confidence threshold (LOD = 6.21) and fewer mismatching loci than all other potential parents. Sixty-nine of the offspring assigned one parent were juveniles of known age, none of which assigned a parent from the wrong cohort.

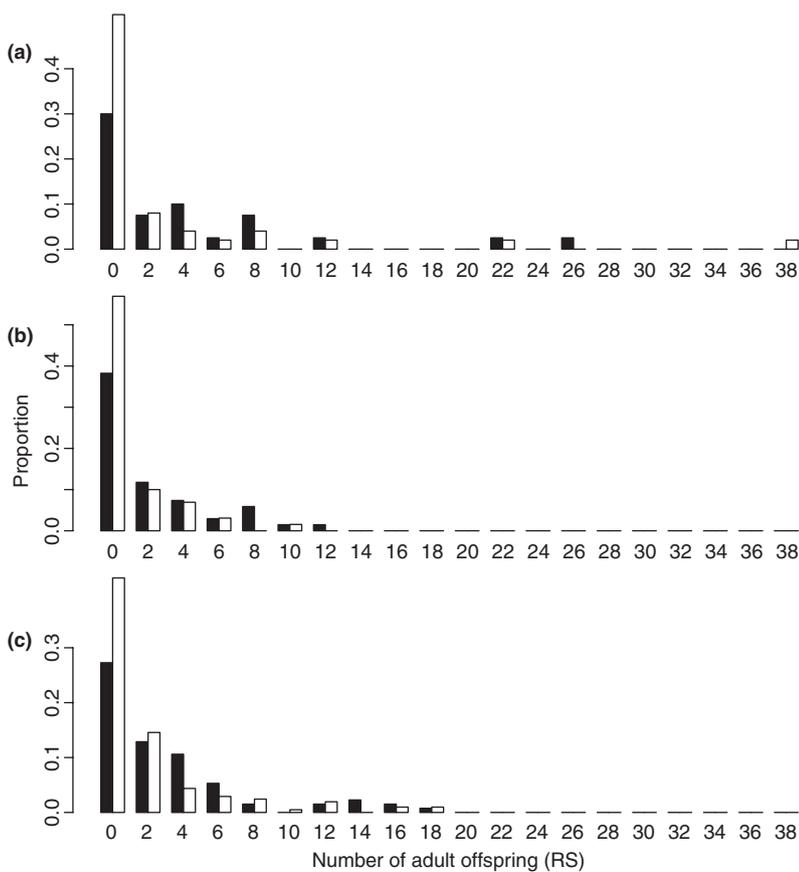
For each of the three juvenile offspring cohorts, over 92% of analysed samples assigned at least one parent and the vast majority assigned two (range = 84–94%, Table 2), indicating that the rate of failure to assign parentage was low. The proportion of returning adults assigned to at least one parent was lower than the juveniles and tended to increase in subsequent years (range = 20–74%), and similar to the juveniles, more adults assigned two parents than one parent (Table 2). Finally, 62 of the 480 adult offspring assigned parentage were 2-year olds and the rest were 3-year olds.

Reproductive success, defined as the number of returning adult offspring, showed a strikingly consistent pattern across years. Many fish produced zero or few offspring, and a small number produced many offspring (Fig. 1). Within each year, a higher proportion of males (range = 43–57%) than females (range = 27–38%)

Table 2 Results of parentage assignments, with the number of offspring analysed and those falling into one of four assignment categories: zero parents, mother only, father only, and two parents

Class	Brood year	Return year	Analysed	Zero parents	Mother only	Father only	Two parents
Juvenile	2003	—	329	9	41	2	277
	2004	—	571	17	16	1	537
	2005	—	194	15	8	1	170
Adult	—	2005	169	135	2	3	29
	—	2006	190	105	6	4	75
	—	2007	141	36	6	4	95
	—	2008	360	104	6	27	223

Brood year was assigned based on body size and date of capture for juveniles (see methods), but age could not be determined for adults failing to assign parentage so they are listed by return year (which includes both 2- and 3-year olds).

**Fig. 1** The number of adult offspring (both 2- and 3-year olds) produced by males (white) and females (black) for (a) 2003, (b) 2004 and (c) 2005 parental cohorts of coho salmon.

produced no returning adult offspring (Fig. 1). The opportunity for selection (I) was much larger for males than females in each year, and was greatest in the first year for both sexes (Table 1).

The date of arrival to the breeding grounds had a strong but variable influence on reproductive success, with very similar patterns for both sexes. We observed a significant directional or stabilizing selection gradient in all 3 years for males and 2 years for females (Table 3). However, 2003 showed a much different

pattern than 2004 and 2005, which were similar to each other. In 2003, early salmon produced the most adult offspring (Fig. 2a), and directional selection gradients were negative and significant in both sexes (Table 3). In 2004 and 2005, we observed stabilizing, rather than directional, selection. Fitness curves for both sexes were dome-shaped (Fig. 2c and e); quadratic selection coefficients were significantly negative for males in 2004 and 2005, and females in 2004 (Table 3). In each of the 3 years, the date of maximum

Table 3 Selection gradients for body size and date of arrival to the breeding grounds in colonizing coho salmon, with one standard error in parentheses

Sex	Year	N	β : Linear (directional)		γ : Quadratic (stabilizing/disruptive)		Bivariate
			Length	Date	Length ²	Date ²	Length*Date
Males	2003	25	0.0588 (0.382)	-1.01* (0.382)	0.249 (0.876)	1.37 (0.824)	0.419 (0.449)
	2004	65	0.426* (0.197)	-0.00778 (0.197)	0.00804 (0.292)	-0.626* (0.306)	-0.0493 (0.211)
	2005	103	0.702*** (0.193)	-0.212 (0.193)	0.413 (0.353)	-1.24* (0.549)	0.185 (0.228)
Females	2003	20	0.529 (0.314)	-0.674* (0.314)	-0.161 (0.737)	1.32 (0.846)	-0.319 (0.370)
	2004	34	0.348 (0.217)	-0.194 (0.217)	-0.525 (0.487)	-0.858* (0.356)	0.218 (0.305)
	2005	66	0.538*** (0.137)	-0.00556 (0.137)	-0.161 (0.268)	-0.245 (0.447)	0.138 (0.148)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

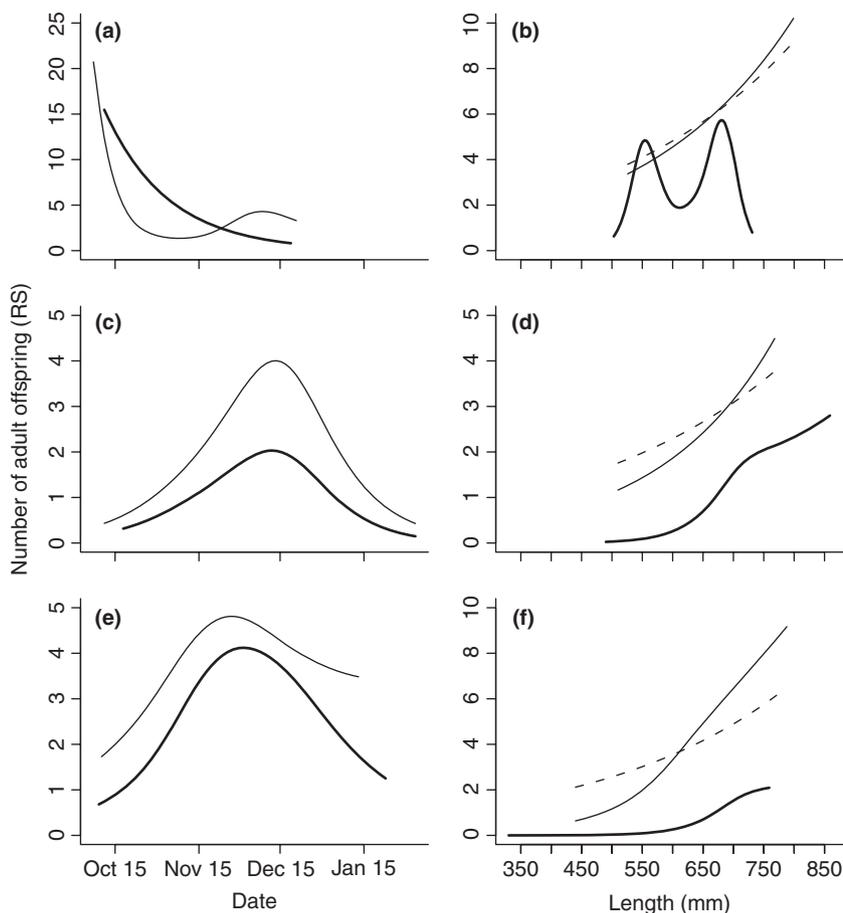


Fig. 2 Cubic splines of date of arrival to the breeding grounds (a, c, e) and body size (b, d, f) vs. reproductive success for 2003 (a and b), 2004 (c and d) and 2005 (e and f) coho salmon. Observed data are shown as thick dark lines for males and thin black lines for females. Thin dashed lines are model-predicted estimates of female reproductive success assuming fecundity (number of eggs) was the sole determinate of fitness. Note the different scales on the y axes.

reproductive success was nearly identical between the sexes (Fig 2a, c and e).

Both sexes showed directional selection favouring larger salmon. The magnitude of the effect increased progressively from no advantage for large males in 2003, to a significant advantage in 2004 and an even stronger advantage in 2005 (Table 3). Although the cubic splines showed two peaks for male reproductive success in

2003 (Fig. 2b), there was no evidence for disruptive selection based on the quadratic selection gradient (Table 3). There was also no indication of disruptive selection in 2005 (Table 3, Fig. 2f), when the size class typical of sneaker males (<500 mm) was present. Females were much more consistent than males in the large-size advantage, both in terms of the range in selection gradients (Table 3) and the shape of the fit-

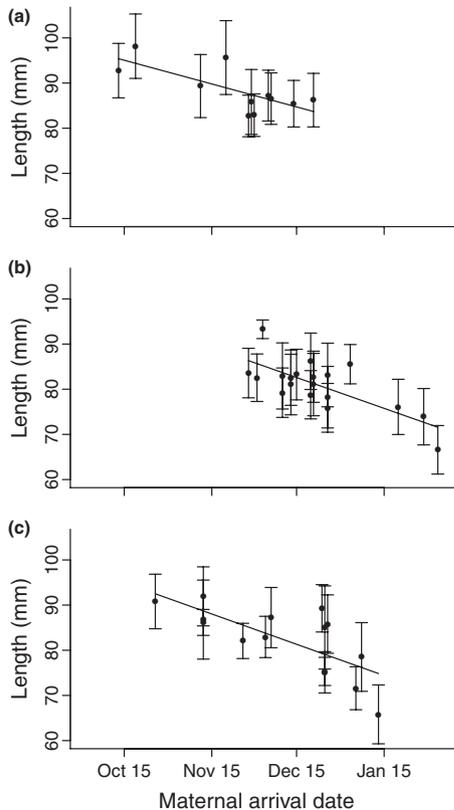


Fig. 3 Later arriving females in (a) 2003, (b) 2004 and (c) 2005 produced stream-rearing juvenile offspring that were smaller at the end of the summer. Each point represents the average length of a family sharing a mother, with the whiskers representing one SD. Length of juveniles collected over a range of dates was standardized to a common date at the end of the growing season (Sept 15, see section 'Methods' for details).

ness curves (Fig. 2b, d and f). There was also some evidence, primarily from 2005, that large females had an advantage beyond increased egg production. Female body size directional selection coefficients (Table 3) exceeded the coefficients resulting from an analogous linear regression assuming model-predicted number of eggs determined offspring production (2003: $\beta = 0.275$, 2004: $\beta = 0.219$, 2005: $\beta = 0.296$, all $P < 0.0001$). In addition, cubic splines for observed reproductive success showed steeper slopes than the fecundity model predictions, and this difference was greatest in 2005 (Fig. 2b, d and f).

The data on progeny as juveniles helped to explain the variation in RS inferred from samples of the progeny as adults. Specifically, the juvenile offspring of earlier migrating parents were larger at a common date than those of later parents (Fig. 3). We regressed (linear, ordinary least squares) juvenile body size averaged within maternal half-sibling families vs. maternal arrival date with year as a fixed factor in an ANCOVA for

families with at least four sampled juvenile offspring. Both length ($t_{39} = -2.66$, $P = 0.011$; overall model $r^2 = 0.58$) and weight ($t_{39} = -2.89$, $p = 0.0064$; $r^2 = 0.56$) were negatively related to maternal arrival date. Neither the year nor the year \times maternal arrival date terms was significant for either length or weight analyses ($P > 0.10$), indicating that the regression coefficients (slopes in Fig. 3) were indistinguishable among years.

Discussion

Our measurements of selection on breeding date and body size in coho salmon during the very first generation of colonization provided two primary conclusions. First, selection was not consistent for either trait across years: selection on breeding date changed in form, while selection on body size changed in magnitude. Second, comparisons with other studies employing similar methodology indicated that selection was strong during colonization. We consider potential mechanisms driving the observed patterns and the implications of selection during colonization to local adaptation and the conservation of Pacific salmon.

Early breeding fish had a distinct advantage in 2003, but intermediate spawning dates were favoured in 2004 and 2005. In coastal drainages where coho salmon predominate, extreme fluctuations in river discharge during fall and winter are common but the timing is unpredictable. The dynamic habitat conditions vary widely from year to year, and can dramatically affect spawning success, as well as the survival of incubating embryos (Holtby & Healey 1986) and over-wintering juveniles. In addition to variation in the physical environment, timing likely had a strong influence on the availability of mates and number of breeding competitors. At the beginning and end of the spawning period when instantaneous densities were lowest, encounter rates may have been stochastic. A few salmon that arrived early in 2003 were very successful; the males may have gained fertilizations when few competitors were present. Furthermore, juvenile offspring from earlier adults were consistently larger at the end of the summer (Fig. 3), and this advantage may have translated to higher survival during the subsequent freshwater and marine periods (Holtby *et al.* 1990; Ebersole *et al.* 2006). The earliest adults in 2003 also produced a large number of salmon that matured at age-2 rather than the more common age-3, suggesting their offspring enjoyed growth or size advantages (Vøllestad *et al.* 2004). Indeed, the most successful individual from all 3 years was the first male to ascend the ladder in 2003, and 28 of his 37 offspring matured as 2-year olds.

In contrast, early arriving salmon (i.e., October) in 2004 and 2005 were unsuccessful (Fig. 2c and e).

Although the mechanism is difficult to determine, arrival during the middle of the breeding season may have increased the probability of finding a mate or reduced the risk that embryos were destroyed by high winter stream flows. In addition, biotic conditions likely changed from year to year; early emerging offspring in 2004 and 2005 (but not 2003) may have suffered high predation (Brännäs 1995). Early spawning appeared to carry consistent long term offspring growth advantages, but also increased the risk that unfavourable breeding, incubation or emergence conditions limited reproductive success.

Regardless of the mechanism, shifting optima, coupled with the relatively high heritabilities observed for migration and maturation date in salmon (Carlson & Seamons 2008), probably maintain significant genetic variation for breeding date. Protracted spawning might therefore be common for salmonids inhabiting rain-dominated rivers where physical factors affecting recruitment are unpredictable among years and variable in timing within years. Consistent with this hypothesis, Seamons *et al.* (2007) observed shifts in the form of selection on breeding date in steelhead trout (*O. mykiss*) spawning over a protracted period where stream flow fluctuations were common. Shifting selection patterns among years have been documented in many taxa (reviewed by Siepielski *et al.* 2009); this might explain evolutionary stasis despite strong selection in one or a few years (Merilä *et al.* 2001).

Selection on body size also changed over time, but in a different manner. The same general form of selection was observed in all 3 years, but the magnitude of the advantage for large males increased markedly in subsequent years. Large males are generally dominant in direct contests (Foote 1990; Fleming & Gross 1994), and thus availability of receptive females probably structured the mating system (Emlen & Oring 1977) and the strength of selection on male body size. Low densities and a scarcity of females probably created scramble competition that favoured males with traits unrelated to size. For example, successful males may have arrived on the spawning grounds at the right time, had greater reproductive longevity or searched for mates more efficiently (Lane *et al.* 2009). As densities increased, big, dominant males were probably more successful at defending access to females, and the mating system transitioned to female defence polygyny (Emlen & Oring 1977). Although the density changes within and between years were small, we had previously found detectable changes in male movement behaviour based on the availability of few additional females (Anderson & Quinn 2007).

Interestingly, there was little evidence for disruptive selection on male body size despite the hypothesized

importance of this process to life history diversity of coho salmon (Gross 1985). The success of the small male sneaking strategy may require a threshold density that was much greater than those observed in our study. Fleming & Gross (1994) only found disruptive selection on male body size at the highest densities in their experiment (10 coho salmon per m²).

Selection also favoured large females, and there was some evidence that the advantage of large size exceeded the simple benefit of increased egg production. Intrasexual breeding competition, resulting from both acquisition of territories and defence of nests from superimposition by later spawning females, is a major source of natural selection on body size (van den Berghe & Gross 1989; Fleming & Gross 1994). Our metric of fitness integrated both adult breeding and offspring survival, and thus large females may also have produced larger eggs resulting in larger fry (Einum *et al.* 2004; Quinn *et al.* 2004) or dug deeper nests that were less susceptible to scour from high stream flows (Steen & Quinn 1999). The greatest divergence between the observed and fecundity-model predicted curves (Fig. 2b, d and f) occurred in the year of the highest density (2005), suggesting that competition, either amongst adults during breeding or juveniles during rearing or both, was greater at higher densities.

One hypothesis of colonization ecology is that selection is initially strong due to phenotypes that are poorly suited to the new environment, and this promotes adaptive evolution (Reznick & Ghalambor 2001). To evaluate this hypothesis, we compared the absolute value of our selection coefficients with others in the literature. All but one of the body size $|\beta|$ values we measured exceeded the 80th percentile of Kingsolver's (2001) review of phenotypic selection in the wild. Furthermore, the directional breeding date gradients in 2003 for both sexes were above the 90th percentile. For comparisons of γ , we conservatively multiplied Kingsolver's (2001) $|\gamma|$ values by two, noting that many γ values in their database may have been erroneously calculated as half their true value (Stinchcombe *et al.* 2008). Even after this adjustment, all four $|\gamma|$ measured for breeding date in 2004 and 2005 ranked above the median, and two ranked above the 85th percentile.

We also compared our selection gradients with similar studies of an established population of coho salmon (two cohorts, Ford *et al.* 2008) and a long term study of a congener with a similar breeding and juvenile rearing ecology (19 cohorts, Seamons *et al.* 2007). For directional selection on body size, our values (median = 0.48) were similar to Ford *et al.*'s (2008) body weight β values (median = 0.49) but greater than Seamons *et al.*'s (2007) length gradients (90th percentile = 0.264).

Directional selection on breeding date (2003) was stronger than any measured by Ford *et al.* (2008) or Seamons *et al.* (2007). Gradients of stabilizing selection on breeding date were also large, as the median $|\gamma|$ for 2004 and 2005 colonists (0.74) was greater than all $|\gamma|$ reported by Ford *et al.* (2008) and most $|\gamma|$ reported by Seamons *et al.* (median = 0.345, 85th percentile = 0.651). Combined, these comparisons suggest that selection was strong.

However, poorly adapted phenotypes seem unlikely as the only explanation for strong selection. The source of most colonists was probably the lower Cedar River system immediately downstream of the dam, therefore it seems improbable that maladaptation can entirely explain the observed selection because the colonists probably came from the same river. Unfortunately, a lack of data on the lower river population prevented us from comparing colonist traits to those of the source population. Although the precise mechanisms remain unclear, strong selection may have resulted from the opportunity for population growth, which is often associated with adaptive evolution during colonization (Reznick & Ghalambor 2001).

Could the observed selection on body size and breeding date have adaptive significance for the colonizing population? Philopatry, combined with generations of natural and sexual selection, has promoted the evolution of intraspecific divergence of many traits in salmon, with each population adapted to its local environment (Taylor 1991). Thus adaptation is a common theme in salmon, and both body size and breeding date vary among populations. Evolution of larger body size is often inhibited by physiological constraints or viability costs of fast growth (Blanckenhorn 2000), so the population may not evolve larger body size. The adaptive significance of migration and breeding date seems clearer, as the timing of reproduction varies widely between populations and has evolved to maximize fitness in the local environment (Ricker 1972; Brannon 1987). Timing responds to environmental change (Quinn & Adams 1996), and selection on reproductive timing can accelerate the evolution of other traits during colonization (Quinn *et al.* 2000). If adaptive evolution does occur, it could increase population fitness and the likelihood of colonization success (Kinnison & Hairston 2007).

Pacific salmon face threats throughout their native ranges, but prime among them is lost access to suitable spawning and rearing habitat due to dams, culverts, and other migration barriers (National Research Council 1996). In our case, salmon volitionally entered the new habitat as soon as it was available (Anderson & Quinn 2007). Females (which limit the spatial and numerical expansion of the population) produced, on average,

more than two returning adult offspring in each of the first 3 years (Table 1). Thus the population was above replacement and sustained itself in the very first generation, providing evidence that salmon are apt colonizers that can successfully establish populations in new areas to which they are given access via restoration action. Furthermore, we found evidence for strong selection on breeding date and body size, traits that may have adaptive significance to the long term persistence of the population, and this underscores the importance of natural and sexual selection during colonization.

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This work is a component of Joseph Anderson's Ph.D. dissertation regarding the evolutionary ecology of salmon colonization. Paul Faulds is a senior environmental analyst focusing on the implementation of anadromous fish research under the Cedar River Watershed Habitat Conservation Plan. William Atlas is a graduate student interested in the role of predators in freshwater communities. George Pess is a supervisory research fishery biologist whose work addresses the relationship between landscape processes and salmonid evolutionary ecology. Thomas Quinn is a professor with research interests in the ecology, behaviour, evolution and conservation of salmon and trout.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Microsatellite loci used for parentage of coho salmon

Table S2 Population genetic analysis of adult coho salmon samples

Table S3 Population genetic analysis of juvenile coho salmon samples

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