

Local adaptation, dispersal, and gene flow
in a metapopulation of sockeye salmon

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Chapter 1

Local adaptation limits gene flow in a metapopulation of sockeye salmon

Abstract

Gene flow within a metapopulation depends on the reproductive success of dispersers after immigration, but few empirical studies have measured the reproductive contributions of dispersers from distinct natal populations. The local adaptation frequently observed within metapopulations of anadromous salmonids could exert strong selection against immigrants with phenotypes adapted to ecologically distinct habitats. Here, we used genetic parentage analysis to directly measure the reproductive success of dispersers from multiple natal habitats relative to philopatric individuals within a group of geographically proximate but ecologically and genetically distinct subpopulations of stream- and beach-spawning sockeye salmon (*Oncorhynchus nerka*). Our results indicate that dispersers between stream-spawning populations and philopatric individuals experienced similar reproductive success, while immigrants from the beach habitat to the streams produced fewer returning adult offspring than either philopatric individuals or stream-to-stream dispersers. The difference in reproductive contribution between beach-to-stream dispersers and the other two dispersal categories was estimated to be one returning adult offspring per individual. Thus, the difference in reproductive success of dispersers between habitat types represents a strong barrier to gene flow between these sockeye salmon ecotypes.

We further investigated whether differences between dispersers in morphological or behavioral traits correlated with differences in their reproductive success. Dispersers between stream-spawning populations did not experienced consistent differences in reproductive success

compared to philopatric individuals with respect to body shape, in-stream residency period, entry date and mean location, suggesting that the selectively optimal phenotype may vary among years in these streams. On the other hand, the reproductive contribution of immigrants from the beach population was limited by a shorter in-stream residency period compared to philopatric individuals, possibly reflecting their dissatisfaction with the stream spawning habitat.

Introduction

The importance of metapopulation theory in understanding population dynamics has become well accepted, and it forms the basis of many wildlife management and conservation programs (McCullough 1996). Indeed, the “portfolio effect” of asynchronous subpopulation productivity plays an important role in buffering metapopulations against annual variation in population abundance (Hilborn *et al.* 2003, Schindler *et al.* 2010). However, gene flow between subpopulations on an evolutionary time scale can also influence species resilience by offsetting local adaptation in distinct environmental niches, spreading mutations, and reducing inbreeding (Slatkin 1987, Garant *et al.* 2007, Hanski *et al.* 2011). Thus, the patterns of gene flow in a metapopulation, as well as the factors that control those patterns, are of great interest to researchers and managers of wild animal populations.

Gene flow is caused by the reproduction of individuals that disperse between habitats, and many factors can influence the rate, direction and genetic consequences of dispersal within a metapopulation. Importantly, gene flow depends on the reproductive success of dispersers after immigration. Empirical data from a wide variety of vertebrates including birds, mammals, and lizards have shown that while dispersers often face an increased risk of mortality while in transit between habitats, they can exhibit equal, higher or lower reproductive success than their

philopatric (non-dispersing) counterparts once settled in the recipient habitat (reviewed by Béchichon *et al.* 1996, Doligez & Pärt 2008). Explanations of immigrant superiority in reproductive success in birds and mammals often involve heterosis (outbreeding vigor) or facultative inbreeding avoidance (Pusey 1987), while explanations of philopatric superiority often involve fitness associated with territory acquisition and defense (e.g. Parn *et al.* 2009). Unfortunately, most previous work could not directly measure total lifetime reproductive success, which is the only way to precisely assess the genetic contribution of dispersers to the recipient population. Moreover, of the studies that have generated such data, few have attempted to identify the natal origins of the dispersers; most have grouped all non-philopatric individuals into a single category, ignoring the diversity of source populations that could contribute to the disperser group. However, great reed warbler immigrants from distant populations had lower reproductive success than immigrants from nearby populations (Hansson *et al.* 2004), suggesting that not all dispersers experience the same fitness consequences. An experimental study on aphids found that dispersers between host plants of different species experienced much lower survival and reproductive success than dispersers between hosts of the same species (Via *et al.* 2000). The aim of the present study was to identify any population-level traits that influence gene flow by quantifying the relative reproductive success of dispersers among a set of three of well-characterized sockeye salmon populations.

In anadromous salmonid metapopulations, empirical estimates of subpopulation connectivity from tagging studies of dispersal are often higher than those inferred from genetic differentiation, indicating that dispersers are not as reproductively successful as philopatric individuals (e.g. Tallman & Healey 1994). These differences in reproductive success are unlikely to arise from an advantage of philopatric individuals in familiarity with the local environment

because both dispersers and philopatric individuals arrive in the spawning region for the first time as adults shortly before spawning (Quinn 2005). However, local adaptation, demonstrated in many salmonid species (Taylor *et al.* 1991), could reduce the reproductive success of dispersers between ecologically distinct habitats, thus reducing gene flow (Nosil *et al.* 2005). For example, a study of 51 populations of Atlantic salmon found that environmental temperature differences were positively correlated with genetic differentiation but not with dispersal rates, suggesting that adaptation to local thermal regimes acts as a selective pressure against dispersers in that system (Dionne *et al.* 2008).

Here, we directly tested the hypothesis that local adaptation limits the reproductive success of dispersers between populations spawning in ecologically distinct habitats. The focal populations for our study were two small, proximate populations of stream-spawning sockeye salmon (*Oncorhynchus nerka*) in the Wood River system of Southwest Alaska (Figure 1). The spawning areas, A Creek and C Creek, drain into the same lake 1.5 km apart and are similar physically. Each population is composed of 150-600 spawning adults each summer and both populations have been surveyed comprehensively every year since 2004. A previous study that genotyped a sample of individuals found that the populations are genetically distinct at neutral microsatellite loci (Lin *et al.* 2008). Further work that quantified individual reproductive success in these populations using genetic pedigree reconstruction found that the populations are exposed to comparable selection regimes for many morphological and behavioral traits (Lin 2012). Populations of sockeye salmon also spawn on lake beaches that are located < 100 m from the mouth of each stream, and these fish differ both morphologically and genetically from the stream-spawning populations (Lin *et al.* 2008). Differences between stream- and beach-spawning populations in body size and shape (Quinn *et al.* 2001b), egg size (Quinn *et al.* 1995), and

migration timing (Doctor *et al.* 2010) are commonly observed in this and other systems and are likely maintained by variation in selection regimes between the spawning habitats (Quinn *et al.* 2001b, Carlson *et al.* 2007). We therefore hypothesized that dispersers between beach- and stream-spawning populations would be maladapted to their recipient habitat and experience lower reproductive success than locally adapted individuals.

To distinguish the general effects of dispersal from the population-specific effects of local adaptation, we compared the reproductive success of dispersers between ecologically similar habitats (streams) with that of dispersers between ecologically distinct habitats (streams and beaches). Individual reproductive success was quantified by counting the number of adult offspring assigned to each potential parent using genetic parentage reconstruction, and the dispersal status of each individual in the parental generation was inferred with genetic population assignment tests. We further investigated whether differences between dispersers from different natal populations in morphological or behavioral traits might explain any differences in reproductive success between those groups.

Materials and Methods

Sample Collection

Our goal was to determine the reproductive success of all adults spawning in A and C Creeks during 2004 and 2005 (F0 cohorts). Sockeye salmon in these streams almost always return to spawn as adults either four or five years after being laid as eggs (Quinn *et al.* 2001b), so the offspring of the F0 cohorts returned in 2008, 2009 and 2010 (F1 cohorts). Exhaustive sampling of A and C Creek adult spawning populations was conducted every year from 2004 through 2010. We walked the full length of both streams every day during the spawning season

(late July through early September), tagging any newly observed (i.e., untagged) fish and noting the location of each previously tagged fish. To expedite tagging during times of peak abundance, fish in the lake at the mouth of each stream were captured by beach seine and tagged before they entered the streams. A fin clip was taken from each fish upon tagging for genetic analysis. Sex, body length (mid-eye to hypural plate), and body depth (anterior insertion of the dorsal fin to the belly) were also measured and recorded for each newly tagged individual (following Quinn *et al.* 2001b). When a dead fish was found in or near either stream, the tag ID was recorded and the cause of mortality was determined by visual inspection (generally either bear kill or senescence). If a dead fish was found untagged, a fin clip was taken and length and depth were measured if the carcass was intact. While 100% sampling was not possible given the fact that bears remove some fish from the stream (Quinn *et al.* 2009) and others enter and depart the streams without ever being observed, previous work suggests that we sampled the majority (> 80%) of spawning individuals (Hauser *et al.* 2011).

Fish present on the beach spawning habitats adjacent to A and C Creeks were captured with a beach seine on a weekly basis throughout the spawning season. Capturing fish in those deeper habitats is much more difficult than in the streams, and many beach-spawning fish were never sampled. Nevertheless, we were able to obtain a large sample (> 50%) of the individuals spawning at those sites.

Laboratory Methods

All adults observed in either A or C Creek in 2004, 2005, 2008, 2009 and 2010 were genotyped at 11 tetranucleotide repeat microsatellite loci (mean heterozygosity in all samples = 0.87 ± 0.04 SD, 266 independent alleles; loci developed by references in Hauser *et al.* 2011;

Table 1). All individuals sampled in 2010 were genotyped and included in the parentage analysis to account for the 5-year-old offspring of the adults that spawned in 2005, even though most individuals that returned in 2010 were likely spawned in 2006. All individuals sampled in the beach habitats in 2004 and 2005 were also genotyped in order to provide a genetic baseline for that population to be used in identifying dispersers in the F0 generation. DNA was extracted from the samples using DNeasy extraction columns following the manufacturer's instructions (Qiagen, Valencia CA, USA). Microsatellite loci were amplified in three multiplex panels using Qiagen multiplex PCR kits (Qiagen, Valencia CA, USA), with varying primer concentrations and annealing temperatures (Table 1). Forward primers were fluorescently labeled and amplified fragment sizes were measured with a MegaBACE 1000 DNA Analysis System (GE Healthcare Life Sciences, Piscataway, NJ, USA). To quantify genotyping error rates, 146-203 individuals were re-amplified and re-genotyped at each locus.

Error Checking

Departures from Hardy-Weinberg and linkage equilibrium at each locus in each subpopulation were tested by permutation tests with the program FSTAT (Goudet 1995). Estimates of effective population size (N_e) for each subpopulation in each year were calculated based on observed linkage equilibrium using the program LDNe (Waples & Do 2008). The presence of null alleles and large-allele dropout were assessed with the programs ML-Null (Kalinowski & Taper 2006) and Microchecker (Van Oosterhout *et al.* 2004). Genotyping error was quantified using the program Pedant (V1.0, Johnson & Haydon 2007), which uses Bayesian methods to separately estimate allelic dropout and false allele rates. 92 individuals genotyped at fewer than 8 of 11 loci were removed from the dataset because parentage assignments were not

reliable in this group.

Pedigree Reconstruction

Parent-offspring relationships between the F0 generation (stream spawners in years 2004 and 2005) and their F1 offspring (stream spawners in years 2008-2010) were reconstructed using the parentage analysis software package Colony (version 2.0; Jones & Wang 2010). Genotypes for all F0 individuals were considered to be either potential dams or sires (according to their observed sex) for all F1 offspring genotypes, and F0 individuals of unknown sex were included as both potential dams and potential sires. Data from both stream populations and all years were incorporated into a single pedigree reconstruction in order to account for dispersal between streams in the offspring generation, as well as the variation in age-at-return of those offspring. Individuals that spawned on the beach were not included in the parentage reconstruction because our sampling was too limited in that habitat to produce a reliable pedigree. Colony accounts for the fact that not all parents may be sampled, and we conservatively set the estimated probability of a stream-spawning parent being sampled at 0.7, as this was the proportion of adults sampled in 2008 assigned to parents that were sampled in 2004 in the A Creek population by Hauser *et al.* (2011). A sensitivity analysis of this parameter indicated that it had negligible effects on the parentage assignments. Only assigned parent-offspring relationships with a confidence score greater than 95% were included in subsequent analyses (following Hauser *et al.* 2011 and Lin 2012). From this confidently assigned subset of the pedigree, the total number of stream-spawning offspring assigned to each F0 individual was tabulated. This value represents the reproductive success of each individual within the stream populations, but does not account for any offspring that returned as adults to the beach population. Thus, while it may not capture the

full reproductive success of an individual in an overall sense, it does measure the genetic contribution of dispersers to the focal stream-spawning populations.

Identification of Dispersers

The spawning location of each individual in the parental generation was inferred from field observations. Fish that entered a single stream were classified as A or C Creek spawners, depending on the stream where they were observed. If a fish entered both streams, the stream in which it was seen more often was considered to be its chosen spawning habitat. The accuracy of these spawning location classifications was assessed by comparing the spawning locations of mates inferred by parentage analysis. Fish that never entered either stream but were observed on redds in the beach were classified as beach spawners (as in Lin *et al.* 2008).

The most likely natal population for each individual in the parental generation was estimated using a Bayesian genetic assignment method (Rannala & Mountain 1997) implemented in the software application GeneClass2 (Piry *et al.* 1999). F0 genotypes were grouped into A Creek, C Creek, and beach populations based on the observationally inferred spawning location of each individual. We consider the two sampled beach spawning sites as one population because beach-spawning aggregations throughout the entire lake exhibit very low genetic and morphological differentiation (Lin *et al.* 2008). A leave-one-out procedure was utilized to assign each individual to its most likely natal population (Piry *et al.* 1999), with the two stream populations and the beach population considered as the only potential natal populations. The number of immigrants from un-sampled populations in our sample was likely small because no other populations spawn within four kilometers of the focal streams.

Dispersal status for each stream-spawning individual was assigned by comparing its

genetic population assignment with its inferred spawning location. Fish were classified as philopatric if they spawned in the stream of their natal population assignment, stream dispersers if they spawned in the stream that was not assigned as their natal population, and beach dispersers if they spawned in either stream but were genetically assigned to the beach population. Dispersal status within the beach-spawning group was not investigated because sampling was insufficient to estimate reproductive success for those individuals

To assess the accuracy of the assignment method, 10 pools of simulated genotypes of the same size as the original data set were produced and source populations were assigned to each individual using the same leave-one-out Bayesian assignment method as above. Simulated genotypes were produced by combining multilocus haploid “gametes” from random individuals within each population, thereby preserving any linkage disequilibrium present in the data (as in Paetkau *et al.* 2004). The assignment algorithm in GeneClass2 produces a probability of each multilocus genotype belonging to each population, which was used as a confidence score for each assignment. The accuracy of the assignment method for simulated individuals from each population was quantified by calculating the proportion of individuals assigned to each of the three populations under two approaches: 1) accepting the most likely population of origin for each individual regardless of statistical confidence and 2) only accepting the most likely population of origin when it was assigned with 95% confidence or greater.

Because of the likelihood that philopatric fish would greatly outnumber dispersers, we were concerned that even low misassignment rates of philopatric individuals could produce a large ratio of spurious dispersers to true dispersers. We therefore conducted a second verification of the overall accuracy of assigned dispersal categories by comparing the results of population assignments in an offspring cohort with the results of our parentage analysis. Parentage

reconstruction is often a much more accurate method to identify dispersers (in the offspring generation) between genetically similar populations than population assignment tests (e.g. Saenz-Agudelo *et al.* 2009), so it offers an excellent empirical assessment of the validity of our dispersal assignments (Hauser *et al.* 2006). We compared parentage with dispersal assignments in the 2009 cohort because for those individuals we had samples from both contributing parental cohorts (2004 and 2005), allowing a high rate of parentage assignment. We expected that assigned philopatric individuals would have parents from their observed spawning stream, assigned stream dispersers would have parents from the other stream, and assigned beach dispersers would have no parents in the sample. We therefore evaluated the accuracy of the dispersal categories inferred from the population assignment method by calculating the proportion of each group's parents that spawned in the expected population.

Comparison of Reproductive Success between Dispersers and Philopatric Individuals

The relationship between individual dispersal status and reproductive success was characterized with generalized linear regression models. Many F0 individuals did not have any offspring, and we observed overdispersion in the number of offspring per parent (such distributions are typical of salmon reproductive success; e.g. Seamons *et al.* 2007, Anderson *et al.* 2010). We therefore used zero-inflated negative binomial (ZINB) models in order to accommodate the distribution of the response variable, reproductive success (Zeileis *et al.* 2008, following Kodama *et al.* 2012 and Lin 2012). Validation of this model choice using AIC values indicated that the ZINB model provided a better fit to the data than a standard glm with a normal or Poisson distribution. ZINB models use a mixture of two distributions to model count data: a binomial distribution of zeros versus counts and a negative binomial distribution of counts. Thus,

two types of zeros are assumed to exist in the data: those that arise from the same process that creates the distribution of the non-zero integer counts (the count component) and additional zeros that occur with an independent probability (the inflation component, Zuur *et al.* 2009). The ZINB regression model estimates the effect of the predictor variable(s) on both the probability of obtaining an independent zero value and the count mean, but also produces a single expected response mean that accounts for both zero inflation and count distribution processes. Our ZINB regression models were implemented with the “pscl” package (v1.04.1, Zeileis *et al.* 2008) implemented in the R statistical environment (v2.13.2, R Development Core Team 2011).

We estimated two ZINB models to quantify the difference between dispersal groups in reproductive success: one using the full dataset and one using only those individuals that were assigned to a natal population with at least 95% statistical confidence. In both cases the response variable was the total number of offspring per F0 individual and the predictor variable of interest was dispersal category (philopatric, disperser from the other stream population, or disperser from the beach population). We also included as additional covariates sex, spawning stream and spawning year. All pairwise interactions between these four categorical variables were evaluated for inclusion in the model using the stepwise model selection procedure advocated by Zuur *et al.* (2009). The resulting model was used to calculate the difference in mean number of offspring between dispersal categories after accounting for the effects of spawning population and year. Statistical confidence in the differences in reproductive success between dispersal categories was assessed by bootstrapping individuals in each dispersal category per sex and stream, re-parameterizing the regression model and recalculating differences between groups 1000 times (as recommended by Keele 2008). Differences in reproductive success were considered statistically significant if the range between the 2.5% and 97.5% quantiles of the estimates did not include

zero.

Effects of Differences Between Dispersal Groups in Morphological and Behavioral Traits

We wanted to determine whether any observed differences in reproductive success between dispersal groups could be explained by differences in readily measurable morphological and behavioral traits. For example, if beach dispersers had longer bodies than philopatric fish, and longer fish tended to have more offspring (after accounting for the covarying effect of dispersal status), we would infer that some mechanism associated with body length increased the reproductive success of beach dispersers relative to philopatric individuals. These inferences would depend on both the differences in trait value distributions between dispersal groups *and* the effect of a given value of each trait on an individual's reproductive success. We investigated both of these factors individually and then considered the two together, as described below.

Differences between the three dispersal groups were identified for the following traits: sex, body length, body shape (body depth / body length ratio, Quinn & Foote 1994), residency period on the spawning grounds (total number of days observed in the stream), missing observations (number of days not observed between first and last in-stream observation, an indicator of elusive behavior), death fate (bear-killed, senescent or unknown), stream entry date, mean position within each stream (distance upstream from the lake) and mean net movement per day (mean difference in position between consecutive observations). The dataset was divided by sex and stream to create four groups, within each of which trait distributions were compared between dispersal categories. Significant differences between dispersal categories in mean trait values were evaluated with Fisher's exact tests for categorical variables and generalized linear models for continuous variables. In the case of the glms, the morphological or behavioral trait of

interest was the response variable and dispersal category was the predictor variable. The best distribution for each continuous trait was chosen by evaluating the AIC for models assuming normal, Poisson, and gamma distributions and a simple intercept as the only predictor.

The association between the measured traits and individual reproductive success was estimated with another set of ZINB regression models in which the number of offspring per individual was the response variable and the individual-level traits from above were predictor variables along with dispersal category assignments and the population-level covariates stream, year and sex. All possible interactions between the focal continuous traits and the categorical population-level covariates and dispersal status were evaluated within a model selection framework. Quadratic terms for each continuous trait were also evaluated to account for non-linear relationships. Forward and backward selection algorithms were used to determine the optimal model based on AIC. The coefficients estimated from the resulting model indicated which individual-level traits influenced reproductive success and allowed us to generate predictions for individual reproductive success given an individual's set of trait values. Importantly, by including both dispersal assignments and individual-level traits as predictors in the same model, we were able to distinguish the effects of dispersal status from any effects of the measured morphological and behavioral traits on individual reproductive success.

Finally, the empirical trait distributions of the three dispersal groups and the regression-derived effect sizes of each trait on individual reproductive success were combined to quantitatively predict the effect of the differences in trait distributions between dispersal groups on the differences in mean reproductive success between dispersal groups. We conducted this analysis separately for each stream and sex combination. To isolate the effect of each trait in turn we created a separate, duplicate dataset for each trait within which the focal trait retained its

measured values for each individual but all other traits were homogenized to their mean value (for that sex and stream). We then used the model parameterized above to calculate a predicted number of offspring for each individual in each focal trait dataset. Because we used an additive model, any resulting model-predicted differences in reproductive success between individuals within each dataset were based entirely on the relationship of the focal trait with reproductive success. We then used the predicted individual reproductive success values to calculate the mean expected differences in reproductive success between dispersal categories for each dataset. Thus, we were able to estimate the differences in reproductive success of the three dispersal classes attributable solely to differences in their distributions of each trait. Statistical confidence in these estimates was calculated by performing the following procedure 1000 times: bootstrapping individuals within each dispersal category per sex and stream, re-parameterizing the regression model, creating the focal trait datasets from the bootstrapped full dataset, predicting the expected number of offspring for each individual in each focal trait dataset, and recalculating the differences in reproductive success between dispersal groups due to each trait. Differences between groups were considered statistically significant if the range between the 2.5% and 97.5% quantiles of the estimated differences did not include zero.

Mate Choice with Regard to Dispersal Status

We also wanted to determine whether reproductively successful dispersers mated with other dispersers or with philopatric individuals. We therefore calculated the proportion of successful dispersers that mated with another disperser. In order to test whether the observed pattern was significantly different from expectations under random mating, we performed a Fisher's exact test on a table of the number of inferred matings between males and females from

each dispersal category. This test was conducted with and without the 95% assignment confidence cutoff.

Results

We observed a total of 4473 individuals in A Creek and C Creek in 2004, 2005, 2008, 2009 and 2010, and 166 individuals that were settled on the beach habitat in 2004 and 2005. The overall sex ratio of all individuals sampled in this study was 66% female, but this value ranged from 58% to 71% over populations and years. Of these 4639 individuals, 4377 were fin-clipped and 4350 were successfully genotyped at eight or more loci (Table 2). Departures from Hardy-Weinberg equilibrium were found in 100 of the 132 tests (Appendix A), probably because of small population sizes. There was evidence for null alleles at three loci (One100a, One106, and One115a; as found by Hauser *et al.* 2011 and Lin 2012). Linkage disequilibrium was statistically significant for all pairs of loci within each stream subpopulation, but only 3 of 55 locus pairs were significantly linked in the beach population. Estimated effective population sizes (N_e) were lower for the stream subpopulations than for the beach subpopulation (Table 3). The ratios of the estimated N_e values to the census sizes (0.13-0.44) are similar to those reported for other salmonid populations (Waples 2004), suggesting that the observed linkage is caused by small N_e rather than physical linkage between loci.

Colony identified 552 unique, confidently assigned parent pairs (sire and dam) and 127 unique, confidently assigned single parents in 2004 and 2005. Only 17 parent-offspring assignments were discarded for < 95% confidence. Both parents were sampled in the same year in 96% of inferred triads (offspring and both parents) and in the same stream in 98% of triads. Of 711 F1 individuals sampled in 2008, 461 (65%) were assigned to a parent pair and 124 (17%)

were assigned to a single parent. Of 1167 F1 individuals sampled in 2009, 1031 (88%) were assigned to a parent pair and 89 (8%) were assigned to a single parent. Of 725 F1 individuals sampled in 2010, 179 (25%) were assigned to a parent pair and 58 (8%) were assigned to a single parent, likely because the 4-year-old cohort (whose parents we did not sample) dominated in this year. Reproductive success was extremely variable between individuals, with the number of inferred offspring ranging from 0 to 36 (mean 3.4) for males and 0 to 24 (mean 1.6) for females (Table 4). 49% of males and 68% of females were assigned no offspring.

Individuals assigned to all three potential source populations were sampled in both streams (Table 5), and 83% of assignments were given confidence scores of at least 95%. Our simulations indicated that 88-95% of population assignments were correct with no confidence cutoff, and 95-99% were correct with the 95% confidence cutoff (Table 6). Furthermore, parentage results for the 2009 cohort indicated that 44-46% of putative dispersers identified by population assignment were true dispersers when no confidence cutoff was used, and 64-79% of putative dispersers were true dispersers when a 95% assignment confidence cutoff was used (Figure 2). Nevertheless, despite the higher accuracy of assignment with the 95% cutoff, we present results with and without that cutoff limit because sample sizes of dispersers were much smaller when using the cutoff (101 versus 192).

The reproductive success of stream dispersers did not differ significantly from that of philopatric individuals, but dispersers from the beach population had significantly lower mean reproductive success in the streams than did philopatric fish in the streams (Figure 3). Moreover, dispersers from the beach population had lower reproductive success than dispersers between stream populations. The point estimates of this difference (both with and without the cutoff) indicated that beach dispersers produced about one fewer offspring on average than stream

dispersers, but this difference was statistically significant only when the assignment cutoff was not used.

Beach dispersers differed from philopatric individuals in many traits, but stream dispersers were indistinguishable at most traits from philopatric individuals (Supplementary Figures 1-8). We used the gamma distribution for all traits because it always produced the lowest AIC. The only differences observed between stream dispersers and philopatric individuals were in body shape (C Creek males were deeper than A Creek males) and stream entry date (C Creek fish entered earlier than A Creek fish). Beach dispersers were longer and deeper-bodied, had shorter residency periods in the stream, and entered later than philopatric individuals. Few differences were observed between beach dispersers and philopatric individuals in the number of missing observations, daily movement or stream position. The distributions of death fates were not significantly different between stream dispersers and philopatric individuals, but beach dispersers were less likely to be killed by bears and more likely to have an unknown fate (relative to the proportion dying of senescence) than philopatric individuals (although this difference was only statistically significant for females).

When calculating the regression model with individual- and population-level traits together, all measured morphological and behavioral traits (except body length for males) exhibited a positive association with number of offspring (Supplementary Figures 9-14). Including the following interaction terms between individual- and population-level traits improved the fit of the model: spawning stream by entry date, spawning stream by stream position, spawning stream by residency period duration, spawning stream by number of missing observations, sex by body length, sex by residency period duration and sex by number of missing observations. No individual-level trait exhibited a significant interaction with spawning year or

dispersal status. Forward and backward stepwise interaction selection algorithms converged on the same model. We were unable to include mean net movement per day as a predictor in the model because we could not calculate this value for the large number of fish that were only observed once, but we did not find any evidence that dispersers differed from philopatric individuals in movement within the stream. Associations between the measured individual-level traits and reproductive success were very similar with and without the 95% cutoff.

Several traits significantly affected the differences in reproductive success between dispersal groups (Figure 4, Supplementary Figures 15-18). In-stream residency period exhibited the greatest effects, generally raising the expected reproductive success of stream dispersers and reducing that of beach dispersers. Stream entry date was also consistently important, raising the expected success of the beach dispersers and dispersers from A Creek to C Creek and reducing that of dispersers from C Creek to A Creek. Body shape exhibited significant effects for males, increasing the success of dispersers from C Creek to A Creek and beach immigrants in C Creek, while lowering that of dispersers from A Creek to C Creek. Stream position also produced substantial mean effects on differences in reproductive success between dispersal categories spawning in C Creek, although none of these effects was statistically significant. Body length and the number of missing observations exhibited little effect on the relative reproductive success of the three dispersal categories.

After accounting for the effects of the above morphological and behavioral traits on individual reproductive success, differences in the mean reproductive success between the three dispersal categories were reduced relative to those inferred from the simpler regression model (Figure 5). However, beach dispersers identified without the 95% cutoff still exhibited significantly lower reproductive success than philopatric individuals, suggesting that factors

beyond the differences in morphological and behavioral traits that we measured may contribute to the observed differences in reproductive success between dispersal categories.

Almost all reproductively successful dispersers mated with a philopatric individual rather than another disperser. Of 136 female and 56 male dispersers identified without the assignment cutoff, 30 females and 18 males had at least one offspring, collectively participating in 91 inferred mate pairs. 68 female and 33 male dispersers were identified when the 95% assignment cutoff was used, of which 11 females and 10 males were reproductively successful, contributing to 39 mate pairs. Of these successful dispersers, the ratio of beach to stream dispersers was nearly equal (21:27 with no assignment cutoff and 10:11 with the 95% confidence cutoff). Only one mating between dispersers was inferred, indicating that the proportion of successful disperser mating events that involved a philopatric mate was 0.97 or 0.99 (calculated with and without the assignment cutoff respectively). Fisher's exact tests provided no evidence for departure from random mating with respect to dispersal status (with assignment cutoff: $p = 0.65$, without assignment cutoff: $p = 0.92$).

Discussion

The goal of this study was to compare the reproductive success of philopatric individuals, immigrants from ecologically similar stream habitats, and immigrants from ecologically distinct beach habitats in two focal stream-spawning sockeye salmon populations. Our results indicated that stream-to-stream dispersers and philopatric individuals had similar reproductive success, while immigrants from the beach habitat to the streams produced fewer returning adult offspring than either other group. Both disperser classes differed from philopatric individuals in several morphological and behavioral traits. Many of these differences in trait distributions affected

differences in reproductive success between dispersal categories, although evidence for an unexplained effect of dispersal status remained after those effects had been accounted for. Dispersers between stream-spawning populations experienced both positive and negative effects from differences with philopatric individuals in body shape, in-stream residency period, stream entry date and mean stream position, while the reproductive contribution of immigrants from the beach population was strongly limited by a reduced in-stream residency period relative to philopatric individuals. These findings suggest that local adaptation to specific habitat types limits gene flow between ecologically distinct subpopulations, a conclusion that is supported by evidence from other studies on patterns of genetic, morphological and behavioral differentiation in this system (Quinn *et al.* 2001a, Lin *et al.* 2008, McGlaufflin *et al.* 2011).

Reproductive Success

Our approach to immigrant identification relied on genetic population assignments, and our simulations indicated that these were only 40-80% accurate in our system. However, this imperfection in dispersal category assignment should only reduce our power to detect differences between philopatric and dispersing individuals, rendering our results conservative. Further measures that we tested to improve assignment accuracy, such as using population exclusion tests (as performed by Lin *et al.* 2008), lacked the power to identify many of the true dispersers inferred from parentage verification. We therefore balanced assignment accuracy and sample size, maximizing our power to compare mean reproductive success between dispersal categories while acknowledging that these category assignments are imperfect.

Comparisons of reproductive success between individuals further relied on inferences of parent-offspring relationships from the genetic data, which could not have been perfect given

that the assignment of some offspring to parents that returned in different years and that we did not sample 100% of each population. However, the vast majority of inferred mate pairs were observed in the same stream during the same year, indicating that most of our assignments are compatible with biological reality. Moreover, the performance of parentage assignment using this microsatellite marker set in this system has been directly verified with an independent marker set of single nucleotide polymorphisms, suggesting that our power to correctly identify parent-offspring relationships was high (Hauser *et al.* 2011). A small proportion of incorrect assignments or missing parents is unlikely to strongly affect our results, and should produce more conservative estimates of differences in reproductive success between groups.

Our finding that some disperser groups had lower reproductive success than philopatric individuals while other disperser groups did not mirrors the mixed results of the literature in avian and mammalian systems (Bélitchon *et al.* 1996). However, unlike most previous studies, we can attribute this difference between groups to the relationship between the dispersers' natal and spawning populations. Furthermore, rather than an effect of geographical distance (as found by Hannsson *et al.* 2004), adaptation to ecologically distinct habitat types is likely responsible for the observed differences in reproductive success. Beach and stream spawning habitats differ in many physical environmental factors, and several previous studies have reported differences in the selection regimes they impose on local salmon populations (e.g. Quinn *et al.* 2001b, Carlson *et al.* 2007). Local adaptation to these distinct selection regimes would thus be expected to reduce the fitness of dispersers between habitat types (Nosil *et al.* 2005). We found a mean difference in reproductive contribution of one offspring per individual between beach dispersers and stream dispersers, which represents 23% to 150% of the observed overall mean reproductive success per individual, depending on the sex, stream and year. The substantial difference in the

reproductive contribution between these two dispersers classes appears to be a strong reproductive barrier between stream-spawning and beach-spawning populations, and may be crucial to the maintenance of the morphologically and genetically recognizable stream and beach ecotypes.

Differences in Morphological and Behavioral Traits

Fish from the beach-spawning population differed in morphological and behavioral traits from stream-spawning fish, but we also found differences between the two stream-spawning populations. Male body shapes and stream entry dates showed the clearest distinctions, possibly reflecting differences in long-term selection regimes for those traits between the two streams. However, beach spawners were more different from stream spawners than stream spawners from different streams were from one another, as has been found by several previous studies (e.g. Quinn *et al.* 2001b, Lin *et al.* 2008). Thus, our assumption that beach-to-stream dispersers were more likely to exhibit different phenotypes from philopatric individuals than stream-to-stream dispersers was supported.

Sexual dimorphism in sockeye salmon is well known (Quinn & Foote 1994), and the morphological and behavioral differences between sexes could affect the overall reproductive effect of dispersal if selection acts differently between the sexes. Indeed, we found the relationships between reproductive success and the traits body length, residency period duration and the number of missing observations were significantly different for males and females (Figure 4). Therefore, we might expect dispersal to be more favorable for males or females depending on the overall trait differences between two populations, possibly leading to the evolution of sex-biased dispersal on a population-by-population level. However, we found no

evidence of sex-ratio differences between philopatric individuals and dispersers, which matches the results of most previous studies of dispersal in Pacific salmon (Hendry *et al.* 2004).

Nevertheless, the realized level of gene flow between populations may be strongly affected by sex ratio of the dispersers, which may vary annually even without a behavioral bias due to external factors (e.g. selective fishing, Kendall & Quinn 2012).

Mechanisms Explaining the Difference in Reproductive Success Between Dispersal Groups

Several assumptions were important to our regression-based predictions of the effects of individual morphological and behavioral traits on the differences in reproductive success between dispersal groups. Most crucially, we relied on the correct estimation of the relationship between each trait and individual reproductive success, which we assumed here to be either linear or quadratic in form. This assumption may miss more complex non-linear relationships but visual examination of the regression residuals provided no evidence of any such errors.

Another important assumption in our analysis is that we obtained a representative sample of trait value distributions for each group. This is clearly not the case for stream, sex and dispersal category combinations that had small sample sizes, which were as low as 1 and 3 for stream and beach dispersers among A Creek males when using the 95% assignment confidence cutoff. For these subsets of the data, our estimates of the distributions of trait values are not precise, and the estimated trait effects in these groups with should be interpreted with caution. We therefore attempted to draw generalizations about the importance of specific traits by examining trends that are reflected in both streams as well as with and without the assignment cutoff.

Differences between dispersal groups in stream entry date and body shape contributed to

differences in reproductive success. Later entry timing was inferred to improve the reproductive success of immigrants from later-spawning populations (beach and A Creek), possibly because their redds were less vulnerable to superimposition by subsequent females (McPhee & Quinn 1998). Deeper body shape similarly improved the reproductive success of male immigrants from deeper-bodied populations (beach and C Creek), likely reflecting a competitive advantage of deep bodies in attracting females and intimidating other males (Quinn & Foote 1994). Population means for these traits likely reflected the long-term optimal phenotype for their spawning habitat (Quinn *et al.* 2001a), but annual variability in environmental selective forces may produce advantageous conditions for immigrants in some years, at least with respect to individual traits.

However, despite the above evidence for immigrant advantages in two traits, we also observed that dispersers from the beach population exhibited shorter residency periods than philopatric stream-spawning individuals and between-stream dispersers, which reduced reproductive success for beach immigrants. Beach dispersers had a lower proportion of bear-killed fish and a higher proportion of fish with no recorded fate than philopatric individuals, suggesting that voluntary departure rather than bear predation is the mechanism limiting the in-stream residency period of beach dispersers. These fish may have been searching for their natal habitat or “prospecting” a novel habitat for potential dispersal, a behavior that has been observed in other sockeye salmon populations (e.g. Griffith *et al.* 1999). Both inherited spawning habitat preference and olfactory imprinting likely reduce the tendency of beach dispersers to spawn in stream habitats (Dittman & Quinn 1996), but those mechanisms may not reduce the spawning tendency of dispersers between similar habitats. Thus behavioral differences between ecotypes may strongly influence the conversion of dispersal into gene flow.

In addition to the above effects of measured morphological and behavioral traits, we

found some evidence that beach dispersers experienced an additional, unexplained reduction in reproductive success (in the stream populations) relative to philopatric stream-spawners. This effect could be explained by factors that we were not able to address in this study, including elements of mate choice and offspring survival and return. Because philopatric individuals make up the vast majority of each stream-spawning population, preferential choice against individuals from a foreign subpopulation could easily reduce immigrant mating opportunities. However, studies of mate choice in salmonids have indicated a preference for mates that are genetically distinct at the MHC locus (Landry *et al.* 2001, Consuegra & Garcia de Leaniz 2008). Previous work in this system has reported differentiation between the beach and stream populations at MHC loci (McGlaufflin *et al.* 2011), leading to a possible mating advantage for immigrant fish.

Selection against immigrants could alternately act via lower survival of their offspring, especially if the immigrants come from populations adapted to distinct environments. Studies of multiple salmonid species at a wide range of life stages have found extensive evidence for adaptation by populations to their local environmental conditions and reduced survival of experimentally introduced foreign individuals (reviewed by Fraser *et al.* 2011). However, juvenile sockeye salmon from both stream- and beach-spawning populations congregate in the same lake immediately after emergence from the gravel, so opportunities for local selection after mating are limited to the incubation and emergence stages. Moreover, almost all successfully reproducing dispersers mated with philopatric individuals, thereby incorporating locally adapted alleles into the genomes of their offspring. On the other hand, outbreeding depression may reduce the fitness of these hybrid offspring (as found in pink salmon by Gilk *et al.* 2004), although a meta-analysis of the fitness effects of outbreeding in fish did not reveal a clear trend of lower fitness in outbred offspring (McClelland & Naish 2007).

A third factor that may reduce the estimated reproductive success of dispersers is the dispersal of their offspring back to their immigrant parent's natal habitat (Doligez & Pärt 2008). In hatchery-reared Chinook salmon, offspring from hybridized matings between local and foreign individuals were less likely to return to their rearing site than offspring from matings between two local parents, although in this case the higher straying rate was only 6% (Candy & Beacham 2000). This concern does not apply to dispersers between streams because individuals returning to both streams were sampled in all years, allowing us to incorporate any back-migrating offspring into the estimates of reproductive success of their parents, but any offspring that returned to the beach populations were not included in our parentage assignments. Thus, straying rates from the stream populations in the F1 generation may have been higher for offspring of beach immigrant parents, reducing the apparent reproductive success of that dispersal category. However, it is important to note that if offspring of immigrants return to the natal population of their immigrant parent, there would be no gene flow into the recipient population - in fact gene flow would be reversed, because these offspring would likely be hybrids between immigrant and resident fish.

Conclusion

Our results showed that the reproductive success of immigrants depended on their habitat of origin. While differences from philopatric individuals in some morphological or behavioral traits may be advantageous for immigrants, differences in stream residency period limit the reproductive contribution of dispersers between geographically proximate but ecologically distinct subpopulations of sockeye salmon. Thus, the factors determining an individual's decision to spawn in a certain habitat, as well as the potential effects of mate choice and offspring survival

on the reproductive success of dispersers, may greatly influence how rates of gene flow within a metapopulation vary on ecological and evolutionary timescales.

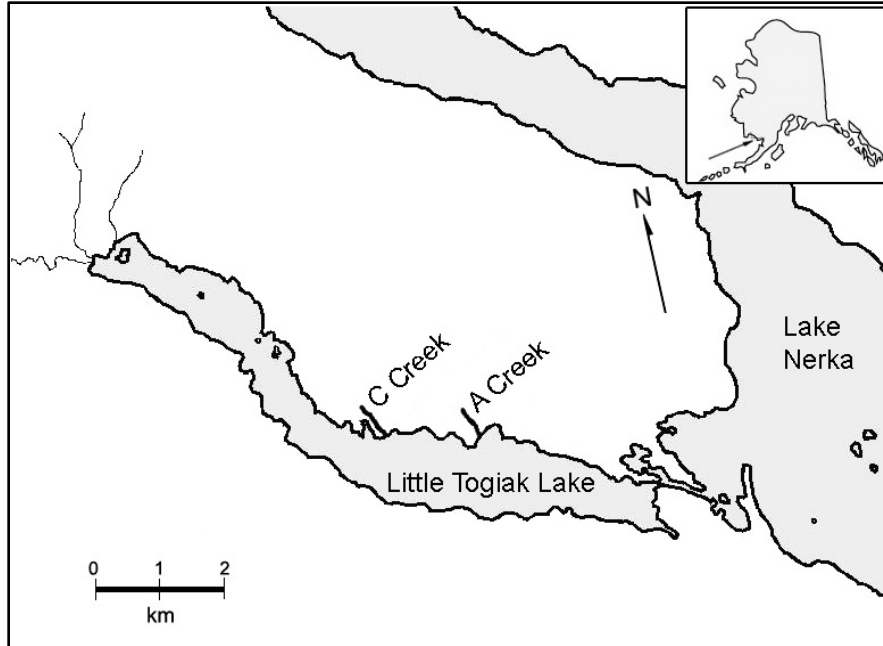


Figure 1: Map of study sites. Beach spawning sites are located within 100 meters of the mouth of each creek. Arrow in inset map indicates location within Alaska.

Table 1: Microsatellite loci, PCR conditions and statistics on variability and error rates. Panels 1a and 1b were amplified separately but run together for fragment size analysis.

Locus	Conc. (uM)	Panel	T_A (°C)	H_E	# All	F_{IS}	Dropout	False	Null
ONE100a	0.1	1a	63	0.853	23	0.137	0.022	0.000	0.073
ONE102	0.05	3	57.5	0.845	17	-0.013	0.014	0.000	0.007
ONE103	0.08	1b	58.5	0.926	43	0.018	0.007	0.007	0.007
ONE106	0.05	1b	58.5	0.874	32	0.097	0.068	0.021	0.047
ONE108	0.05	3	57.5	0.864	24	0.015	0.002	0.006	0.010
ONE109	0.05	2	60	0.802	16	0.010	0.021	0.003	0.011
ONE110c	0.08	1a	63	0.833	19	0.001	0.004	0.000	0.003
ONE111	0.05	2	60	0.755	30	-0.009	0.021	0.000	0.004
ONE114	0.05	2	60	0.880	25	0.009	0.006	0.000	0.005
ONE115a	0.05	3	57.5	0.896	19	0.179	0.015	0.019	0.101
OTS103	0.05	1a	63	0.859	18	-0.005	0.011	0.004	0.004
Average				0.853	24.2	0.040	0.017	0.005	0.024

Conc., final PCR concentration of forward and reverse primers; T_A , annealing temperature, H_E , expected heterozygosity; # All, total number of observed alleles; F_{IS} , average of F_{IS} estimates from each population and year (FSTAT); Dropout, estimated large allele dropout rate (Pedant); False, estimated false allele rate (Pedant); Null, estimated null allele frequency (ML-Null).

Table 2: Number of individuals successfully genotyped in each population and each year, with the total number observed in parentheses. Beach fish were sampled in 2008-2010 but were not genotyped for this study.

	2004	2005	2008	2009	2010
A Creek	517 (573)	458 (480)	229 (243)	655 (668)	428 (433)
C Creek	371 (481)	304 (311)	482 (498)	510 (528)	253 (258)
Beach	95 (118)	48 (48)	0 (213)	0 (155)	0 (166)

Table 3: Effective population size estimates (from LDNe) of each population in each year. Ranges represent estimates assuming lowest allele frequencies of 0.05, 0.02 and 0.01, with the minimum and maximum of the three 95% confidence intervals in parentheses.

	2004	2005	2008	2009	2010
A Creek	93-97 (85-103)	73-92 (67-98)	72-93 (64-100)	92-116 (85-123)	74-85 (68-90)
C Creek	54-69 (49-73)	73-105 (65-112)	65-99 (59-104)	98-127 (89-134)	76-92 (67-98)
Beach	64-156 (53-185)	170-612 (92-Inf)			

Table 4: Mean and variance in reproductive success. Proportion of zero offspring indicates the fraction of individuals that did not produce any offspring.

Population	Sex	Year	N	Mean rep. success	Var. in rep. success	Prop. zero offspring
A Creek	f	2004	362	0.66	2.60	0.79
A Creek	f	2005	287	2.49	11.34	0.49
A Creek	m	2004	141	1.56	7.63	0.65
A Creek	m	2005	169	4.24	24.88	0.32
C Creek	f	2004	271	1.68	16.66	0.77
C Creek	f	2005	174	2.23	16.80	0.66
C Creek	m	2004	95	4.32	60.75	0.55
C Creek	m	2005	128	3.34	27.50	0.51

Table 5: Number of parental individuals assigned to each population and observed in each habitat.

A: No assignment cutoff used

Assigned to:	Sampled in A Creek		Sampled in C Creek	
	Females	Males	Females	Males
A Creek	601 (93%)	293 (95%)	34 (8%)	21 (9%)
Beach	29 (4%)	7 (2%)	54 (12%)	18 (8%)
C Creek	19 (3%)	10 (3%)	357 (80%)	184 (83%)

B: 95% assignment cutoff

Assigned to:	Sampled in A Creek		Sampled in C Creek	
	Females	Males	Females	Males
A Creek	554 (96%)	270 (99%)	22 (6%)	16 (9%)
Beach	16 (3%)	3 (1%)	23 (7%)	13 (8%)
C Creek	7 (1%)	1 (0%)	291 (87%)	138 (83%)

Table 6: Simulated probabilities of assignment to each of the three focal populations. Cell values are the means of ten iterations of the simulation, with standard deviations in parentheses. Of the three F1 cohorts, we assessed the assignment accuracy in 2009 because we had both parental cohorts for those offspring, allowing verification of the assignment accuracy with parentage.

A) F0 Cohorts (2004 & 2005)

No assignment cutoff

True pop.:	Assigned to:		
	A Creek	Beach	C Creek
A Creek	0.949 (0.005)	0.019 (0.003)	0.033 (0.005)
Beach	0.018 (0.006)	0.910 (0.015)	0.072 (0.013)
C Creek	0.055 (0.006)	0.065 (0.010)	0.880 (0.010)

95% assignment cutoff

True pop.:	Assigned to:		
	A Creek	Beach	C Creek
A Creek	0.982 (0.003)	0.009 (0.002)	0.009 (0.003)
Beach	0.010 (0.007)	0.972 (0.015)	0.018 (0.011)
C Creek	0.026 (0.008)	0.029 (0.007)	0.945 (0.010)

B) F1 Cohort (2009)

No assignment cutoff

True pop.:	Assigned to:		
	A Creek	Beach	C Creek
A Creek	0.949 (0.006)	0.008 (0.005)	0.042 (0.006)
Beach	0.008 (0.005)	0.928 (0.020)	0.065 (0.017)
C Creek	0.080 (0.013)	0.045 (0.009)	0.875 (0.018)

95% assignment cutoff

True pop.:	Assigned to:		
	A Creek	Beach	C Creek
A Creek	0.989 (0.003)	0.002 (0.002)	0.009 (0.002)
Beach	0.005 (0.004)	0.980 (0.009)	0.015 (0.009)
C Creek	0.043 (0.008)	0.022 (0.007)	0.935 (0.009)

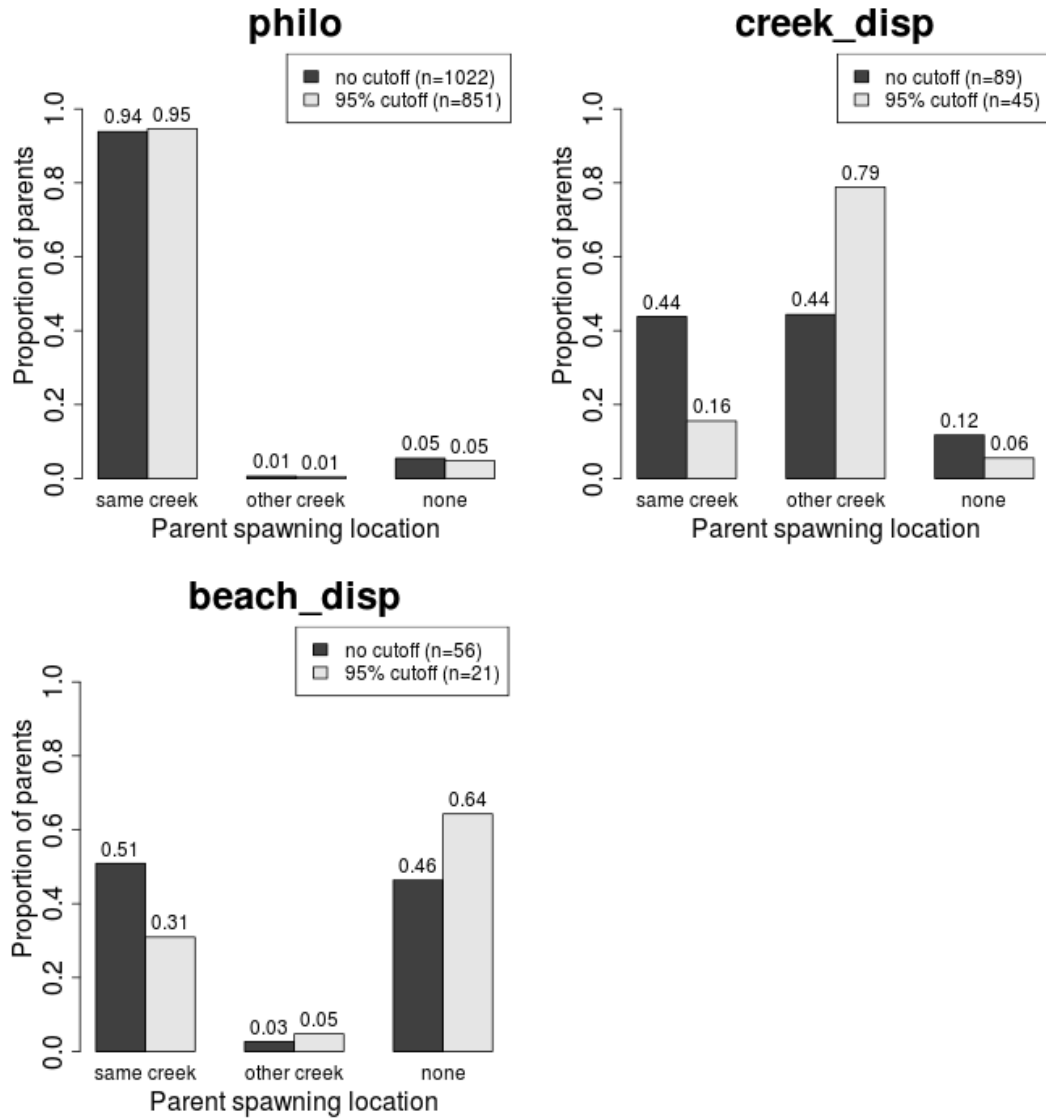


Figure 2: Distributions of observed parent locations for offspring (2009 cohort) in each assigned dispersal category. Bar height indicates the proportions of parents from the same creek in which the offspring were also observed (“same creek”), the other creek, or no parent assigned (“none”). These proportions are plotted for individuals from 2009 in each assigned dispersal category (“philo” = philopatric individuals, “creek_disp” = dispersers from one creek to the other, and “beach_disp” = dispersers from the beach to the creeks). All individuals sampled in A or C Creek in 2009 were included, whether they had 0, 1 or 2 parents assigned. Parents are expected to be from the same creek for putative philopatric individuals and from the other creek for putative creek dispersers. No parents are expected to be assigned for putative beach dispersers.

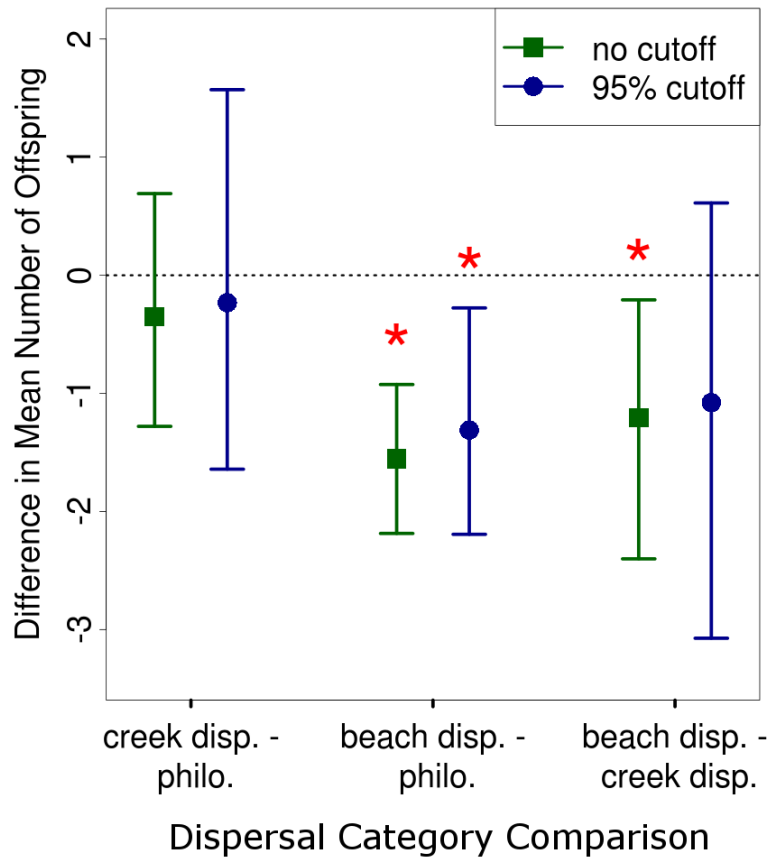


Figure 3: Differences between dispersal categories in mean reproductive success per individual. Values represent the model-predicted effect of dispersal category on number of offspring after accounting for differences in reproductive success between creeks, sexes and years. Error bars indicate 95% confidence intervals.

* indicates a difference that is statistically significant after bootstrapping (95% CI does not include zero).

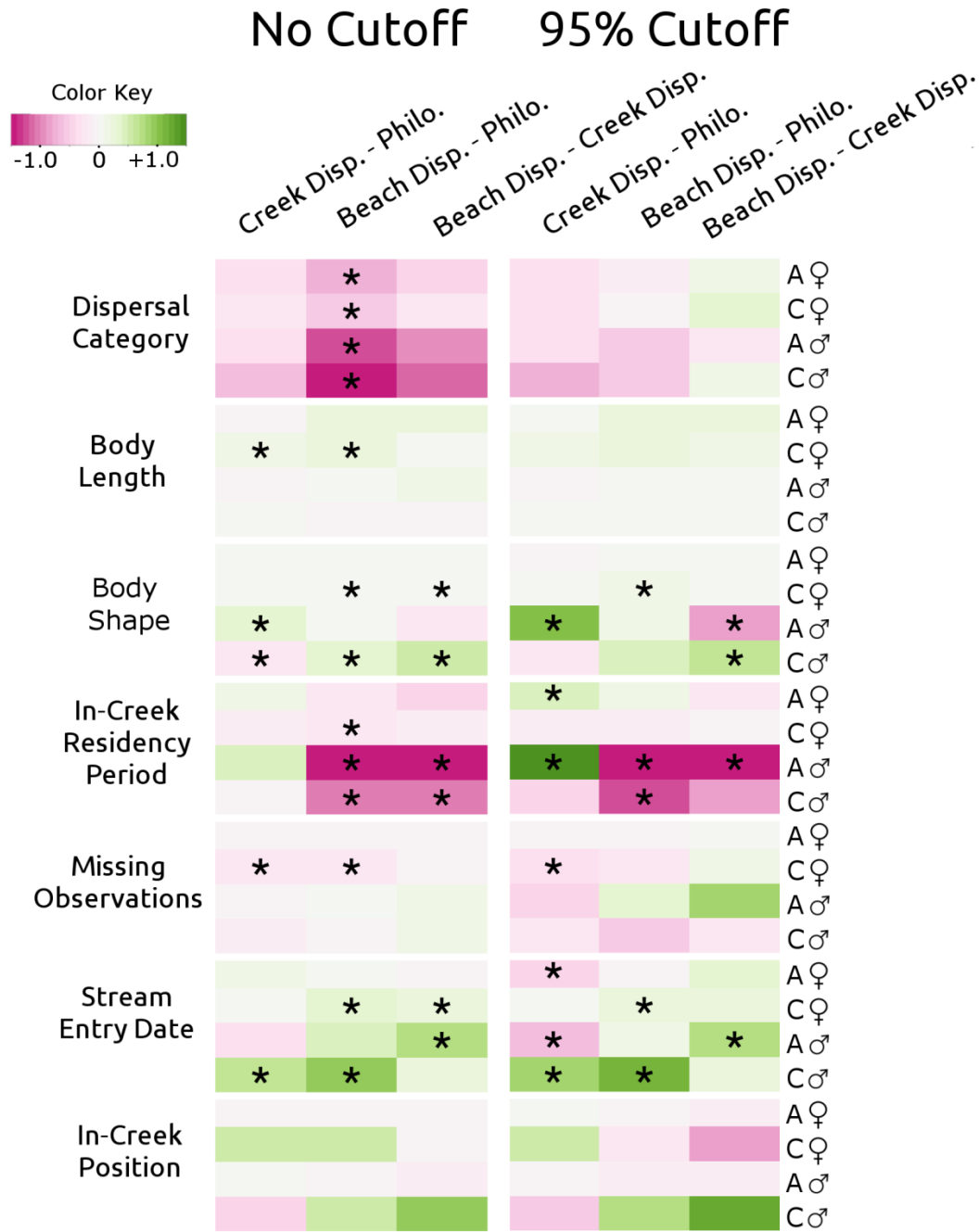


Figure 4: Effects of differences in trait distributions between dispersal categories on differences in reproductive success. Cell colors represent the model-predicted difference in mean reproductive success per individual based on the distributions of the focal trait in the two dispersal groups. Negative effects are purple and positive effects are green. Columns correspond to comparisons between dispersal categories, and rows represent spawning populations by sex.

* indicates a statistically significant effect (95% CI from bootstrapping does not include zero).

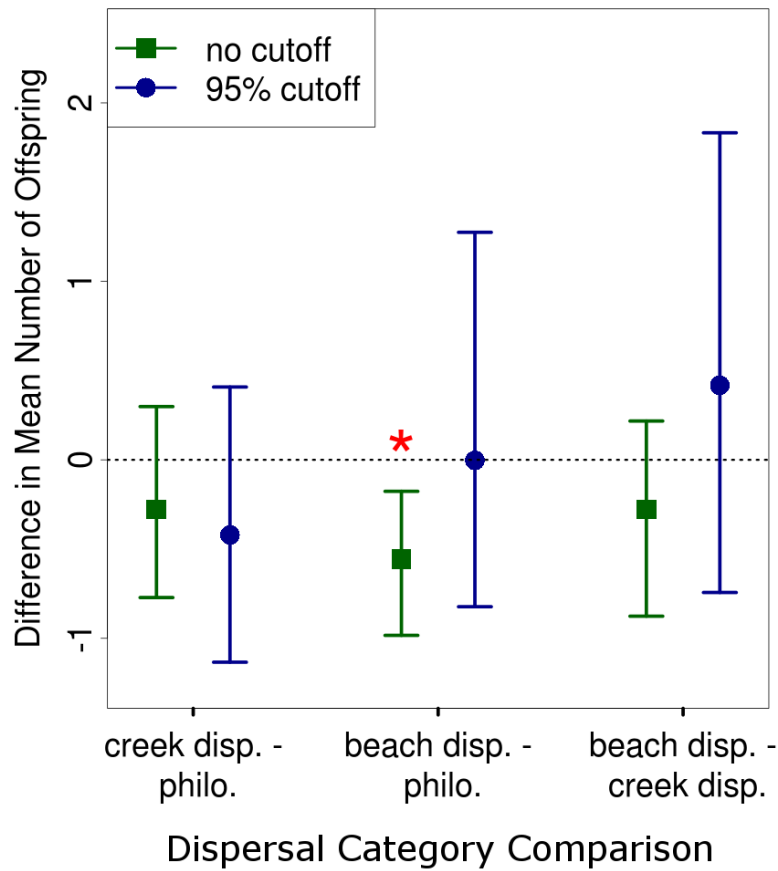


Figure 5: Residual differences between dispersal categories in mean reproductive success per individual after accounting for the effects of the following individual-level traits: body length, body shape, creek entry date, in-creek residency period, mean creek position and mean daily movement. Values represent model-predicted differences between categories in mean number of offspring per individual. Error bars indicate 95% confidence intervals from bootstrapping.

* indicates a difference that is statistically significant (95% CI does not include zero).

Chapter 2

Exploratory behavior of dispersers within a metapopulation of sockeye salmon

Abstract

In anadromous fishes, most individuals return (home) to their natal habitat to spawn. However, experimental and observational studies of the homing and spawning behavior of hatchery-raised salmon have indicated that their search for high-quality spawning habitat can overcome their homing tendency. Nevertheless, the extent to which dispersal between populations is motivated by habitat selection versus navigational errors during the homing process is not well understood, especially in wild populations. Here we examined whether dispersing individuals exhibited more exploratory behavior than philopatric individuals within a metapopulation of wild sockeye salmon, which would suggest that dispersal may be influenced by comparisons between potential spawning areas. We tracked the daily locations of all adult salmon spawning in two proximate streams and determined the dispersal status for each individual by comparing its chosen spawning stream with that of its parents (as determined by genetic parentage reconstruction). Dispersers were often observed in their natal stream (8-11% of individuals) or at its mouth (29-58% of individuals) before spawning in the other stream, whereas philopatric individuals were rarely observed in their non-natal stream (0-2% of individuals) or at its mouth (1-7% of individuals). These results suggest either that the mechanism of dispersal encourages exploration or that individuals that explore are more likely to disperse. In either case, dispersers are exposed to multiple spawning habitats, potentially allowing annual variation in local environmental or demographic conditions to influence the patterns of gene flow within a metapopulation.

Introduction

Gene flow within a metapopulation is caused by the dispersal and subsequent reproduction of individuals between subpopulations, but the factors that determine whether any given individual disperses can be complex. In many animals, dispersal decisions are influenced by environmental and social cues such as habitat quality, intraspecific competition and mate availability (i.e., conditional dispersal; reviewed by Clobert *et al.* 2009). Therefore, environmental and demographic variation can affect the rate and directions of dispersal within a metapopulation (e.g. Dugger *et al.* 2010). The resulting effects on gene flow can have significant evolutionary consequences, including influencing the scale of local adaptation or the prevalence of inbreeding depression (Garant *et al.* 2007, Hanski *et al.* 2011).

For anadromous fishes, philopatry involves precise navigation from the ocean to the natal freshwater habitat to breed. It is well established that most populations of anadromous salmonids exhibit high levels of successful natal homing (Dittman & Quinn 1996), but a low level of dispersal between geographically proximate populations is also consistently observed (Hendry *et al.* 2004). It is unclear, however, to what extent this dispersal is caused by failure of the learning, memory or homing mechanisms versus facultative choice to spawn in a non-natal habitat after the natal site has been identified. Given the complex nature of the olfactory imprinting processes associated with homing (Nevitt *et al.* 1994, Dittman *et al.* 1997) as well as the challenge of distinguishing between similar environments whose chemical cues may change temporally (Dittman & Quinn 1996), some instances of dispersal might easily result from “mistakes” during the homing process (Hendry *et al.* 2004). On the other hand, we cannot rule out the possibility that some individuals disperse because they prefer the local environmental or social conditions in a non-natal habitat to those in their natal habitat (Quinn 2005).

Physical habitat features including water depth, temperature and velocity, substrate size, cover availability and hyporheic exchange rate all strongly influence spawning site selection across multiple spatial scales (Beechie *et al.* 2008). The abundance and sex ratio of conspecifics may also influence site selection because an individual's ability to reproduce may be limited by a scarcity of potential mates or an excess of competitors (Semenchenko 1988, Quinn *et al.* 1996). If an individual is able to identify that the physical or demographic conditions at its natal site are unfavorable, it may explore alternative spawning sites outside of the range of its natal population (McMahon & Matter 2006).

Multiple lines of evidence suggest that the factors associated with spawning site selection can motivate dispersal between populations of salmonids. Behavioral experiments on mature, hatchery-reared Chinook salmon (*Oncorhynchus tshawytscha*) showed that while the fish exhibited a strong homing preference towards their home water over a non-natal water source, introduction of a contaminant (volcanic ash) into the natal water source substantially reduced that preference (Whitman *et al.* 1982). Adding ash to both the natal and non-natal water sources restored the preference for the natal water, indicating that the foreign material did not hinder the natal water recognition mechanism but influenced preferences. Moreover, a recent analysis of homing patterns of Chinook salmon raised in hatcheries but briefly held at distinct acclimation sites (outdoor holding ponds) before release showed that the proportion of individuals that returned to and spawned at their acclimation site was much lower for sites providing low-quality spawning habitat than for sites providing high-quality spawning habitat (Cram *et al.* 2012). These findings suggest that an individual's spawning habitat requirements may influence its homing tendency on a fine scale, although little information is available concerning the tradeoffs between these factors in wild populations (Quinn 2005).

Studies of multiple stages of the return migration of salmon to freshwater indicate that fish have the opportunity to visit and evaluate non-natal spawning habitats while homing. Individual fish from multiple salmonid species have been observed following non-direct homing paths; behaviors include temporary presence in non-natal spawning habitats and overshoot of natal stream junctions (Chinook salmon, Keefer *et al.* 2008; sockeye salmon (*O. nerka*), Burger *et al.* 1995, Young & Woody 2007). Moreover, studies of individual movement within continuous spawning grounds have revealed that exploratory behavior *after* successful homing can be extensive (male sockeye salmon, Rich *et al.* 2006) and is influenced by physical habitat characteristics (Chinook salmon, Neville *et al.* 2006). The relationship between exploratory behavior and dispersal between discrete populations remains unclear, however, because few studies combine natal population assignment, movement data and ultimate spawning habitat choices (Griffith *et al.* 1999).

Genetic assignment is now a widely used method of natal site identification for salmonids (e.g. Bekkevold *et al.* 2004, Neville *et al.* 2006), in part because it avoids the difficulty of capturing and tagging alevins just after emergence from the substrate. Moreover, recent advances in parentage analysis allow very confident assignments when multiple generations are sampled and genotyped (Hauser *et al.* 2006, Saenz-Agudelo *et al.* 2009). However, previous studies have not combined genetic information with observational data to investigate the behavior of dispersers between populations of salmon.

We hypothesized two reasons to expect an association between exploratory behavior and dispersal: 1) the mechanism of dispersal encourages habitat exploration (e.g. if dispersers have “forgotten” their natal habitat), or 2) habitat exploration encourages dispersal (e.g. if fish can be “tempted” away from their natal habitat by attractive non-natal habitats). Alternately, we would

not expect an association between dispersal and exploratory behavior if dispersal were caused by an error during navigation, mistaking a non-natal habitat for the natal habitat or if it were under direct genetic control.

The objective of this study was to examine whether dispersing individuals exhibited more exploratory behavior than philopatric individuals within a metapopulation of wild sockeye salmon. To address this question, we monitored the daily locations of all individuals in two geographically proximate subpopulations of stream-spawning Alaskan sockeye salmon. We determined the natal origin, and thus the dispersal status, of all fish by genetic parentage analysis with the previous generation of spawning adults. To test whether dispersal was associated with habitat exploration in this system, we compared the relative proportions of dispersers and philopatric individuals that were observed in or at the mouth of the stream in which they did not spawn (the proportion of “exploring” individuals). To test whether any dispersers failed to visit their natal stream, we compared the relative proportions of dispersers and philopatric individuals that were observed at the mouth of their natal stream.

Materials and Methods

Study Area and Sample Collection

The focal populations for our study were two small, geographically proximate populations of stream-spawning sockeye salmon in the Wood River system of southwestern Alaska (Figure 1). A Creek and C Creek both drain into Little Togiak Lake 1.5 km apart and each population is composed of 150-600 spawning adults in most years. Previous work has found that the populations are genetically differentiated, although they do exchange dispersers (Lin *et al.* 2008). The two streams experience similar water temperature and flow regimes, and

both offer the gravel size that stream-spawning salmon prefer (Pess 2009). However, C Creek is slightly deeper and has more woody debris and undercut banks than A Creek (Pess 2009), providing somewhat greater protection from predators (Carlson *et al.* 2007, Lin 2012). In the present study, we determined the natal origin of all adults spawning in A and C creeks during 2008 and 2009 (F1 cohorts). Sockeye salmon in these streams almost always return to spawn as adults either four or five years after being laid as eggs (Quinn *et al.* 2001), so the parents of our F1 cohorts returned in 2003, 2004 and 2005, although we were only able to sample the individuals that returned during 2004 and 2005 (F0 cohorts).

Exhaustive sampling of A and C Creek adult spawning populations was conducted every year between 2004 and 2009. We walked the full length of both streams every day during the spawning season (late July through early September), tagged any newly observed fish with individually labeled disc tags, and noted the location of each previously tagged fish. A fin clip was taken from each fish upon tagging for genetic analysis. Sex, body length (mid-eye to hypural plate), and body depth were also measured and recorded for each newly tagged individual (following Quinn *et al.* 2001). When a dead fish was found in or near the streams, the tag ID was recorded and the cause of mortality was determined by visual inspection (bear-killed, gull-killed or senescent). If a dead fish was found untagged, a fin clip was taken and length and depth were measured if the carcass was intact. While 100% sampling was not possible given the fact that bears remove some fish from the stream (Quinn *et al.* 2009) and others enter and depart the streams without ever being observed, previous work suggests that we sampled the majority (> 80%) of spawning individuals (Hauser *et al.* 2011).

To expedite tagging during times of peak abundance, fish “milling” (*sensu* Young & Woody 2007) in the lake within 10 m of the mouth of the streams were captured by seine net and

tagged before they entered the streams. All individuals without a tag were tagged, and the identities of all previously tagged fish were recorded. In 2008, these stream mouth surveys were conducted between July 26 and August 18, with 11 surveys of A Creek mouth and 8 surveys of C Creek mouth during that period. In 2009, stream mouth surveys were conducted between July 26 and August 12, with each stream mouth surveyed 7 times. However, only fish that were observed at least once in one of the two streams were included in the analyses.

Genotyping Methods

To reconstruct parent-offspring relationships, all adults observed spawning in either A or C Creek in 2004, 2005, 2008 and 2009 were genotyped at 11 tetranucleotide repeat microsatellite loci (mean heterozygosity = 0.87 ± 0.04 SD, 266 independent alleles; loci developed by references in Hauser *et al.* 2011; Table 1). DNA was extracted from the samples using DNeasy extraction columns following the manufacturer's instructions (Qiagen, Valencia CA, USA). Microsatellite loci were amplified using Qiagen multiplex PCR kits (Qiagen, Valencia CA, USA), with the primer concentrations and annealing temperatures given in Table 1. Forward primers were fluorescently labeled and amplified fragment sizes were measured with a MegaBACE 1000 DNA Analysis System (GE Healthcare Life Sciences, Piscataway, NJ, USA).

Departures from Hardy-Weinberg and linkage equilibrium at each locus in each subpopulation and year were tested by permutation tests with the program FSTAT (Goudet 1995). Estimates of effective population size (N_e) for each subpopulation in each year were calculated based on observed linkage equilibrium using the program LDNe (Waples & Do 2008) to investigate whether small population size might account for linkage disequilibrium. The presence of null alleles and large-allele dropout were assessed for each subpopulation and year

with the programs ML-Null (Kalinowski & Taper 2006) and Microchecker (Van Oosterhout *et al.* 2004). Genotyping error was quantified using the program Pedant (V1.0, Johnson & Haydon 2007), which uses Bayesian methods to separately estimate the frequencies of allelic dropout and incorrect alleles from 146-203 individuals per locus that were re-amplified and re-genotyped. Thirty-seven individuals genotyped at fewer than 8 of 11 loci were removed from the dataset because parentage assignments were less likely to be reliable in this group (following Hauser *et al.* 2011).

Pedigree Reconstruction

Parent-offspring relationships between the F0 generation (stream spawners in years 2004 and 2005) and their F1 offspring (stream spawners in years 2008 and 2009) were reconstructed using the parentage analysis software package Colony (version 2.0; Jones & Wang 2010). All F0 individuals were considered to be either potential sires or dams (according to their observed sex) for all F1 offspring, and F0 individuals of unknown sex were included as both potential sires and potential dams. Data from both stream populations and all years were incorporated into a single pedigree reconstruction in order to account for dispersal between streams in the offspring generation, as well as the variation in age-at-return of those offspring. Colony accounts for the fact that not all parents may be sampled, and we conservatively set the estimated probability of a stream-spawning parent being sampled at 0.7, as this was the proportion of adults sampled in 2008 assigned to parents that were sampled in 2004 in the A Creek population by Hauser *et al.* (2011). A sensitivity analysis of this parameter indicated that it had negligible effect on the parentage assignments (data not shown). Only assigned parent-offspring relationships with a

confidence score greater than 95% were included in subsequent analyses (following Hauser *et al.* 2011 and Lin 2012).

Identification of Dispersers and Philopatric Individuals

To examine natal dispersal in the F1 generation, we determined the spawning location of all individuals in both the F1 and F0 generations. Fish of either generation that entered a single stream were considered to have spawned in that stream. If a fish entered both streams, the stream in which it was seen more often was considered to be its chosen spawning habitat. The accuracy of these spawning location classifications was assessed in the F0 generation by comparing the spawning locations of mate pairs inferred by parentage analysis. F1 individuals were classified as philopatric if they spawned in the same stream as their parents, and as dispersers if they spawned in the other stream. We only considered F1 individuals for whom both parents were confidently assigned and observed in the same stream.

We tested for sex-biased dispersal by comparing the sex ratio of dispersers and philopatric individuals using Fisher's exact test.

Analysis of Exploratory Behaviors

To investigate the prevalence of habitat exploration by dispersers and philopatric individuals, we calculated the proportion of individuals in both categories that were observed in each stream. We also calculated the proportion of individuals in both categories that were observed "milling" in the lake within 10 m of each stream (hereafter "at the mouth"). We analyzed the latter type of habitat visit because individuals in this system usually hold at the mouth of a stream for multiple days, possibly evaluating the environment of the stream during

that time. The statistical significance of the differences in the proportions of dispersers and philopatric individuals observed in or at the mouth of each stream was evaluated with Fisher's exact tests.

Results

We sampled and genotyped a total of 3526 individuals from A and C Creeks in 2004, 2005, 2008 and 2009 (Table 2). Departures from Hardy-Weinberg equilibrium were found in 100 of the 132 tests (Appendix A), probably because of small population sizes. There was evidence for null alleles at three loci (One100a, One106, and One115a; as found by Hauser *et al.* 2011 and Lin 2012). Linkage disequilibrium was statistically significant for all pairs of loci within each population and year. Effective population sizes (N_e) estimated from linkage disequilibrium were between 54 and 105 for each stream in each year (Table 3). The ratios of the estimated N_e values to the census sizes (0.13-0.44) are similar to those reported for other salmonid populations (Waples 2004), suggesting that the observed linkage is caused by small N_e rather than physical linkage between loci.

Colony identified 526 confidently assigned parent pairs (sire and dam) and 126 confidently assigned single parents in the F0 generation. In 98.1% of inferred parent pairs, both parents were sampled in the same year, and in 97.9% of inferred pairs, both parents were classified from in-stream observations as spawning in the same stream. In 5 of the remaining 11 parent pairs, one parent was seen in each stream once so the natal stream of all offspring was assumed to be the spawning stream of the unambiguous parent. Of 711 F1 individuals sampled in 2008, 461 (65%) were assigned to a parent pair and 124 (17%) were assigned to a single parent. Of 1167 F1 individuals sampled in 2009, 1031 (88%) were assigned to a parent pair and 89 (8%)

were assigned to a single parent. Offspring ages in the 2009 cohort, as inferred from the difference between parent pair and offspring sampling year, were 86% age-4 and 14% age-5. We could not assign ages in the 2008 cohort because we did not sample the parents of age-5 fish in that cohort.

Almost all (99%) of both F1 and F0 individuals were observed in only one stream. Of the 1486 F1 offspring with confident parent pair assignments, 756 were classified as A Creek spawners and 729 were classified as C Creek spawners (Table 7); one F1 individual was observed once in each stream and omitted from further analysis because its spawning location was ambiguous. 1434 (96.6%) of the F1 individuals were classified as philopatric and 51 (3.4%) were classified as dispersers. Of the 51 dispersers, 50 dispersed from the A Creek population to the C Creek population and only a single individual dispersed from the C Creek population to the A Creek population. The dispersal rate for fish born in A Creek into C Creek was therefore 6.2%, while that of fish born in C Creek into A Creek was 0.1%. Due to this unexpected asymmetry in dispersal rates, we only examined the homing behavior of the 50 dispersers from A Creek to C Creek.

The overall sex ratio of all individuals sampled in this study was 66% female, but this value ranged from 58% to 71% over populations and years. A higher proportion of females dispersed than males (females: 39/988, 3.9%; males: 12/485, 2.5%), but this difference was not statistically significant ($p = 0.17$).

A substantially higher proportion of dispersers explored the creek in which they did not spawn compared to philopatric individuals in both 2008 and 2009. The percentage of dispersers observed in their natal stream was 8% in 2008 and 11% in 2009, and those percentages increased to 58% and 29% when considering observations at the mouth of the natal stream (Figure 6). In

contrast, the proportion of philopatric individuals observed in the stream in which they did not spawn ranged from 0.3% to 2% across streams and years, and the proportion of philopatric individuals observed at the mouth of the stream in which they did not spawn ranged from 0.8% to 7%. All statistical comparisons between proportions of dispersers and philopatric individuals were statistically significant ($p < 0.05$) in 2009. Statistical power for the 2008 data was lower due to a smaller number of dispersers. Nevertheless, the proportion of dispersers observed at the mouth of the stream in which they did not spawn was significantly greater than that of philopatric individuals from both streams in that year ($p < 0.05$).

The percentage of philopatric individuals observed at the mouth of their natal stream was higher in 2008 than in 2009 (89% versus 57%), likely because of more frequent sampling in 2008. In both years, the proportion of dispersers that were observed at the mouth of their natal stream was significantly lower than that of philopatric individuals (Figure 7).

Discussion

We found that dispersers between a pair of proximate streams often visited their natal stream before spawning in their non-natal stream, whereas philopatric individuals very rarely explored their non-natal stream but simply aggregated near the mouth of their natal stream and then ascended it. However, dispersers were less likely to be observed at the mouth of their natal stream than philopatric individuals, suggesting that some individuals disperse without exploring their natal habitat.

We observed very low levels of exploratory behavior by philopatric individuals from both streams. Thus, we can rule out the hypotheses that either population had an innate exploratory tendency or that non-natal stream exploration was a part of the standard homing

process in this system. The observed exploratory behavior of dispersers was therefore likely related to the causes of dispersal itself.

Two hypotheses could account for the fact that dispersers were more likely than philopatric individuals to be seen in or near the stream in which they did not spawn: either the mechanism of dispersal encouraged exploration or individuals that explored were more likely to disperse. In either case, the fact that dispersers were exposed to multiple potential spawning habitats suggests that dispersal decisions may have been influenced by direct comparisons between the local conditions in different spawning areas. The physical and social indicators salmon use to compare sites within streams (reviewed by Beechie *et al.* 2008) might also be used to evaluate the suitability of candidate spawning streams, potentially influencing dispersal decisions between genetically differentiated populations.

On the other hand, it is possible that dispersers merely represented individuals that chose a random spawning location. However, spawning habitat choices of salmon appear to be non-random even in the absence of homing cues. For example, returning adult Coho salmon (*O. kisutch*) whose olfactory pits had been occluded (to remove any homing cues) preferentially chose to ascend the main stem of the study creek rather than its tributary, regardless of whether they had originally been captured in the main stem or the tributary (Wisby & Hasler 1954). Further evidence against random spawning site selection by dispersers comes from the strong asymmetry observed in dispersal between A and C creeks (as well as in Chapter 1 for the parent generation), a result suggesting either that C Creek was more attractive than A Creek to individuals choosing a spawning location or that A Creek fish were 50 times more likely than C Creek fish to choose a spawning location randomly. The greater dispersal to C Creek was unlikely to have been motivated by convenience because C Creek is farther than A Creek from

the outlet of Little Togiak Lake, although sockeye salmon homing movements within rearing lakes can be indirect (e.g. Young & Woody 2007), making it difficult to predict which stream a fish will encounter first. While we cannot definitively identify the characteristics that salmon used to evaluate the suitability of spawning streams in this system, the better protection from bear predation afforded by C Creek's greater depth, woody debris and undercut banks may have attracted dispersers from A Creek. Alternately, dispersal could be more frequent from A Creek than from C Creek because of genetic differences between the populations or differences in the strength of olfactory cues in the two habitats.

Despite the observed association between exploratory behavior and dispersal in this system, dispersers were less likely to be observed at the mouth of their natal stream than philopatric individuals, suggesting that not all dispersers engage in exploratory behavior before dispersing. However, an alternate explanation for this pattern is that dispersers spent less time at the mouth of their natal stream than did philopatric individuals, lowering their chances of being observed in our infrequent surveys. Moreover, the proportion of dispersers observed at the mouth of their natal stream was roughly half that of philopatric individuals, indicating that successful homing to the natal stream mouth was common. It remains unclear, however, to what extent individuals evaluate a spawning stream when holding at its mouth, as well as what factors might cause a fish to leave its natal stream in search of another spawning habitat.

We did not consider exploratory behavior outside of this study's two focal streams, despite the fact that exploration of potential spawning habitat likely occurred throughout the extensive freshwater migration of individuals from A and C creeks (as in Keefer *et al.* 2008). However, because we were interested in whether spawning site selection could influence dispersal between these two proximate populations, we restricted our definition of exploratory

behavior to that which would allow an individual to directly compare A Creek with C Creek. Nevertheless, we hope that our findings may inform analyses of dispersal at greater distances that are more difficult to confidently quantify but are nonetheless very important evolutionarily.

It should be noted that the overall percentage of dispersers (3.4%) was less than the nominal error rate for parentage assignment (5%), suggesting that many or all of our putative dispersers could represent misassigned philopatric individuals. However, this error rate corresponds to a parent-offspring pair assignment. We only classified as dispersers individuals who were assigned both parents from the creek in which they did not spawn. Thus the likelihood of an individual being spuriously categorized as a disperser was much lower than the probability of assigning a single erroneous parent to that individual. Moreover, we found that the spawning locations of the two parents differed in only 1% of inferred pairs, indicating that our parent-offspring pair assignment accuracy was in fact higher than 95%. Finally, any incorrect disperser classifications should have had the effect of rendering the behavior of the putative disperser and philopatric groups more similar within each spawning population, reducing our power to detect differences between them in exploratory behavior. On the other hand, errors in natal population assignment would be expected to increase the differences between putative dispersers and philopatric individuals from the same natal population. Therefore, our observation that dispersers did not always visit their natal habitat is vulnerable to this effect, and not conservative.

Our relatively limited sampling scheme provides only an initial, broad investigation of disperser behavior in this system. We conducted only one survey per day of each stream, and we may therefore have missed many brief incursions into streams made by exploratory individuals. We also probably underestimated the proportion of individuals that visited stream mouths due to the less frequent and less exhaustive surveys we made at those locations. Thus, our estimates of

8-11% for the natal stream and 29-58% for the natal stream mouth may be far lower than the actual proportion of dispersers who successfully home before spawning in a non-natal habitat. Errors in reading tag IDs may also have produced spurious observations of exploratory behavior, although these errors should not affect the comparisons of exploratory behavior between dispersers and philopatric individuals. Sampling bias between the streams or cohorts may also have introduced errors into the analysis, but, again, these errors should have been spread evenly between dispersers and philopatric individuals, leaving our tests unbiased.

Further work with more comprehensive temporal sampling (e.g. acoustic tags) could provide a precise estimate of the proportion of dispersers that never reach their natal habitat. More detailed movement data could also reveal whether exploratory dispersers usually visit their natal stream before or after their first visit to the non-natal spawning habitat, and whether that order is associated with likelihood of dispersal. This information could also offer insight into the relative importance of natal habitat dissatisfaction versus failure of the natal habitat recognition mechanism in the motivation of exploratory dispersers.

In summary, we found that dispersers exhibited more exploratory behavior than philopatric individuals, suggesting that habitat selection may influence dispersal decisions within a metapopulation of wild sockeye salmon. Because salmon are highly sensitive to local physical habitat and demographic factors, annual variability in the conditions of proximate subpopulations could strongly affect the amount and direction of gene flow between them. This prediction could be tested empirically by quantifying the relationship between temporal and spatial variation in habitat quality (perhaps measured by mean reproductive success), competition and the rate of dispersal into and out of a local population.

Table 7: Observed locations for all adults spawning in A or C creeks in 2008 and 2009. The stream in which each individual spawned was determined by observational data (see Methods). The natal stream of each individual was determined by parentage reconstruction and observational data for the parents.

Natal Stream	Spawning Stream	Total Individuals	Observed in Spawning Stream Only	Observed in Other Stream	Observed at Other Stream Mouth	Observed in Other Stream and at Mouth
A	A	755	720	9	24	2
A	C	50	31	1	14	4
C	A	1	1	0	0	0
C	C	679	654	1	23	1

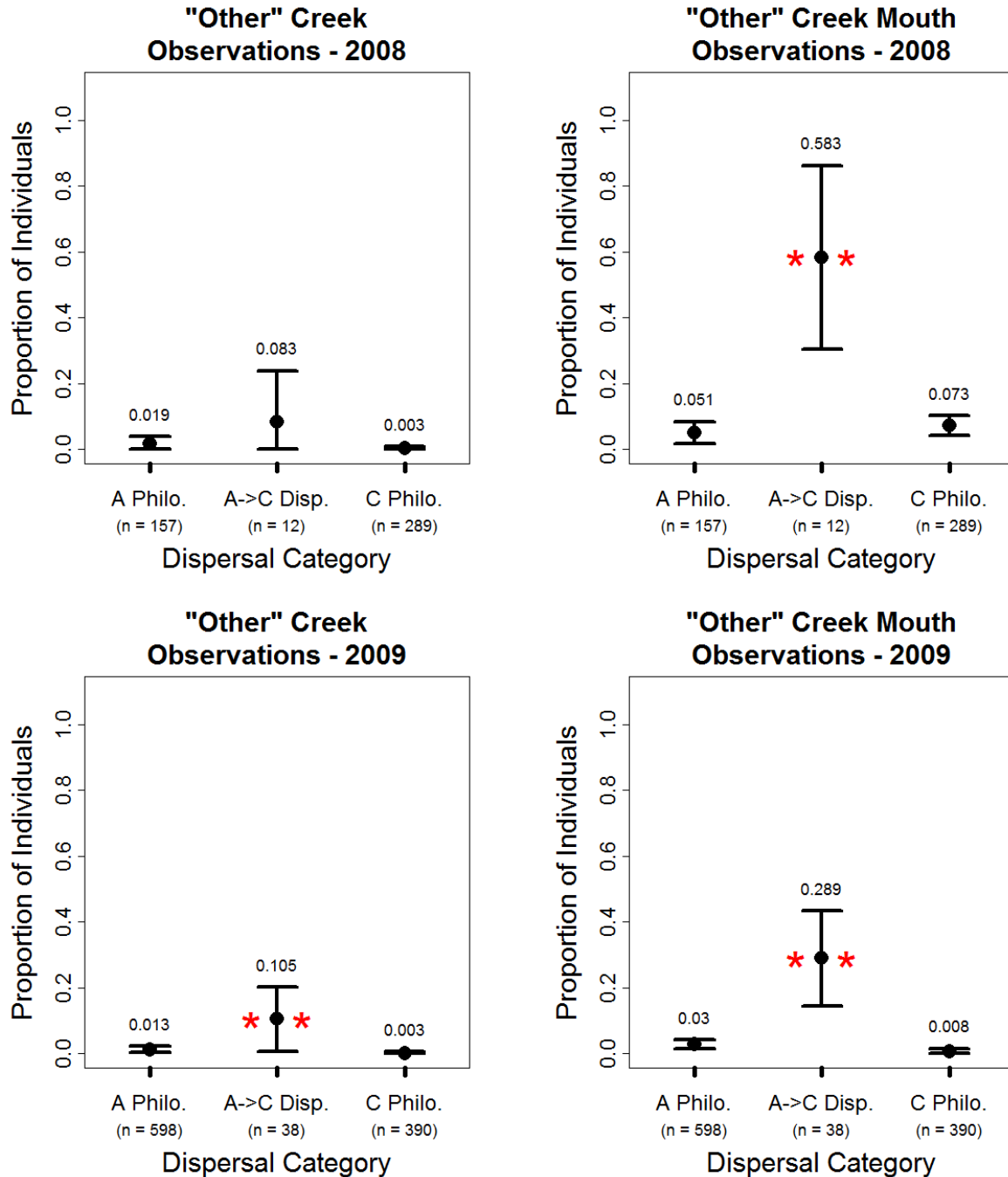


Figure 6: Proportion of stream-spawning individuals that returned in 2008 (top) and 2009 (bottom) that were observed in the stream in which they did not spawn (left) or at the mouth of that stream (right). Error bars indicate 95% confidence intervals for the observed proportions (calculated using the Wald formula for the standard error of a proportion, Zar 2009).

* indicates that the proportion of dispersers was significantly different from that of philopatric individuals in A Creek or C Creek (A on the left, C on the right), as determined by a Fisher's exact test.

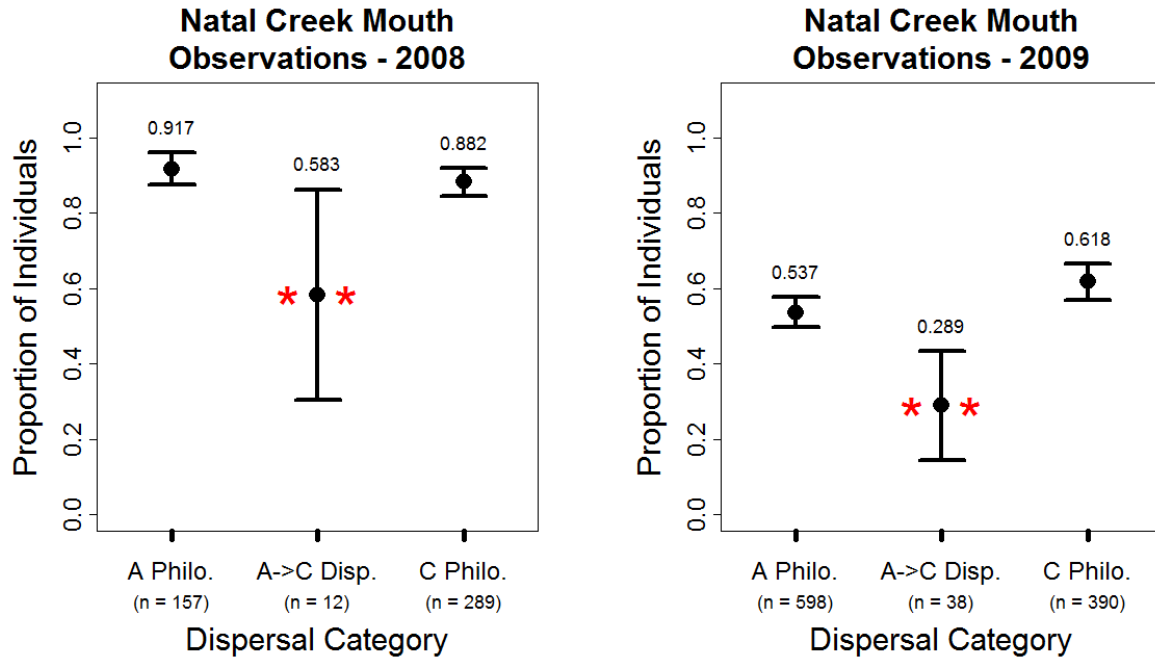


Figure 7: Proportion of stream-spawning individuals that returned in 2008 (left) and 2009 (right) that were observed at the mouth of their natal stream, as inferred from parentage. Error bars indicate 95% confidence intervals for the observed proportions (calculated using the Wald formula for the standard error of a proportion, Zar 2009).

* indicates that the proportion of dispersers was significantly different from that of philopatric individuals in A Creek or C Creek (A on the left, C on the right), as determined by a Fisher's exact test.

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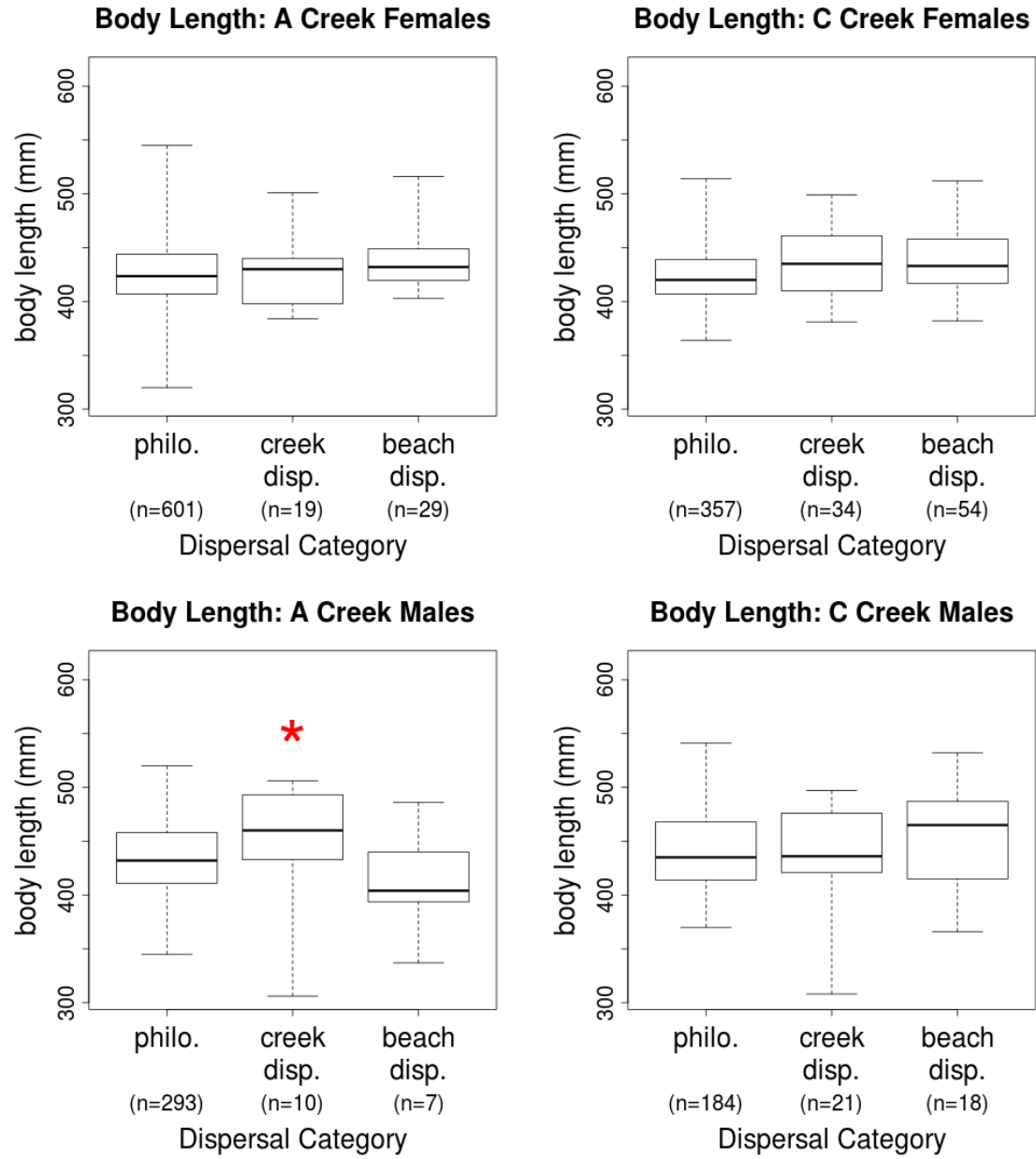
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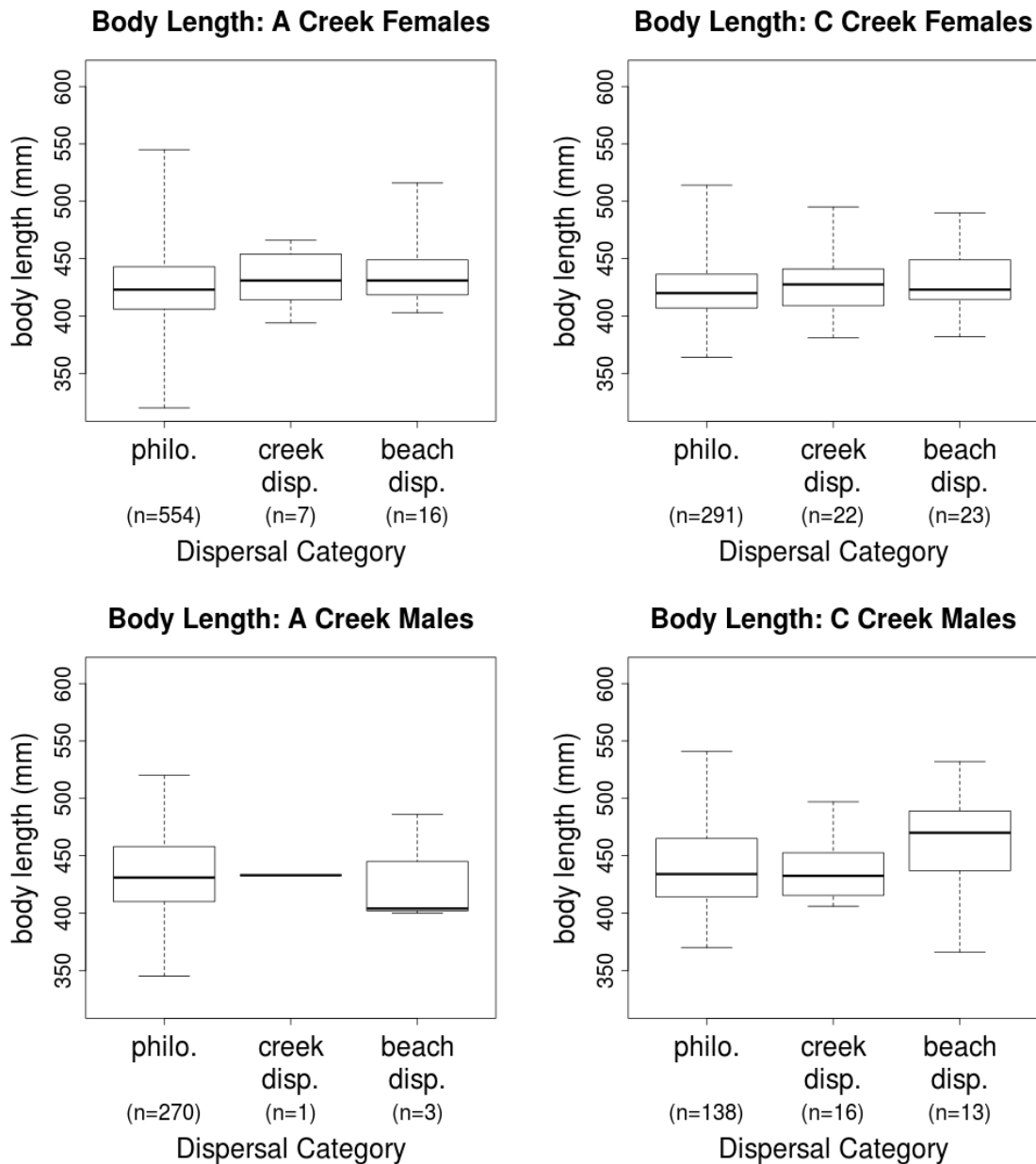
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Supplementary Figures

A: No assignment cutoff used



B: 95% assignment cutoff

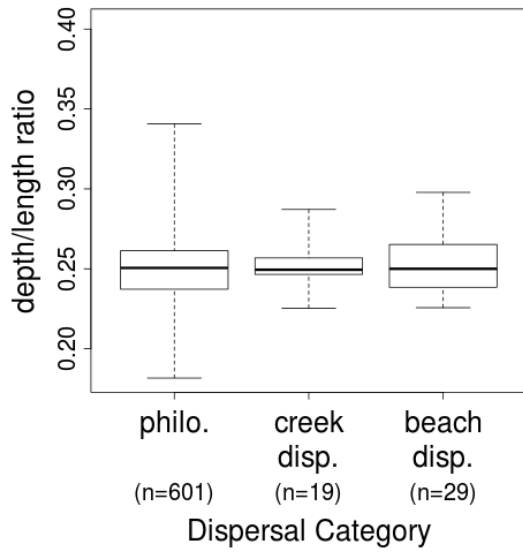


Supplementary Figure 1: Body length distributions by dispersal category for each creek and sex. Boxplots of continuous variables show the median (dark horizontal line), 25% and 75% quartiles (the box) and the entire range (the whiskers).

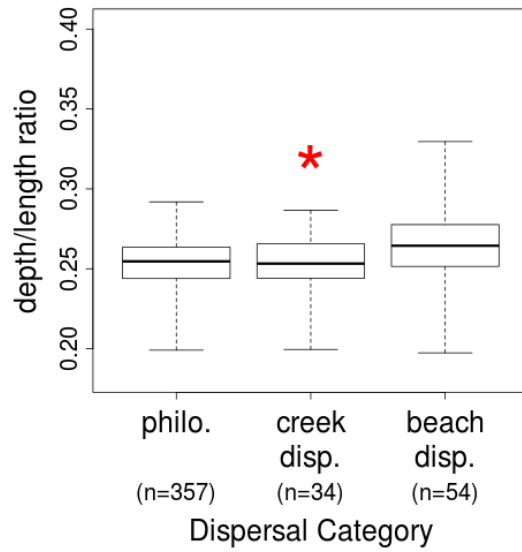
* indicates a statistically significant difference between the mean of that disperser group and that of philopatric individuals based on generalized linear models assuming a gamma distribution.

A: No assignment cutoff used

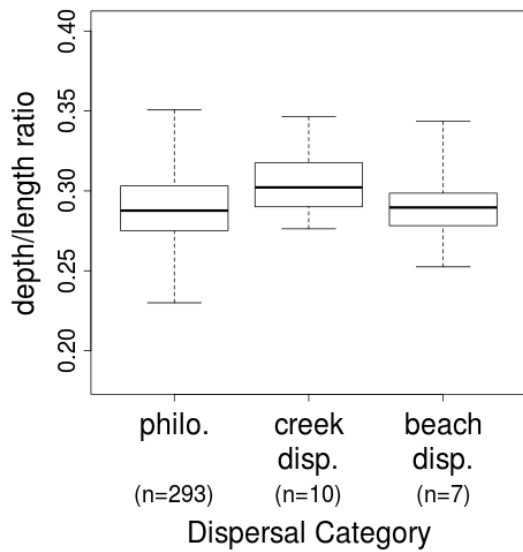
Std. Body Depth: A Creek Females



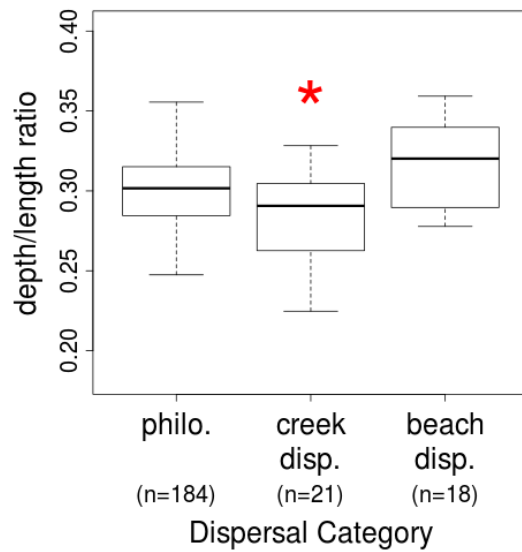
Std. Body Depth: C Creek Females



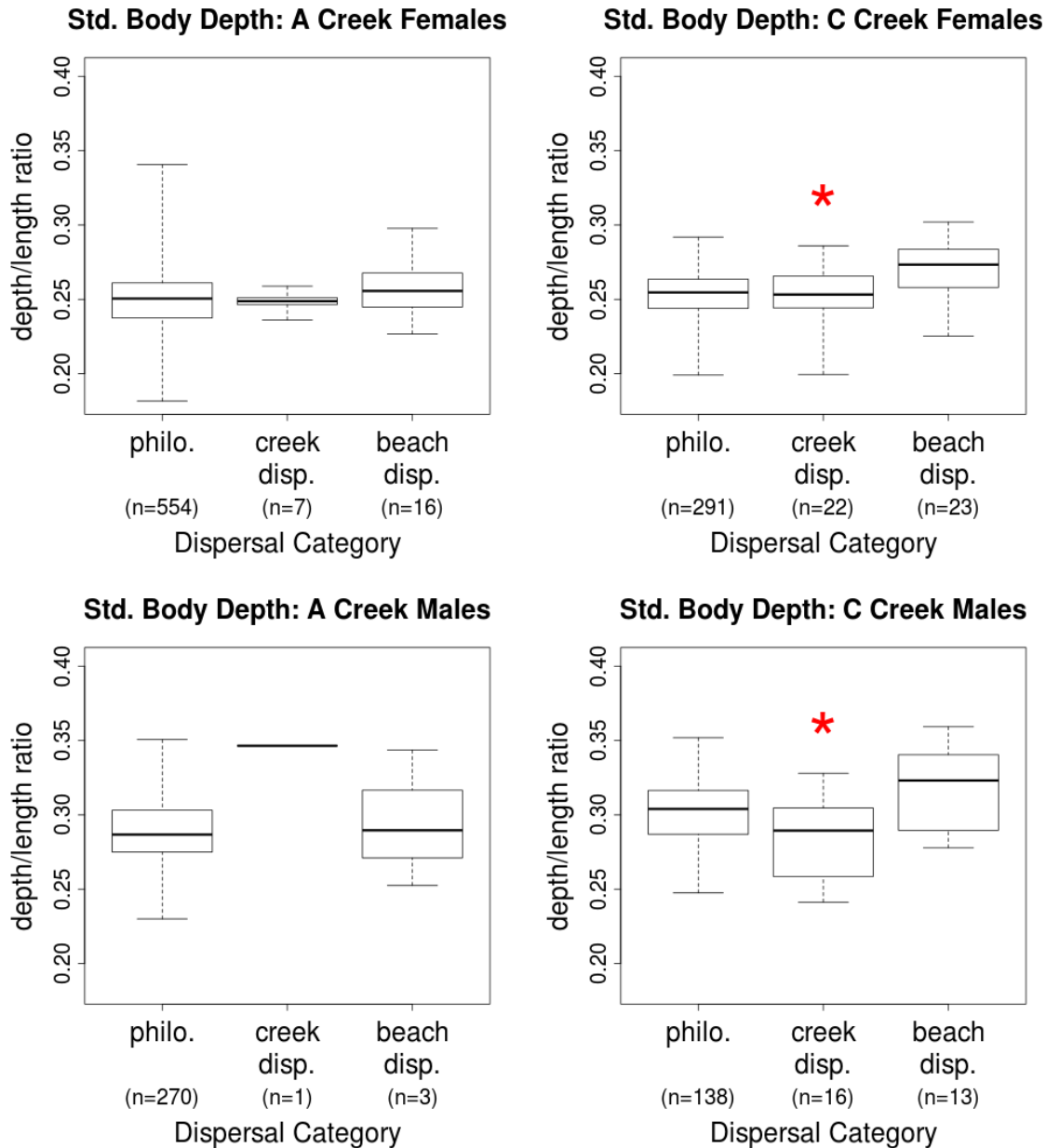
Std. Body Depth: A Creek Males



Std. Body Depth: C Creek Males



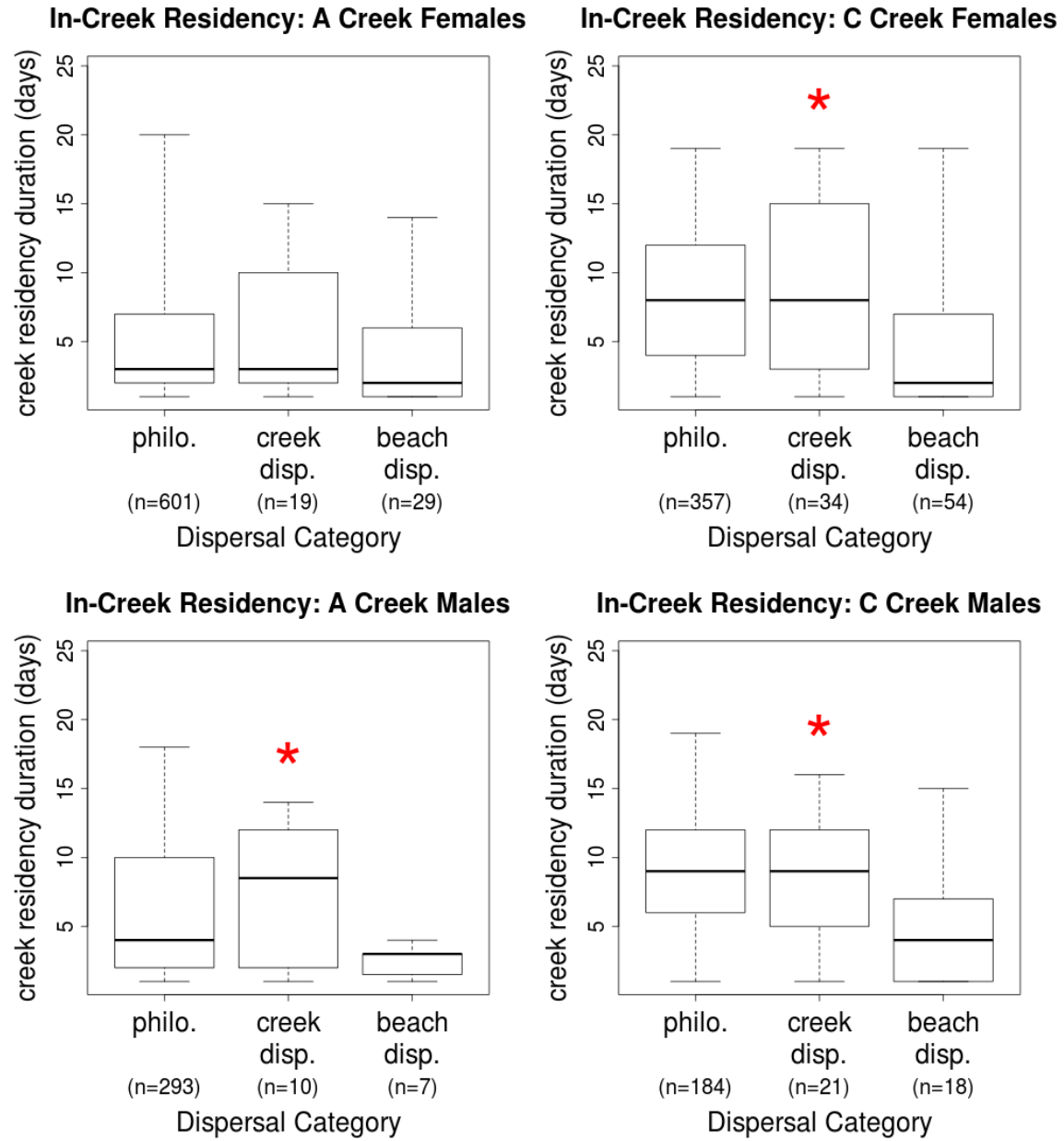
B: 95% assignment cutoff



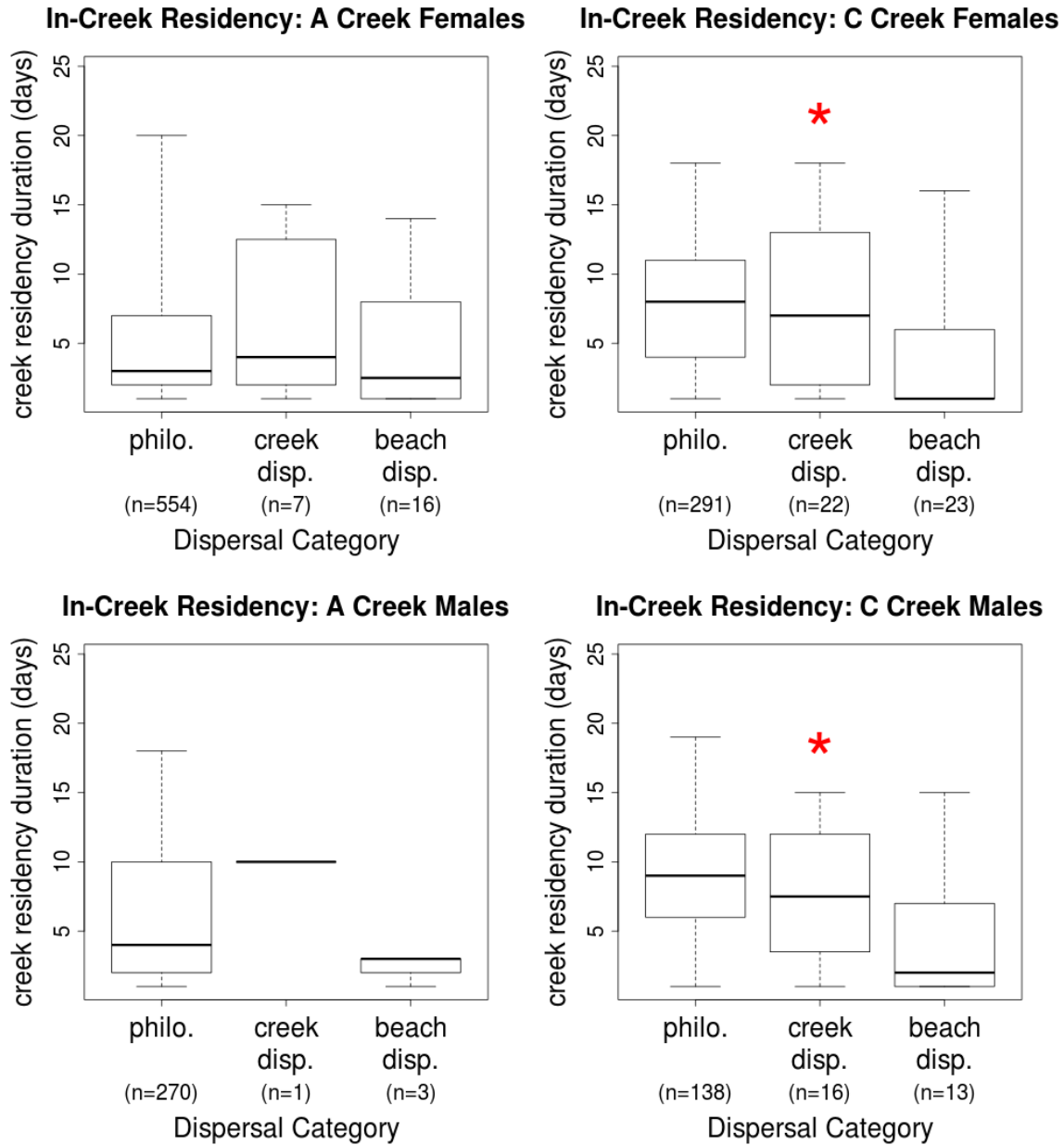
Supplementary Figure 2: Body shape (body depth / body length ratio) distributions by dispersal category for each creek and sex. Boxplots of continuous variables show the median (dark horizontal line), 25% and 75% quartiles (the box) and the entire range (the whiskers).

* indicates a statistically significant difference between the mean of that disperser group and that of philopatric individuals based on generalized linear models assuming a gamma distribution.

A: No assignment cutoff used



B: 95% assignment cutoff

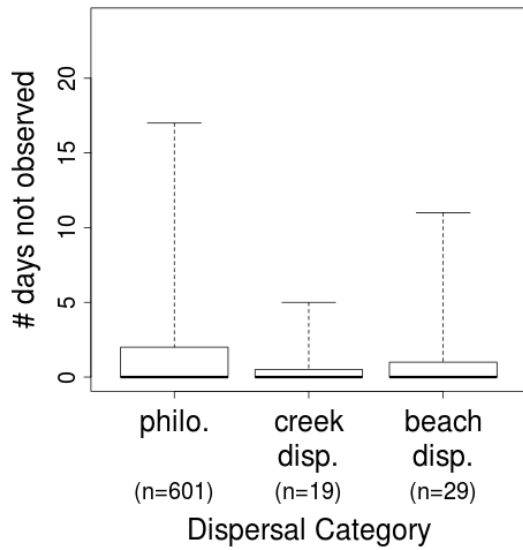


Supplementary Figure 3: In-creek residency period distributions by dispersal category for each creek and sex. Boxplots of continuous variables show the median (dark horizontal line), 25% and 75% quartiles (the box) and the entire range (the whiskers).

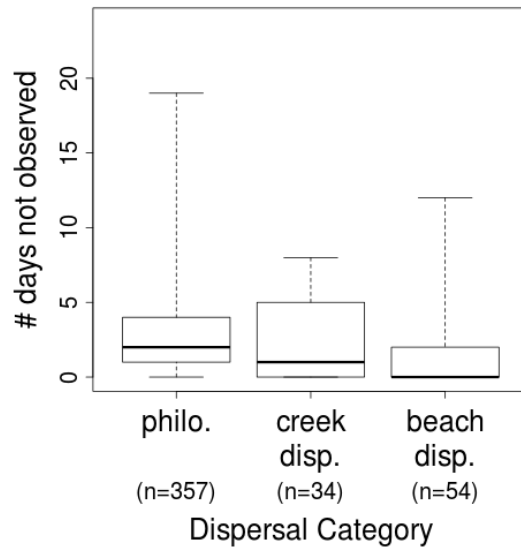
* indicates a statistically significant difference between the mean of that disperser group and that of philopatric individuals based on generalized linear models assuming a gamma distribution.

A: No assignment cutoff used

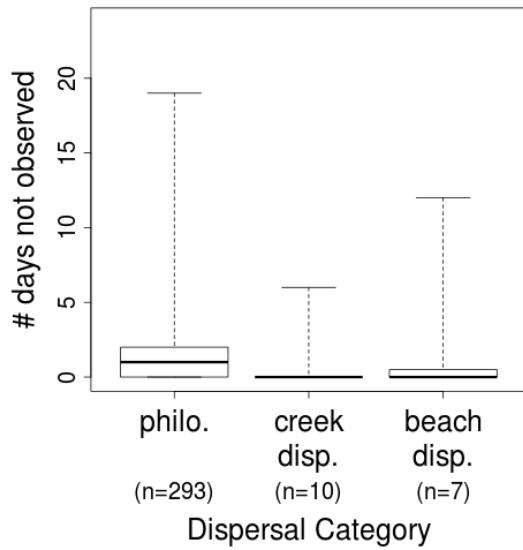
Missing Observations: A Creek Females



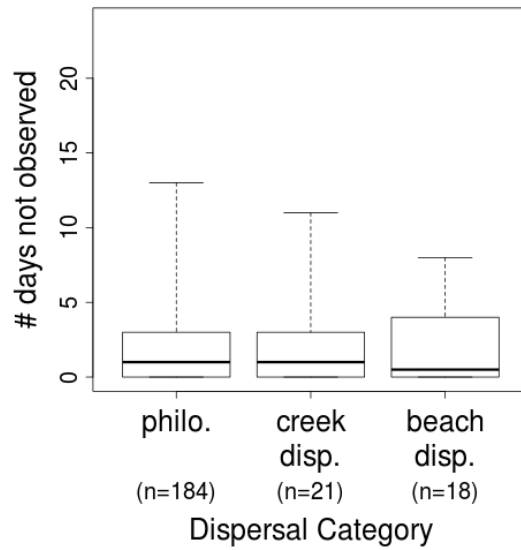
Missing Observations: C Creek Females



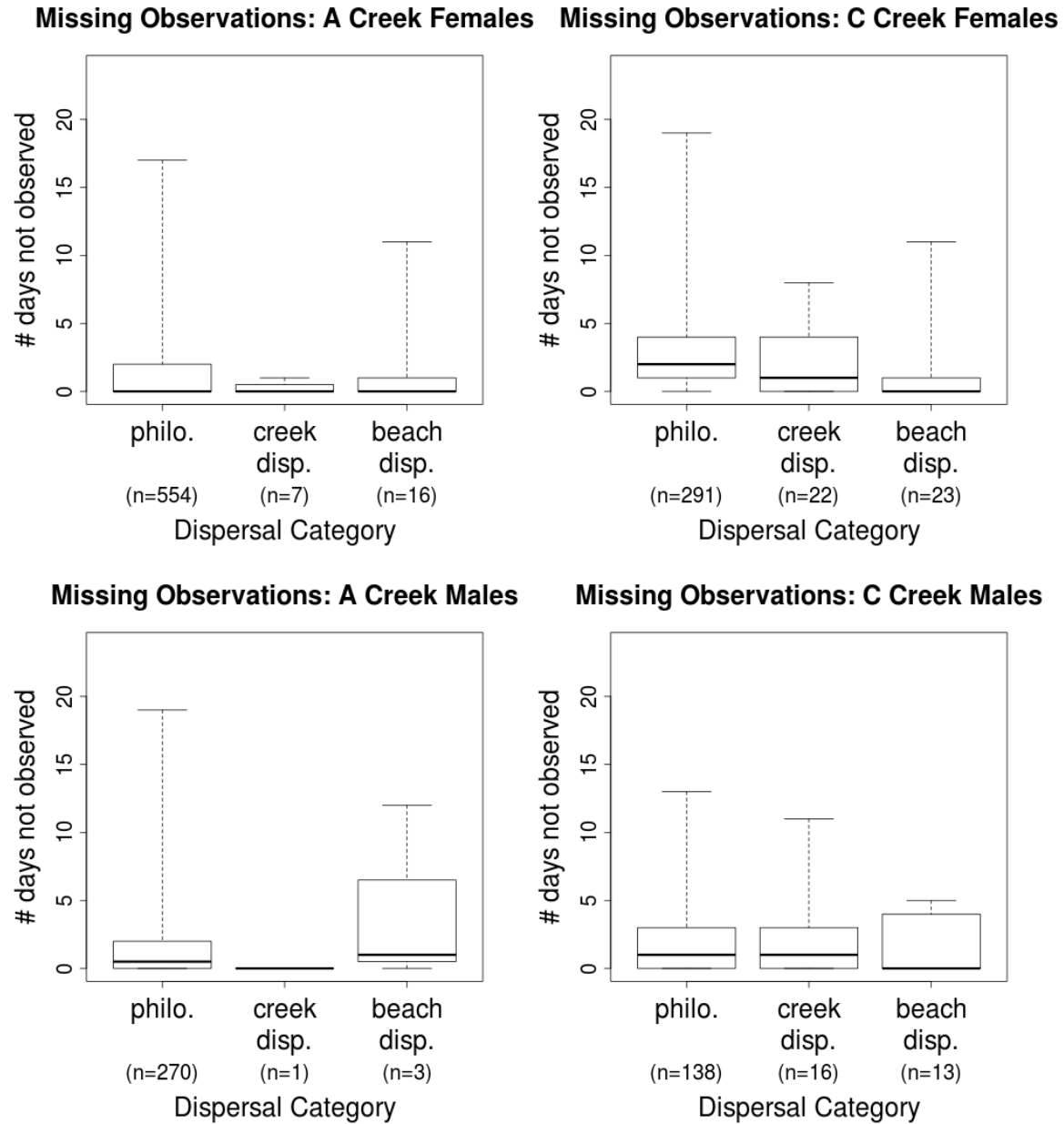
Missing Observations: A Creek Males



Missing Observations: C Creek Males



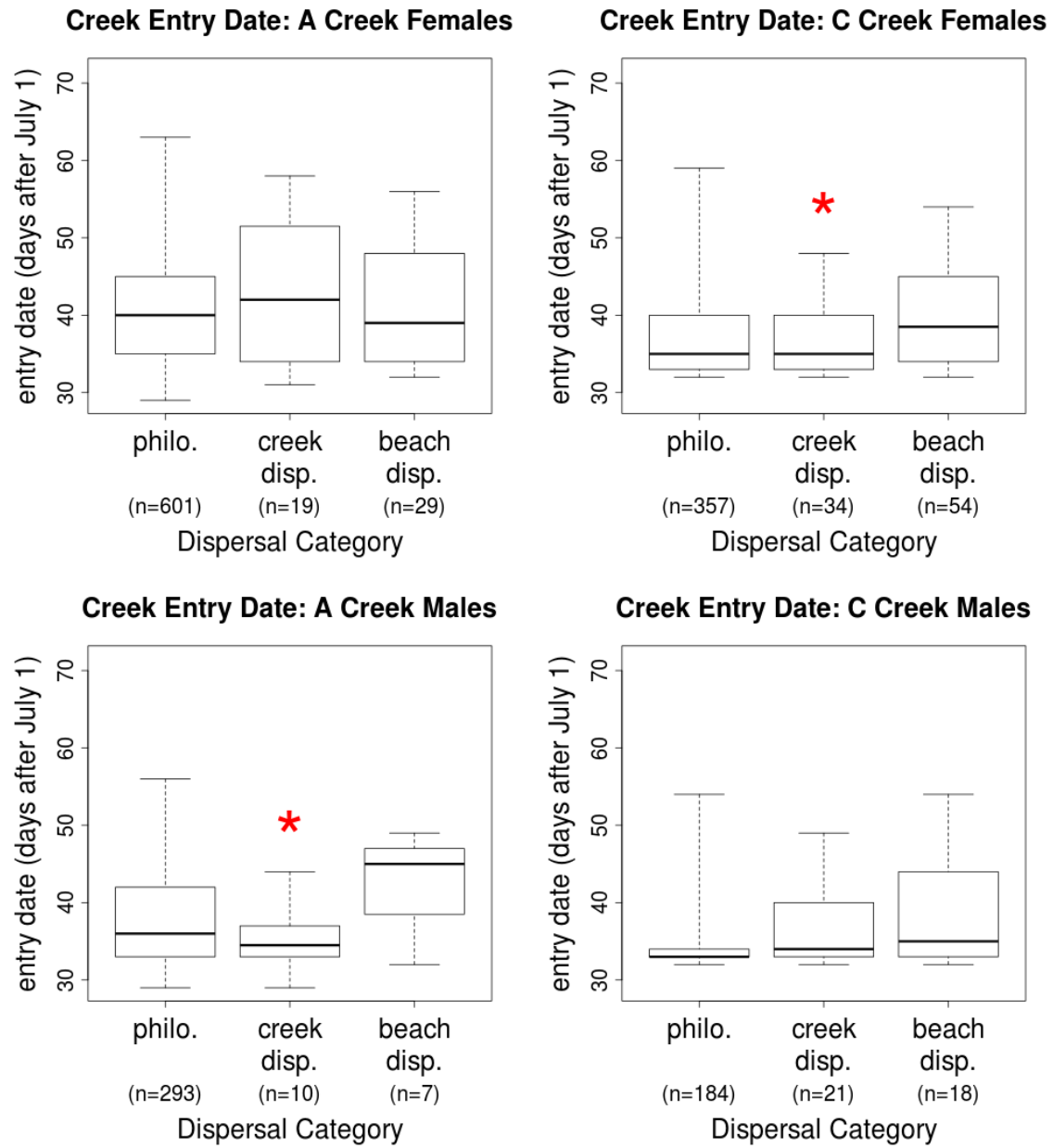
B: 95% assignment cutoff



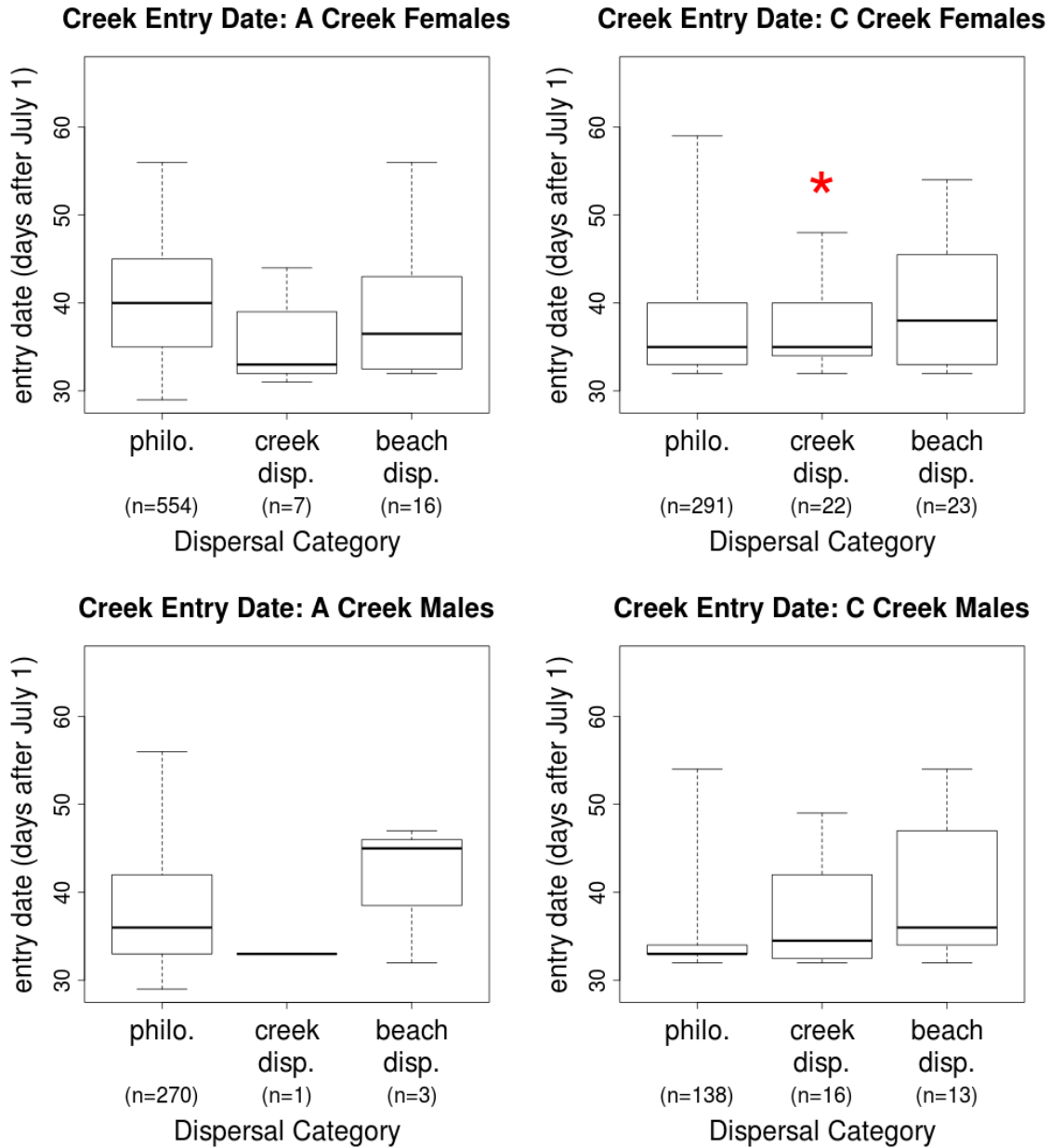
Supplementary Figure 4: Distributions of number of missing observations by dispersal category for each creek and sex. Boxplots of continuous variables show the median (dark horizontal line), 25% and 75% quartiles (the box) and the entire range (the whiskers).

* indicates a statistically significant difference between the mean of that disperser group and that of philopatric individuals based on generalized linear models assuming a gamma distribution.

A: No assignment cutoff used



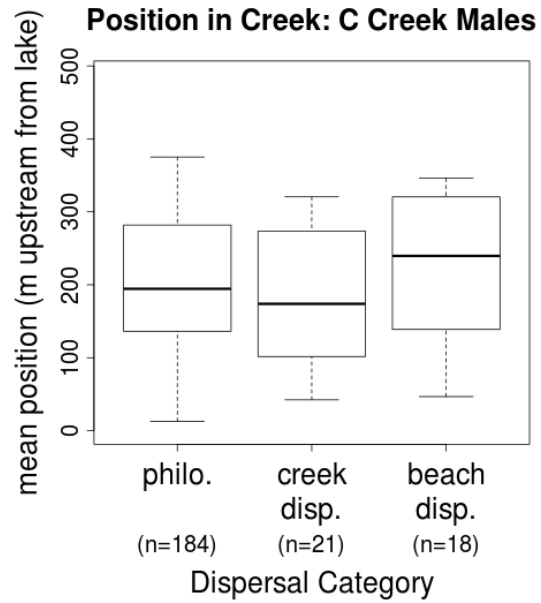
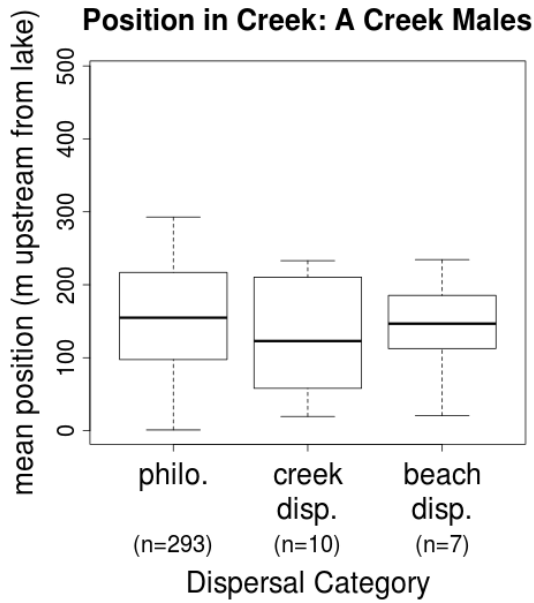
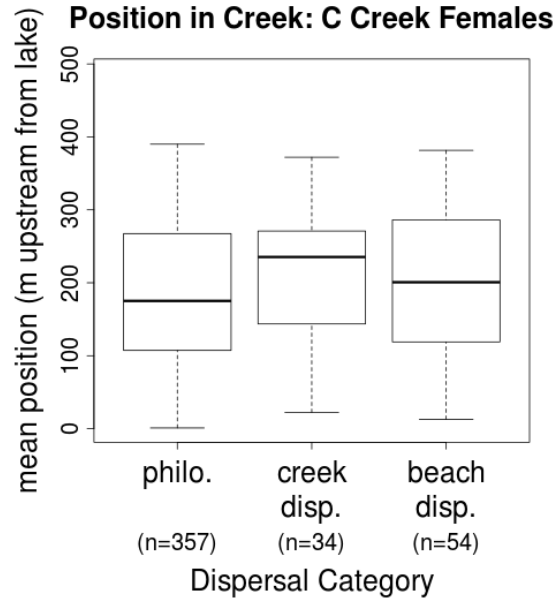
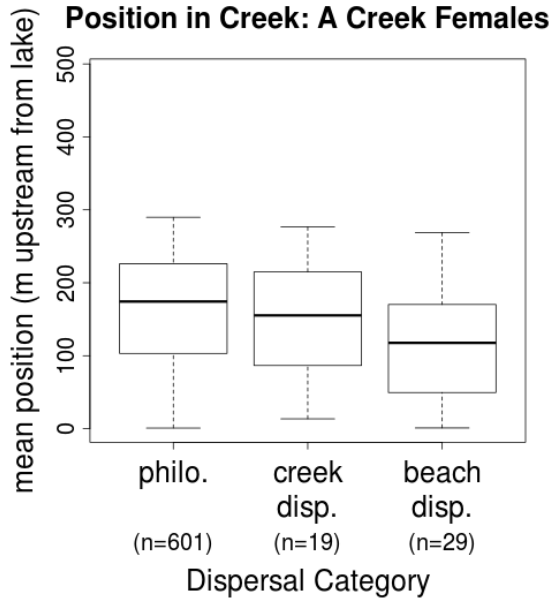
B: 95% assignment cutoff



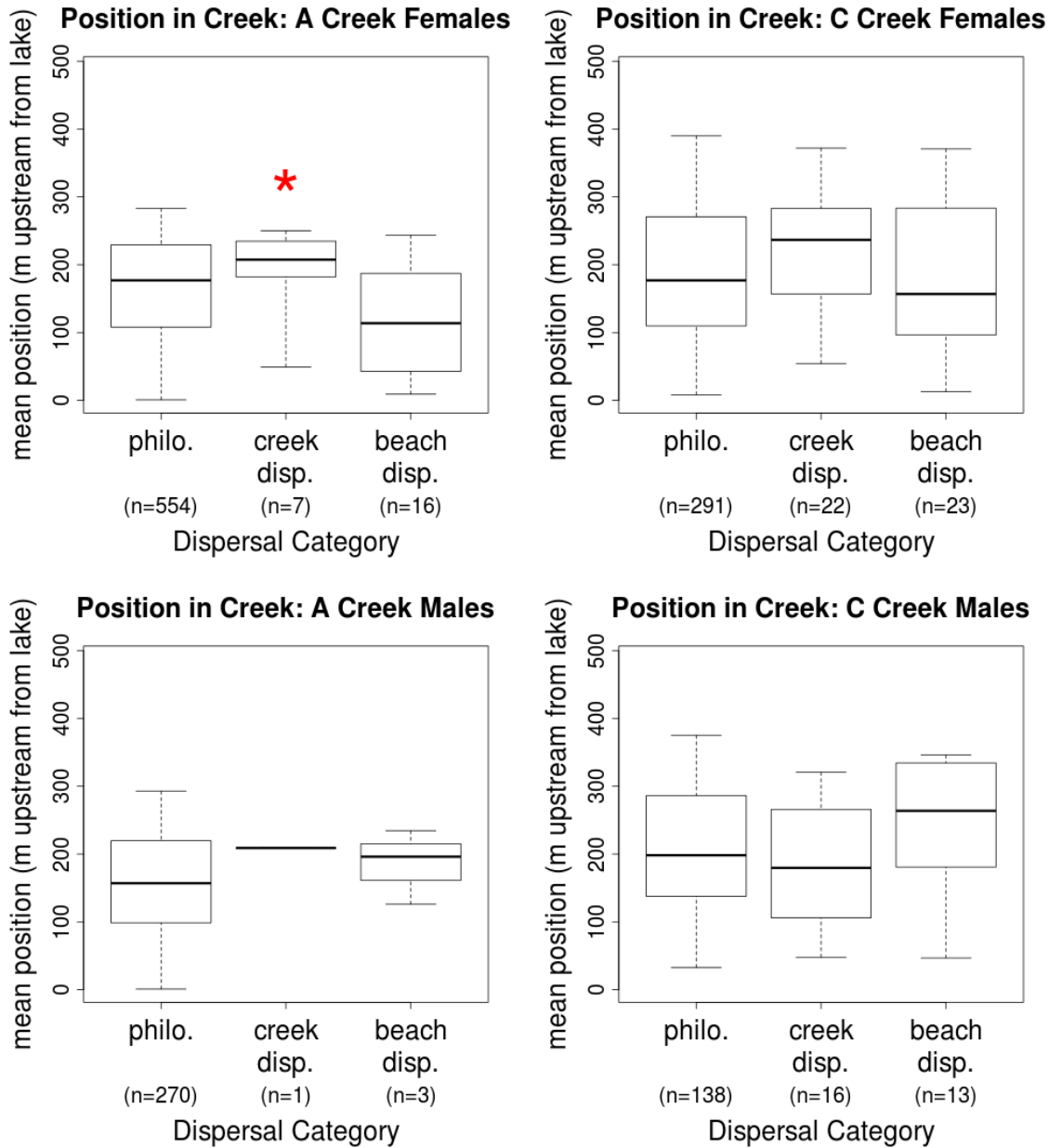
Supplementary Figure 5: Creek entry date distributions by dispersal category for each creek and sex. Boxplots of continuous variables show the median (dark horizontal line), 25% and 75% quartiles (the box) and the entire range (the whiskers).

* indicates a statistically significant difference between the mean of that disperser group and that of philopatric individuals based on generalized linear models assuming a gamma distribution.

A: No assignment cutoff used



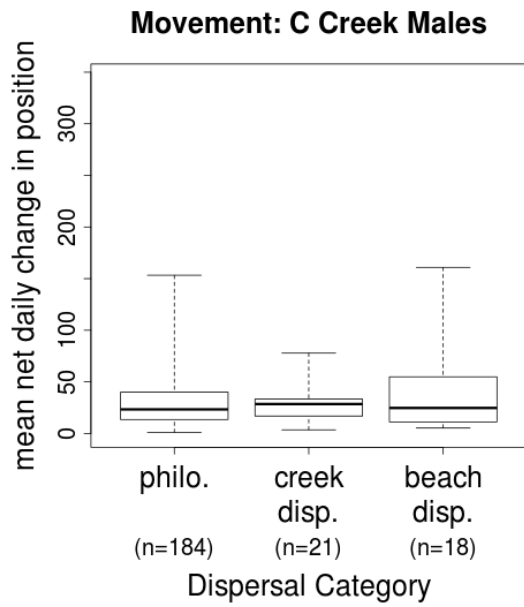
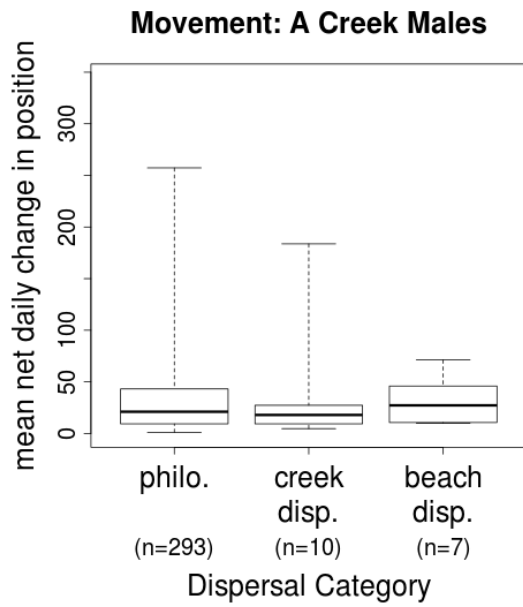
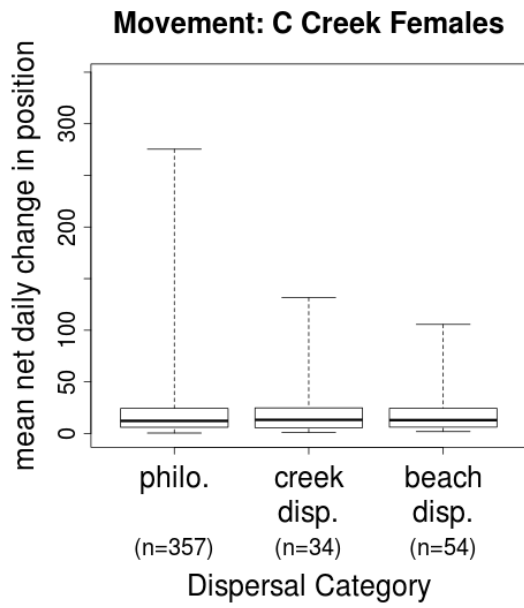
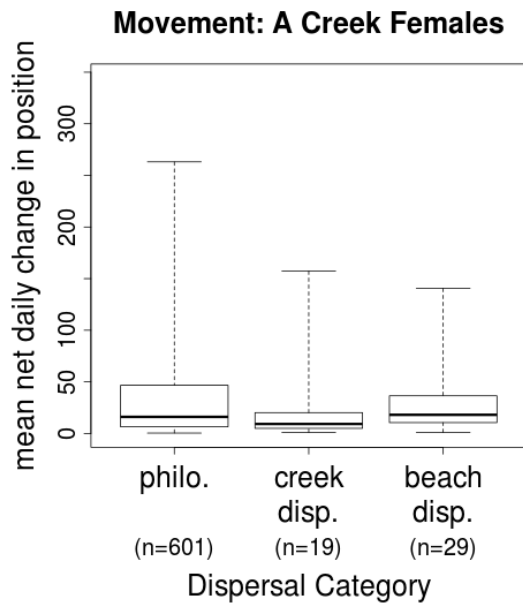
B: 95% assignment cutoff



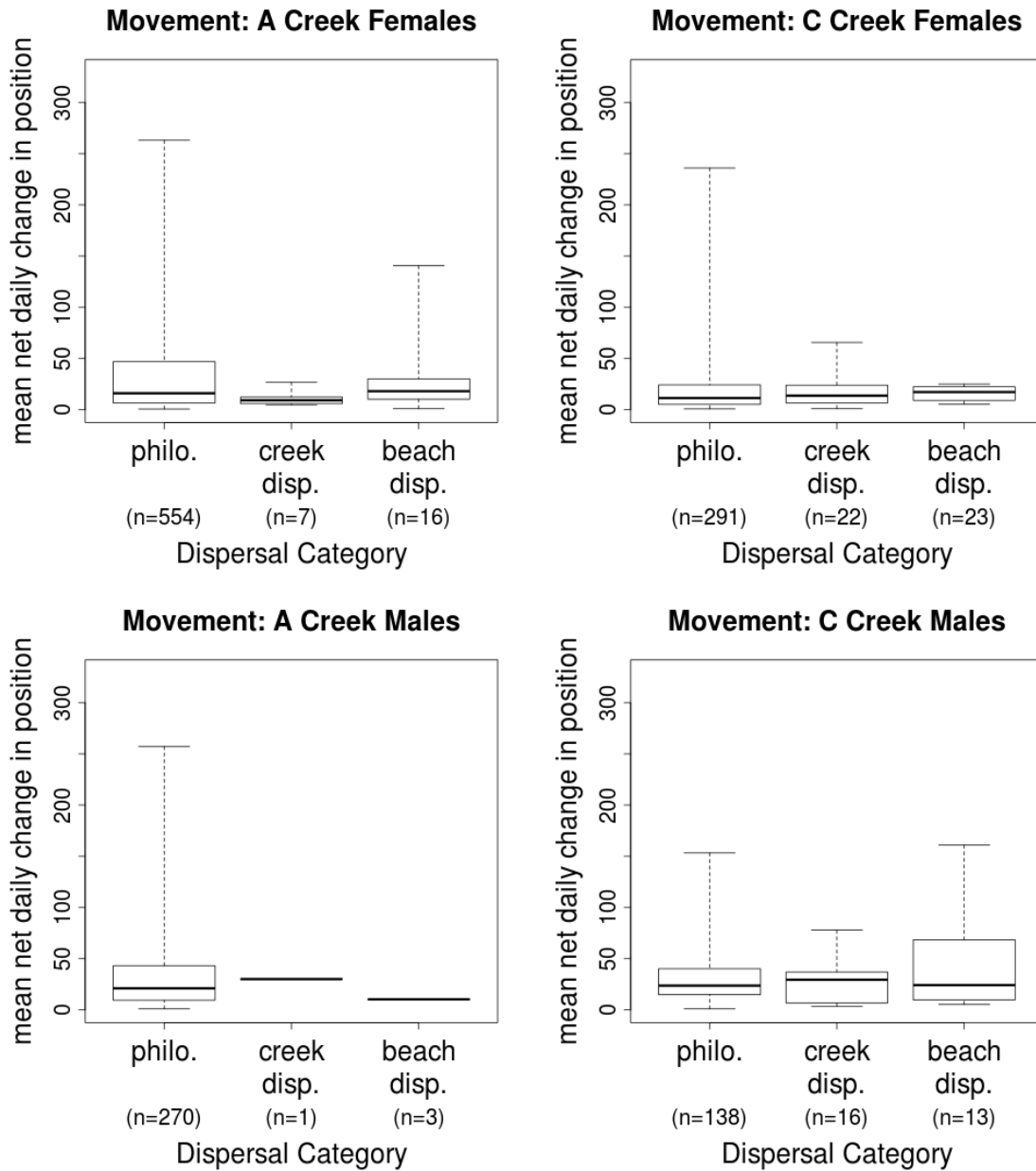
Supplementary Figure 6: Creek position distributions by dispersal category for each creek and sex. Boxplots of continuous variables show the median (dark horizontal line), 25% and 75% quartiles (the box) and the entire range (the whiskers).

* indicates a statistically significant difference between the mean of that disperser group and that of philopatric individuals based on generalized linear models assuming a gamma distribution.

A: No assignment cutoff used



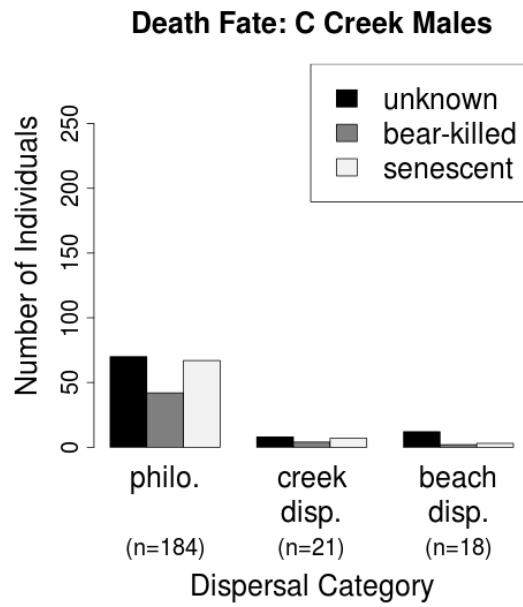
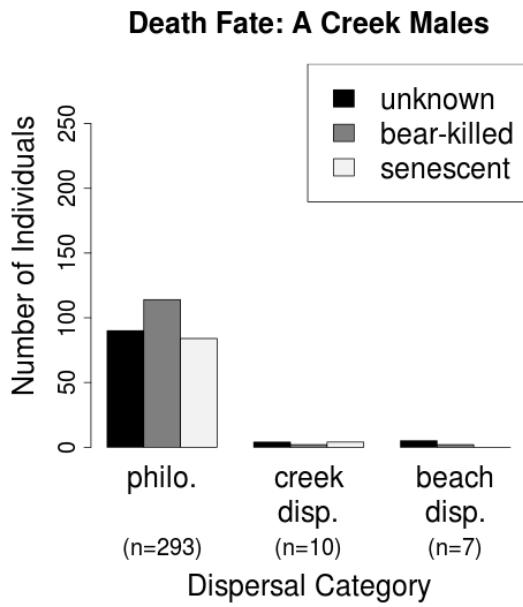
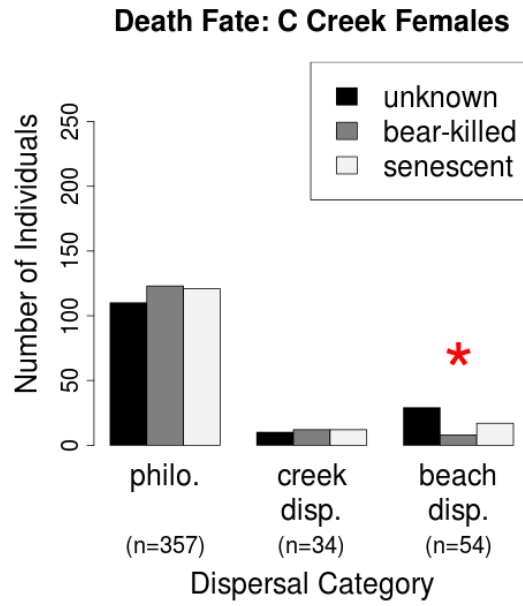
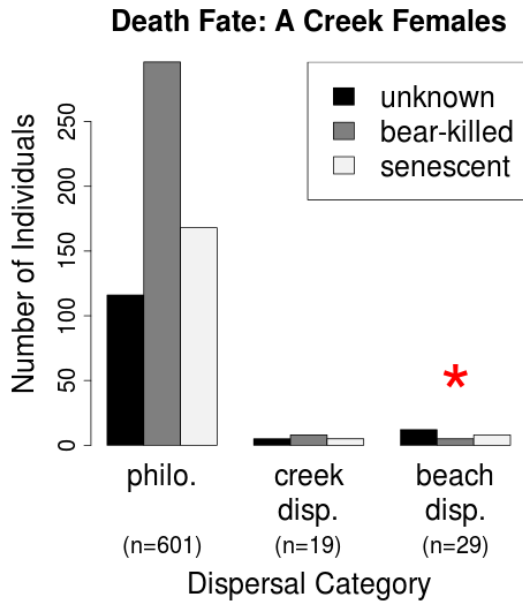
B: 95% assignment cutoff



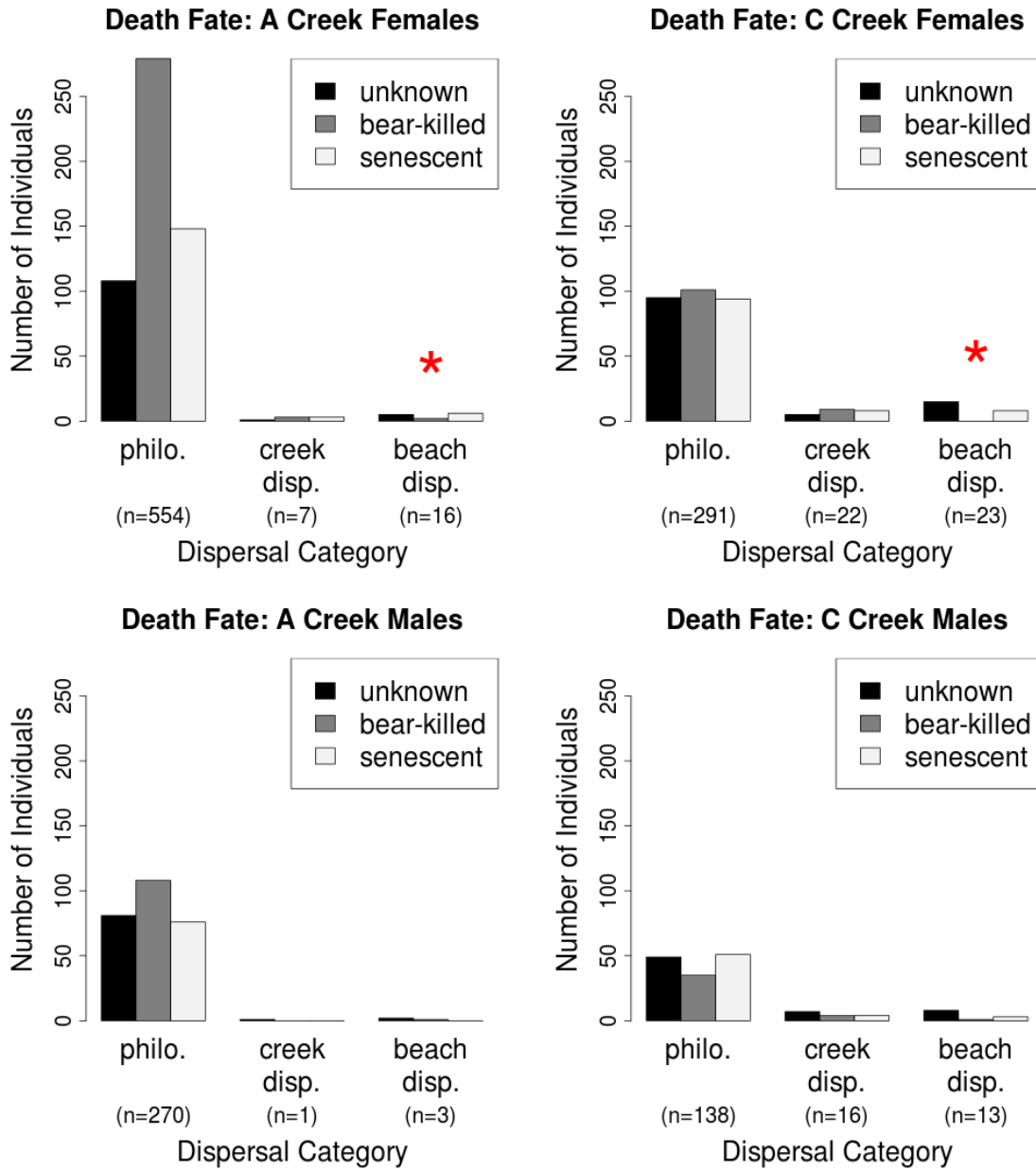
Supplementary Figure 7: Mean daily movement distributions by dispersal category for each creek and sex. Boxplots of continuous variables show the median (dark horizontal line), 25% and 75% quartiles (the box) and the entire range (the whiskers).

* indicates a statistically significant difference between the mean of that disperser group and that of philopatric individuals based on generalized linear models assuming a gamma distribution.

A: No assignment cutoff used

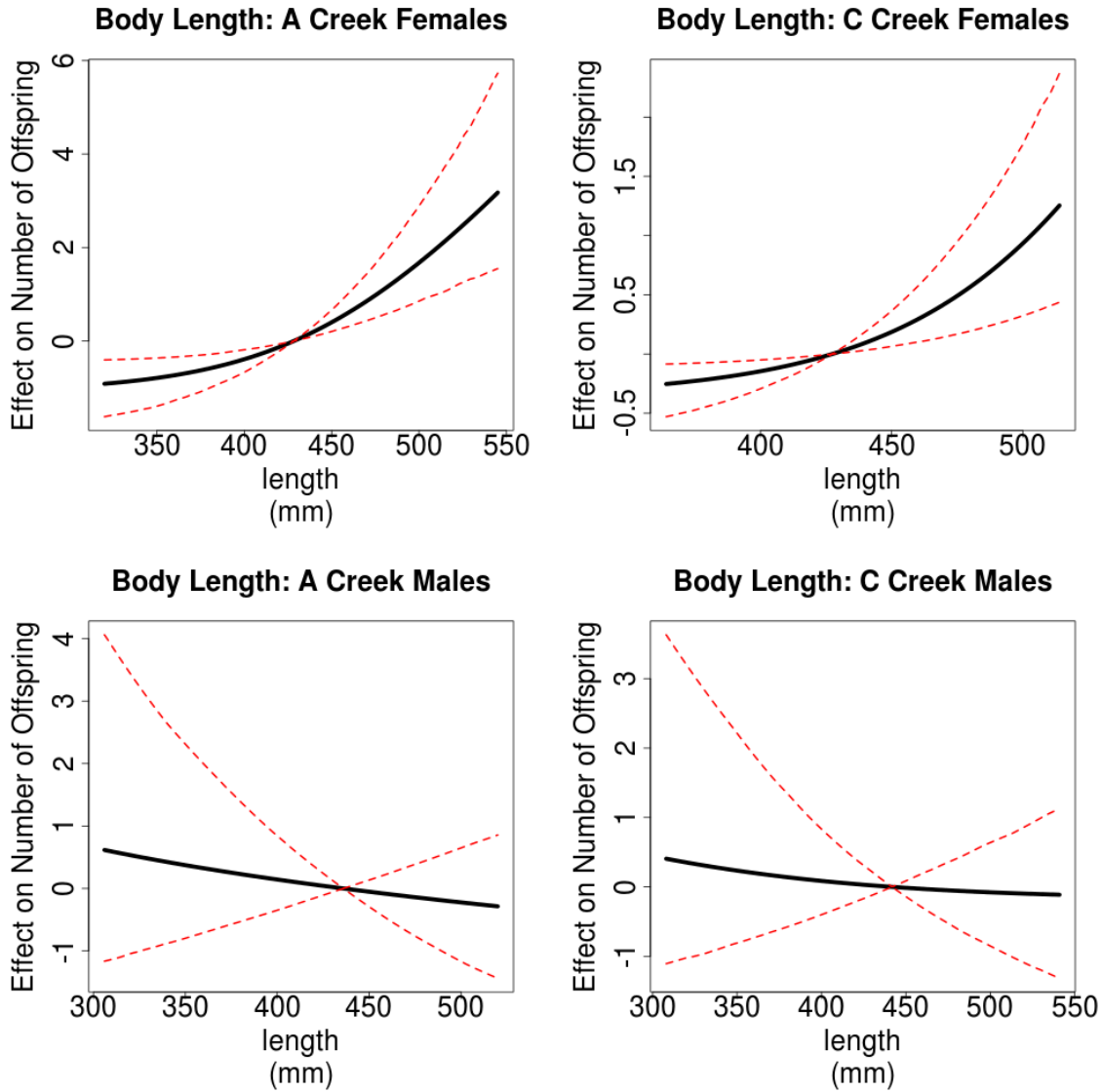


B: 95% assignment cutoff

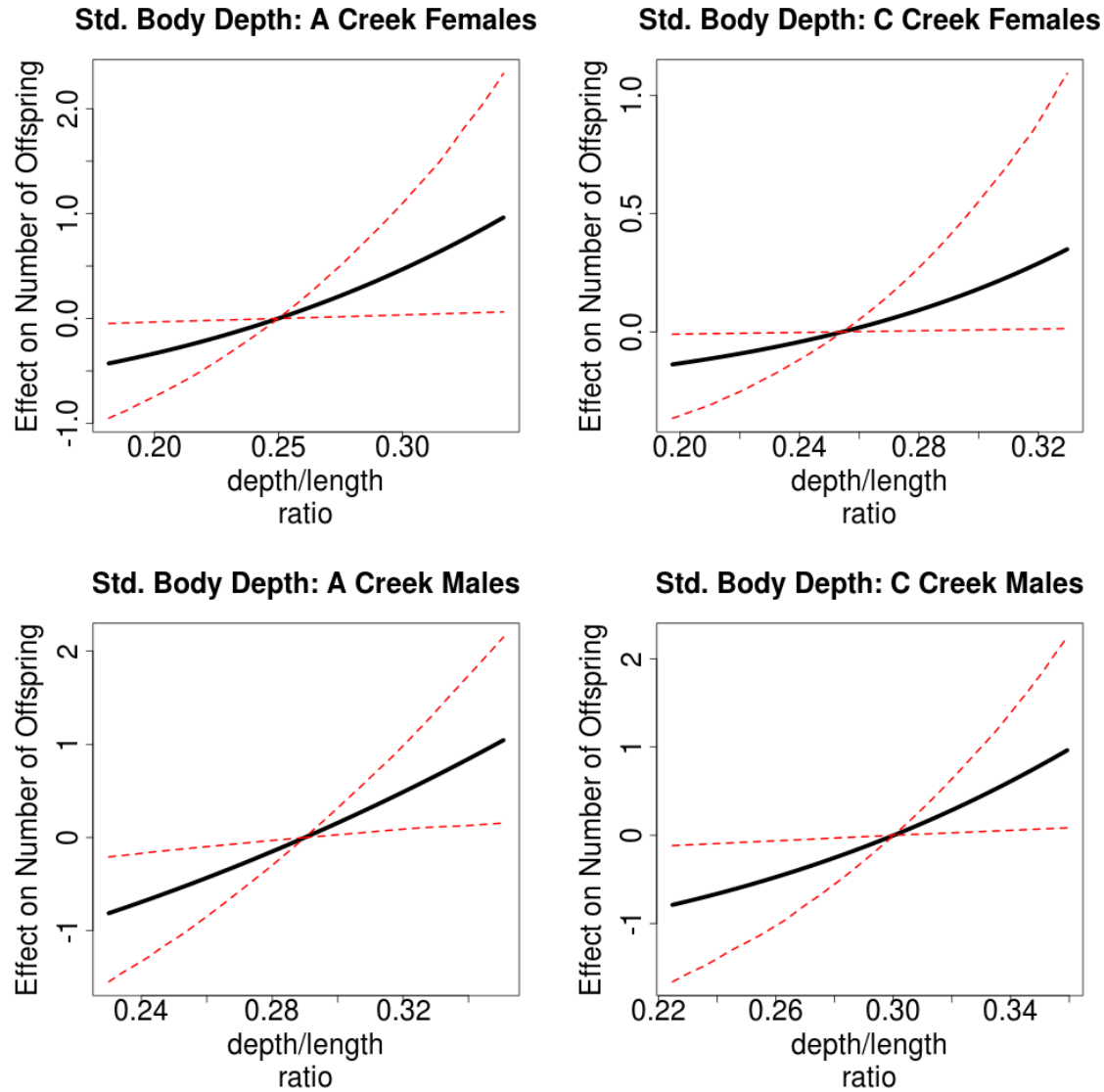


Supplementary Figure 8: Death fates by dispersal category for each creek and sex. Boxplots of continuous variables show the median (dark horizontal line), 25% and 75% quartiles (the box) and the entire range (the whiskers).

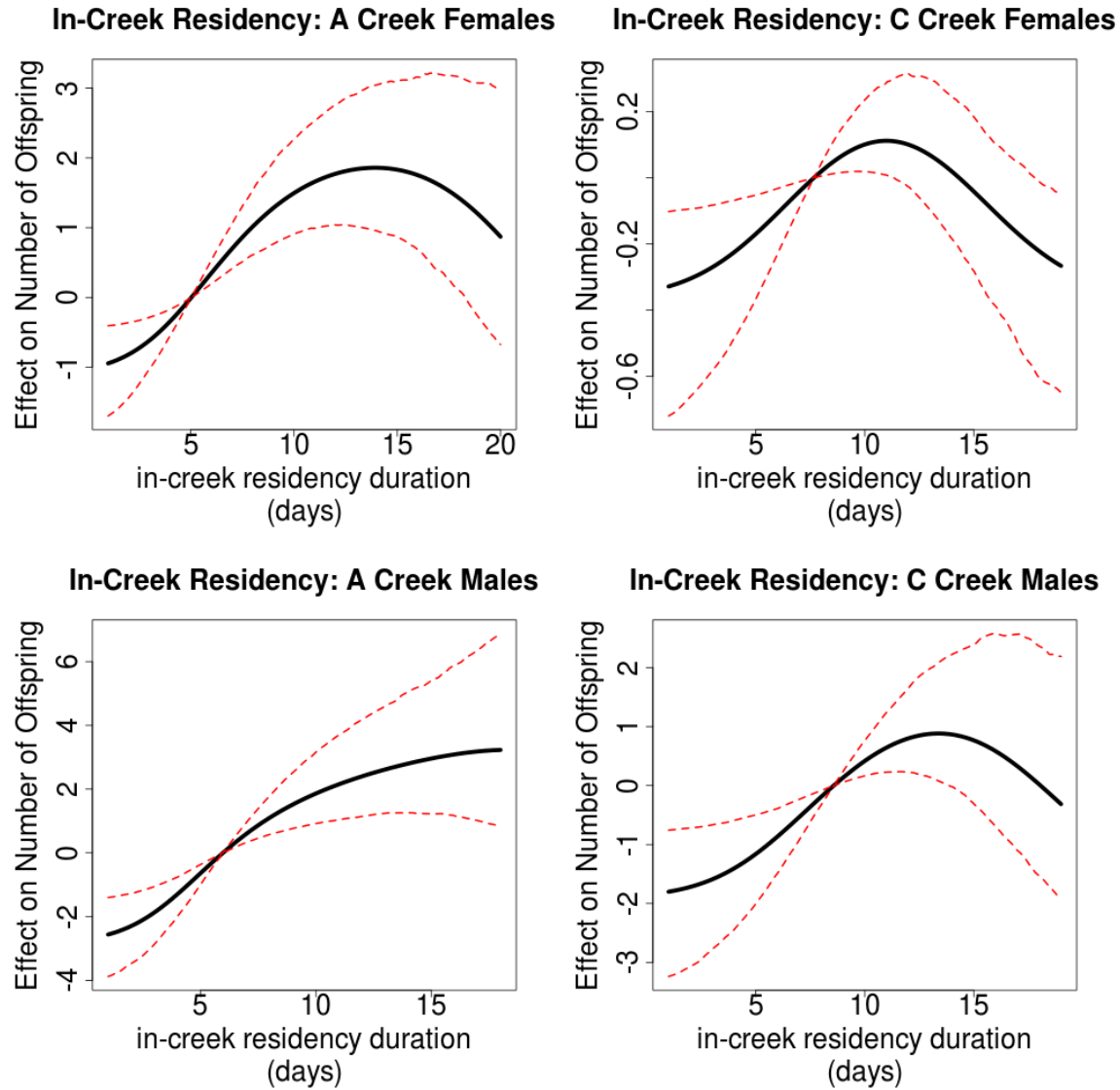
* indicates a statistically significant difference between the distribution of the disperser group and that of philopatric individuals (Fisher's exact test).



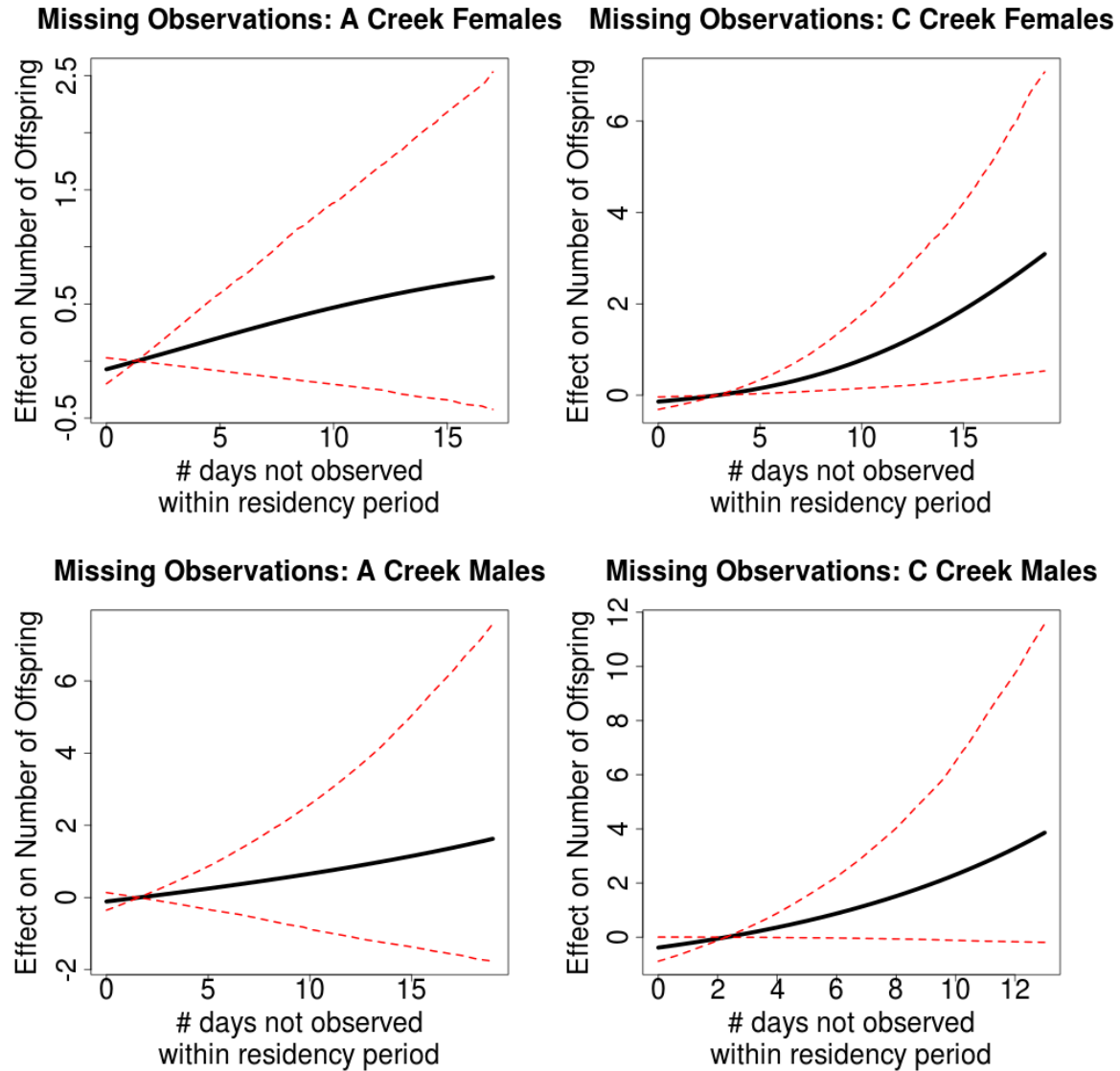
Supplementary Figure 9: Regression-derived relationship between body length and individual reproductive success for each creek and sex. Y-axis values indicate the expected difference in mean number of offspring between individuals with a given trait value and individuals with the mean trait value for that sex and creek. Solid black lines indicate mean effects and dashed red lines indicate 95% confidence intervals. The above plots show results using the full dataset, but results with the 95% assignment cutoff were nearly identical.



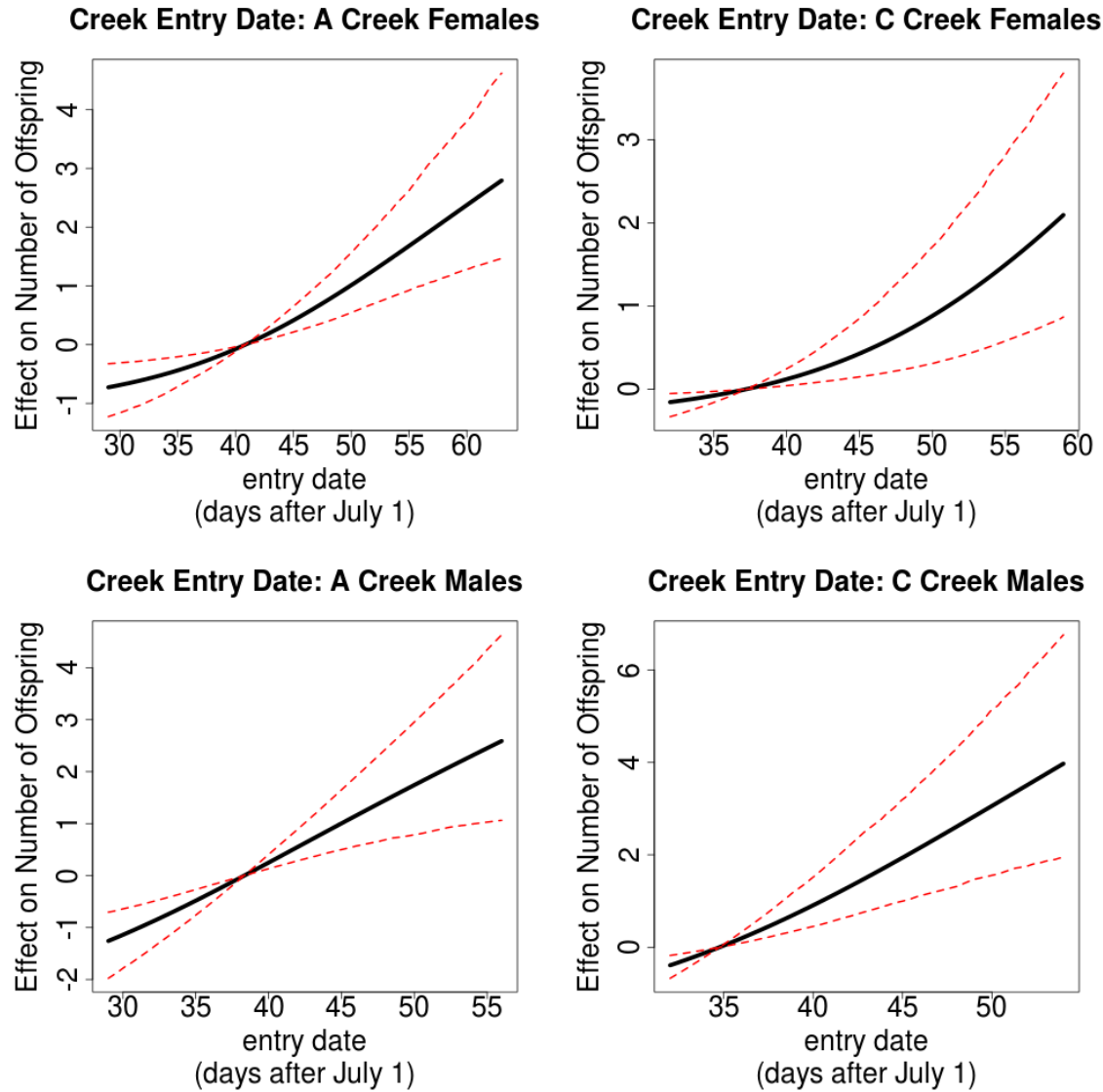
Supplementary Figure 10: Regression-derived relationship between body shape and individual reproductive success for each creek and sex. Y-axis values indicate the expected difference in mean number of offspring between individuals with a given trait value and individuals with the mean trait value for that sex and creek. Solid black lines indicate mean effects and dashed red lines indicate 95% confidence intervals. The above plots show results using the full dataset, but results with the 95% assignment cutoff were nearly identical.



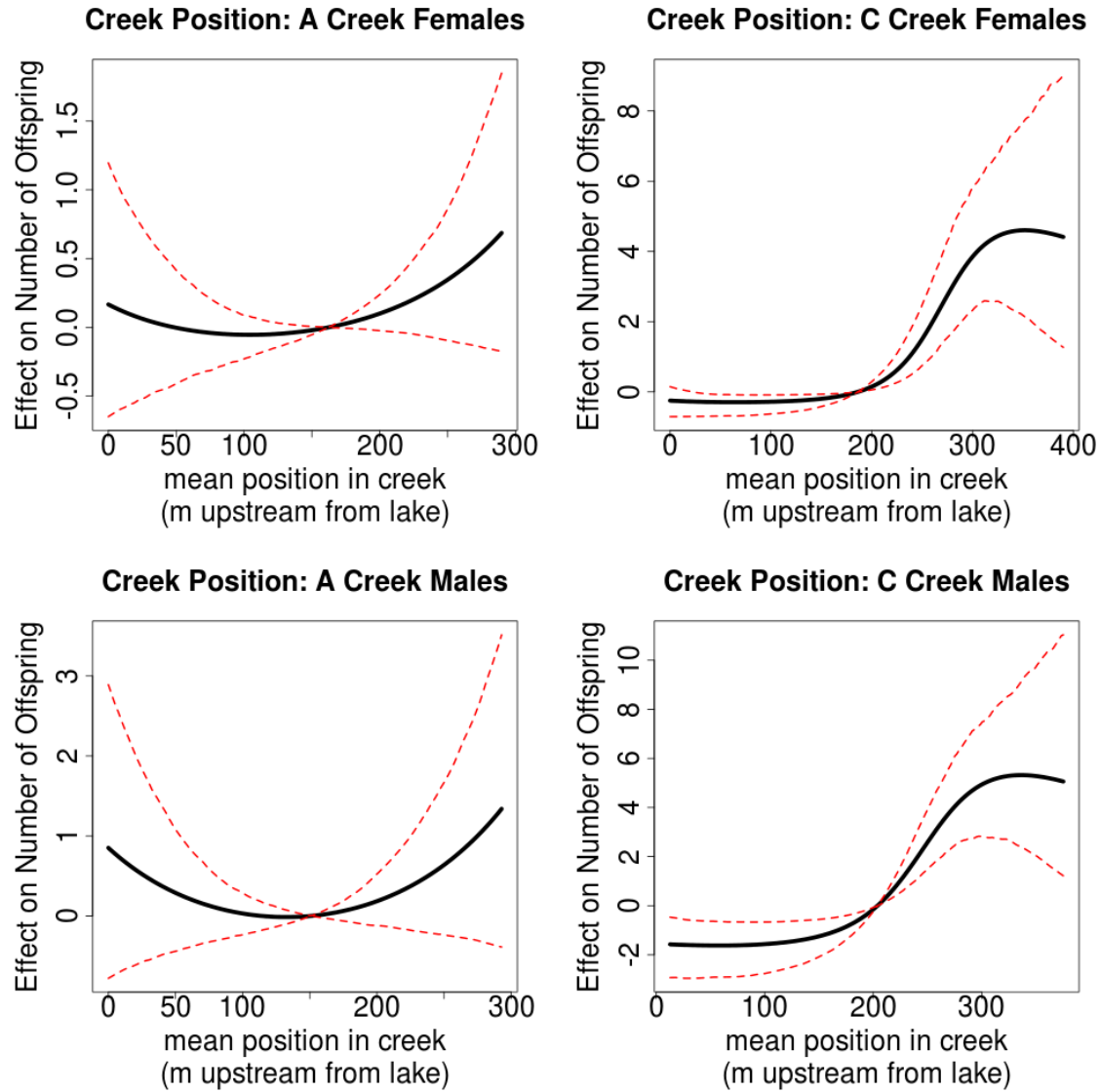
Supplementary Figure 11: Regression-derived relationship between in-creek residency period and individual reproductive success for each creek and sex. Y-axis values indicate the expected difference in mean number of offspring between individuals with a given trait value and individuals with the mean trait value for that sex and creek. Solid black lines indicate mean effects and dashed red lines indicate 95% confidence intervals. The above plots show results using the full dataset, but results with the 95% assignment cutoff were nearly identical.



Supplementary Figure 12: Regression-derived relationship between number of missing observations and individual reproductive success for each creek and sex. Y-axis values indicate the expected difference in mean number of offspring between individuals with a given trait value and individuals with the mean trait value for that sex and creek. Solid black lines indicate mean effects and dashed red lines indicate 95% confidence intervals. The above plots show results using the full dataset, but results with the 95% assignment cutoff were nearly identical.

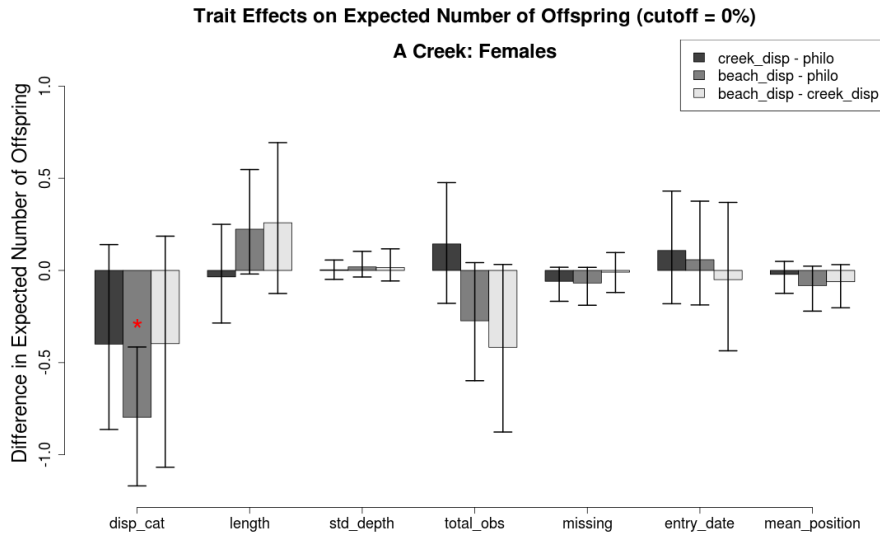


Supplementary Figure 13: Regression-derived relationship between creek entry date and individual reproductive success for each creek and sex. Y-axis values indicate the expected difference in mean number of offspring between individuals with a given trait value and individuals with the mean trait value for that sex and creek. Solid black lines indicate mean effects and dashed red lines indicate 95% confidence intervals. The above plots show results using the full dataset, but results with the 95% assignment cutoff were nearly identical.

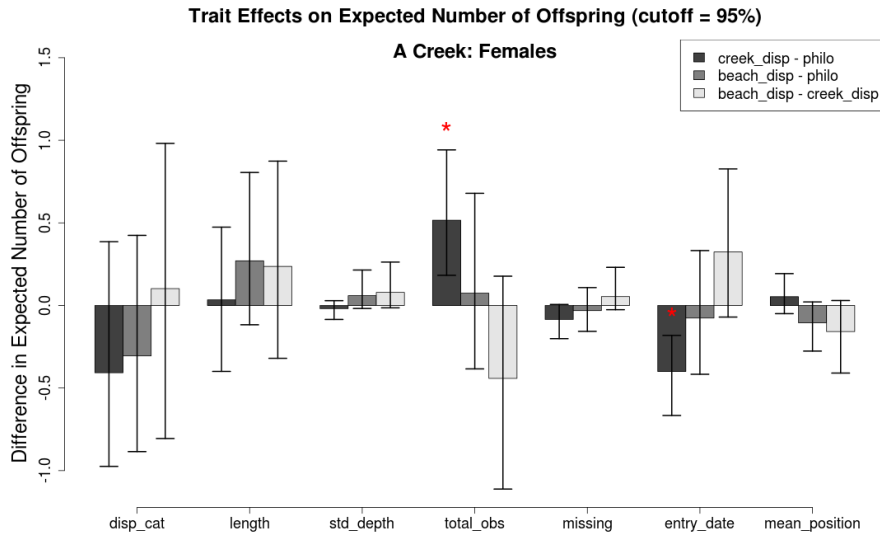


Supplementary Figure 14: Regression-derived relationship between mean observed creek position and individual reproductive success for each creek and sex. Y-axis values indicate the expected difference in mean number of offspring between individuals with a given trait value and individuals with the mean trait value for that sex and creek. Solid black lines indicate mean effects and dashed red lines indicate 95% confidence intervals. The above plots show results using the full dataset, but results with the 95% assignment cutoff were nearly identical.

A: No assignment cutoff used



B: 95% assignment cutoff

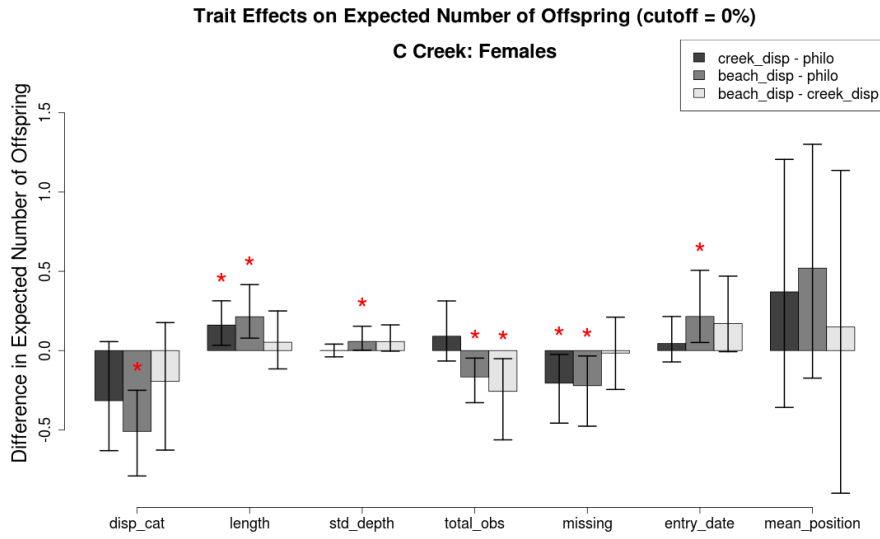


Supplementary Figure 15: For A Creek females, effects of differences in trait distributions between dispersal categories on differences in reproductive success between dispersal categories. Filled bars indicate the mean predicted effect and error bars indicate 95% confidence intervals from bootstrapping.

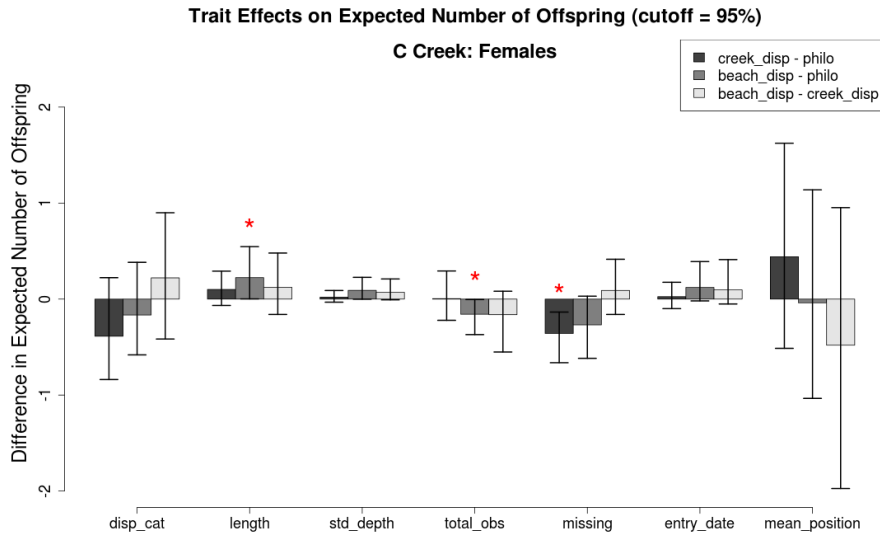
“disp_cat” = dispersal category, “total_obs” = length of in-creek residency period (number of days observed in the creek), “missing” = number of days not observed between first and last in-creek observation.

* indicates a statistically significant effect

A: No assignment cutoff used



B: 95% assignment cutoff

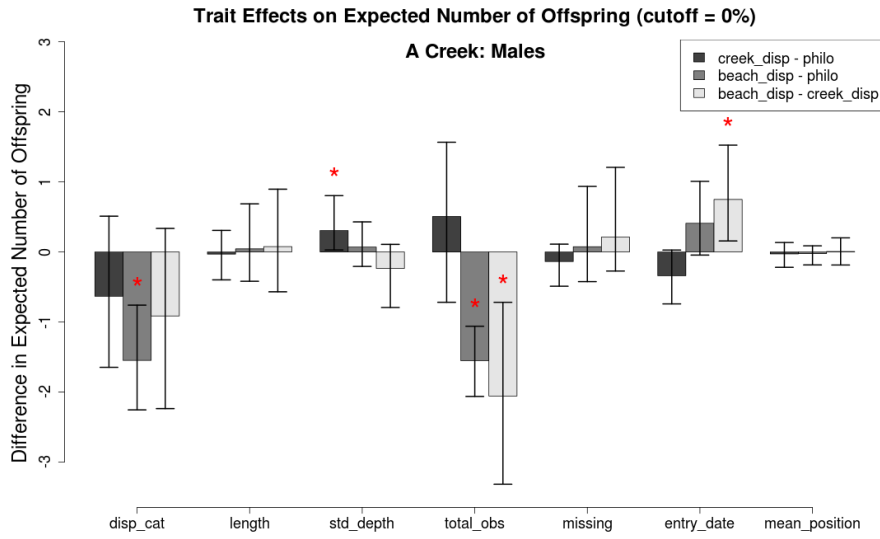


Supplementary Figure 16: For C Creek females, effects of differences in trait distributions between dispersal categories on differences in reproductive success between dispersal categories. Filled bars indicate the mean predicted effect and error bars indicate 95% confidence intervals from bootstrapping.

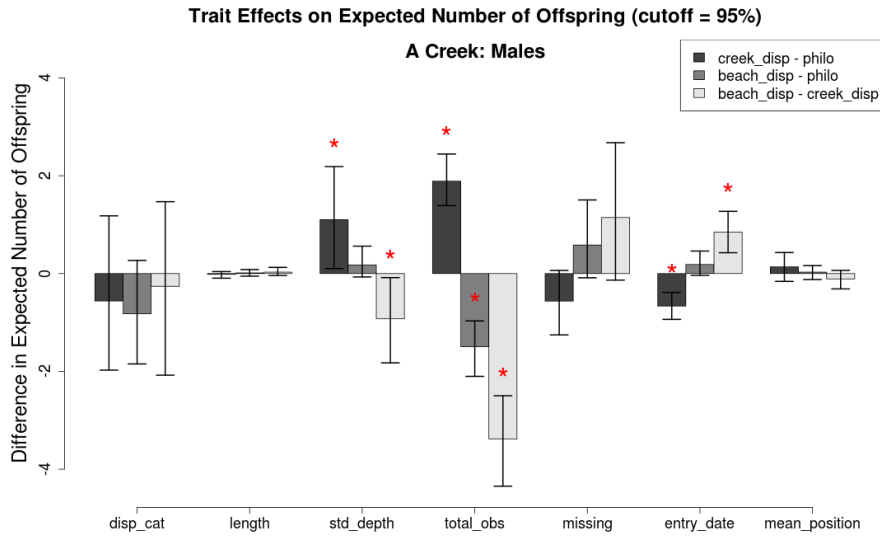
“disp_cat” = dispersal category, “total_obs” = length of in-creek residency period (number of days observed in the creek), “missing” = number of days not observed between first and last in-creek observation.

* indicates a statistically significant effect

A: No assignment cutoff used



B: 95% assignment cutoff

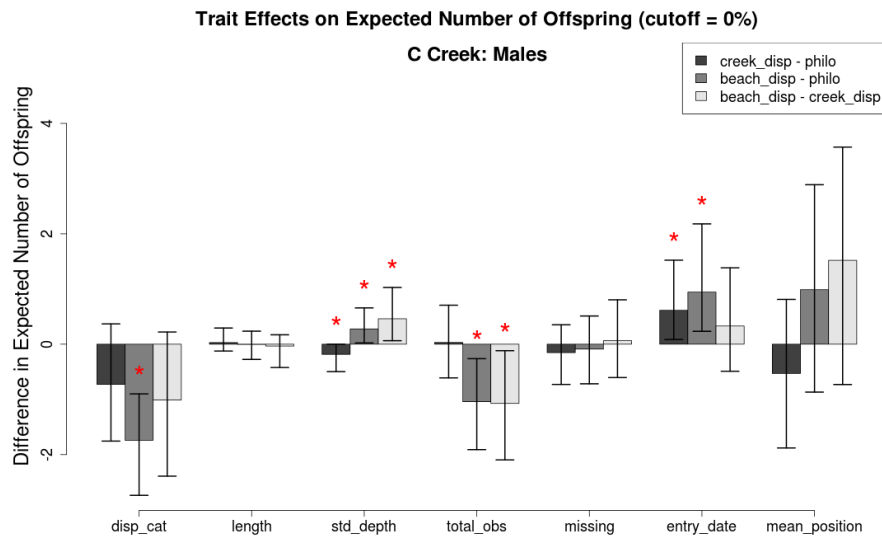


Supplementary Figure 17: For A Creek males, effects of differences in trait distributions between dispersal categories on differences in reproductive success between dispersal categories. Filled bars indicate the mean predicted effect and error bars indicate 95% confidence intervals from bootstrapping.

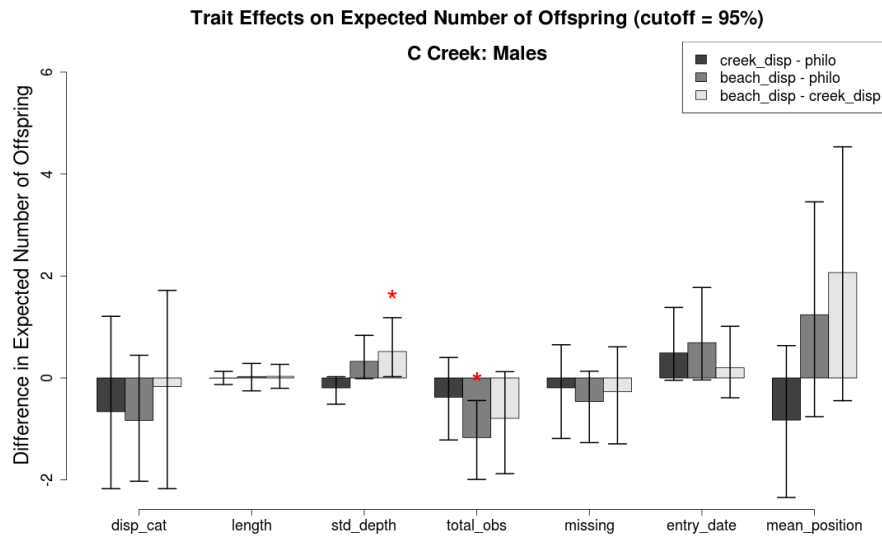
“disp_cat” = dispersal category, “total_obs” = length of in-creek residency period (number of days observed in the creek), “missing” = number of days not observed between first and last in-creek observation.

* indicates a statistically significant effect

A: No assignment cutoff used



B: 95% assignment cutoff



Supplementary Figure 18: For C Creek males, effects of differences in trait distributions between dispersal categories on differences in reproductive success between dispersal categories. Filled bars indicate the mean predicted effect and error bars indicate 95% confidence intervals from bootstrapping.

“disp_cat” = dispersal category, “total_obs” = length of in-creek residency period (number of days observed in the creek), “missing” = number of days not observed between first and last in-creek observation.

* indicates a statistically significant effect

Appendix A

Statistics for each locus and population on number of alleles (# All), observed heterozygosity (H_O), expected heterozygosity (H_E), F_{IS} , probability of departure from Hardy-Weinberg equilibrium (HWE), the proportion of null alleles inferred by ML-Null (Null), and whether evidence for stuttering or null alleles was found by Microchecker (MC).

Locus	Population	# All	H_O	H_E	F_{IS}	HWE	Null	MC
ONE100a	2004ACREEK	15	0.698	0.814	0.142	0.00	0.083	stutter, null
ONE100a	2004BEACH	18	0.800	0.910	0.121	0.01	0.057	null
ONE100a	2004CCREEK	17	0.747	0.854	0.126	0.00	0.072	null
ONE100a	2005ACREEK	15	0.668	0.810	0.176	0.00	0.084	stutter, null
ONE100a	2005BEACH	15	0.896	0.912	0.018	0.74	0.008	-
ONE100a	2005CCREEK	20	0.753	0.868	0.132	0.00	0.063	null
ONE100a	2008ACREEK	12	0.671	0.829	0.190	0.00	0.102	null
ONE100a	2008CCREEK	17	0.729	0.877	0.169	0.00	0.092	null
ONE100a	2009ACREEK	12	0.635	0.819	0.225	0.00	0.120	null
ONE100a	2009CCREEK	17	0.771	0.865	0.109	0.00	0.064	null
ONE100a	2010ACREEK	15	0.611	0.795	0.232	0.00	0.131	stutter, null
ONE100a	2010CCREEK	19	0.880	0.880	0.000	0.07	0.001	-
ONE102	2004ACREEK	13	0.830	0.839	0.011	0.00	0.009	-
ONE102	2004BEACH	12	0.842	0.802	-0.051	0.06	0.004	-
ONE102	2004CCREEK	14	0.855	0.845	-0.012	0.00	0.010	-
ONE102	2005ACREEK	13	0.903	0.864	-0.045	0.01	0.000	-
ONE102	2005BEACH	13	0.907	0.849	-0.069	0.37	0.000	-
ONE102	2005CCREEK	13	0.867	0.852	-0.018	0.02	0.000	-
ONE102	2008ACREEK	13	0.830	0.858	0.032	0.25	0.020	-
ONE102	2008CCREEK	15	0.857	0.839	-0.022	0.00	0.000	-
ONE102	2009ACREEK	13	0.818	0.857	0.045	0.01	0.022	null
ONE102	2009CCREEK	13	0.875	0.859	-0.019	0.00	0.007	-
ONE102	2010ACREEK	12	0.799	0.816	0.022	0.18	0.007	-
ONE102	2010CCREEK	13	0.885	0.855	-0.036	0.01	0.000	-
ONE103	2004ACREEK	29	0.877	0.910	0.036	0.00	0.011	null
ONE103	2004BEACH	29	0.926	0.949	0.024	0.00	0.000	-
ONE103	2004CCREEK	29	0.943	0.929	-0.016	0.00	0.000	-
ONE103	2005ACREEK	28	0.909	0.913	0.004	0.00	0.000	-
ONE103	2005BEACH	26	0.933	0.957	0.025	0.19	0.000	-
ONE103	2005CCREEK	31	0.934	0.932	-0.002	0.00	0.003	-
ONE103	2008ACREEK	29	0.873	0.913	0.044	0.00	0.022	null
ONE103	2008CCREEK	31	0.884	0.935	0.054	0.00	0.022	null
ONE103	2009ACREEK	27	0.929	0.915	-0.015	0.00	0.002	-
ONE103	2009CCREEK	32	0.917	0.930	0.014	0.00	0.004	-

ONE103	2010ACREEK	30	0.919	0.915	-0.005	0.00	0.000	-
ONE103	2010CCREEK	28	0.867	0.911	0.049	0.00	0.019	null
ONE106	2004ACREEK	23	0.780	0.854	0.087	0.00	0.044	null
ONE106	2004BEACH	26	0.698	0.912	0.236	0.00	0.118	null
ONE106	2004CCREEK	22	0.719	0.859	0.163	0.00	0.079	null
ONE106	2005ACREEK	23	0.837	0.868	0.037	0.00	0.017	null
ONE106	2005BEACH	22	0.841	0.929	0.096	0.03	0.050	null
ONE106	2005CCREEK	26	0.863	0.864	0.002	0.01	0.000	-
ONE106	2008ACREEK	24	0.827	0.859	0.037	0.00	0.008	-
ONE106	2008CCREEK	27	0.598	0.866	0.310	0.00	0.154	null
ONE106	2009ACREEK	18	0.844	0.867	0.027	0.00	0.013	-
ONE106	2009CCREEK	24	0.872	0.868	-0.004	0.00	0.000	-
ONE106	2010ACREEK	23	0.852	0.880	0.031	0.00	0.021	-
ONE106	2010CCREEK	21	0.737	0.856	0.140	0.00	0.064	stutter, null
ONE108	2004ACREEK	14	0.848	0.846	-0.002	0.00	0.000	-
ONE108	2004BEACH	16	0.758	0.870	0.129	0.01	0.048	null
ONE108	2004CCREEK	20	0.858	0.874	0.019	0.00	0.006	-
ONE108	2005ACREEK	17	0.851	0.843	-0.010	0.00	0.007	-
ONE108	2005BEACH	13	0.896	0.884	-0.014	0.02	0.000	-
ONE108	2005CCREEK	17	0.863	0.860	-0.004	0.00	0.008	-
ONE108	2008ACREEK	15	0.846	0.863	0.020	0.00	0.016	-
ONE108	2008CCREEK	18	0.888	0.870	-0.020	0.00	0.003	-
ONE108	2009ACREEK	12	0.847	0.842	-0.007	0.02	0.006	-
ONE108	2009CCREEK	17	0.863	0.875	0.014	0.00	0.009	-
ONE108	2010ACREEK	14	0.843	0.880	0.042	0.00	0.018	stutter, null
ONE108	2010CCREEK	15	0.857	0.866	0.010	0.00	0.000	-
ONE109	2004ACREEK	10	0.768	0.765	-0.004	0.00	0.000	-
ONE109	2004BEACH	13	0.832	0.847	0.018	0.19	0.007	-
ONE109	2004CCREEK	11	0.811	0.810	-0.001	0.03	0.005	-
ONE109	2005ACREEK	12	0.829	0.796	-0.041	0.14	0.000	-
ONE109	2005BEACH	13	0.891	0.854	-0.044	0.25	0.000	-
ONE109	2005CCREEK	11	0.778	0.821	0.053	0.05	0.021	null
ONE109	2008ACREEK	13	0.771	0.779	0.010	0.00	0.011	-
ONE109	2008CCREEK	13	0.766	0.813	0.059	0.00	0.028	null
ONE109	2009ACREEK	13	0.818	0.799	-0.023	0.30	0.000	-
ONE109	2009CCREEK	12	0.840	0.823	-0.021	0.00	0.000	-
ONE109	2010ACREEK	12	0.691	0.723	0.044	0.00	0.035	-
ONE109	2010CCREEK	11	0.743	0.798	0.068	0.04	0.019	null
ONE110c	2004ACREEK	11	0.836	0.797	-0.049	0.00	0.000	-
ONE110c	2004BEACH	17	0.916	0.897	-0.021	0.31	0.000	-
ONE110c	2004CCREEK	14	0.842	0.829	-0.015	0.04	0.000	-
ONE110c	2005ACREEK	12	0.775	0.787	0.016	0.10	0.009	-

ONE110c	2005BEACH	13	0.896	0.884	-0.013	0.64	0.000	-
ONE110c	2005CCREEK	12	0.841	0.842	0.002	0.22	0.000	-
ONE110c	2008ACREEK	12	0.824	0.839	0.018	0.02	0.003	-
ONE110c	2008CCREEK	17	0.837	0.848	0.013	0.03	0.000	-
ONE110c	2009ACREEK	12	0.799	0.781	-0.023	0.05	0.003	-
ONE110c	2009CCREEK	13	0.841	0.841	0.000	0.04	0.001	-
ONE110c	2010ACREEK	14	0.815	0.832	0.021	0.01	0.007	-
ONE110c	2010CCREEK	12	0.769	0.818	0.060	0.00	0.008	null
ONE111	2004ACREEK	14	0.739	0.792	0.067	0.00	0.030	stutter, null
ONE111	2004BEACH	21	0.820	0.807	-0.016	0.57	0.000	-
ONE111	2004CCREEK	22	0.666	0.683	0.025	0.00	0.004	-
ONE111	2005ACREEK	21	0.815	0.798	-0.021	0.08	0.000	-
ONE111	2005BEACH	15	0.689	0.744	0.075	0.46	0.000	-
ONE111	2005CCREEK	23	0.718	0.721	0.004	0.00	0.001	-
ONE111	2008ACREEK	19	0.868	0.807	-0.076	0.03	0.000	-
ONE111	2008CCREEK	25	0.772	0.746	-0.035	0.01	0.002	-
ONE111	2009ACREEK	18	0.823	0.801	-0.027	0.08	0.000	-
ONE111	2009CCREEK	19	0.820	0.761	-0.077	0.38	0.000	-
ONE111	2010ACREEK	18	0.703	0.691	-0.017	0.14	0.010	-
ONE111	2010CCREEK	20	0.717	0.713	-0.005	0.15	0.000	-
ONE114	2004ACREEK	18	0.899	0.882	-0.020	0.00	0.000	-
ONE114	2004BEACH	16	0.863	0.912	0.054	0.22	0.014	-
ONE114	2004CCREEK	17	0.852	0.870	0.021	0.01	0.002	-
ONE114	2005ACREEK	19	0.855	0.854	-0.001	0.01	0.005	-
ONE114	2005BEACH	15	0.915	0.913	-0.002	0.26	0.000	-
ONE114	2005CCREEK	18	0.877	0.882	0.006	0.10	0.000	-
ONE114	2008ACREEK	17	0.838	0.886	0.055	0.01	0.016	null
ONE114	2008CCREEK	19	0.850	0.867	0.019	0.00	0.000	-
ONE114	2009ACREEK	18	0.877	0.840	-0.044	0.11	0.000	-
ONE114	2009CCREEK	18	0.875	0.881	0.006	0.00	0.007	-
ONE114	2010ACREEK	21	0.851	0.872	0.024	0.00	0.010	-
ONE114	2010CCREEK	18	0.908	0.903	-0.006	0.01	0.005	-
ONE115a	2004ACREEK	14	0.696	0.895	0.223	0.00	0.124	null
ONE115a	2004BEACH	16	0.878	0.910	0.035	0.24	0.014	-
ONE115a	2004CCREEK	17	0.816	0.892	0.085	0.00	0.046	null
ONE115a	2005ACREEK	16	0.637	0.900	0.293	0.00	0.171	stutter, null
ONE115a	2005BEACH	17	0.854	0.896	0.048	0.19	0.039	-
ONE115a	2005CCREEK	17	0.716	0.896	0.201	0.00	0.105	stutter, null
ONE115a	2008ACREEK	16	0.756	0.889	0.150	0.00	0.079	null
ONE115a	2008CCREEK	17	0.797	0.897	0.112	0.00	0.057	null
ONE115a	2009ACREEK	14	0.633	0.898	0.296	0.00	0.173	stutter, null
ONE115a	2009CCREEK	17	0.777	0.900	0.136	0.00	0.077	stutter, null

ONE115a	2010ACREEK	15	0.541	0.890	0.393	0.00	0.242	stutter, null
ONE115a	2010CCREEK	15	0.735	0.891	0.175	0.00	0.086	null
OTS103	2004ACREEK	13	0.847	0.850	0.004	0.00	0.000	-
OTS103	2004BEACH	14	0.937	0.892	-0.051	0.70	0.000	-
OTS103	2004CCREEK	15	0.835	0.856	0.024	0.00	0.019	-
OTS103	2005ACREEK	14	0.826	0.841	0.017	0.03	0.003	-
OTS103	2005BEACH	14	0.957	0.891	-0.075	0.80	0.000	-
OTS103	2005CCREEK	15	0.841	0.856	0.018	0.00	0.002	-
OTS103	2008ACREEK	13	0.873	0.878	0.005	0.01	0.006	-
OTS103	2008CCREEK	18	0.852	0.861	0.011	0.00	0.010	-
OTS103	2009ACREEK	14	0.847	0.843	-0.005	0.00	0.000	-
OTS103	2009CCREEK	16	0.835	0.833	-0.003	0.04	0.001	-
OTS103	2010ACREEK	13	0.876	0.876	0.000	0.00	0.004	-
OTS103	2010CCREEK	15	0.836	0.836	0.000	0.00	0.000	-