Mark-recapture estimates of stickleback population sizes in Paxton and Priest Lakes in 2016

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Introduction

We conducted a mark-recapture survey of population (census) sizes of the stickleback species pairs in Priest and Paxton lakes in spring 2016. Trapping success over the years by the Schluter lab and people in other labs had suggested that total numbers were high. Nevertheless, current population sizes were unknown. The present project aimed to remedy this situation.

Previous knowledge of population sizes of fish in these lakes comes from a mark-recapture study carried out in Paxton Lake in May, 2005 (M. Nomura and D. Schluter, unpublished). The results of this effort are shown in Table S1 of the Supplement. These estimates are now stale, being more than 10 years out of date. The estimates were based on relatively small samples, leading to highly uncertain estimates (wide confidence intervals).

Population sizes of the Priest Lake (Vananda Creek) species pair have never been estimated directly (previous numbers are based on extrapolating from Paxton Lake estimates according to area differences between Priest Lake and Paxton Lake).

Methods

Study populations

Population size was estimated in four populations from Texada Island, BC. These included the Paxton Lake Benthic Threespine (*Gasterosteus aculeatus*), the Paxton Lake Limnetic Threespine (*Gasterosteus aculeatus*), the Vananda Creek Benthic Threespine (*Gasterosteus aculeatus*) from Priest Lake, and the Vananda Creek Limnetic Threespine (*Gasterosteus aculeatus*) from Priest Lake. All four populations are federally listed as Endangered under the Species at Risk Act (SARA),

Access difficulties prevented us from conducting similar studies in Spectacle (Balkwill) Lake and Emily Lake (Vananda Creek), which also contain stickleback species pairs. Population sizes in these lakes have yet to be estimated directly.

Dates of study

The study was carried out in late April and early May, 2016. These dates were thought ideal because they represent the start of the breeding season, when stickleback move to the shallow margins of the lake to build nests and lay eggs. It is our experience that the breeding season is the time of year during which stickleback enter traps most readily. This is especially true of individuals of the Limnetic species, which can be difficult to catch using traps at other times of the year.

Trapping methods and locations

The field work was carried out by a group led Dr. Thor Veen (postdoctoral fellow), and consisting of other members of the Schluter lab: Marius Roesti (postdoctoral fellow), Seth Rudman (PhD student), Carling Gerlinsky, MSc (lab technician), Mackenzie Kinney (volunteer), Jeff Groh (UBC undergraduate student), and Brian Lohman (PhD student, University of Texas). All personnel had experience working with stickleback and were able to recognize the species in the hand, and were trained on the marking methods used.

Two main rounds of trapping were carried out in each lake. The first round constituted the *mark* phase, when captured fish were marked and released. In the second, *recapture* phase, captured fish were inspected for marks and then released. Dates of each activity are shown in Table 1. We used 1/4” and 1/8” mesh galvanized steel “Gee’s” minnow traps baited with a small amount of cheddar cheese. Traps were approximately evenly spaced around the margins of each lake, with higher numbers of traps set where shallow substrate was more extensive. Traps were placed along the lake bed from a row boat at a range of water depths between about 0.5 m and 2.5 m. Latitude and longitude of each trap was recorded using a Garmin eTrex 30 GPS device, and are accurate to within approximately 3 m. The number of traps used and the mean number of hours traps were active on each day are shown in Table 1. Maps of trap locations on each date, as determined using GPS coordinates, are provided in Supplemental Figure S1.

Table 1. Dates of activities, the number of traps used, and the mean number of hours traps were active.

		Number of traps used per activity			**Mean hours**
Lake	**Date**	**Mark**	**“Test recapture”**	**Recapture**	**per trap**
Paxton	2016-04-15	86			8.0
Paxton	2016-04-16	90			5.8
Paxton	2016-04-17	80			9.4
Paxton	2016-04-21		69		6.8
Paxton	2016-04-22		62		6.4
Paxton	2016-04-23		50		3.8
Paxton	2016-05-05			119	6.9
Priest	2016-04-18	63			8.1
Priest	2016-04-19	61			9.4
Priest	2016-04-20	48			8.8
Priest	2016-05-03			116	9.7
Priest	2016-05-04			109	10.4

To minimize disturbance to the breeding populations, traps were checked regularly, at most every three hours, and fish were released at the point of capture. During the mark phase of the study, fish caught in a trap were gently decanted into a pail of clear lake water. Fish were manually taken from the pail one at a time and marked by removing a small section of caudal fin using surgical scissors (see below). The same approach was used during the recapture phase, except that instead of marking, fish were individually inspected to determine whether they had been marked previously.

In Paxton Lake only, we carried out a "test recapture" shortly after the mark phase was completed to compare with the recapture results obtained later. Our purpose was to determine whether the proportion of marked fish showed an apparent decline between the time shortly after marking and the later recapture date, which is expected if more time results in better mixing of marked fish with the unmarked population in the shallow areas of the lake subjected to trapping. This effect was confirmed, as we show in the Supplement. We feel that population size estimates based on the recapture results are more reliable than those based on the test recaptures, and we present only these estimates in the Results.

We recorded each individual as either benthic or limnetic, with different marks applied to each species to speed classification during the recapture phase. Processing time was short, and mistakes in classification were undoubtedly made. The percentage 0.87% of marked individuals was judged upon recapture to have been classified as the wrong species (i.e., benthics initially scored as limnetics, or the reverse). An individual was scored as a "hybrid" if species status was difficult to determine because of an intermediate phenotype. Only 0.19% of individuals captured throughout the entire study were classified as "hybrid". This is less than the true fraction of hybrids in the lake (approximately 1-2% of individuals are first-generation (F1) hybrids, and a similar number of individuals are backcross hybrids between F1's and limnetics and benthics, based on a preliminary genetic analysis; Rennison et al. unpublished). As a result, most F1 and backcross hybrids have been mistakenly classified as benthic or limnetic depending on their degree of resemblance to those species.

With one exception, all captured fish were processed to ensure that every section of the lake received approximately equal effort. However, this was not possible in Paxton Lake for one of the three rounds of trap checks during the "capture" session on April 17, 2016, because too many fish were captured to process in the daylight hours available. In this one case we processed fish only for each 4th trap, whereas fish in the other three traps were released unmarked. It was felt that marking fish from a uniform proportion of the traps would not subsequently bias the estimation of the proportion of marked individuals in the lake.

Marking methods

Fish were marked by holding the individual in the hand and taking a small proportion of the caudal fin using a pair of surgical scissors. In the case of limnetics, the fin clip was taken from the top part of the caudal fin. The clip was taken from the bottom part of the caudal fin in the case of benthics. "Hybrids" had a fin clip removed from both the top and bottom part of the caudal fin.

When marking and recapturing individuals we did not record the sex or likely age of individuals, in contrast to the 2005 study by M. Nomura and D. Schluter (unpublished). This was mainly because the current study began early in the reproductive season, when it was difficult to distinguish males not yet in breeding colours from females. In addition, ignoring age and sex sped up processing time and permitted an increased sample size. In the case of limnetics, all captured individuals are about 1 year old. (We have not seen evidence of 2-year old limnetics in the wild, as judged by body size. They often live to 2 years of age in the lab, but in the wild the limnetic species in Priest and Paxton Lake appears to have an annual life history.) Benthics were a mixture of juveniles (≥ 1 year old) and adults.

Table 2 summarizes the numbers of fish captured and marked in this study.

Table 2. Number of marked and unmarked fish caught in each trapping session. Marked fish caught during the Mark sessions represent individual fish that were re-caught while marking was under way. These individuals were not used in population size estimates.

		Paxton Lake			**Priest Lake**	
Species	**State**	**Mark**	**“Test recapture”**	**Recapture**	**Mark**	**Recapture**
Benthics	Unmarked	882	1663	1234	4458	5767
Benthics	Marked	11	121	51	69	227
Limnetics	Unmarked	4401	2517	2340	2211	1789
Limnetics	Marked	22	97	29	22	37
“Hybrids”	Unmarked	23	1	4	5	18
“Hybrids”	Marked	0	3	0	0	0

Analysis

Analyses use the counts obtained from two trapping sessions in each lake. In the first (mark) session, n_1 individuals were caught, marked, and released. In the second (recapture) session, n_2 individuals were captured and the number of individuals found to be previously marked, r, was counted. We recorded the numbers of individuals captured and recaptured in each trap. The counts n_1, n_2 and r are sums over all traps used in the session.

Two methods were used to estimate population size. The first was the Lincoln-Petersen method (Krebs 1999), which treats individual fish as independent observations. In other words, the fish caught in each trap are assumed to represent a random sample from a single, well-mixed population. In this case the estimated proportion $\hat{p}$ of marked individuals in the population is obtained from the recapture data as

$$\hat{p} = r/n_2.$$

Estimated population size $\hat{N}$ is then

$$\widehat{N} = n_1/\hat{p}.$$

To obtain a confidence interval, we use the fact that the number of recaptures r in a random sample of n_2 individuals from a population in which n_1 individuals are marked follows a hypergeometric distribution (Gazey and Staley 1986). We used the *dhyper* function in *R v*3.3.1 (R Core Team 2016) to calculate the log-likelihood of all feasible values of N. Log-likelihood values falling within 1.92 units of the maximum value (the value 1.92 is the critical value $\chi^2_{1(0.05)}/2$) determine the likelihood-based 95% confidence interval for N (Whitlock and Schluter 2015).

The second, regression-based method to estimate population size allowed the true proportions of marked and unmarked fish to differ between traps, owing to likely heterogeneity in the proportions of marked individuals sampled. We used least squares regression to estimate the mean and variance in the proportion of marked individuals in traps. This approach treats the trap, rather than the individual fish, as the independent observation. This was judged to be the most reasonable assumption because stickleback often aggregate in schools, and the fish caught in a given trap might be made up mainly of individuals from a single school. The assumption of independence of individuals (the assumption of the Lincoln-Petersen method) is violated if marked and unmarked fish do not mix freely among schools after marking. Especially in the case of limnetics from Paxton Lake, we observed high variance among traps in the proportion of marked individuals when large numbers of fish were caught simultaneously. This suggested to us that schools were not freely mixed aggregations of marked and unmarked individuals. Hence we prefer the second, regression-based method.

This regression method estimated the mean proportion $\hat{p}$ of marked individuals in the population as the slope of a linear regression in which each data point is a trap. The y-variable in the analysis was the number of previously marked individuals caught in each recapture trap, whereas the x-variable was the total number of individuals caught in the same trap. Traps that caught no fish were not included. The regression line was fitted through the origin and was weighted by assuming that the variance of the residuals is proportional to the total number of individuals caught in a recapture trap. The regression slope $\hat{p}$ was estimated using the *lm* function in *R v*3.3.1, which also yielded a 95% confidence interval for the true slope p, the mean proportion of marked individuals in the population. The lower and upper bounds of a 95% confidence interval for population size N was obtained by separately plugging the lower and upper limits of the 95% confidence interval for p into the formula above for $\hat{N}$.

The regression method was also used to test whether the proportion of fish recaptured differed between traps with coarse mesh (1/4”) and those with fine mesh (1/8”). Two regression lines were fitted through the origin, one for each mesh type, again weighted by assuming that the variance of the residuals was proportional to the number of fish caught in recapture traps. Effect of mesh type on the recapture proportions was tested by comparing the slopes of the two regressions using the *lm* function in R. No differences were detected between trap types in either species (all $P > 0.05$) in both the recapture session and the “test recapture” session (Paxton Lake only), and the results are not included herein.

Both the Lincoln-Petersen and regression methods assume that mark and recapture sessions are close enough in time that no individuals have died in the interim; that no fish have been born; and that there has been no immigration or emigration. Some stickleback reproduction had probably occurred between mark and recapture sessions, but young of the year were rarely caught in traps (these were released immediately and left uncounted). Immigration to Paxton Lake is not possible, and emigration (over a dam) was unlikely. Immigration from, and emigration to, Spectacle Lake from Priest Lake was possible but unlikely. Fin clips used to mark fish regrow eventually, but not over the time frame of the study.

However, mortality was possible between sampling sessions. If mortality of marked individuals was higher than that of unmarked individuals, then our methods will tend to overestimate population size. Population sizes will also tend to be overestimated if marked fish are more reluctant to enter traps during the recapture sessions than unmarked fish. In contrast, population size will be underestimated if some classes of fish in the population are reluctant to enter traps at all. For example, past experience suggests that female limnetics in Priest Lake are much more difficult to trap than female limnetics in Paxton Lake. This will have the effect of biasing downward the estimated population size if males and females are not distinguished in the analysis.

Graphs and maps were drawn in *R v*3.3.1. Maps were drawn using the *ggmap v2.6.2* package (Kahle and Wickham 2013).

Permits

The study was conducted under the permit SARA 16-PPAC-00004 from the Department of Fisheries and Oceans, permit MRSU16-229407 from the Ministry of Forests, Lands and Natural Resources Operations of British Columbia, and Animal Care Protocol A16-0044 from the University of British Columbia, to Dolph Schluter.

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Results and Discussion

Population size estimates calculated using the regression method are shown in Table 3. Values for $\hat{N}$ are similar to those based on the Lincoln-Petersen method (Table S2), but the confidence limits from the regression method are wider. Of the two methods used, we believe that the confidence limits based on the regression method (Table 3) more reliably indicate the uncertainty of estimates of population size than those based on the Lincoln-Petersen approach.

Table 3. Population size estimates of limnetics and benthics in Priest and Paxton lakes. These results are based on the regression method to estimate population size, which treats the trap as the independent replicate rather than the individual fish. Corresponding Lincoln-Petersen estimates are given in Table S2.

Lake	**Species**	$\boldsymbol{n_1}$	$\hat{p}$	$\hat{N}$	**95% confidence interval** **lower**	**upper**
Priest	Benthic	4,458	0.0378	**118,058**	101,351	141,358
Priest	Limnetic	2,211	0.0200	**110,612**	78,068	189,684
Paxton	Benthic	882	0.0397	**22,191**	17,544	28,991
Paxton	Limnetic	4,401	0.0119	**368,885**	236,137	842,518

These are the first direct estimates of population sizes of benthics and limnetics in Priest Lake (Table 3). Numbers of both species appear to be high, over 100 thousand. The number of limnetics was estimated to be only slightly higher than the number of benthics (in contrast to Paxton Lake, where the limnetics appear to be much more common than benthics). A possible explanation is the higher density of native cutthroat trout in Priest Lake compared to Paxton Lake, which observers have seen attacking limnetics in the open water, but this difference between lakes is anecdotal – trout densities have not been quantified in the species pair lakes.

Another possibility is that the number of limnetics in Priest Lake might be underestimated. Our anecdotal observations over the years suggest that male limnetics in Priest Lake enter traps more readily than female limnetics. If so, then the estimated proportion of marked limnetics in the lake ($\hat{p}$ = 0.020; Table 3) might be inflated, if a large segment of the population is avoiding the traps. This would cause us to underestimate total population size of limnetics in Priest Lake. Priest Lake male and female benthics are not noticeably different in their tendency to enter traps.

In Paxton Lake, the population size estimate for benthics in 2016 (~22.2×10^3) is lower than the estimate from 2005 (~ 30×10^3; Table S1, based on the combined categories). However, the confidence intervals for benthic population sizes in the two years overlap extensively ([17.5K – 30×10^3] in 2016 vs [23.5×10^3 – 40×10^3] in 2005), which means that the differences observed are not statistically significant. Nevertheless, caution concerning the possibility of relatively low numbers of benthics in Paxton Lake is warranted.

In 2016, unlike 2005, we did not distinguish mature males from other individuals and so we are unable to divide the estimated population size into categories. In 2005, approximately 10% of Paxton Lake benthics were estimated to be mature males, leading to the expectations that 20% of the population represented reproductively mature individuals, assuming an equal sex ratio of reproductive individuals.

There is clearly a large population of limnetics in Paxton Lake, but even so, an estimated population size of 369×10^3 seems extraordinary (Table 3). We do not have a satisfactory explanation for this high estimate. Any increased mortality of limnetics as a consequence of marking will cause us to underestimate the proportion of marked individuals in the lake, and consequently overestimate population size. At present we have no information on mortality caused by marking, but we do not believe that it is significant.

During the recapture phase of the study in Paxton Lake we noticed that a small number of traps contained large numbers of limnetics having a low fraction of marked individuals. We considered the possibility that these were fish from relatively stable schools of limnetics that were largely missed during the capture phase of the study. Attempts to correct for this effect by using only traps with <100, <60, and <40 individuals and re-estimating the proportion of marked individuals in the lake did not yield population size estimates less than ~150,000. But the very existence of large schools of limnetics with a low proportion marked individuals would imply that even this estimate of population sizes must be an underestimate. We conclude that the population sizes of limnetics in Paxton Lake are probably very large, but exactly how large remains uncertain.

Finally, we attempted to detect spatial variation in the density of limnetics and benthics within each lake by plotting the rate per hour at which individuals were caught in traps. The results are shown on maps of the lakes in Figure S2. Mark, recapture, and "test recapture" sessions were plotted separately (each session might represent the results of 1-3 consecutive days of trapping). The results suggest that the species are fairly evenly distributed around the lake. The exception is the Paxton Lake limnetic, which showed very high spatial variability in capture rates. This variability might be contributing to the uncertain estimates of population size in this species.

Data files

All analyses are based on the data set provided as an attachment to this report, "PaxtonPriestMarkRecaptureData2016.v1.2.csv". Table S5 includes a description of the variables in the data file.

References

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