GREAT LAKES FISHERY COMMISSION

2002 Project Completion Report[1]

Genetic assignment of larval parentage as a means of assessing mechanisms underlying adult reproductive success and larval dispersal

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GLFC RESEARCH PROJECT COMPLETION REPORT

March 27, 2002

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Introduction

Historically, sea lamprey control has been achieved through use of barriers to prevent upstream migration of spawning adults and by chemical applications designed to kill larvae in streams prior to metamorphosis into the parasitic life history stage. Increased costs and concern over the use of chemicals has led to greater support for alternative methods of control such as release of sterile males, and enhanced trapping methods, and increased use of barriers (GLFC 1992). The efficacy of many control efforts is based on assumptions pertaining to fundamental, though as yet unresolved aspects of the species breeding behavior, male and female reproductive success, and patterns of larval dispersal and survival. Given the difficulty of directly observing these aspects of lamprey biology and behavior, novel application of molecular genetic markers may help elucidate important aspects of the species' biology that can be used to validate and/or enhance control measures.

The Great Lakes Fishery Commission's (GLFC's) Integrated Management of Sea Lamprey (IMSL) program hopes to rely heavily on new technologies in programmatic areas of lampricide control, adult assessment, and male sterilization. Work completed during this study has combined expertise of both principal investigators and their respective students and staff in areas of population ecology, biometry, behavioral ecology, and molecular biology. We used innovative molecular technologies by applying novel genetic markers to adult and larval samples obtained in a spatially explicit manner, in the context of ongoing field ecological studies.

The primary purpose of this study was to examine the feasibility of using adult-progeny genotypic arrays to characterize larval dispersal and adult reproductive success. Specifically, the null hypotheses to be addressed in this study were: 1) larval dispersal from the nest is random and unconstrained with respect to distance and larval density in the stream substrate, and 2) all adult male and female lamprey present in a spawning population contribute equally to the number of age-0 and age-1 larvae produced. We determined parentage and examined larval dispersal in stream habitats over multiple sampling periods from shortly after the animals dispersed their nests and again >1 year post-hatching. We estimated the mean and variance of male and female reproductive success of adults released into long-term research streams.

The project was an unequivocal success. We successfully addressed all stated objectives in the proposal. The project realized numerous collateral benefits as well. Our multi-disciplinary approach has built upon ongoing GLFC-sponsored research programs and has provided critical data facilitating the examination of issues of critical research and management importance in areas of compensation, larval early life history, and adult reproductive ecology. Below we detail the major results from the study.

Component 1 Larval dispersal

Background

Little is known about the early life history of sea lamprey ammocetes. In particular, the dispersal of age-0 ammocetes from nests into suitable larval rearing habitat has never been investigated. Taken over an entire drainage, larval rearing habitat is not likely to be limiting and thus compensatory (density dependent) effects on larval survival may be minimal. However, are all potential larval rearing habitats accessible? Is density-dependent larval survival during early life-history stages likely given larval dispersal capabilities and the juxtaposition of spawning and rearing habitats? At present little is known regarding dispersal capabilities or whether, and by which means, dispersion of larvae changes over time, either by sequential bouts of dispersal (e.g., due to unsuitable or "crowded" habitat), or by differential mortality. By collecting genetic information on spawning adults, eggs in nests, and age 0 larvae in streams, it should be possible to attribute larvae captured in stream habitats to specific source nests. This will allow us to determine the range of larval dispersal distances from nests at various times during their first and second growing season. This information will in turn help us to determine the degree to which the juxtaposition of suitable habitats for successive life stages might be a constraint on larval recruitment. How close together do nesting and early larval habitats have to be for the latter to be limiting and thus of potential importance as a limiting factor to larval survival?

Methods

Molecular Methods

Marker development – A size-selected partial genomic DNA library was constructed for sea lamprey as described by Ostrander et al. (1992) and was screened for microsatellite containing regions. Ten microsatellite loci that were found to be polymorphic and conformed to Mendelian expectations (Bryan et al. in review) were employed for analyses.

DNA extraction and microsatellite genotyping - DNA was extracted from adults and ammocetes using PureGene (Gentra Systems, Inc.) or QiaGen (Qiagen, Inc.) extraction kits. DNA samples were quantified by flourimetry and standard working stocks of 20ng/µl were established for all individuals. Microsatellite loci were amplified using polymerase chain reaction (PCR) protocols developed for each locus (Bryan et al. in review) using flourescently labeled primers. PCR products for each locus were run onto 6% denaturing polyacrylamide gels and scanned using an FM BIO II (Hitachi, Inc.) scanner. Genotypes were assigned based on molecular size standards and by running individuals of known genotype run on each gel as controls.

Determination of Parentage- Given the relatively small number of males and females that were planted in each stream (see below) we anticipated that all but the true parents would be excluded for most ammocetes. If more than one male and/or female could not be excluded as possible parents, then estimates of the likelihood of maternal and paternal parentage were made for all parental combinations using maximum likelihood methods of Thompson (1976) and Meagher and Thompson (1986) as implemented in program CERVUS (Marshall et al. 1998). Determination of most likely parental combinations was based on log-likelihood's (LOD scores). Estimates of statistical confidence in parental assignments were based on simulations of random parental genotypic combinations. Analyses presented below are based on larvae whose parentage was based either on total exclusion or at the 95% level of statistical confidence, conditional on offspring and putative parental multi-locus genotypic data.

Field Methods

Collection of samples – Data were obtained from 2 sets of streams. First, we used streams currently under investigation as part of the compensatory mechanisms study, also funded by the GLFC, to study larval dispersal: Ogemaw Creek in Michigan and Carp River in Ontario. To investigate recruitment at relatively small spawner population sizes (an objective of the Compensatory Mechanisms Study), 12 pairs of adult lamprey were introduced into Ogemaw Creek, and 7 pairs into the Carp River. Due to the presence of the lamprey barriers on these streams, all lamprey present in the study sections were there as a result of deliberate introductions of adult lamprey.

Tissue samples were taken from all adults prior to release for later genetic analysis. We monitored both streams for nests and once spawning activity appeared to be complete to obtain stream locations for all potential sources of larvae.

Nests were located on both streams. Preliminary data on larval distributions suggested that age-0 larvae seemed to be within 200m of nests (Derosier, 2001). We created three sampling regions below each nest. Zone 1 extended from the nest downstream for 50 m. Zone 2 extended from 55-155 m downstream of the nests. The third zone included habitats between 160-210 m downstream of the nest. On three occasions during the summer and early fall, we collected age 0 ammocetes from all streams using a suction dredge device from each of the three stream zones on each stream. Sampling occurred in Ogemaw Creek on July 20-22 (event 1), August 12-13 (event 2), and September 5-6 (event 3). Sampling on the Carp River occurred on August 2-5 (event 1), August 23-24 (event 2), and September 24-25 (event 3). Dredge-sampled small plots (< .25 m^2) were randomly chosen from Type I habitats downstream of documented nest locations. All age-0 larvae were preserved in 70% ethanol, identified to species and measured.

The second set of study streams comprise seven of the eight streams used in the SMRT Long Term Study. Streams include the Big Garlic, Rock, Misery and Middle along the southern (U.S.) shore of Lake Superior and the Wolf, Carp and Stokely along the northern (Canadian) shore. In the spring of 1999, small numbers of adult lamprey were introduced above barriers into each of these streams. The total numbers of adults released were: Middle (100), Misery (70), Big Garlic (14), Rock (48), Carp (14), Stokely (14), Wolf (20). These releases were part of a pilot study for a further investigation of SMRT effects on lamprey recruitment in which the emphasis was to determine recruitment at very low spawning stock sizes.

Prior to their release, tissue samples (fin clips) were taken from each adult for genetic analysis. Samples from each adult were placed into individually marked 1.5 ml eppendorf tubes containing high-salt buffer.

Larval lampreys that were presumed to be produced by the spawning year class introduced into each stream were collected during the fall of 1999 (as age-0) and during the following summer (as age-1). Larval collections were conducted coincident with annual larval stream assessments by U.S. Fish and Wildlife Service personnel. Sampling protocols combined a transect-based, habitat-stratified electrofishing procedure with a stream-level habitat inventory to yield estimates of densities and size distribution of age-0 and age-1 larvae in each stream. Field personnel sampled larvae from Type I larval habitat while walking the stream between each of the pre-defined sampling transects.

Microsatellite analysis of these larvae together with the data from the released adults facilitated estimates of the proportion of potential parents that actually contributed to the larval population. Because all larval collections in both stream sets were spatially referenced (by transect separated by, on average, 50 m intervals), we also use these data to examine how widely dispersed progeny of individual parental pairs becomes (assuming pairs spawn in only a single nest).

U.S. Fish and Wildlife Service personnel provided size distributions of larvae consistent with presumed age status for each stream. Upon return to the lab, measurements of larva length and weight were taken for each individual. Lengths are recorded using a dissection microscope that is linked to a computer. The program "OPTIMUS" was used to estimate larval length to the nearest 0.01mm. Animals were subsequently blotted to remove alcohol and individuals were weighed to the nearest 0.001g using an analytical balance. All individuals were subsequently dissected. A portion of each sample was used for DNA extraction.

Statistical Analysis –Larval parentage was determined as described above based on conditional maximum likelihood measures as implemented in program CERVUS (Marshall et al. 1998).

Component 2 –Adult reproductive success

Field Methods

Collection of samples - This segment of the project focused exclusively on the second set of seven study streams used in the SMRT Long Term Study described above. Sampling of adults and larvae at age-0 and age-1 were conducted as described above.

Statistical analyses - We determined the number of ammocetes produced by each stocked male and female using adult/ammocete genotype comparisons and likelihood-based determinations of parentage as described above based on program CERVUS (Marshall et al. 1998). The null hypothesis was that all males and females contribute equally to larvae produced in each stream. Thus, during the i^{th} time period (where the i^{th} period corresponds to fall and spring sampling for age 0 and age 1 larva, respectively) each of N adult males and females is expected to produce x_i/N progeny where x_i is the total number of larva sampled during the i^{th} period.

Results

Multi-locus genotype data were generated for 280 adults, 473 age-0 larvae and 1434 age-1 larvae from the SMRT streams. Samples from the Middle River were not analyzed. Sample sizes of age-0 and age-1 varied greatly across the SMRT streams (Table 1) and from Ogemaw Creek and the Carp River. Age-0 and age-1 larvae collected from several streams were determined to be native lamprey (*Ichthyomyzon and Lampetra*) and not *P. marinus* on the basis of multi-locus genotype profiles (Table 1). Non-*Petromyzon* larvae (range 0-88% for age-0 and 0-60% for age-1 across streams for larvae screened genetically; Table 1) were removed from analyses. Using likelihood-based estimators described in Marshall et al. (1998) and implemented in program CERVUS, we excluded all possible males and female adults whose genotypes are inconsistent with those of each larvae. Even though all larvae genotyped from the approximately 2000 samples were within the size range consistent with larval age-1 status based on stream-specific profiles (M. Twohey, personal communication), large numbers of larvae were determined to be older than age-1 (31.2-70% of *Petromyzon* larvae across streams) and were also eliminated from analyses. We assumed that larvae with multi-locus genotypes that were inconsistent with any potential male and female pairs (i.e., all planted adult males and females totally excluded from analysis), were not produced from the adults stocked and thus must be from previous year's cohorts. The presence of large numbers of larvae from native lamprey and lamprey produced from cohorts in earlier years decreased our sample size considerably, and thus our ability to conduct analyses of dispersal and parentage for all streams.

We restrict our analyses of adult mating systems and larval dispersal to data obtained from 4 of the 7 SMRT streams (Big Garlic, Carp, Stokely, and Wolf). Large numbers of adults were stocked into the Middle River (N=100) and Misery River (N=70). Due to the low levels of polymorphism (2-7 alleles per locus; data not shown) characterizing the 10 loci used, we were unable to obtain unambiguous estimates of parentage for larvae from the Middle and Misery Rivers. Only 24 of 143 (17%) of larvae from the Rock River could be assigned to parental pairs based on total exclusions or at $\geq$95% statistical confidence. We use data from the Rock River to estimate larval dispersal but not in summaries of male and female mating success. We estimate that with the loci now available, parentage can be assigned to most progeny in situations where 40 or fewer adults contribute to progeny. Additional statistical power would be possible if adults are pre-screened for variation to maximize genotypic variation, or if additional loci were developed.

Adult mating system

Most adults contributed to reproduction. Across 4 streams (Big Garlic, Carp, Stokely, and Wolf) 50 of 62 adults stocked (80.6%) were found to contribute at least one offspring. Variation in male and female reproductive success varied greatly across adults for age-0 (specific data not shown) and age-1 larvae (Fig. 1). In most of the streams, male and female reproductive success was decidedly non-uniform. Reproductive success among males and females was highly correlated. The most successful males and females in each stream typically mated with one another. We confine our analysis to estimates of parentage for age-1 larvae. However, preliminary analyses for age-0 larvae obtained from the Big Garlic River and Carp River suggest that the same parental pairs were assigned as parents responsible for the age-1 larvae. Restricted sample sizes and limited spatial dispersion of age-0 samples from each stream precluded formal analyses and quantitative comparisons of age-0 larval parental contributions to parental contributions to age-1 larvae sampled the following year.

We document evidence of polygyny and polyandry in all streams surveyed (Table 2). Most males and females mate with more than a single individual (Table 2), and matings with 3 or more individuals were common in all streams. Data on larval parentage were not obtained from individual nests but rather from larvae distributed in each stream. As such, our ability to determine whether multiple matings were due to purposeful or directed behaviors or to passive events must be inferred from the distribution of reproductive success and dispersion of full and half-sib larvae. For example, if a male produced a large number of larvae with each of several females, this suggests the male was a territorial male that enticed multiple females to breed with him. In each stream, we observed females to have produced multiple offspring with each of several males and in different sections of stream (based on different spatial distributions of larvae from different matings) suggesting that females may choose to mate with multiple males. In many situations in each stream we also observed situations where females and males mate with multiple individuals but appear to have produced comparatively fewer progeny. This could indicate either differential reproductive success, differential larval survival after leaving the nest, or adult use of alternative reproductive strategies. Females could choose to lay different numbers of eggs with different males. Females could lay eggs in nests with single or multiple males present. Alternatively, males could use alternative behaviors (e.g., cuckholdry). Even passive modes of insemination are possible. Sperm viability following release into the water column is among the longest of freshwater fishes (Ciereszko et al. 2002).

Predisposition of females to breed with multiple males and of male to do likewise has significant implications for use of alternative control strategies, and provides additional sources of inference into how compensatory recruitment or survival may be mediated. There appears to be considerable plasticity of behaviors and such variation was observed in each of the streams surveyed.

Larval dispersal

Preliminary analyses of age-0 larval data sampled down-stream from known nests on Ogemaw Creek and the Carp River (Derosier 2001) revealed that half- and/or full-sib larvae can distribute themselves over $\geq$900 m of stream habitat down-stream from nests within 2 months following emergence. Limited spatial dispersion of age-0 larvae samples from the Big Garlic River precluded analyses of other data.

Age-1 larvae after a year in the stream are widely distributed. Larvae of common parentage (full- and/or half-sibs) are more likely to be co-distributed non-randomly over relatively short distances (<500 m; Fig. 2). In 3 of the 4 SMRT streams, summaries of all pair-wise inter-individual distances (Fig. 2) revealed that approximately 37% of all larvae of common parentage were within 500m of one another. For a fourth stream where reproduction was dominated by a single pair of adults (Big Garlic River), approximately 69% of larvae of common parentage were within 500 m of one another.

Despite the skew in inter-larval dispersal distances, larvae from a single reproductive event sometimes distribute themselves over large segments of each stream. We found evidence that larvae produced from a single mating were separated by $\geq$5 km of stream. Data suggest that larvae are capable of extensive movements and can over the course of a single year access most downstream habitats.

Discussion

Adult mating system

Departures from equitable progeny production among adults within a stream provide support for differential recruitment by males and females. Of particular interest, the genotypic data were consistent with variance in female and male reproductive success due both to total failure vs. success and to variation in numbers of progeny of successfully reproducing adults (Fig. 1).

Genetic data (Table 2) show unequivocally that the sea lamprey mating system is characterized by both polygyny and polyandry. Analyses of larval parentage in all streams surveyed revealed that the majority of males and females mated with multiple partners. Spatially explicit sampling of larvae also facilitated comparisons of parental contributions to larvae in different sections of the stream. In some instances progeny from the same female but sired by different males were found in different sections of the stream suggesting that female actively sought multiple matings by different males. Heterogeneity across streams may be attributed to aspects of the Long-Term Study relative to historical experimental stocking rates.

Larval dispersal

Larvae were more likely to be found in close proximity to other siblings (Fig. 2). We find a decided skew in pair-wise inter-individual locations towards short distances. Some heterogeneity was observed across streams (e.g., Rock River). However, patterns of larval spatial orientation in the Rock River are attributed to the small number of larvae with known parentage in this stream. Data on inter-larvae distances revealed that larvae from the same reproductive event (and inferentially from the same nest) can be distributed over 900 m of stream shortly (< 2 months) after hatching (Derosier 2001; specific data not shown), and over 5 km of stream after a single year. We were not able to document effective dispersal distances from specific nests. However, consistent observations of large inter-larval distance among siblings for all streams surveyed suggest that larvae are capable of accessing large expanses of habitat. There is considerable potential for larvae even in early life history stages (age-0 and age-1) to exhibit plasticity in dispersal behavior, for example in response to varying densities and/or quality and distribution of larval rearing habitat. Long-distance dispersal potential also implies that juxtaposition of spawning and larval rearing habitat is not a critical prerequisite for successful recruitment.

Potential versus actual spawners

Debates over the efficacy of alternative control techniques that seek to reduce reproductive success center on our lack of understanding of the importance of compensatory processes in lamprey populations. Recently, various investigators have begun an integrated program of research and assessment designed to improve our understanding of compensatory mechanisms during early (i.e., pre-parasitic) life history stages. The rationale underlying the "Compensatory Mechanisms" study is that if demographic processes are controlled in a density dependent manner, and if density dependence is manifested primarily in early life history stages, then reductions in the number of viable offspring due to controls on reproduction may be compensated for by enhanced survival in post-control streams. Thus, if the success of alternative control measures is to be judged as a function of the number of parasites produced, a variety of variables must be assessed throughout the early life history stages.

Knowledge of relationships between estimated numbers of adult spawners and larval recruitment is critical. Observations during lamprey telemetry studies and other compensatory mechanisms research suggest that many potential spawners do not participate in reproduction (O'Connor and Kelso in review). In contrast, when stocked in low densities as simulated in this study, we find that although male and female reproductive success is not uniformly distributed among parents, the majority of adults contribute progeny. The variance in fitness is expressed both as spawning failure and other as yet unidentified factors (Fig. 1). Additional research focusing on physiological or phenotypic variables of adults that correlate with reproductive success would be valuable. Further studies are recommended that are designed to investigate differences in larval growth and survival that can be related to the effects or interactions of environment and genotype (i.e., parentage).

Collateral benefits

Additional collateral benefits from these findings were realized in the forms of genus and perhaps species-specific molecular genetic keys for native lamprey species that can be utilized in situations were morphological classification is not possible. Secondly, we were able to examine the efficacy of using length-frequency data to estimate larval age. Work in this area is still ongoing as part of a graduate research project.

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Table 1. Samples sizes of sea lamprey larvae obtained from each of the seven SMRT Long Term Study Streams.

Stream	Number of Adults stocked[a]	Number age-0 larvae Collected	Genetically determined number non-*Petromyzon* age-0 larvae	Total number age-1 larvae collected	Number of Non-*Petromyzon* in sample of age-1 larvae
Middle River	100	50	not sampled	348	not sampled
Misery River	70	8	not sampled	233	3
Big Garlic River	14	59	0	284	0
Rock River	48	67	not sampled	169	25
Carp River	14	115	15	273	10
Stokely Creek	14	299	264	321	165
Wolf River	20	2	not sampled	154	0
Total	280	600		1782	200

[a] Equal numbers of males and females were introduced into each stream.

Table 2. Incidence of multiple matings by male and female sea lamprey in four Lake Superior streams.

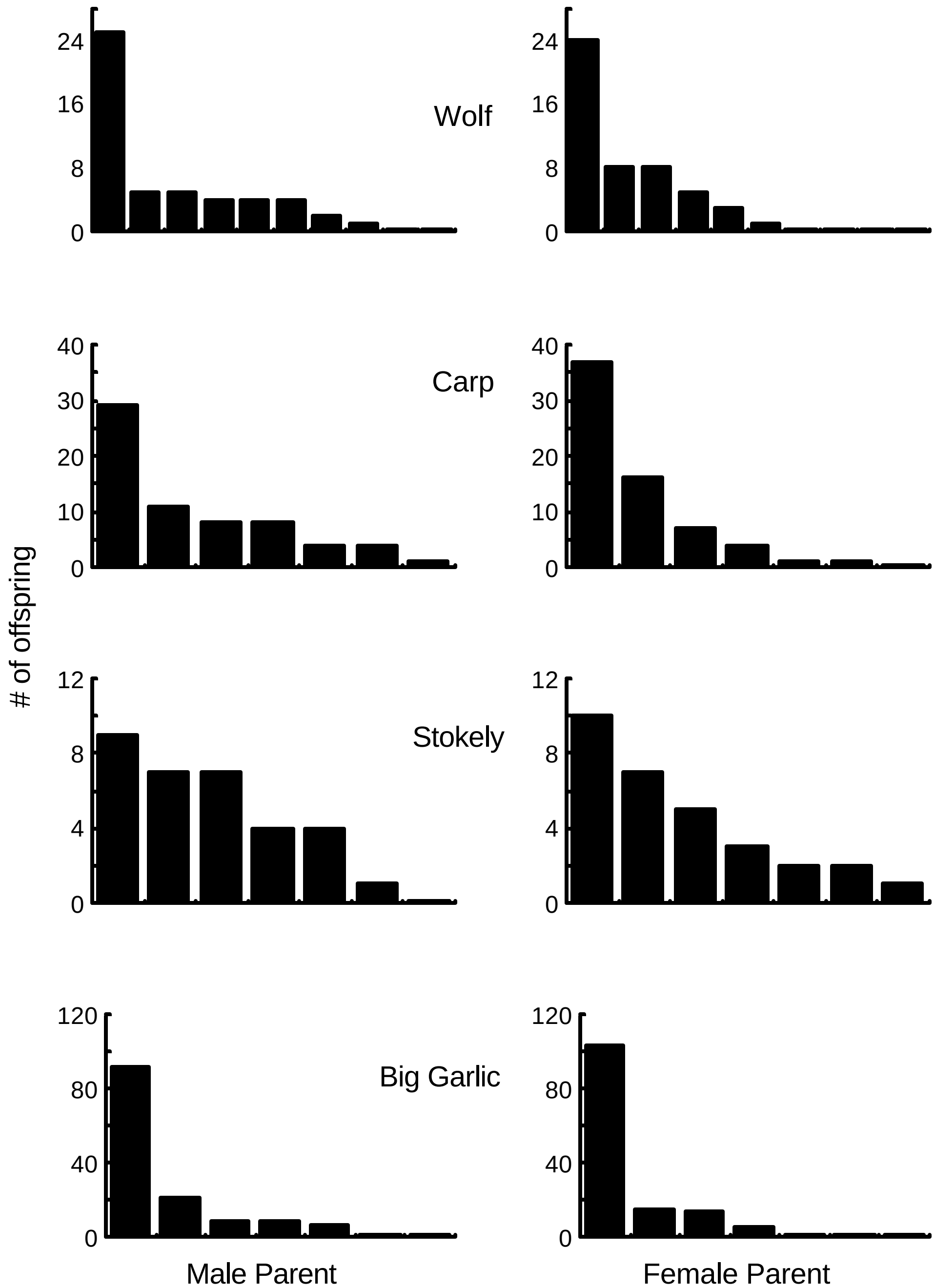
Stream	Number of mates males (polygyny)					Number of mates females (polyandry)					Total
	0	1	2	3	>3	0	1	2	3	>3	
Big Garlic	2	0	2	2	1	2	1	1	2	1	7
Carp	0	3	2	1	1	1	2	1	1	2	7
Stokely	1	1	1	2	2	0	1	3	2	1	7
Wolf	2	3	2	1	2	4	1	0	4	1	10

Note: 81 of 455 age 1 larvae could not be assigned to parents

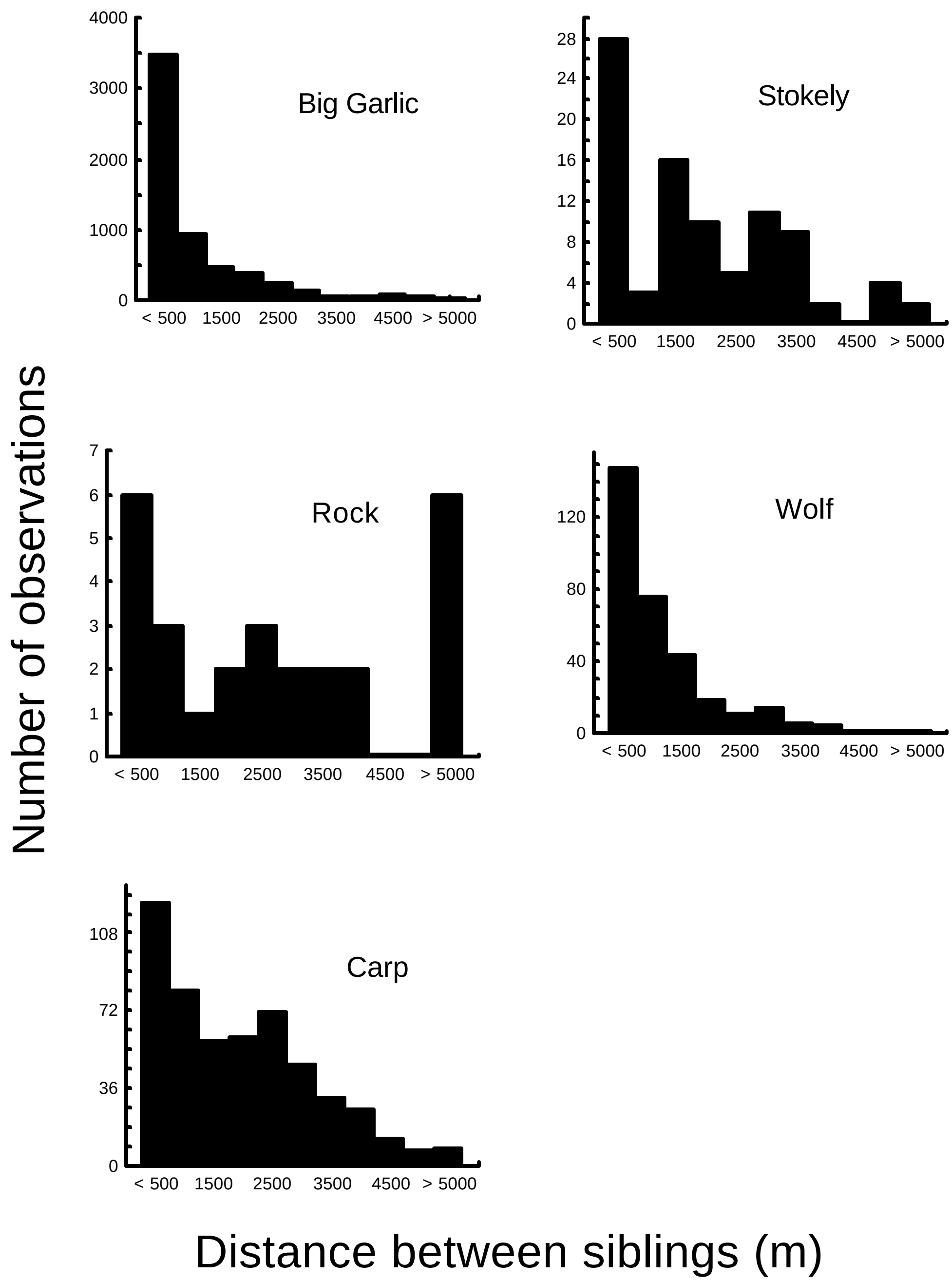
Figure Captions

Fig. 1 Observed number of age-1 larvae produced by each male and female stocked into each of 4 SMRT study streams. For the Wolf River, 10 pairs of adults were present. For the other 3 streams, 7 pairs were planted

Fig. 2. Distributions of the distances separating full- and half-sibling larvae in five SMRT study streams, based on all possible pairs of siblings of known parentage.



Wolf
Carp
Stokely
Big Garlic
24
16
8
0
40
30
20
10
12
4
120
80
# of offspring
Male Parent
Female Parent


Big Garlic
Stokely
Rock
Wolf
Carp
Number of observations
Distance between siblings (m)
< 500
1500
2500
3500
4500
> 5000