SHORT COMMUNICATION

Founding population size of an aquatic invasive species

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Abstract Non-native species of fish threaten native fishes throughout North America, and in the Rocky Mountains, introduced populations of lake trout threaten native populations of bull trout. Effective management of lake trout and other exotic species require understanding the dynamics of invasion in order to either suppress non-native populations or to prevent their spread. In this study, we used microsatellite genetic data to estimate the number of lake trout that invaded a population of bull trout in Swan Lake, MT. Examination of genetic diversity and allele frequencies within the Swan Lake populations showed that most of the genes in the lake trout population are descended from two founders. This emphasizes the importance of preventing even a few lake trout from colonizing new territory.

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Introduction

Invasive species threaten the biodiversity of aquatic ecosystems worldwide (Vitousek et al. 1997), and are considered the second greatest threat to biodiversity loss in North America (Mooney and Cleland 2001). Non-native fishes have homogenized fish faunas throughout North America, impacting many species and populations of native fishes through competition, predation, and introgressive hybridization (Rahel 2000). Invasions of introduced taxa often disrupt the structure and function of ecosystems, reduce biological diversity among native species, and impose huge economic costs (Mack et al. 2000). Therefore, understanding how exotic species invade ecosystems is critical for conserving many native aquatic species.

Lake trout (*Salvelinus namaycush*) are large, long-lived, top-level predators native to deep, cold, oligotrophic lakes of Canada and northern parts of the United States, including the Great Lakes (Behnke 2002). During the late 19th and early 20th century, lake trout were widely introduced into lakes and reservoirs outside their native range (Crossman 1995). More recently, the species is expanding its range in the western United States through dispersal and unauthorized translocations (Behnke 2002). While lake trout occupy an important ecological niche as a top-level predator in lakes where they are native, they have often become predators and competitors with native fishes in lakes where they have been introduced (Ruzycki et al. 2003; Koel et al. 2005; Martinez et al. 2009).

Lake trout recently colonized Swan Lake, MT and now threaten a native population of bull trout (*S. confluentus*). The objective of this study was to determine the number of lake trout that founded the Swan Lake population. Lake trout are unique relative to other aquatic

invasive species because they are long-lived and have a high age at first reproduction. Thus, understanding the effects of founding population size on the rate of population expansion will provide useful information for ecologists wanting to control the expansion of lake trout in lentic ecosystems.

Study area and populations

Swan Lake is 10.8 km^2 and located in the Seeley-Swan Valley of western Montana. The lake morphometry is characteristic of glaciated lakes in the region. However, Swan Lake is relatively shallow compared to other lakes that contain lake trout in the Intermountain West. The native population of bull trout in Swan Lake is one of the most robust extant populations in the United States (Fraley and Shepard 1989; Rieman et al. 1997). Bull trout are listed as "threatened" with extinction under the US Endangered Species Act, which provides a legal mandate to protect the Swan Lake population. Unfortunately, lake trout were discovered in Swan Lake in 1998 (Vernon 1998). Bull trout and lake trout are both top-level predators (Donald and Alger 1993), and lake trout consistently displace native bull trout in lake systems where they have become established (Donald and Alger 1993; Fredenberg 2002). The population of lake trout in Swan Lake appears to be growing rapidly. Approximately 7,000 lake trout >270 mm were estimated to be in Swan Lake in 2008, and 94% of these fish were between 3 and 5 years old (Cox and Guy unpublished).

The source of the lake trout invasion in Swan Lake was most likely Flathead Lake. Flathead Lake is the largest freshwater lake in the western coterminous United States and has had a large lake trout population since lake trout were introduced in 1905. During the 20th century, lake trout spread throughout the Flathead Drainage Basin (Meeuwig 2008), and were first caught in the Swan Lake basin in 1998. Swan Lake is connected to Flathead Lake by the Swan River which drains Swan Lake and enters Flathead Lake 23 km downstream, and this is a plausible invasion route for lake trout. Fish movement between Flathead Lake and Swan Lake was impeded by Bigfork Dam, which was built in 1902, but the dam was retrofitted with a fish ladder that was operational from 1959 to 1993. Lake trout could have entered Swan Lake via the fish ladder or illegally introduced. If lake trout were introduced illegally, Flathead Lake is the most plausible source, as it is only a 10 km drive from Flathead Lake to Swan Lake. The second closest possible source is Whitefish Lake, which is 66 km by road from Swan Lake. The exact mechanism of how lake trout entered Swan Lake is unknown, but it is likely they have been in the system for several years because invasive species are often

established before being sampled using standard sampling methods (Munro et al. 2005).

Methods

Tissue samples of lake trout were collected from Swan Lake ($N = 389$) in 2006 and 2007, and from Flathead Lake ($N = 15$) in 2007. Special care was taken to sample fish randomly from Swan Lake. In 2006, lake trout in Swan Lake were sampled using a random design with 2.54-cm bar mesh sinking monofilament gill nets. In 2007, a stratified random sampling design was used to sample lake trout. The sampling stratum was designated as all of the lake at or below thermocline depth (~ 18 m), where lake trout were expected to occur during lake stratification. Three sinking monofilament gill nets were combined to form a gang 1,371.6-m long with 2.54, 3.18, 3.81, 4.45, and 5.08-cm bar mesh.

Eleven polymorphic microsatellite loci (Sna_MSU01, Sna_MSU02, Sna_MSU03, Sna_MSU05, Sna_MSU06, Sna_MSU07, Sna_MSU08, Sna_MSU10, Sna_MSU11, Sna_MSU12, Sna_MSU13) were genotyped using protocols described by Rollins et al. (2009). Genotypes were tested for agreement with Hardy–Weinberg expectations using the probability test of Guo and Thompson (1992) implemented by the computer program GENEPOP (Rousset 2008). The amount of genetic diversity within Swan Lake and Flathead Lake was measured with expected heterozygosity, H, allelic richness, N_a, and private allelic richness, N_u. Because the number of alleles present in a sample is expected to increase as sample size increases, rarefaction was used to compare allelic and private allelic richness between samples (Kalinowski 2004, 2005). One tailed paired t-tests were used to assess statistical significance for the difference between Swan Lake and Flathead Lake for each of these three measures (H, N_a, N_u).

The size of the founding population was estimated by comparing the expected heterozygosity of the Swan Lake sample with the expected heterozygosity of the Flathead Lake sample. If we assume that the Swan Lake population was founded from N individuals from Flathead Lake, and that the Swan Lake population grew rapidly after it was introduced, we can estimate the effective number of founding individuals, N_f, from the following relationship (Hedrick 2005, Eq. 6.3a)

$$H_{\text{Swan}} = H_{\text{Flathead}} \left(1 - \frac{1}{2N_f} \right) \tag{1}$$

where H_{Flathead} is the heterozygosity in Flathead Lake and H_{Swan} is the post-colonization heterozygosity of Swan Lake. A 95% confidence interval for N_f was constructed by bootstrapping across loci 10,000 times.

Results and discussion

Hardy–Weinberg tests revealed no statistically significant departures from expected genotype counts at the 0.05 level of significance. The average heterozygosity in the Swan Lake sample was much lower than the Flathead Lake sample ($H = 0.68$ vs. 0.88; $P < 0.0001$). In addition, there were fewer alleles in the Swan Lake sample than in the Flathead Lake sample ($N_a = 3.6$ vs. 9.9 alleles per locus per ten individuals; $P < 0.00001$). The genetic diversity present in Swan Lake appears to be a subset of the genetic diversity present in Flathead Lake—there were few unique alleles present in Swan Lake, but many for Flathead Lake ($N_u = 1.5$ for Swan Lake and $N_u = 7.8$ for Flathead Lake; $P < 0.00001$). The estimated effective size of the founding population, N_f, was 2.3, with a 95% confidence interval of (1.7, 3.3).

These results are consistent with the Swan Lake population of lake trout being founded from a very small number of individuals from Flathead Lake. We estimated that the founders of the population had an effective population size of 2.3. To obtain this estimate, we assumed that the population of lake trout in Swan Lake expanded to a large size in the generation immediately after the population was founded, and lost genetic diversity only during the initial bottleneck. If this did not happen, and the Swan Lake population of lake trout was small for more than one generation, Eq. 1 will underestimate the true number of founders. There are an infinite number of founding population sizes and population growth trajectories that could cause heterozygosity to decline from 0.88 to 0.68. For example, this decline in heterozygostiy is consistent with two generations of genetic drift with an effective population size of 4.3 or three generations of drift with an effective population size of 6.3 (see Eq. 6.3b in Hedrick

2005). We do not know enough about the history of the population to choose from among these scenarios using historical information, but there are additional ways to analyze the genetic data to help discriminate among them.

Equation 1 estimated the effective number of founders to be 2.3. This suggests that the Swan Lake population might have been founded from two individuals. The "two founders" hypothesis makes two testable predictions: (*i*) there should be no more than four alleles at any locus, and (*ii*) each allele in the population should have a frequency of 0.25, 0.50 or 0.75. The first prediction of the "two founder" hypothesis is not met: there are two loci with five alleles and two loci with six alleles in the sample of fish from Swan Lake (Table 1). However, the second prediction is nearly met. Of the 47 alleles observed in Swan Lake, 35 have a frequency distributed around 0.25, three have a frequency of approximately 0.5, and one has a frequency of approximately 0.75. There are also eight rare alleles, each with a frequency of less than 2% (Table 1; Fig. 1).

A plausible explanation for these data is that the population of lake trout in Swan Lake population was founded from three individuals, but two individuals were responsible for the great majority of reproduction. The data fit this scenario well. Despite the fact there are an average of 12 alleles per locus in the Flathead Lake sample, there are no more than six alleles at any locus in Swan Lake. Furthermore, loci that have five or six alleles in Swan Lake have only four common alleles (Table 1).

These data suggest that two founders were responsible for most of the reproduction and that one or more additional fish contributed a small amount of genes to the population. This may have occurred in at least a couple ways. The third fish may have been an original founder and may have only contributed a small number of gametes to the first cohort in Swan Lake. Alternatively, this third founder may have

Table 1 Number of alleles observed (N_a) in Swan and Flathead Lake, and allele frequencies in Swan Lake

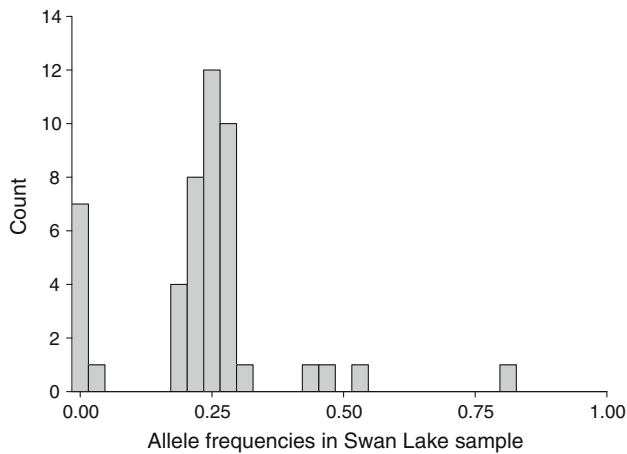| Locus	N_a		Allele frequencies in Swan Lake					
	Flathead	Swan						
Sna_MSU01	13	4	0.2935	0.2558	0.2273	0.2234		
Sna_MSU02	6	4	0.2863	0.2642	0.2604	0.1891		
Sna_MSU03	10	4	0.2723	0.2618	0.2435	0.2225		
Sna_MSU05	11	4	0.2642	0.2604	0.2578	0.2176		
Sna_MSU06	21	5	0.2791	0.2674	0.2339	0.2183	0.0013	
Sna_MSU07	13	6	0.2816	0.275	0.2329	0.1921	0.0158	0.0026
Sna_MSU08	9	2	0.8119	0.1881				
Sna_MSU10	13	5	0.5390	0.2701	0.1753	0.0143	0.0013	
Sna_MSU11	10	4	0.4367	0.2984	0.2636	0.0013		
Sna_MSU12	14	3	0.4664	0.2661	0.2674			
Sna_MSU13	14	6	0.2631	0.2579	0.2461	0.2291	0.0026	0.0013

Fig. 1 Histogram of the frequencies of alleles in the Swan Lake sample. Alleles in the bin labeled "0.00" had a frequency greater than zero but less than 0.016

immigrated to Swan Lake after the initial founding of the population and made a relatively small genetic contribution to the population because the population was already large. It is also possible that Swan Lake was colonized by many lake trout, but only a couple fish reproduced.

We have assumed that lake trout in Swan Lake originated from Flathead Lake. Genetic data can be used to check this assumption, but the analysis is complicated by the genetic bottleneck created by the founding event. The value of θ (Weir and Cockerham 1984) between the Swan Lake and Flathead Lake samples is 0.15. This is a substantial amount of genetic differentiation, but may not be higher than we would expect from a severe genetic bottleneck. We can calculate the expected value of θ created by a founding event, $\theta_{\text{bottleneck}}$, if we assume Hardy–Weinberg equilibrium within populations

$$\theta_{\text{bottleneck}} = \beta = \frac{\frac{1}{2}(F_{\text{Flathead}} + F_{\text{Swan}}) - F_{\text{Flathead}}}{1 - F_{\text{Flathead}}},$$

where F is the homozygosity within populations and β is a version of θ that assumes populations are in Hardy–Weinberg equilibrium (Cockerham and Weir 1987). Given the homozygosities within Swan Lake and Flathead Lake, we would expect $\theta_{\text{bottleneck}}$ to equal 0.11. This is a bit less than observed, but if we construct a 95% confidence interval for $\theta_{\text{bottleneck}}$ by bootstrapping over loci, it extends to 0.15, so we conclude that the observed amount of genetic differentiation between Swan Lake and Flathead Lake could be caused by a bottleneck.

We have concluded that the Swan Lake population of lake trout is descended from approximately two founders and that these founders were from Flathead Lake. We supported this conclusion with four lines of evidence: reduced heterozygosity in Swan Lake, the lack of unique alleles in Swan Lake, allele frequencies in Swan Lake that

were distributed among multiples of 0.25, and genetic differentiation between Swan and Flathead lakes that is consistent with a population bottleneck. Considered independently, none of these pieces of evidence is especially convincing. However, considered together, this provides strong support for the history that we have proposed.

Conservation of native biota requires understanding the invasion patterns and the mechanisms that promote invasion success. In North America, many populations of native salmonids are threatened by competitive interactions with non-native salmonids, yet prior to this study, few studies have assessed the founding population size of the invasive taxon. Our genetic data collected from nonnative lake trout in a recently invaded system show that the lake trout population was established by approximately two individuals. Additionally, the allele frequencies at four microsatellite loci in the Swan Lake sample were consistent with those found in nearby Flathead Lake, indicating that Flathead Lake is likely the original source population. Combined, these data suggest that only a few individuals are needed to establish a self-sustaining population of lake trout.

Lake trout have been intentionally, illegally or invasively established in over 200 waters in the western United States (Martinez et al. 2009). In many cases, introduced lake trout prey on and/or compete for resources with native fishes, resulting in cascading impacts within and beyond the affected water bodies and terrestrial communities. Our data demonstrate the remarkable invasive capability of lake trout and how few fish it takes to found a population. Eradication or suppression of nonnative populations, therefore, may be required as an effective management strategy for reducing the spread of nonnative lake trout to protect and conserve native fish populations in Swan Lake and elsewhere.

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