Post-Perturbation Genetic Changes in Populations of Endangered Virgin River Chubs

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Abstract: A 34-kilometer reach of the Virgin River, Utah-Arizona-Nevada, was poisoned with rotenone in an attempt to eradicate non-native red shiners (Cyprinella lutrensis), a species implicated in the decline of native fish populations in the American West. An error in detoxification resulted in lethal concentrations of pesticide passing through an additional 50 kilometers of stream. We used allozyme electrophoresis to analyze genetic variation among pre- and post-poison samples of endangered Virgin River chubs (Gila seminuda). Pre-poison samples indicated a single panmictic population in the river. In contrast, fish subsequently produced through natural recruitment in poisoned reaches exhibited deviations from the original pattern of genetic variation. A genetic bottleneck caused by severe reduction in the number of spawning adults was indicated. The altered pattern persisted 2.5 years post-poisoning, indicating unexpectedly slow recolonization from the unpoisoned reach upstream. Genetic variation among hatchery-produced young was similarly unrepresentative of the original pattern because of the small number of brood fish used in propagation. Because of their small numbers and/or restricted distribution, endangered species are particularly vulnerable to natural or anthropogenic catastrophes. Assessment of the genetic impact of such events is essential but requires that baseline data are available.

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Cambios genéticos post-perturbación en poblaciones amenazadas de “Virgin River chubs” (Gila seminuda).

Resumen: Un tramo de 34 kilómetros del Río Virgin, Utah-Arizona-Nevada, fue envenenado con rotenon en un intento de erradicar “red shiners” (Cyprinella lutrensis) no nativos, una especie implicada en la declinación de peces nativos en el Oeste Americano. Un error en la detoxificación resultó en concentraciones letales de pesticida que penetraron en 50 km adicionales del río. Usamos electroforesis de aloenzimas para analizar la variación genética entre muestras de Gila seminuda en peligro de extinción obtenidas antes y después del envenenamiento. Las muestras previas al envenenamiento indicaron una única población panmítica en el río. Por el contrario, los peces producidos subsequentemente por medio de reclutamiento natural en los tramos envenenados exhibieron desviaciones del patrón de variación genética original. Esto indicó un cuello de botella genético debido a una severa reducción en el número de adultos descendientes. Este patrón persistió por 2.5 años después del envenenamiento, lo que indica una recolonización insperadamente lenta a partir de los tramos no envenenados de río arriba. La variación genética entre jóvenes producidos en criaderos fue igualmente no representativa del patrón original debido al bajo número de crías de los peces usados en la propagación. Las especies en peligro de extinción son particularmente vulnerables a catástrofes naturales o antropogénicas debido a su baja abundancia y/o distribución restringida. La evaluación del impacto genético de tales eventos es esencial pero requiere que los datos de base estén disponibles.
Introduction

Management plans for conservation of endangered fishes may include a number of options, such as translocation to refugia, captive breeding, or removal of detrimental, non-native competitors and predators. With regard to the last option, nonselective poisons (piscicides) have long been used by fisheries managers to eradicate unwanted fishes (Lennon et al. 1970; Eschmeyer 1975), and desired species, usually sportfishes, are reintroduced or allowed to recolonize from adjacent populations. These same techniques are now being applied in conservation efforts designed to save imperiled fishes (Rinne & Turner 1991) for which no or only small adjacent populations may exist.

The use of poisons for management purposes may constitute a major biotic disturbance, equivalent, for example, to natural perturbations such as regional drought or volcanism or anthropogenic disasters such as chemical spills. Clearly, such a catastrophe can affect the genetic structure of future generations. We report substantial genetic changes in the Virgin River chub (*Gila seminuda*; formerly *G. robusta seminuda* [see DeMarais et al. 1992]), an endemic, endangered fish subject to a piscicide applied to eradicate an exotic species, and we assess the value of genetic data for quantifying the effects of environmental perturbations.

The Virgin River System

Native western fishes have long suffered from ever-increasing regional aridity. Modern expansion of humans has devastated them further through habitat destruction accompanying water-resource development. Fishes of the Virgin River are among the most imperiled due to water diversions and invasions by non-native species (Deacon 1979, 1988). Thirteen species of non-native fishes have been introduced to the system (Cross 1985). Among these, the red shiner (*Cyprinella lutrensis*) has been implicated in the decline of indigenous fishes throughout the region (Minckley & Deacon 1968; Minckley 1973; Greger & Deacon 1988). A native of the Rio Grande/Mississippi River drainages, it was first introduced into the Colorado River basin in the 1950s as a bait and forage fish (Hubbs 1954; U.S. Fish and Wildlife Service [USFWS] 1980).

Red shiners invaded the lowermost Virgin River by the 1960s, but their upstream progress was blocked for two decades by a normally dry, high-gradient canyon (Fig. 1). In 1984, they advanced upstream above the gorge to populate the river below Washington Fields Diversion, Utah, an enhanced natural barrier that delayed further dispersal (Heckmann et al. 1987). In response to the threat of further invasion, the USFWS and Utah Department of Wildlife Resources used rotenone to eradicate red shiners from a reach below Washington Fields Diversion (Fig. 1). Artificial downstream barrier dams were proposed to impede reinvasion following treatment (USFWS 1989).

Rotenone is nonselective at recommended concentrations, so detoxification (using potassium permanganate as an oxidant) was planned to prevent downstream mortalities. Fishes from the untreated upstream reach were expected to recolonize. Prior to poisoning, about 1600 Virgin River chubs were removed to an upstream pond, and 160 of these were transferred to Dexter Na-
tional Fish Hatchery (NFH) and Technology Center, New Mexico, for use as broodstock if reintroductions were deemed necessary (USFWS 1989).

A 10-kilometer reach immediately below Washington Fields Diversion was first poisoned in September 1988. No detoxicant was applied; natural oxidation and low discharge precluded a downstream fish kill. In October, although discharge was greater and downstream barriers were not yet completed, rotenone was applied to the lower 24 kilometers of the target area. Detoxification failed because of human error (Stefferud 1988). Dead and dying fishes were recorded over the next two days at Littlefield, Arizona, and at Mesquite, Nevada, more than 30 and 50 river-kilometers, respectively, below the detoxification point (USFWS 1989). Collecting efforts immediately following poisoning failed to obtain any chubs near Littlefield (USFWS 1988). The entire 34-kilometer reach was again poisoned in late October. Downstream barriers were then in place and, based on bioassays, detoxification succeeded (USFWS 1989).

An additional, unexpected perturbation occurred in January 1989, when a dam impounding Quail Creek Reservoir (Fig. 1) failed, sending a flood estimated at twice any previously recorded through the river channel. Direct influence on native fishes was probably minor. Flash floods characterized by explosive increases in discharge are common in the region, and native fishes exhibit behaviors that preclude downstream displacement. Little direct effects on native species have been detected for floods of similar or greater magnitude in other regional streams (Minckley & Meffe 1987). Potential for displacement was strong only in the narrow gorge; wide floodplains both above and below these "narrow" would have provided numerous refuges for fishes. Even prior to poisoning, fishes were rare in the gorge because it was ephemeral in summer. Furthermore, any significant transfer of fishes would have tended to homogenize the population rather than promote genetic differences between upstream and downstream fishes, the latter of which proved to be the case. It is therefore implausible that the flood was responsible for the changes we observed.

### Materials and Methods

Wild and hatchery samples of Virgin River chubs (Table 1) were analyzed to assess potential genetic changes following the perturbations and hatchery manipulations described below. Wild-caught chubs consisted of two pre-poison (one of mixed year-classes from near Littlefield, Arizona, collected in 1987, \( N = 17 \), and another of young-of-1988 from just below Washington Fields Diversion, Utah \( N = 50 \)) and three post-poison samples (young-of-1989 fish from the same two localities collected as juveniles in 1989 [Washington Fields Division, \( N = 16 \); Littlefield, \( N = 32 \) and 1989 year-class fish (age confirmed by examination of otoliths) collected as adults from Littlefield in 1991 \( N = 21 \) ). Hatchery samples included two samples of young-of-1989 fish from Dexter NFH, one produced by manual stripping of gametes \( N = 50 \) and the other resulting from unassisted (volunteer) spawning in a hatchery pond \( N = 50 \).

There is little likelihood that closely related individuals were over-represented in samples. All wild fish were taken by seining substantial areas of habitat. The pre-poison sample from Washington Fields Diversion was selected only by year-class and frozen alive from more than 1000 fish taken during the initial rotenone application. Hatchery samples were selected randomly from 100 to 200 fish provided by Dexter NFH personnel.

Following capture, chubs were immediately frozen on dry ice and subsequently stored at -80°C. Muscle was homogenized in distilled water and gene products were resolved by electrophoresis of homogenates through 12% starch gels using the histidine-citrate pH 8.0 buffer system (Brewer 1970). The following three polymorphic loci were chosen for analysis because allele frequencies were of appropriate magnitudes to provide reasonable statistical reliability for moderate sample sizes: calcium-binding protein (Cbp-1, nonspecific), creatine kinase (Ck-A, EC 2.7.3.2), and phosphoglucomutase (Pgm-A, EC 5.4.2.2). Correspondence of observed genotypes of Hardy-Weinberg expectations was

### Table 1. Observed genotypes for the three marker loci and tests of deviations from Hardy-Weinberg equilibrium (G-values).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cbp-1</th>
<th>Ck-A</th>
<th>Pgm-A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aa</td>
<td>ab</td>
<td>bb</td>
</tr>
<tr>
<td>WFD-1</td>
<td>13</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>WFD-2</td>
<td>0</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>LA-1</td>
<td>3</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>LA-2</td>
<td>16</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>LA-3</td>
<td>11</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>DNHF-1</td>
<td>11</td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>DNHF-2</td>
<td>17</td>
<td>25</td>
<td>8</td>
</tr>
</tbody>
</table>

*Wild samples from Washington Fields Diversion (WFD) and Littlefield, Arizona (LA), are numbered sequentially in order of collecting date. Samples from Dexter NFH represent artificially spawned (DNHF-1) and volunteer (DNHF-2) fish. Significant deviations from Hardy-Weinberg expectations are indicated by asterisks \( \ast \text{P} < 0.025; \ast \ast \text{P} < 0.001 \).
tested using log-likelihood goodness-of-fit tests (G-test), and allelic or genotypic frequencies were compared using log-likelihood contingency tests (Zar 1974).

**Results and Discussion**

**Pre- and Post-Perturbation Wild Samples**

The two pre-perturbation samples showed no significant within-sample deviations from Hardy-Weinberg expectations (Table 1) and no between-sample differences in allele frequencies (Fig. 2). An earlier genetic survey of Virgin River chubs (Rosenfeld & Wilkinson 1989) provided similar allele frequencies at these loci; therefore, there was no evidence of either spatial or temporal genetic divergence within the river.

Post-poison data contrasted sharply with pre-poison results. Allele frequencies differed significantly between populations, and genotypic frequencies deviated from expected Hardy-Weinberg proportions. Although allele frequencies of chubs were similar to the original condition at Washington Fields Diversion (Fig. 2), an excess of heterozygotes was detected at the Cbp-1 locus (Table 1). Genetic changes in chubs near Littlefield were far more dramatic. Large shifts in allele frequencies occurred at the Cbp-1 and Pgm-A loci (Fig. 2A and C), and a significant excess of heterozygotes was present at Ck-A (Table 1). Population subdivision was documented by the significant allele frequency differences at Cbp-1 and Pgm-A between the two post-poison samples (Fig. 2A and C). Allele frequencies of chubs collected at Littlefield in 1991, nearly 2.5 years after poisoning, were similar to those of the earlier post-poison sample. These fish similarly exhibited a significant excess of heterozygotes at Ck-A (Fig. 2A–C; Table 1). Annulli, read from otoliths taken from the two largest and two smallest individuals in the 1991 sample, indicated that they were two-year-olds and therefore members of the 1989 year-class. Therefore, it is likely that the two post-poison samples from Littlefield were of the same year-class produced by the same parents, and their genetic equivalence is not surprising.

Thus, the genetic composition of chubs differed dramatically after poisoning. There are three alternative explanations for these results: (1) differential reproduction or migration of specific genotypes; (2) selection favoring or eliminating specific genotypes; or (3) a severe reduction in effective population size.

The first is unlikely because chubs were originally panmictic, and the probability that specific genotypes migrated up- or downstream seems remote. Furthermore, pre-poison chubs from Washington Fields Division were all of a single year-class yet genetically identical to those from Littlefield, which were composed of various year-classes, suggesting a substantial proportion of the population rather than a few individuals reproduced each year.

The second alternative also is unlikely because allele frequencies from the post-poison samples from Little-
field and Washington Fields Diversion, although of the same year-class, did not vary in concert. The possibility of different selection pressures at the two localities seems remote, especially given the genetic identity of the two pre-poison samples.

A severe reduction in number of spawning adults remains the only plausible alternative. Changes in allele frequencies and deviations from expected Hardy-Weinberg proportions are consistent with the occurrence of a genetic bottleneck, because random fluctuation is more likely to be significant in small populations. Theoretically, for example, post-poison genotypic distributions at Littlefield could result from only two matings involving three fish. Interbreeding of two individuals homozygous for alternative alleles (aa and bb) at the Ck-A locus would produce only heterozygous progeny. If the bb fish also spawned with a heterozygous (ab) fish, one half the progeny would be homozygous bb and the other half heterozygous. In this model, no aa homozygotes would be produced. Assuming an equal contribution of progeny from these hypothetical crosses, the expected distribution of genotypes are statistically indistinguishable from those observed in the 1989 year-class at Littlefield (Table 2). Given the high frequency of the a allele (0.43) in 53 post-poisoning fish from Littlefield, but the absence of aa homozygotes, it seems unlikely that a mating between any two fish possessing this allele occurred. Estimation of the actual number of spawning adults is impossible, but it is unlikely to have been much greater than three fish because all possible genotypes would otherwise be expected.

Independence of observed genetic variation in chubs from Washington Fields Diversion and Littlefield indicated they were two distinct populations following poison application. Fish at Washington Fields Diversion were genetically more similar to the original state than were the Littlefield fish. Because the reach immediately above Washington Fields Diversion was not poisoned, it was a source of immigrants, and a few migrants likely repopulated the reach to produce the young-of-1989 we sampled. Chubs from Littlefield exhibited extreme genetic deviation from the original state, likely the result of two factors. First, rotenone reached Littlefield and below at sufficient toxicity to kill a substantial portion if not most of the fish fauna (USFWS 1988). Chubs, ecologically restricted to deeper parts of the channel (Cross 1978), must have sustained a maximum impact. Second, unlike Washington Fields Diversion, Littlefield is more than 60 stream-kilometers downstream from potential colonizers, and, unless carried by the January flood (or a subsequent event), adult chubs had at best a few months to reach the area. A major factor limiting potential movement between Washington Fields Diversion and Littlefield was an exceedingly dry spring and summer in 1989, resulting (due to irrigation diversions) in a long, dry reach of channel through the Virgin River Gorge, separating the two sites for much of that year. Recolonization from downstream was unlikely because irrigation diversion dams would impede upstream migration, and the influence of rotenone extended past Mesquite, Nevada, below which chubs were normally rare (Cross 1978).

Genetic equivalence of the two post-poison samples from Littlefield provides strong evidence that substantial recolonization from upstream had not yet occurred by Spring 1991. Both 1990 and Winter-Spring 1991 remained relatively dry, and the barrier formed by intermittent flow through the Virgin River Gorge persisted. The situation at Washington Fields Diversion should differ, reflecting emigration from the immediately adjacent, unpoisoned reach upstream. Unfortunately, permit restrictions have as yet precluded our sampling in that area.

**Table 2. Comparison of observed Ck-A genotypes from post-poison chubs collected near Littlefield, Arizona, with expected frequencies from two hypothetical crosses: aa × bb and bb × ab (matings produce equal numbers of progeny).**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>G-value/Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>aa</td>
<td>0</td>
</tr>
<tr>
<td>ab</td>
<td>45</td>
</tr>
<tr>
<td>bb</td>
<td>8</td>
</tr>
<tr>
<td>Expected</td>
<td>0 39.75 13.25</td>
</tr>
</tbody>
</table>

\(^{1}\) Genotypic frequencies of both post-poisoning samples from near Littlefield, Arizona, did not significantly differ (G = 0.42; P > 0.50) and were combined.

**Hatchery Samples**

Chubs produced at Dexter NFH were analyzed genetically to determine the advisability of using them to augment the wild population. Both stocks differed from their source population. Tests of Hardy-Weinberg expectations indicated significant excesses of heterozygotes at Cbp-1 for artificially-spawned chubs and at Ck-A for volunteer-spawned fish (Table 1). Artificially propagated chubs also exhibited a significant allele frequency shift at Ck-A (Fig. 2B). The volunteer brood was not as different; allele frequencies were similar to those of the source stock (Fig. 2A–C). The absence of the rarest Pgm-A allele in both hatchery samples provided further evidence of genetic divergence. This allele occurred at a frequency of 3.0% in both the source stock and in the sample from an earlier study (Rosenfeld & Wilkinson 1989). The binomial probability of not detecting an allele at this frequency (0.03) in a sample of 50 fish (100 alleles) is small (P = 0.048).

The stochastic nature of genetic changes in hatchery samples mirrored those in post-poison samples from the river; demonstration of a genetic bottleneck in the artificially spawned fish is unequivocal. Six females and
eighteen males were used to produce these fish, making the effective population size ($N_e$) a maximum of only eighteen individuals (Hartl and Clark 1989). For an $N_e$ of this size, 16% of alleles at frequencies of 0.05 would be lost, with probability of loss increasing dramatically for rarer alleles. Our calculation of $N_e$ takes into account only unequal numbers of each sex. If there was large variation in numbers of eggs produced by females or fertilizations obtained by males, our estimate of $N_e$ is too high and the proportion of alleles lost would be substantially higher.

In the case of the volunteers, it is also clear that a nonrandom sample of the 160 available adults actually contributed to the year-class. Volunteer reproduction in ponds likely involved too few adults to produce progeny representative of the source population.

Management Options and Recommendations

The USFWS wisely decided not to augment the Virgin River population with either stock produced at Dexter NFH. If individuals naturally produced in the poisoned reach had been common, augmentation with either of these stocks would have had little impact. If chubs had been rare in the river, however, stocking could have had unforeseen and potentially deleterious effects (Hindar et al. 1991). If future restocking is deemed necessary, a large effective population size of brood fish should be used to maintain natural patterns of genetic variation (Allendorf & Ryman 1987).

Young-of-1989 chubs were abundant near Littlefield in 1991, possibly the consequence of high survival rates in a predator-free and open-niched environment produced by the poisoning; chubs in the lower river may have been as numerous in 1991 as they were prior to poisoning. On one hand, this numerical recovery lessens the likelihood of further losses of genetic variability (Nei et al. 1975). On the other hand, because of the apparent lack of immigration and the small size of the founding population, a high proportion of adult chubs of 1989 year-class at Littlefield may consist of closely related individuals (full-siblings and half-siblings). Thus a high proportion of matings may occur between close relatives, and inbreeding depression is a potential problem in progeny of the 1989 year-class.

Two alternative management options seem viable. The first would involve only continued monitoring of the demographic and genetic status of Littlefield chubs relative to those upstream. This would provide detailed information on the effects of a bottleneck. Yearly samples of both adult and young from Littlefield would provide a measure of recolonization rates and perhaps yield insight into factors affecting gene flow. For example, fluctuations in gene flow rates could reflect yearly hydrologic variations, both natural and anthropogenic.

Whereas such information could prove invaluable for future management, the potential for reduced fitness due to inbreeding depression is a risk associated with this first option. If few or no individuals migrate downstream, prolonged population subdivision and potentially deleterious levels of inbreeding could occur. Probability of the latter would diminish, however, as post-1989 year-classes become reproductive. In the meantime, chubs produced near Littlefield should be compared with cohorts from the unpoisoned reach to assess potential inbreeding effects. Higher frequencies of morphological deformities, especially increased fluctuating asymmetry among bilateral characters, are potentially sensitive measures of decreased developmental stability associated with inbreeding (Van Valen 1962; Leary et al. 1984; Allendorf & Ryman 1987).

The second option involves supplemental stocking of hatchery-produced chubs. While this alternative would preclude measurement of natural recolonization, population recovery could still be gauged by continued genetic monitoring. Its primary benefit would be to reduce inbreeding and increase genetic variability, provided that original levels of genetic variation are represented in the hatchery stock. This option, however, is not entirely without risk. If the wild population is small, supplemental stocking can potentially reduce the effective population size due to the differential reproductive rates of wild and captive parents (Ryman & Laikre 1991).

Other Management Implications

Our results have implications for management of imperiled fishes in addition to those indicated for hatchery propagation, especially with reference to the use of piscicides. The use of chemical agents for enhancement of sport fisheries has a long history (Eschmeyer 1975), some of which is marked by conflict between sportfish and native species proponents (Holden 1991; Rinne & Turner 1991). Most commonly, "rough fish" (often common carp, *Cyprinus carpio*, but also native minnows and suckers) are removed because they are believed to interfere with sport fishes (usually both native and non-native trout in the western United States, but sometimes non-native catfish and bass as well).

In the past, little attention was given to the impact of piscicide on other than targeted taxa. Nontarget species/populations, commonly including a number of nongame fishes (and other organisms), were generally ignored. The potential loss of biodiversity as a consequence of stock destruction is obvious, but, as demonstrated here, incomplete kills may also have dramatic effects on the genetic structure of surviving populations. Genetic changes associated with bottlenecks, the magnitude and consequences of which are unknown, must have oc-
curred in many populations of both native and non-native fishes re-established from survivors of piscicide application. With fishes in well-watered zones, dispersal from adjacent habitats presumably allows re-establishment of the original stock/species, given sufficient time. But species and populations of fish and other organisms in the arid American West may not be available for natural recolonization from elsewhere. For example, Virgin River chub is known only from this river system, woundfin (*Plagopterus argentissimus*) was formerly widespread but is now restricted to the Virgin River, and other species and distinct stocks are endemic to this river (Minckley, unpublished data).

On the other hand, the harmful effects that exotic species may have on native forms are well recognized, and attempts to eliminate exotics are often included in recovery plans. Because of their small size, large numbers, and/or high fecundity, selective removal of most exotic fishes such as the red shiner is seldom a viable option. Once established, they are particularly difficult to eradicate by other than drastic measures.

Despite these problems, we consider the judicious use of piscicides compatible with management of endangered fishes (see Rinne & Turner 1991). When piscicide use is deemed necessary, the potential adverse effects of such action on all native organisms must be taken into account. When natural phenomena (such as drought), anthropogenic catastrophes (such as chemical spills), or a management accident (as discussed here) dramatically affect local populations, concentrated effort should be applied to derive as much information as possible for use in future management situations. Maintenance of unique populations requires special considerations, and accurate assessment of the impact of catastrophic events by quantitative methods such as genetic analysis provides guidance regarding what recovery measures, if any, are necessary.

Allozyme surveys of imperiled species and populations thus seem especially prudent. If spatial and temporal variation are known, genetic data can provide sensitive measures of change in population structure and status immediately following a perturbation, and likewise provide long-term measures of patterns of recolonization and population recovery for imperiled and common species alike. In organisms with high reproductive rates, rapid numerical recovery can be achieved, but the genetic effects of a bottleneck, for example, may remain for a long time. For organisms that are difficult to count, population status may be more readily assessed with genetic data than with comparisons of abundance before and after the perturbation. Measures of genetic variation may also better reflect effective population size than census number. Thus, protein electrophoresis is readily applicable to quantification of perturbation effects in a wide range of circumstances, if baseline data are available.

In the Virgin River, chubs from upstream, indistinguishable from the original population, were predicted to move into the perturbed area (USFWS 1989). The post-poison sample directly below Washington Fields Diversion was more similar to those fish originally present than at Littlefield, providing evidence that this process was already underway. In contrast, 29 months after poisoning significant recolonization of the river near Littlefield had yet to occur, since ripening, two-year-old adults collected in March 1991 were genetically indistinguishable from our earlier sample of juveniles collected in July 1989. No young-of-1990 were observed or collected in our second Littlefield post-poison sample, likely reflecting the scarcity of mature chubs and consequent lack of reproduction. Given the lack of downstream adult colonization, allele frequencies of initial year-classes are expected to more closely resemble post-poison rather than pre-poison proportions, and the time to re-establishment of panmixia in the system may be far longer than anticipated.

In addition to chubs, the Virgin River fish fauna consists of five other native species, including the woundfin, also listed as endangered by the U.S. Department of Interior, and the Virgin spinedace (*Lepidomeda m. mollispinis*), a candidate for listing. The other three native fishes, flannelmouth sucker (*Catostomus latipinnis*), desert sucker (*Pantosteus clarki*), and speckled dace (*Rhinichthys osculus*) are stocks of more widespread species. Woundfin, like chubs, are obligate mainstream fishes, whereas spinedace are most common in tributaries. The suckers and speckled dace show variable levels of mainstream versus tributary use. The effect of the poisoning likely differed among these species because of different habitat preferences and life histories. We plan to continue genetic monitoring of Virgin River chubs over the next several years, and we expect to extend the study to other native fishes of the system.

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