

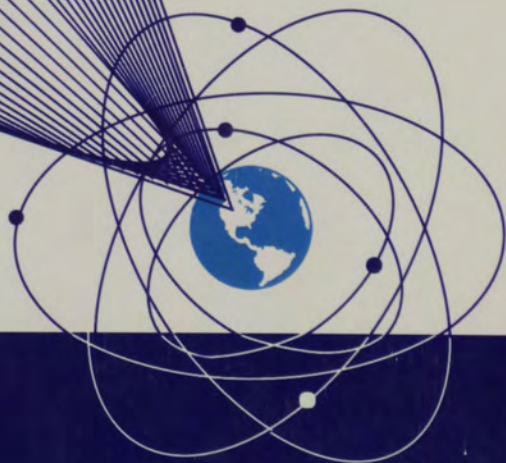
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INTERMOUNTAIN JOURNAL OF SCIENCES

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TOXICITY OF FINTROL® (ANTIMYCIN) AND PRENFISH® (ROTENONE) TO THREE AMPHIBIAN SPECIES

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ABSTRACT

The toxicity of two piscicides, Fintrol® and Prenfish®, to Columbia spotted frogs (*Rana luteiventris*), long-toed salamanders (*Ambystoma macrodactylum*), and Rocky Mountain tailed frogs (*Ascaphus truei*) of varying life stages was determined from 96-hr tests. The 96-hr LC50 values for Fintrol ranged from 13.7 to 192 µg/L, and for Prenfish the range was 0.009 to 9.65 mg/L. Tailed frog larvae were the most sensitive to both piscicides, surviving exposure to Fintrol as low as 3.7 µg/L, and having 10-percent mortality to the lowest test concentration of Prenfish tested (0.005 mg/L). Spotted frog adults survived exposure to Fintrol at concentrations six times the label prescription and survived exposure to Prenfish up to 4.5 times the label prescription. Long-toed salamander larvae survived exposure to Fintrol at levels ~30 percent higher than the label prescription, but had a similar sensitivity to Prenfish as some species of fish. Comparing the results of these tests with tests on fish and other amphibians showed that when used in the field, Fintrol would likely not have an impact on any of the species or life stages tested, and Prenfish would not likely impact adult amphibians but could have an impact on larvae.

Key words: Antimycin, Fintrol®, rotenone, Prenfish®, Columbia spotted frog, long-toed salamander, Rocky Mountain tailed frog, salamander, frog, larvae.

INTRODUCTION

Antimycin and rotenone are active ingredients in piscicides commonly used for fisheries management and commercial aquaculture. However, despite increasing use nationally (Finlayson et al. 2002, McClay 2005), there is limited published information available that evaluates effects of piscicides on non-target organisms such as amphibians (Cutkomp 1943, Schnick 1974a, 1974b, Chandler and Marking 1982), and to our knowledge there is none for the Columbia spotted frog (*Rana luteiventris*), long-toed salamander (*Ambystoma macrodactylum*), and Rocky Mountain tailed frog (*Ascaphus truei*). It has been shown that piscicides generally do not affect amphibians at label-prescribed dosages for fish removal (Schnick 1974a, 1974b). For example, several antimycin field trials conducted by Berger (1964, 1965a, 1965b, 1966a, 1966b) showed no effect on adult and larval frogs,

and evaluations of rotenone-treated lakes in northwest Montana showed in most cases that amphibians persisted after treatment (Grisak 2003). These three amphibian species commonly coexist with trout in lakes and streams in northwest Montana. What effect using piscicides for native trout conservation or to improve sport fisheries in northwest Montana could have on these specific amphibians remains unknown.

Piscicide applications in northwest Montana are typically conducted in the autumn or fall. During this time period, cool water temperature and lower intensity of sunlight help maximize piscicide performance, water flow is typically low and amphibians have generally metamorphosed and migrated to wintering habitats. Long-toed salamanders and Columbia spotted frogs use lakes in the spring for breeding and depositing eggs, then immediately return to forested areas and semi-aquatic

habitat (Marnell 1997). Larvae of both species metamorphose by fall and then follow similar behavior patterns as adults. This life history cycle favors conducting piscicide treatments in the fall when these species are in advanced metamorphic stages and moving away from targeted aquatic environments. Tailed frogs are dependant on streams for their three-year larval stage and adults remain close to streams or in nearby wetted areas for the majority of their life (Werner et al. 2004, Jones et al. 2005). Consequently, a larger portion of the tailed frog population is more likely to be subject to exposure to piscicide-treated water.

The goal of any piscicide treatment is to remove unwanted fish, while minimizing effects to non-target organisms. Here we tested the piscicide brands Fintrol® (antimycin) and Prenfish® (rotenone) on spotted frog adults, long toed salamander adults and larvae, and tailed frog larvae to (1) determine the acute effects of the piscicides on these species and (2) compare these results with toxicity tests on other amphibian and fish species.

METHODS

We used the American Society for Testing and Materials – International Standard Guide for Conducting Toxicity Tests on Test Materials with Amphibians (ASTM 2002) as the basis for our methodology. Tests were conducted at the Montana Fish, Wildlife and Parks facility in Kalispell in 2003 and 2004. Field crews used dip nets to collect spotted frog adults and long-toed salamander adults and larvae from lakes (1750-1850 m elevation) in the Swan Mountain Range of northwest Montana in September. These individuals were segregated, quarantined and acclimated at the test facility in 1850-L tanks for at least 14 days. We fed them a diet of dipteran larvae and arthropods until 48 hr prior to the experiments. Field crews used dip nets to collect tailed frog larvae from Hungry Horse Creek in December. These were held for no longer than 24 hr in 1850-L tanks at the test facility. Tailed frog larvae feed primarily on epilithic periphyton, so prolonged quarantine and feeding in the test environment was not practical.

We used Flathead River water for all quarantine, acclimation, and testing (mean values were: dissolved oxygen, 9.2 mg/L (8.8-10.4); alkalinity, 87 mg/L (86-89); conductivity 179 μ S/cm (174-186); hardness, 92 mg/L (88-96); pH, 8.1 (8.1-8.2); TDS, 98 mg/L (90-103)). The test chambers were 55-L glass aquaria. Because little is known about the sensitivity of these species to the piscicides, we first conducted test concentration range-finding evaluations, and chose the range of test concentrations that were separated by at least 0.6 of the next highest concentration (ASTM 2002). The number of individuals in the tests was constrained by their availability from wild sources. Tests on adult spotted frogs, larval salamanders, and adult salamanders were conducted with five individuals in 3 L of water (3.5, 2.6 and 2.6 g/L loading, respectively). Adult spotted frogs could remain submerged with their nostrils exposed while in a sitting position. Salamander larvae stayed completely submerged, but adults could keep their heads out of water. Tests with tailed frog larvae were conducted with 10 individuals in 6 L of water (1.7 g/L loading). We replicated each concentration of piscicide tested, and tests on each species and life stage included replicated controls. Antimycin testing of adult salamanders was limited to one concentration due to a limited number of test animals. The tests ran for 96-hr using the renewal technique (ASTM 2002) in which we completely replaced treated and control water every 24 hr.

Our target test water temperature was 10 °C to mimic fall water temperatures during a typical piscicide application in Montana. We maintained water temperature by regulating air temperature in the lab using a thermostatically controlled natural gas heater and monitored it (every 15 min) using Onset® Optic Stowaway thermographs that were housed in each control chamber. The mean water temperature during the tests was 9.6 °C (7.7-11.6). The photoperiod of the facility was similar to the natural photoperiod (10:14 hr light: darkness).

We made concentrated stock solutions of antimycin and rotenone daily. Antimycin

consisted of equal proportions of Fintrol-concentrate 23-percent (w/w%) active ingredient and Fintrol diluent, plus nonoxynol-9 (as recommended by the manufacturer to maintain solubility as a surfactant) at a volume of 15 percent that of the Fintrol mixture. Fintrol-concentrate was placed in a warm water bath before mixing to ensure the soy lipids were in solution before making the stock. The rotenone came from Prenfish 5 percent active ingredient liquid formulation. Piscicides were dissolved in distilled water to make stock solutions. We added the appropriate amount of stock solution to test water using Nichiryo® model 5000DG 200-1000 μ l and 10-50 μ l digital micro pipettes to achieve the desired concentrations. For reporting purposes, and to remain consistent with the product labels, references to Fintrol are of active ingredient, and references to Prenfish are of formulation.

Test organisms that were fed did so readily until they were removed from feeding 24 hr before testing and did not appear to be stressed during the quarantine and acclimation period. Over the 96-hr test period we recorded mortalities at scheduled intervals (hr 1, 2, 4, 8, 12, 24) daily. We defined mortality as no movement, no reaction to gentle prodding with a glass instrument and no visual signs of life. During the tests, we observed no large differences in the progression of mortality between each replicated test concentration and all control organisms survived. We combined results of replicated test concentrations to calculate mortality at each concentration and for each species. The median lethal concentration (LC50) for each test was calculated using the Probit Method (Finney 1978) and the Trimmed Spearman-Kärber Method (Hamilton et al. 1977) with the U.S. Environmental Protection Agency's Probit Analysis Program, version 1.5 and the Trimmed Spearman-Kärber Method Program, version 1.5.

RESULTS

Fintrol was toxic to these three amphibian species over a wide range of concentrations. The only behavioral change observed in any of the amphibians exposed to Fintrol was

that adult spotted frogs relaxed their legs and lowered their heads below the water line. We observed no discoloration of any amphibians exposed to Fintrol, as is common in fish (Rosenlund and Stevens 1992). With tailed frog larvae, we first observed mortality after 8 hr of exposure to the highest concentration (300 μ g/L; Fig. 1). Minimum concentration that caused mortality (15%) was 7.5 μ g/L after 96 hr of exposure. The 96-hr LC50 for tailed frog larvae was 13.7 μ g/L (Table 1). Salamander larvae mortality was first observed after 4 hr of exposure to concentrations of 300 μ g/L and greater. Maximum concentration that caused no lethal effects on salamander larvae after 96 hr was 15 μ g/L. The 96-hr LC50 for salamander larvae was 81.7 μ g/L (Table 1). Adult salamanders were exposed to only 7.5 μ g/L of Fintrol, following the same protocol, and all survived the 96-hr period. The first sign of mortality in adult spotted frogs occurred at 250 μ g/L after 52-hr and 96-hr exposure to this concentration, only 70 percent of the specimens died. Maximum concentration level that had no observable effect to adult spotted frogs was 60 μ g/L at 96 hr and the 96-hr LC50 was 192 μ g/L (Table 1).

The amphibians we exposed to rotenone displayed no clinical signs of toxicity such as surface gulping or loss of equilibrium as is common in fish (Brown and Ball 1943, Parker 1970, Loeb and Engstrom-Heg 1971, Teixeira et al. 1984). With the tailed frog larvae, the first sign of mortality occurred at the 8-hr observation interval at Prenfish concentrations of ≥ 0.35 mg/L. The 96-hr LC50 for tailed frog larvae was 0.009 mg/L (Table 1). We first observed mortality in larval salamanders after 2 hr of exposure to ≥ 14.5 mg/L, and all concentrations tested caused complete mortality after 72 hr. Adult salamander mortality was first observed after 2 hr of exposure to 116 mg/L, and the minimum concentration tested (3.5 mg/L) caused 50 percent mortality after 96 hr of exposure. With spotted frog adults, we first observed mortality after 1 hr of exposure to concentrations of ≥ 73 mg/L. The maximum concentration level that had no observable effect on adult spotted frogs after 96 hr was 4.5 mg/L (Fig. 2) and the 96-hr LC50 was 9.65 mg/L (Table 1).

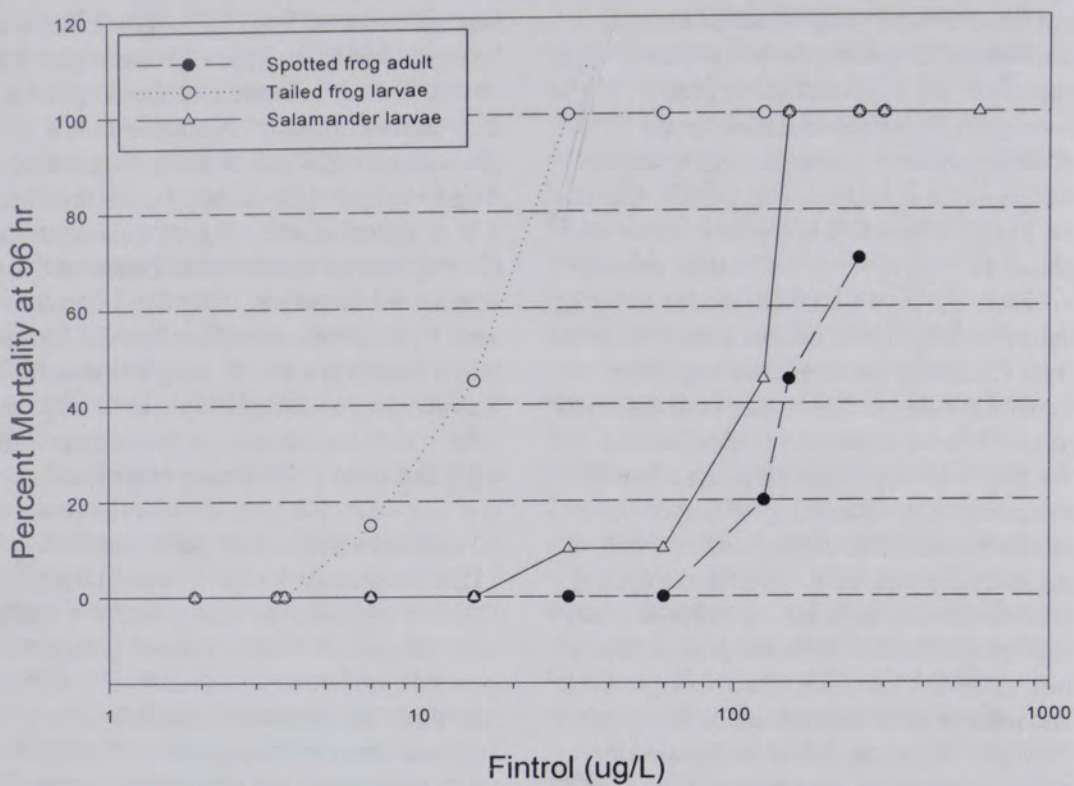


Figure 1. Toxicity of Fintrol (µg/L) to three amphibian species after 96 hours.

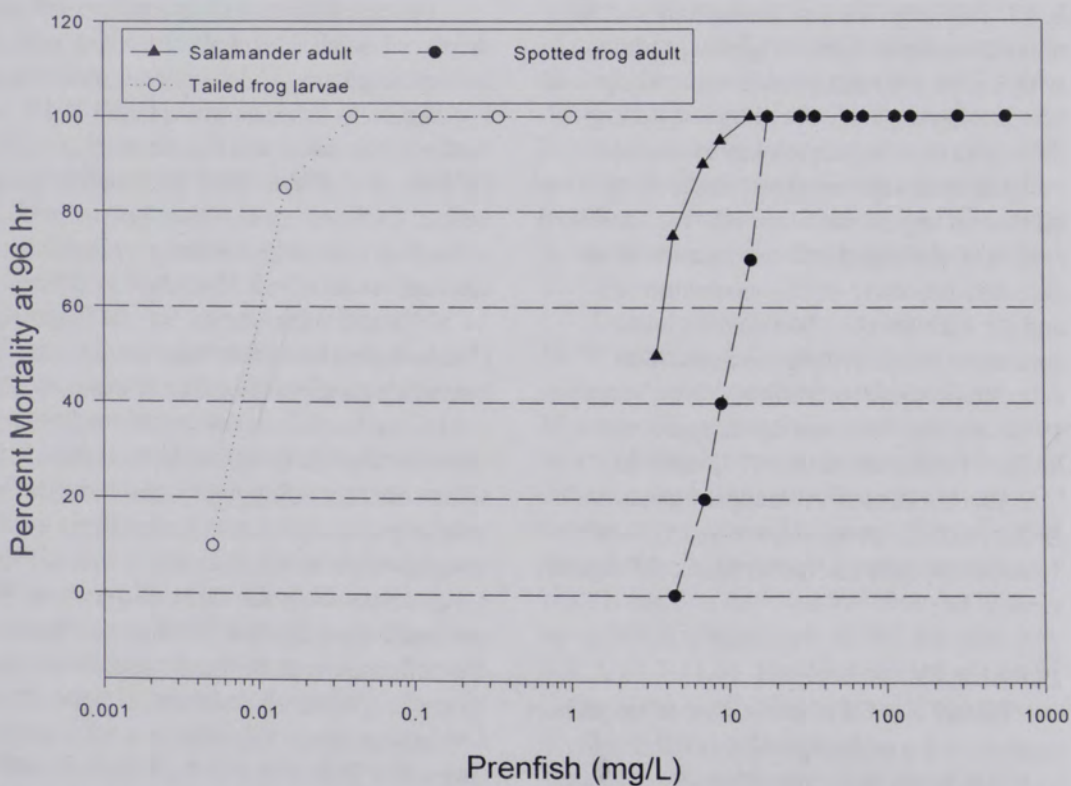


Figure 2. Toxicity of Prenfish (mg/L) to three amphibian species after 96 hours.

Table 1. The 24-hr and 96-hr LC50 values ($\pm$ 95% CI) for Columbia spotted frogs, long-toed salamanders and tailed frogs exposed to Fintrol and Prenfish.

species	life stage	Fintrol ($\mu\text{g/L}$)		Prenfish (mg/L)	
		24 hr	96 hr	24 hr	96 hr
Columbia spotted frog	Adult	>250 a (---)	192 (141-313)	41.5 (33.9-50.9)	9.65 (7.8-12.0)
Long toed salamander	Adult	---	b	8.0 (6.6-9.8)	3.5 (0.8-4.9)
	Larvae	225 (150-300)	81.7 (66.8-99.1)	<0.23 c (---)	<0.23 d (---)
Tailed frog	Larvae	77.6 (66.3-90.7)	13.7 (11.1-16.7)	0.037 (0.030-0.047)	0.009 (0.007-0.012)

- a – no effect at greatest exposure (250) within 24 hr
b – exposed to 7.5 $\mu\text{g/L}$ for 96 hr with no mortality
c – lowest level (0.23) caused 70 percent mortality at 24 hr
d – lowest level caused 100 percent mortality at 72 hr

DISCUSSION

Trout are considered among the most sensitive fish species to antimycin (Walker et al. 1964, Gilderhus et al. 1969) and are the target fish most likely to co-exist with the amphibians we tested. Fintrol label prescription for removing trout is 5-10 $\mu\text{g/L}$ of antimycin. In our tests, spotted frog adults survived Fintrol up to six times that amount, and only 70 percent mortality occurred at 25 times the label prescription. These findings agree with the results of a field trial in Wisconsin that used 10 $\mu\text{g/L}$ of Fintrol and had no effect on adult and larval frogs (*Ranidae* spp.) or salamanders (*Ambystomidae* spp.) (Gilderhus et al. 1969). Comparisons of 96-hr LC50 values for Fintrol showed that adult spotted frogs are 16 times more tolerant than leopard frogs (*Rana pipeins*) (Lesser 1970) and 3840 times more tolerant than rainbow trout (*Oncorhynchus mykiss*) (Marking and Dawson 1972). Long-toed salamander larvae had only 10 percent mortality at the amount prescribed for trout and to attain 100 percent mortality required 15 times that amount. Similarly, adult tiger salamanders (*Ambystoma tigrinum*) survived exposure to 80 $\mu\text{g/L}$ antimycin over 96-hr (Walker

et al. 1964), but succumbed to 600 $\mu\text{g/L}$ in 24-hr (Berger 1966c). Tailed frog larvae were more susceptible to Fintrol than other species tested; even then, only 5 percent of tailed frog larvae died at concentrations specified for trout (5-10 $\mu\text{g/L}$), and this response occurred after 72 hr of exposure and three complete reconstitutions of the test material. By comparison, six species of fish tested by Gilderhus (1972) succumbed to this concentration within 0.5 to 6-hr. On the basis of the LC50 values, tailed frog larvae showed greater tolerance than 21 species of fish, with only the black bullhead being more resilient (Berger et al. 1969, Marking and Dawson 1972).

As with antimycin, trout are considered among the most sensitive fish species to rotenone (Gilderhus 1972). Prenfish label prescription for normal pond use is 0.5 to 1.0 mg/L of formulation, and this range is used in Montana when trout are the target species. Both salamander and spotted frog adults survived 96-hr exposure to these concentrations, but tailed frog larvae did not. Furthermore, the no observable effect concentration for spotted frog adults was 4.5 to nine times greater than the label prescription for normal pond use. Salamander

adults were 15 times more tolerant of Prenfish than the larvae based on 96-hr LC50 (3.5 mg/L and < 0.23 mg/L, respectively) (Table 1). Although this study could not determine the Prenfish level that had no observable effect to larval salamanders or larval tailed frogs, under laboratory conditions apparent sensitivity of these life stages suggests that some mortality could occur during normal field use of Prenfish.

Comparing our findings with 96-hr LC50's of rainbow and brook trout (0.046 mg/L and 0.044 mg/L, respectively; Marking and Bills 1976) showed the respective tolerances of spotted frog adults and salamander adults are 210 and 76 times greater than those fish species. Larval I salamanders were about five times more tolerant than the trout, but larval tailed frogs were about five times more sensitive than the trout.

In conclusion, our findings indicated that when applied in the field according to the label prescription for trout Fintrol would not have an impact on any of the species or life stages tested, Prenfish would not have an impact on the adult amphibians we tested, but could impact larvae if present in the treatment zone. Some of our results showed that achieving an effect on amphibians would require prolonged exposure to Prenfish. Piscicide applicators should also consider that under field conditions, natural factors such as water chemistry, vegetation, and light intensity might greatly reduce persistence of piscicides, which could increase larval amphibian survival (Marking and Dawson 1972, Marking and Bills 1976, Chandler and Marking 1982, Dawson et al. 1991). Implementing piscicide treatments in the fall when adult amphibians typically are not in the treatment zones or after the larvae have metamorphosed will also lessen impacts to non-target amphibians.

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TOXICITY OF FINTROL® (ANTIMYCIN) AND PRENFISH® (ROTENONE) TO SLIMY SCULPIN

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ABSTRACT

In 96-hr acute toxicity tests on slimy sculpins with Fintrol® exposures of 4 to 240 µg/L, the LC50 was 6.1 µg/L; and with Prenfish® exposures of 0.0156 to 1 mg/L, the LC50 was 0.024 mg/L. The highest concentration of Fintrol that had no observable effect was 4 µg/L. Fintrol was less toxic to slimy sculpins than Prenfish at levels prescribed by their labels to completely remove trout. The Fintrol Use Direction Leaflet describes sculpins as sensitive species, but our results showed this species response is similar to highly resilient species such as the channel catfish (96-hr LC50 9.0–21.7). The response of slimy sculpins to Prenfish was similar to other salmonids.

Key words: Antimycin, Fintrol®, piscicide, Prenfish®, rotenone, slimy sculpin, sculpin.

INTRODUCTION

Some conservation programs for native trout species in the western United States use piscicides such as rotenone or antimycin to remove non-native fish from lakes and streams in preparation for native trout re-introductions. Because the habitat and range of several sculpin species overlap those of many western native trout species (Scott and Crossman 1973), use of piscicides for trout conservation should consider potential impacts to other non-target or native species present in a treatment area, such as sculpins. There is no known information available that assesses rotenone toxicity to sculpins and only limited information about antimycin toxicity to sculpins (Gilderhus et al. 1969). The slimy sculpin (*Cottus cognatus*) is a common sculpin species in western Montana (Holton and Johnson 2003) and a good candidate for conducting a toxicity assessment. We conducted this study to assess the acute toxicity of two commercial formulations of piscicide, Fintrol® (10% antimycin) and Prenfish® (5% rotenone) to the slimy sculpin.

METHODS AND MATERIALS

To assess acute toxicity we used the 96-hr renewal technique described by the American Society for Testing and Materials (E 729-96) (ASTM 2002) with some modifications. Because the slimy sculpin is an uncommon species for this type of testing, and availability is limited by wild sources, we used fish from multiple year classes. We collected sculpins from East Spring Creek near Kalispell, Montana, in April 2006 using a battery-powered electrofisher (pulsed DC, 300-V, 30-Hz duty cycle) and immediately transferred them to a Montana Fish Wildlife and Parks laboratory facility in Kalispell. The fish were quarantined in an 1850-L tank for 16 days, and actively fed on a diet of dipteran larvae and arthropods until food was discontinued 48 hr prior to the tests. We observed no negative impacts from electrofishing (Barrett and Grossman 1988, Snyder 2003). Water for quarantine and testing came from East Spring Creek (total alkalinity, 214 mg/L; conductivity, 426 µS/cm; hardness, 224 mg/L; pH, 7.9; mean daily temperature 10.7 °C [9.8–12.1]). During the quarantine and testing dissolved oxygen (DO) was measured twice daily with a digital meter and the mean daily DO was 85 (84–86) percent of saturation. The test chambers were

55-L glass aquaria. We added 20 L of stream water and five sculpins to each chamber (0.7 g/L loading).

To remain consistent with the product labels, references to concentrations of Fintrol and Prenfish are of active ingredient and formulation, respectively. We tested seven concentrations of Fintrol (4, 6, 8, 10, 60, 120, 240 $\mu\text{g/L}$ active ingredient) and one non-treated control, and six concentrations of Prenfish (0.0156, 0.03125, 0.0662, 0.125, 0.25, 1 mg/L formulation) and one non-treated control. Each test concentration and control was replicated once to assess variation in response. We randomly arranged the aquaria in the lab. The primary purpose of the renewal technique was to periodically expose the test organisms to fresh test solution of the same composition (ASTM 2002, section 3.2.3). We did this by replacing the test solution in each chamber every 24 hr with freshly prepared stock solutions and fresh water mixed at the appropriate concentrations. Water in the non-treated controls was similarly replaced every 24 hr.

Mortality inspections occurred each day at 1, 2, 4, 8, 12, and 24 hr. Results from each replicated test concentrations were pooled (five fish replicate = 10 fish concentration) to calculate mortality at each concentration. During the Fintrol test, one fish in a non-treated control chamber died at 96 hr, but this loss was within the acceptable limit described by ASTM (section 13.1.7) (2002). We determined the 96 hr LC₅₀ (median lethal concentration) values and 95-percent confidence intervals (C.I.) using the Probit Method (Finney 1978) and the Trimmed Spearman-Kärber Method (Hamilton et al. 1977) with the U.S. Environmental Protection Agency's Probit Analysis Program, version 1.5 and the Trimmed Spearman-Kärber Method Program, version 1.5.

RESULTS

Our tests showed Fintrol was toxic to slimy sculpins at concentrations ranging from 6 to 240 $\mu\text{g/L}$ (Fig. 1). The highest concentration at which no mortality was

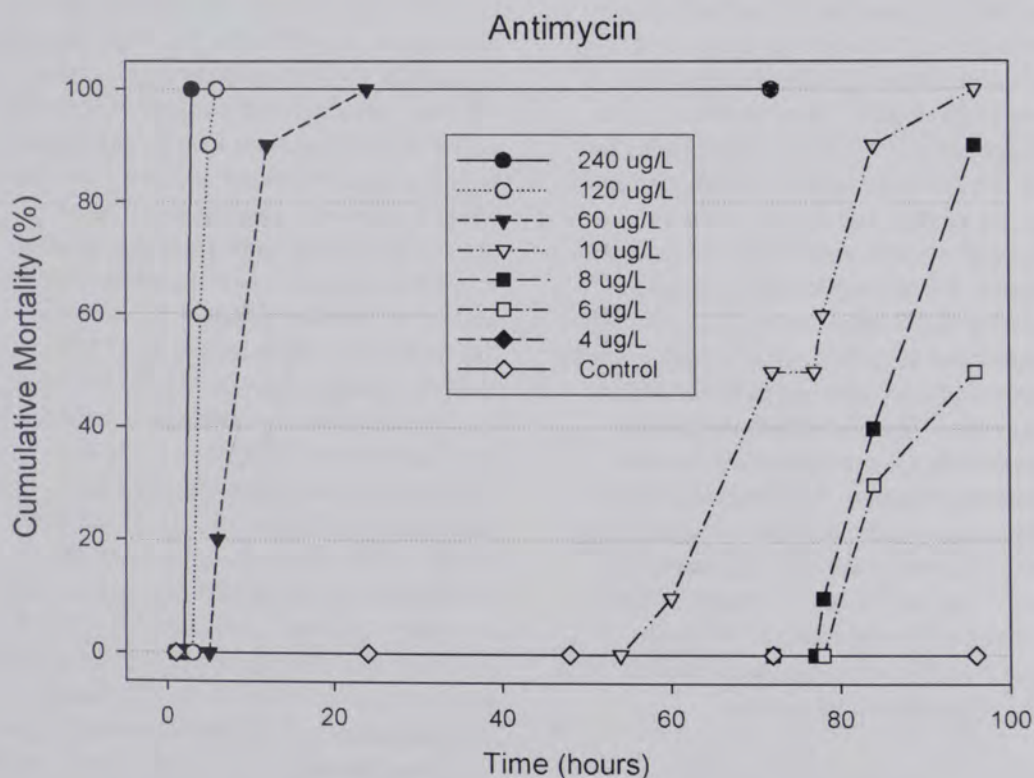


Figure 1. Toxicity of Fintrol (antimycin) to slimy sculpins in 96-hr laboratory tests.

observed was 4 µg/L, and at 10 µg/L the first mortality (10%) occurred after 60 hrs. The 96-hr LC50 for Fintrol was 6.1 µg/L (95% C.I. 5.1-6.9 µg/L). Complete mortality occurred after 8 hr exposure to concentrations of ≥ 120 µg/L, and after 24 hr exposure to 60 µg/L. We observed only slight discoloration of some sculpins exposed to Fintrol, and no apparent differences in swimming or other behavior between the treated and control fish.

Prenfish concentrations of ≥ 0.25 mg/L caused 100 percent mortality after two hours of exposure (Fig. 2). At these concentrations, the fish swam erratically and exhibited surface gulping behavior commonly associated with rotenone toxicity. The lowest exposure used in this test (0.0156 mg/L) caused 10 percent mortality at 96 hr. The 96-hr LC50 for Prenfish was 0.024 mg/L (95% C.I. 0.018-0.031 mg/L).

DISCUSSION

Sculpins are listed on the Fintrol Use Direction Leaflet as a sensitive species that can be completely removed from a water

body with concentrations of 5 to 10 µg/L. Correspondence with the manufacturer during our tests indicated the information on the leaflet has not been updated since 1975 (M. Romeo, Aquabiotics Corp, personal communication, 2006), and we were able to find only a single reference in the scientific literature regarding Fintrol [antimycin] toxicity to the mottled sculpin (*Cottus bairdi*). Gilderhus et al. (1969) reported antimycin applications of 15 µg/L for 5 hr and 10 µg/L for 10 hrs in two Wisconsin streams resulted in complete mortality of mottled sculpins. Our results indicated that 5-10 µg/L would be insufficient to completely remove slimy sculpins during a typical 8-hr stream treatment. Only concentrations >120 µg/L resulted in complete mortality within 8 hr, and at the highest label-recommended concentration (10 µg/L), mortality of slimy sculpins did not start until after 60 hrs of exposure. The results of our tests show that this species response is closer to other non-scaled fishes like the channel catfish (*Ictalurus punctatus*), which

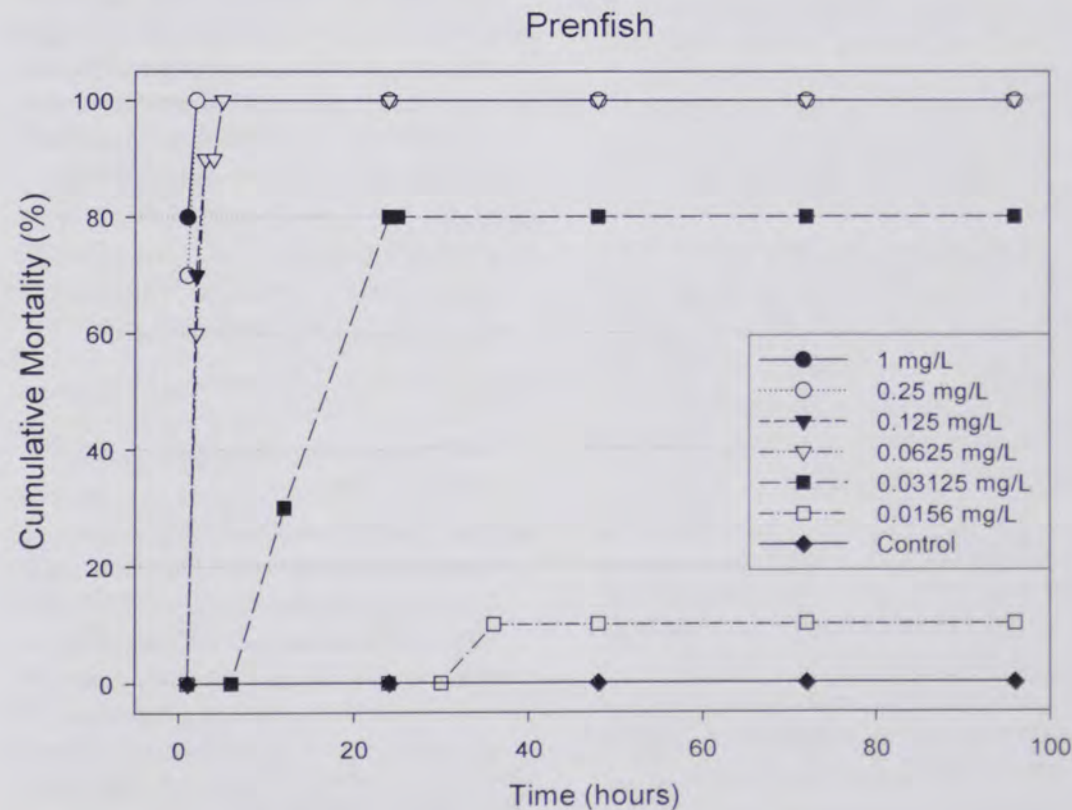


Figure 2. Toxicity of Prenfish to slimy sculpins in 96-hr laboratory tests.

has 96-hr LC50 values ranging from 9.0 to 21.7 $\mu\text{g/L}$ (Berger et al. 1969, Marking and Dawson 1972). When compared to toxicity results of rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*) and lake trout (*S. namaycush*) (Berger et al. 1969), our results show Fintrol is between 87 and 152 times less toxic to slimy sculpins.

The results of our tests and comparisons with toxicity information on other fish species suggest Fintrol would not have an impact on slimy sculpins during a typical 8-hr treatment. As recommended by the Fintrol Use Direction Leaflet, applicators should consider conducting on site assays to evaluate species sensitivity and make adjustments for differences in water chemistry at each site.

We could not find any published literature on the toxicity of rotenone to sculpins.

Marking and Bills (1976) reported toxicity results for twenty-one fish species tested under conditions similar to ours, using similar rotenone formulations. Their 96-hr LC50 values of three salmonid species were: rainbow trout, 0.046 mg/L; brook trout, 0.044 mg/L; and lake trout, 0.026 mg/L. Our results show that Prenfish toxicity to slimy sculpin is similar to lake trout, but Prenfish is nearly twice as toxic to slimy sculpins than it is to rainbow trout and brook trout. On this basis, slimy sculpin would likely be impacted by Prenfish during typical trout removal projects.

ACKNOWLEDGEMENTS

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POPULATION DEMOGRAPHY OF A LAND SNAIL SPECIES OF CONSERVATION CONCERN IN THE BLACK HILLS

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ABSTRACT

Understanding population biology of a species is critical for its successful management. We obtained information on movement, growth, and activity levels from four populations of Cooper's Rocky Mountain snail (*Oreohelix cooperi*) in the Black Hills, South Dakota, during May through September 2005 from a simple mark-recapture set-up. Grid population density estimates for each of the four sites ranged from 145 to 795 individuals. We observed movements up to 7.2 m. Moisture was more important than temperature in determining the presence of Cooper's Rocky Mountain snails within a site. Growth was not continuous across the season, but concentrated in intervals. Different populations maintained distinct differences in shell size. Although we could not statistically explain diameter differences, we hypothesize that population density, range of moisture conditions experienced, or another habitat characteristic, i.e., litter thickness, may influence overall size of individuals. Our results demonstrated that mark-recapture methods can be used for monitoring populations of western land snails as well as answer important demographic, ecological, and life history questions.

Key words: density estimate, growth, mark-recapture, movement, *Oreohelix*, snail

INTRODUCTION

Demography and other ecologically important information are severely lacking for many invertebrates. For those species with management concerns, e.g., land snails, this creates much difficulty for planning. Unfortunately, funding priorities often do not allow for field study to answer the very questions that would make management decisions easier. Thus, we present data to illustrate how even a single-season field study can answer questions vitally important to understanding the ecology of an invertebrate species.

As a case in point, we report on the land snail species, Cooper's Rocky Mountain snail, also known as the Black Hills Mountain snail, (*Oreohelix cooperi* Binney 1858), which resides primarily in the Black Hills of South Dakota and Wyoming although a recent genetic study indicated that isolated populations exist in Montana (Weaver et al. 2006). NatureServe (2006) lists the global status as G5T2Q, meaning they consider it a vulnerable subspecies although they currently list it

as *O. strigosa cooperi*. More information on proper taxonomy for this species appears in Weaver et al. (2006) and Weaver (unpublished data). This species is ranked S2 in South Dakota (Nature Serve 2006), meaning it is vulnerable to extinction. Wyoming Game and Fish Department (2005) includes Cooper's Rocky Mountain snails on their list of Wyoming Species of Greatest Conservation Need (2005). The Black Hills National Forest has designated it a management indicator species. The USDI Fish and Wildlife Service was petitioned to include Cooper's Rocky Mountain snail on the threatened or endangered species list and issued a 90-day ruling that scientific information supporting a listing was not presented (USDI Fish and Wildlife Service 2006). Indeed, lack of information is a problem in evaluating many aspects of the biology and ecology of Cooper's Rocky Mountain snail. For example, a species assessment prepared for the USDA Forest Service lists research priorities that include topics ranging from taxonomy to population size to movement (Anderson 2005).

Our work was part of a study designed to identify and develop a practical mark-recapture protocol to monitor populations of Cooper's Rocky Mountain snails in the Black Hills. We present baseline density estimates. In addition, we incorporated other important questions into the study.

Specifically, we address three main questions. First, how does individual shell size vary over the season? Tracking the range of sizes within a population can help determine the age structure of the population. Tracking growth also might determine whether growth is incremental or continuous. In addition, information on shell size allows for comparison among populations. Other studies (Frest and Johannes 2002, Anderson et al. 2007) report differences among populations in the size of the shell. Frest and Johannes (2002) even suggested that these size differences indicate separate species although genetic data presented in Weaver et al. (2006) disputed this. However, these previous studies utilized specimens collected during a short window of time or from different sites at different times of year. The current study provided an opportunity to track size across the summer season to test whether size differences among populations resulted from timing of collections.

Second, how much horizontal movement occurs within populations? Isolation of populations might cause conservation concerns. Genetic results suggest substantial gene flow among populations, even those separated by distance. That is, haplotypes were present in multiple, non-adjacent populations (Weaver et al. 2006). Gene flow suggested that populations are not isolated; however actual migration has not been observed, and field studies have not examined how much movement occurs. We provide data on the mobility of these snails.

Third, how do activity periods vary by season and microclimate? Activity periods are important not only for feeding and breeding of snails but also may influence long-term growth. For example, if snails are more active at moister sites, these sites might produce larger snails. Anderson et al. (2007) were unable to relate temperature

or moisture to size differences at particular sites based on soil measurements taken at the time of collection although long-term temperature data indicated a correlation to shell size. Including across-season data from our study may show relationships among temperature, moisture, and size. In addition, understanding when snails are aestivating may help managers identify a timeframe when management activities would be least deleterious to snails.

MATERIALS AND METHODS

We set up grids at four locations (Fig. 1) previously scouted for presence of Cooper's Rocky Mountain snail. Sites included rocky slopes with various amounts of tree cover that included ponderosa pine (*Pinus ponderosa*), spruce (*Picea glauca*), birch (*Betula papyrifera*), and aspen (*Populus tremuloides*). Grids consisted of four rows of five sampling stations placed 2 m apart. At each sampling station, we placed a 0.5- x 0.5-m plywood or pressboard board flat on the ground to function as a "trap." Boards were left in place ≥ 24 hrs before the first trapping session.

At each trapping site, we marked individual snails at initial capture with fingernail polish and an individually numbered bee tag (www.bee-works.com). For each snail, we recorded trap location, number of whorls, and shell diameter using calipers. We also recorded and measured individuals at each subsequent recapture. After marking, we returned snails to the location of capture.

We also recorded soil temperature and moisture at each board using a Weksler soil thermometer and a Quick Draw 2900FI Soil Moisture Probe (SoilMoisture Equipment Corp.). Moisture was measured as the soil suction in centibars, so a lower reading indicated higher soil moisture content. When sites became too dry or were too rocky, the moisture probe could not be inserted and readings were not taken. Late in the season the moisture probe malfunctioned, so readings could not be taken on the last sampling visit at some locations.

We initially visited each site on three consecutive days and then at 2-wk intervals thereafter. Sampling did not begin until we

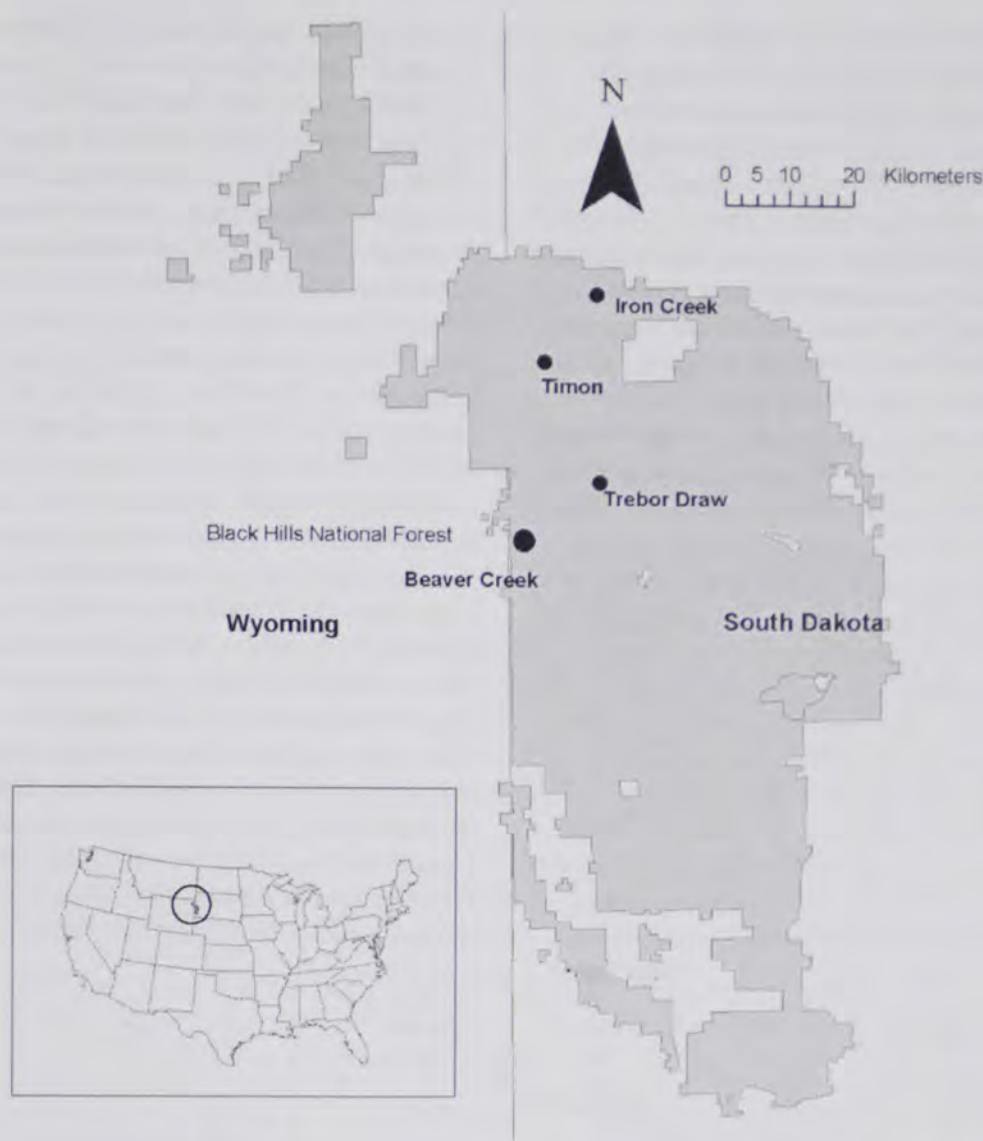


Figure 1. Location of sampling grids used in this study.

observed that snails were “active,” i.e., some snails found moving around on the surface or at least extended out of their shells. Due to cold late spring temperatures, we did not observe snail activity until late May and began trapping then. Snow and closed roads also impacted early access to some sites, so initial sampling days were not the same for all sites. For example, the road leading towards Timon Campground was closed for repairs until very late spring, so we did not begin sampling at that site until late June. Sampling continued at all sites through late September.

General assumptions of mark-recapture analyses include (1) marked individuals were representative of the entire population, (2)

marks, i.e., bee tags, were not lost and did not affect behavior or spatial arrangement of snails, (3) each marked individual had an equal chance of being recaptured, and (4) study duration was short enough assume closure (White et al. 1982). Although these assumptions were not specifically tested, we found no reason to believe that the animals captured and marked did not represent the population as a whole. We used bee tags in a study of another land snail species (i.e., Anderson 2000) and assumed they would be equally reliable for this study.

The grid density estimates we report used closed population models. These models assume birth, death, immigration, and emigration did not occur during sampling.

Other assumptions were relaxed to varying degrees depending on the model that we selected. Because snails aestivate under unfavorable conditions, we expected models in which capture probability varied with time (t) would fit the data better than models in which capture probability was held constant. Capture probability also varied among individuals, so we also considered models that allowed for behavioral (b) variation to capture and individual snail variation (h).

We analyzed mark-recapture data using program MARK (White and Burnham 1999) to obtain population density estimates. Each trapping session was considered a separate sampling occasion. Model selection criteria in program Mark uses a series of goodness-of-fit tests of these models and provided a way to test appropriateness of models for our data.

Some may argue that open models are more realistic for these populations since we observed some mortality and suspect movement off the grid. We could not obtain estimates from an open model, the Jolly-Seber option in Mark, for this data due to lack of numerical convergence to determine if they

were similar to closed estimates. Precedence for using closed models for snail population estimates appears in Anderson (2000). Although some mortality occurred during the study, we likely began sampling after the majority of offspring were born for the year; Anderson et al. (2007) found that most broods were released before June. We also did not observe such extensive surface movement that would indicate snails moved off the grid in large numbers. Therefore, a closed model at this time scale should reliably estimate density. We explored the impact of violations of the closure assumption on the robustness of our density estimates in the discussion section.

Size, whorl number, moisture, and temperature data were analyzed using Microsoft Office Excel 2003 (Microsoft Corp.) and JMP Version 4.0.2 (SAS Institute, Inc.). For individual growth analyses, the first three sampling sessions were lumped. Whorl number and diameter were highly correlated in these samples (Fig. 2) and in a previous study (Anderson et al. 2007), and either measure might be used to examine size. Whorls were difficult to count on the smallest individuals

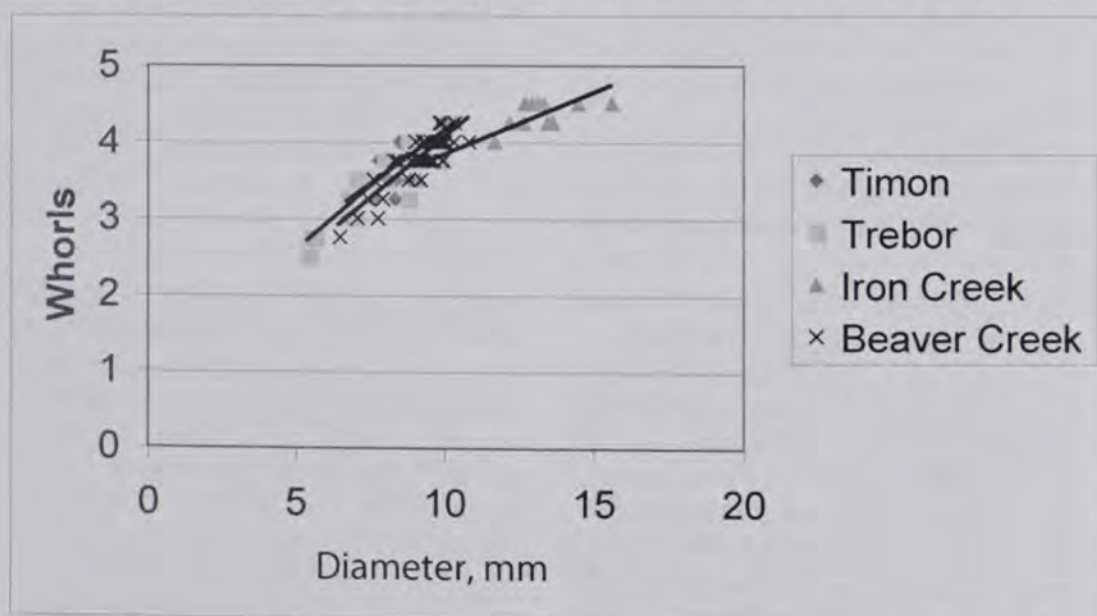


Figure 2. Relationship between number of whorls and shell diameter at each of the sampling locations. Individual snails captured in late June (from sampling dates nearest 23 June from each population) are plotted. Timon (whorls = $0.2842 \text{ diameter} + 1.2768$, $R^2 = 0.66$, $n = 40$), Trebor (whorls = $0.3343 \text{ diameter} + 0.8746$, $R^2 = 0.76$, $n = 12$), and Beaver Creek (whorls = $0.3207 \text{ diameter} + 0.821$, $R^2 = 0.77$, $n = 39$) appear to have a different slope than Iron Creek (whorls = $0.1625 \text{ diameter} + 2.1862$, $R^2 = 0.80$, $n = 14$) although this was not tested due to the small sample size at Iron Creek.

without a microscope without practice. Due to a learning curve for counting whorls, whorl counts during May might be less reliable than those conducted during June or later. Thus, we used diameter for individual growth analyses because it seemed a more precise measure. Some individuals showed negative growth that might have been due to damaged shells, human error, or caliper precision. If the decrease was < 0.1 mm, we assumed it due to caliper precision, and adjusted growth to zero. If the decrease was > 0.1 mm, the cause could not be determined after the fact, so the individual was removed from the individual growth analyses. We examined individual growth in two separate ways. First, average change in diameter of individuals recaptured on subsequent trapping sessions (~ 2 wks apart) was examined for all four sites. At two of the sites that had more recaptures (Beaver and Timon), we plotted total change in diameter of individuals caught at initial capture and subsequently recaptured. Total change in

diameter of all recaptures was not subdivided to a weekly rate because we found evidence that growth was not constant across the season.

We evaluated movement from successive locations of recaptured individuals. Since precise pathways of movement were unknown, we estimated movement distances for those individuals recaptured under different boards. These estimates assumed straight-line distances from the mid-point of the board where the snail was originally captured to the mid-point of the board where it was recaptured.

RESULTS

Grid Density Estimates

The original purpose of the study was to determine if our mark-recapture methods might be useful for monitoring, and we obtained estimates of snail density on each grid. Number of captures, recaptures, and grid density varied among sites (Fig. 3, Table 1). The model selection procedure in the

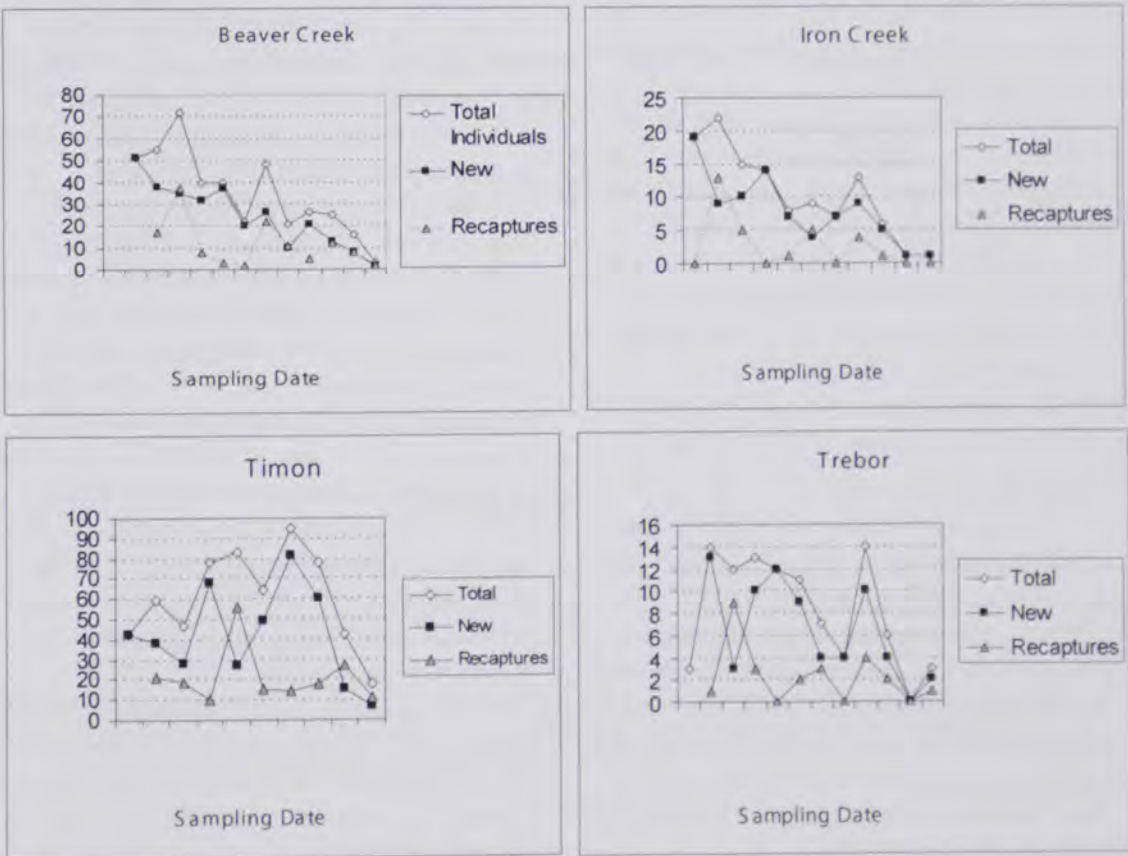


Figure 3. Total individuals captured at each sampling date at each site. Newly captured individuals and recaptured individuals are also shown.

Table 1. Grid density estimates for *Oreohelix cooperi* at four locations.

Site	Estimate (model)	Standard Error	95% confidence interval	Total No. of unique individuals caught	No. of sampling sessions	Date of first sampling session	Recapture rate (No. individuals recaptured/total ind)	No. of dead recoveries
Beaver Creek	483 (M_{th})	272	304-1,740	292	12	26 May	29%	18
Iron Creek	153 (M_{th})	152	90-1,018	86	11	6 Jun	29%	1
Timon Camp	795 (M_{th})	65	687-945	415	10	27 Jun	33%	6
Trebor	145 (M_i)	22	114-202	74	12	26 May	26%	1

CAPTURE option of program MARK chose models that reflected differences in capture rates at different sampling occasions as expected. M_{th} (Burnham's M_{th}) was the most appropriate model (model selection criteria = 1.0, with other models at ≤ 0.89) for two of the sites (Iron Creek and Beaver Creek). For the Trebor site, model M_i (Table 1) had the highest selection criteria value (1.0, with all others being ≤ 0.7).

For the Timon site, the density estimate from model M_{th} (selection criteria 0.91) appears in Table 1. Although the M_{th} model had a higher selection criteria, it produced a much higher estimate (8953) with a much larger standard error (SE = 28,696) than those produced by any other models for this site. These density estimates were most likely to be used as minimum estimates, so we rejected the high M_{th} estimate in favor of that from model M_{th} .

Individual Growth

Change in diameter was not constant across summer but occurred in spurts (Fig. 4). Average changes in diameter of ~ 0.1 mm occurred in early July among three populations. The Trebor population showed a different pattern with a 0.09-mm average change from late May to early June but only a 0.02-mm average change in early July. Small numbers of recaptures at Trebor might have affected our results.

Time of growth also varied by individual with some recaptured individuals showing

virtually no seasonal growth whereas others grew substantially (≤ 0.5 mm; Fig. 5).

Shell Size by Populations

Individuals from all four populations showed a strong correlation between number of whorls and diameter based on analyses of individuals captured at the trapping session closest to 23 June (Fig. 2). Although sample sizes were not similar enough to allow statistical analyses of the slopes of linear relationships, Iron Creek seemingly had a much larger change in diameter as whorl number increased.

We tracked average shell size of all captured individuals across the summer (Fig. 6). From these data, date of collection clearly did not influence our conclusion that shell size varied among populations as similarly reported in previous studies (Frest and Johannes 2002, Anderson et al. 2007). Even if samples were measured at Iron Creek at the point in the season in which average shell size was smallest, they would still be larger on average than samples from other populations at any time during the season, provided more than a small number of samples were measured.

The range in average diameter also differed among populations. We detected only a 0.3-mm difference in average diameter across the season at the Timon site (Fig. 6), whereas the Iron Creek and Trebor sites indicated ranges of > 2.0 mm. A linear regression indicated that average diameter

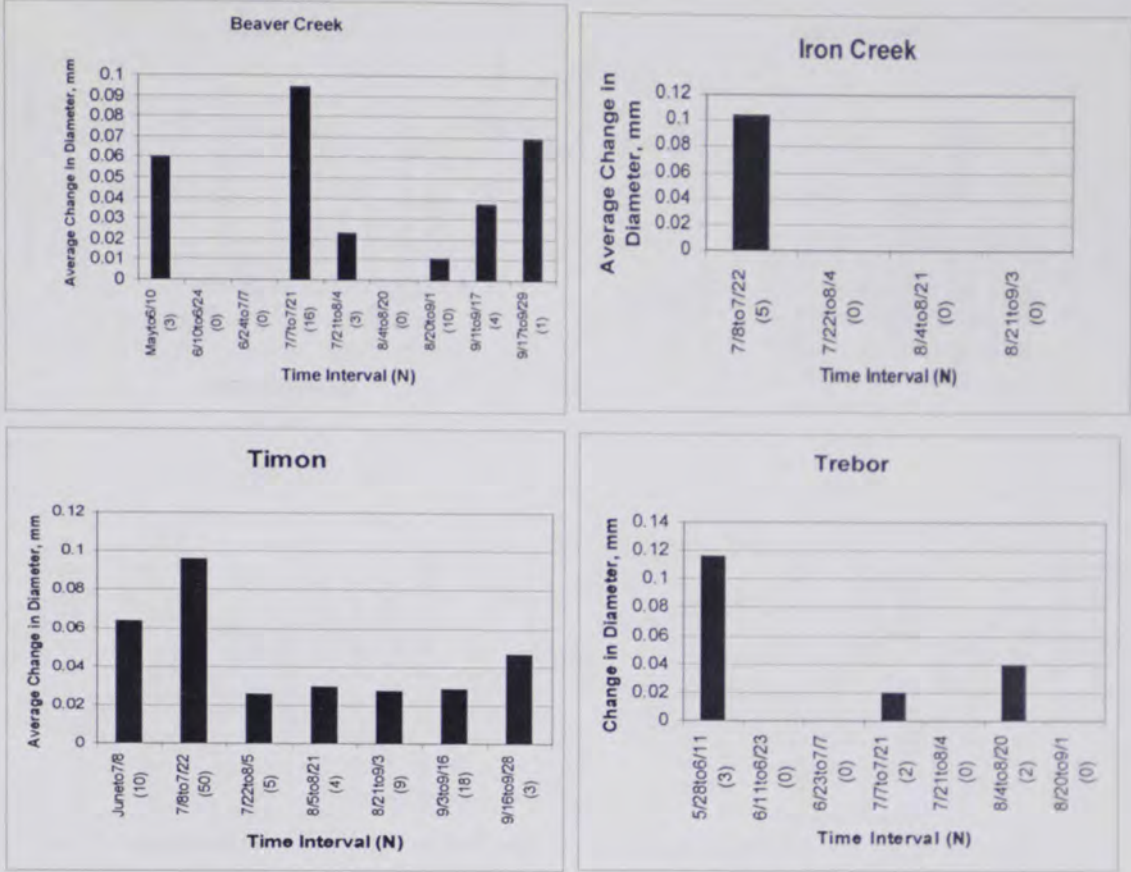


Figure 4. Change in diameter in individual snails captured on subsequent trapping sessions two weeks later. Initial three sampling days are considered one occasion for this chart.

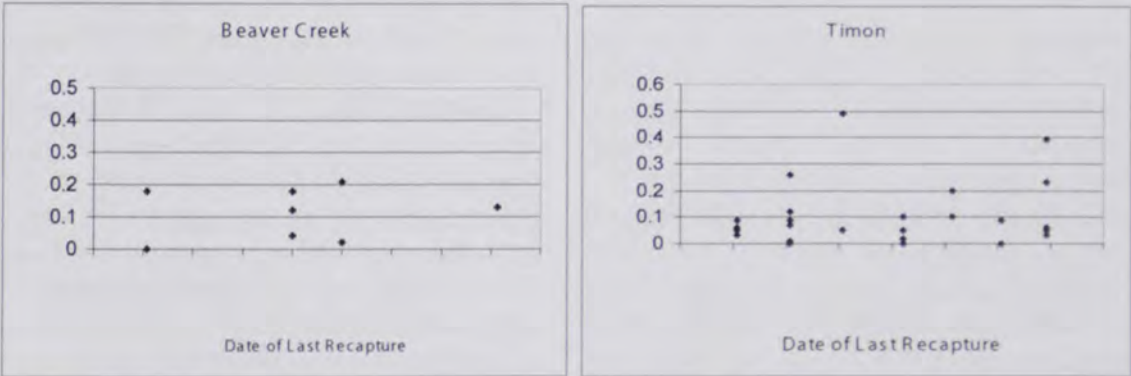


Figure 5. Individual shell growth across the season at the Beaver Creek and Timon sites. Individuals captured on the first day of sampling that were recaptured at any time later in the season are plotted by their change in diameter.

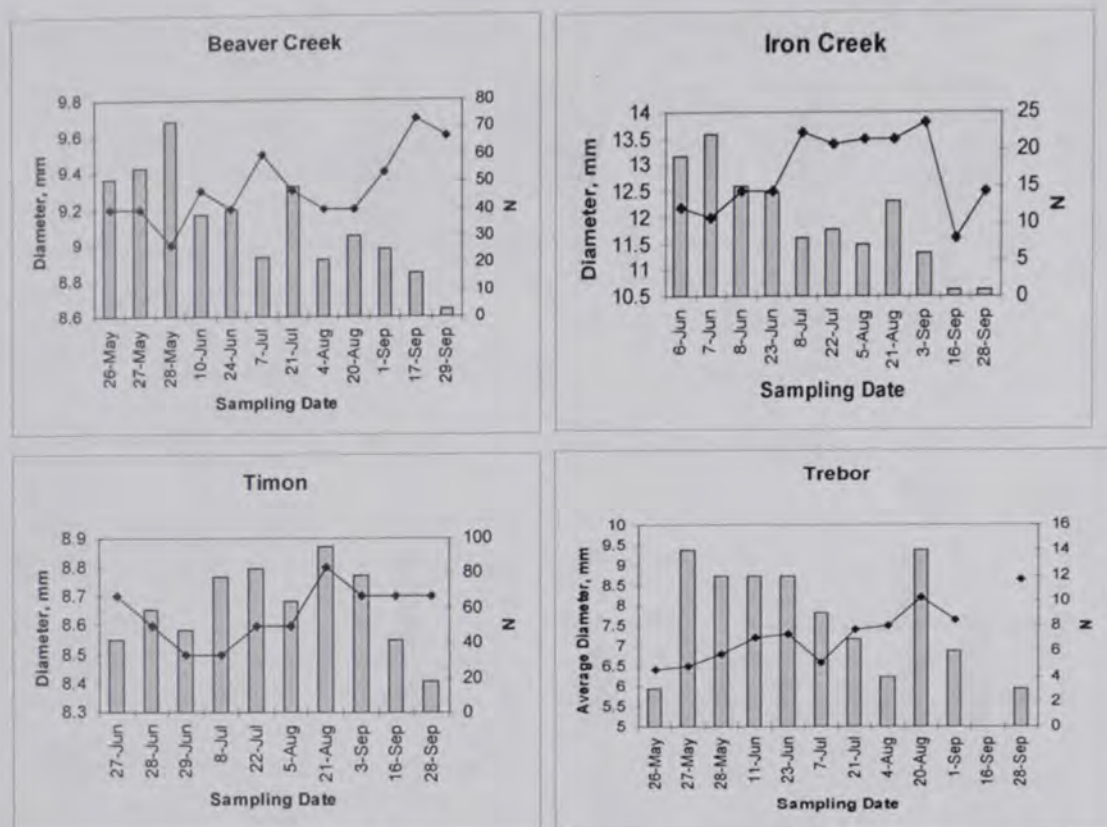


Figure 6. Average diameter of all captured individuals across the season by population. Average diameter is represented by dots and N is represented by columns on each of the graphs. Only individuals with recorded diameter measurements are included, so N may differ than the total number of individuals caught on some occasions.

increased across the summer at Beaver Creek (average diameter = $-108 + 3.66e-8$ date, $R^2 = 0.52$, $P = 0.0082$) and Trebor (average diameter = $-534 + 0.0000002$ date, $R^2 = 0.80$, $P = 0.0002$) sites. The Timon site appeared to flatten out during late summer (average diameter = $-55 + 1.98e-8$ date, $r^2 = 0.38$, linear regression $P = 0.0559$). The late start date at this site may have affected the strength of our results. We did not detect a linear increase at the Iron Creek site (average diameter = $-112 + 3.88e-8$ date, $R^2 = 0.034$, $P = 0.58$). The results on Iron Creek were surely biased by the small sample sizes in September.

Tracking number of whorls of all captured individuals from selected dates also suggested that small individuals became less abundant as the season progressed (Fig. 7). Whorl number suggests that populations shifted towards more mature adults as the season progressed. This provides further evidence that most births occurred early in the season.

Snail Activity

The number of snails caught varied among sampling sessions across the summer (Fig. 3). Snail numbers peaked at all sites in late May or early June except for the Timon Campground site at which we did not begin sampling until late June. Snails apparently became less active as summer progressed although a secondary peak occurred in late July (Beaver Creek) or late August (Iron Creek, Timon, and Trebor) before numbers plummeted in September.

Interpretation of patterns shown by new (unmarked) individuals and recaptured individuals (Fig. 3) was difficult. New snails did not contribute a constant proportion to the population, nor did the percentage of recaptures uniformly increase across the season. Instead some variation in the percentage of new vs. recaptured snails occurred over time.

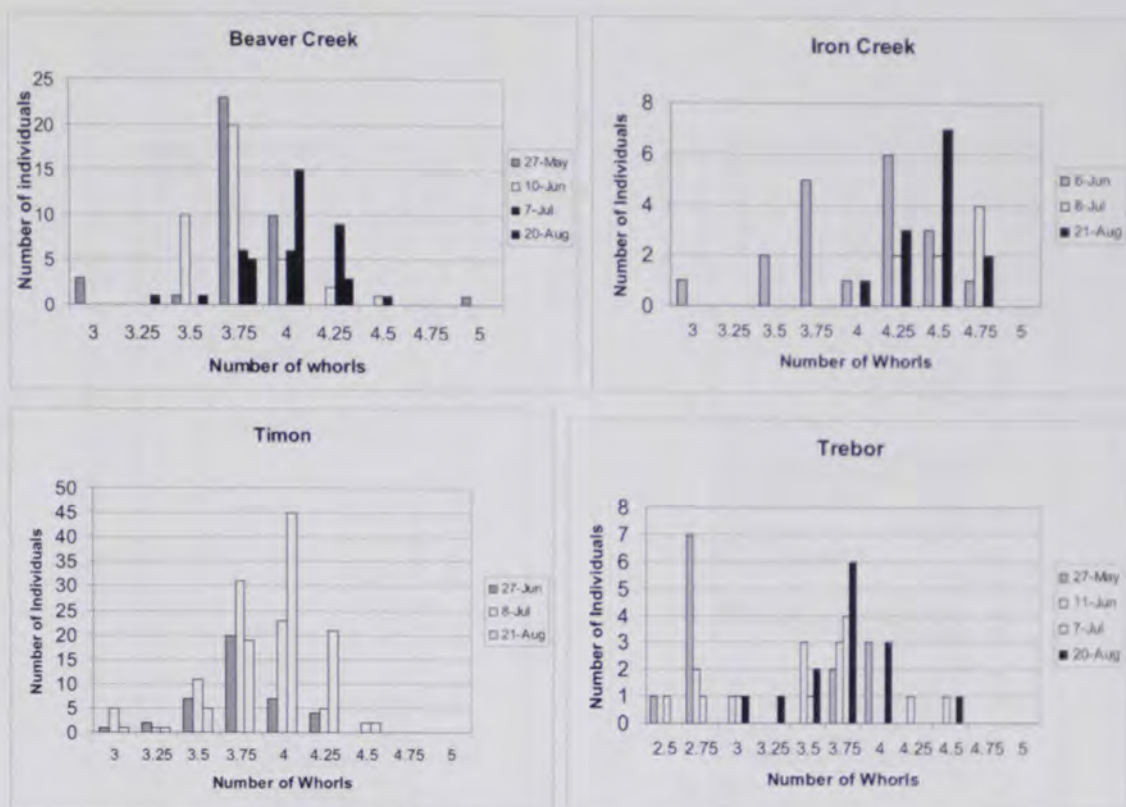


Figure 7. Distribution of whorl number at selected sampling dates. Early, middle, and late summer dates are shown for comparison.

Snails also were not uniformly distributed across a sampling site. Numbers of snails captured differed among the boards on a grid (Fig. 8) that suggested spatial variation. At three sites (Iron Creek, Timon, and Trebor) there were some boards at which we captured no snails on any sampling occasion. This suggested that for some reason, some boards were not as desirable for snail activity as others. Moisture and temperature data provide some insight into this phenomenon as discussed below. Note that at some sampling times, boards were out of place either through animal disturbance or from sliding downhill. When the board had moved, no data were available for that sampling session. Dates and boards affected were 1) Beaver Creek on 24 June (B2 and C2), 4 August (A1, A2, and A3), 20 August (C2), and 29 September (A2), 2) Iron Creek on 8 July (D1), and 3 September (B5), 3) Timon on 27-29 June (B1, C2, D1, and D5), and 4) Trebor on 11 June (C1, D2), 23 June (B1, C1, C2), 7 July (B1, B3), and 20 August (B3).

Movement

Most recaptured individuals remained under the same board as originally captured. However, 20 individuals showed some movement between sampling occasions (Table 2). The number of mobile individuals differed among populations. Percent movement, i.e., number of individuals that moved/total recaptured, ranged from 5 percent at the Timon site to 10 percent at the Iron Creek site.

Observed movements were mostly to adjacent boards, which would be a distance of approximately 2 m. We calculated straight-line distances because the path traveled was not known. We recaptured only six individuals at a board that required movement of a distance > 2 m. The longest movement recorded was by an individual at the Timon site that moved a minimum of 7.2 m between 8 July and 22 July. Interestingly, this individual also showed two other movements > 2 m between other sampling occasions.

Table 2 illustrates horizontal movement, but vertical movement was also likely since

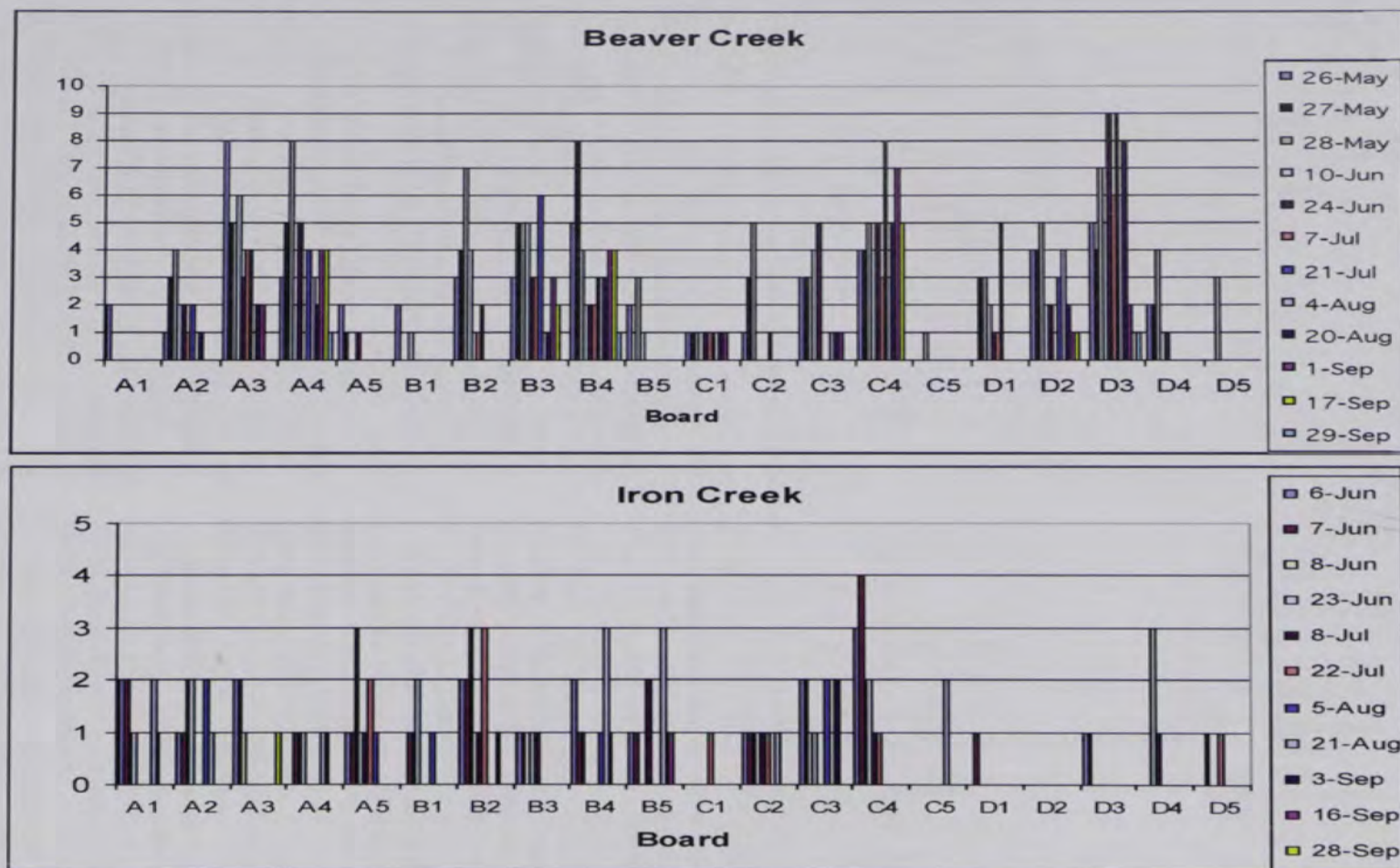


Figure 8. Snails captured at each board on each sampling occasion. Boards were set up in rectangular grids consisting of four rows (A-D) of five boards (1-5), so board A3 refers to the third board in the first row of the grid.

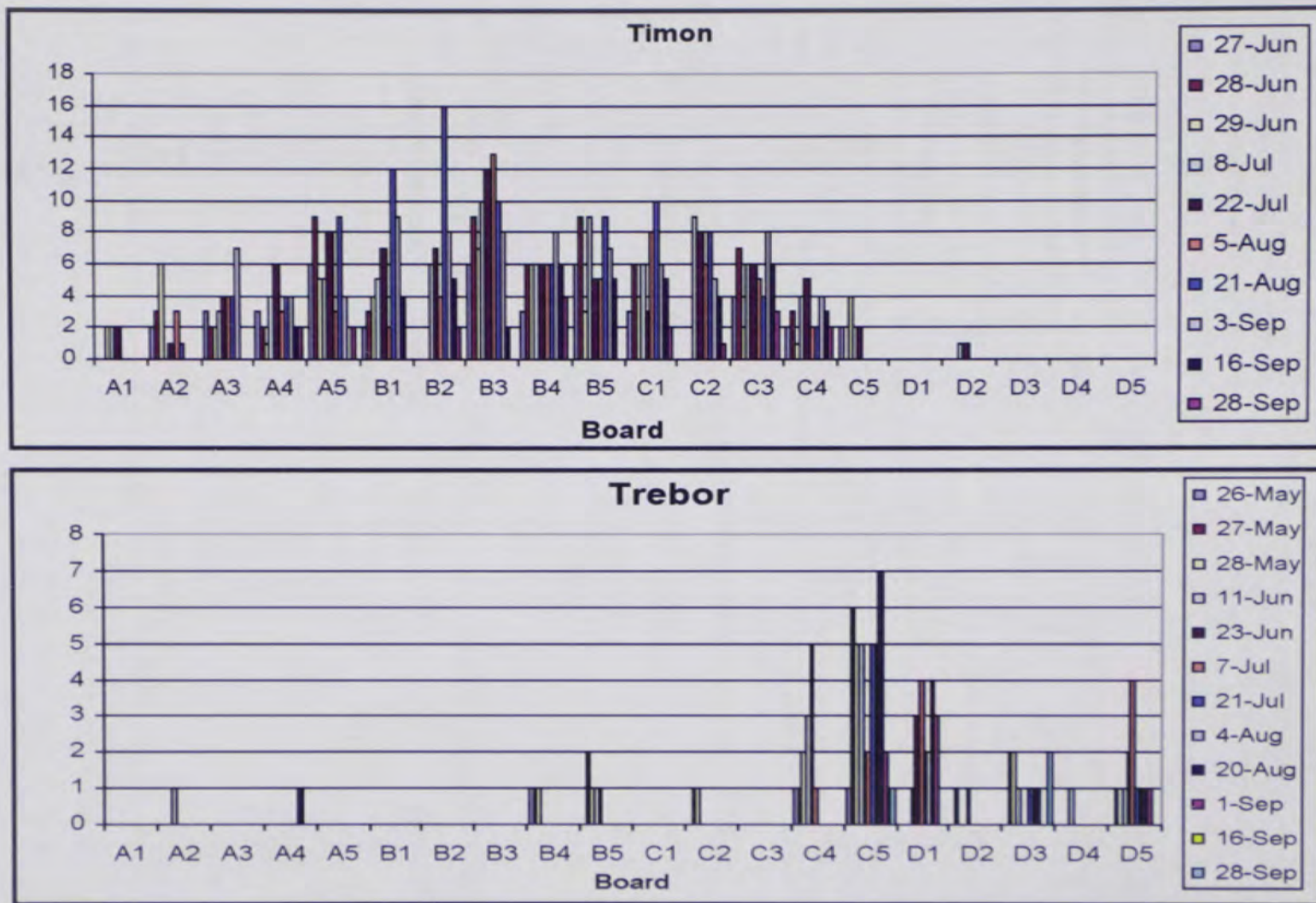


Figure 8. con't.

Table 2. Individuals Recaptured at Different Locations**Trebor Draw** (74 total individuals, 25 recapture events, 8% movement)

Individual	Capture Dates	Locations	Estimated Distance
B11	11 Jun, 7 Jul	C5 to D5	2 m
B12	28 May, 7 Jul	C5 to D5	2 m

Beaver Creek (292 total individuals, 127 recapture events, 7% movement)

Individual	Capture Dates	Locations	Estimated Distance
B28	21 Jul, 4 Aug	B2 to A4	4.5 m
O18	26 May, 21 Jul	B5 to C4	2.8 m
O22	27 May, 7 Jul	B4 to C4	2 m
O43	28 May, 24 Jun	D3 to C3	2 m
O43	24 Jun, 17 Sept	C3 to B3	2 m
O59	28 May, 20 Aug (dead)	A4 to B4	2 m
G7	28 May, 21 Jul	C1 to D1	2 m
G42	10 Jun, 4 Aug	B3 to B4	2 m
G73	24 Jun, 4 Aug	B3 to B4	2 m

Iron Creek (86 total individuals, 29 recapture events, 10% movement)

Individual	Capture Dates	Locations	Estimated Distance
G18	7 Jun, 21 Aug	C4 to C5	2 m
G48	23 Jun, 3 Sept	B3 to B4	2 m
G61	8 July, 22 July	D4 to D5	2 m

Timon Campground (415 total individuals, 191 recapture events, 5% movement)

Individual	Capture Dates	Locations	Estimated Distance
G19	27 Jun, 8 Jul	B3 to C1	4.5 m
G19b	8 Jul, 22 Jul	C1 to A4	7.2 m
G19c	22 Jul, Aug 5 (dead)	A4 to C1	7.2 m
G23	29 Jun, 22 Jul	B3 to A4	2.8 m
G57	28 Jun, 29 Jun	B3 to A1	4.5 m
G67	28 Jun, 3 Sep	B4 to B3	2 m
B18	22 Jul, 21 Aug	A3 to B2	2.8 m
B81	22 Jul, 3 Sept	C3 to B3	2 m
B97	22 Jul, 21 Aug	D2 to C2	2 m

new snails would appear at a board at a higher rate than one would logically expect if horizontal movement rates of only 5 to 10 percent were the only movements that occurred.

Soil Temperature and Moisture

Soil temperature and moisture varied among and within sites (Figs. 9 and 10). A comparison of sites suggests that the range of temperatures experienced by the snails on the grids varied slightly among sites (Table 3).

A regression analysis of the average temperature/board against the average number of captures/board across all sampling dates suggested that temperature did not strongly influence the number of snails captured ($P > 0.05$) at any of four sites. Regressing the average temperature across boards per sampling date against the total captures gave a significant relationship at the Timon site only ($P = 0.0140$), but combining all sites yielded no significant relationship.

Moisture had a slightly stronger influence than temperature on the number

of snails captured. A regression analysis of the average moisture across dates/board against the average number of captures/board was significant only for the Timon site ($P < 0.0001$). Using the average moisture reading across boards/sampling date against the total number of captures per sampling date was not significant for any individual sites. However, when data from all sites were combined, the average moisture reading/sampling date was related to total number of captures/sampling date ($P = 0.0073$). Some of the moisture analyses might have been slightly biased because we did not take moisture readings late in the season after the probe malfunctioned or when sites became too hard to insert the probe. Captures at these times were very low although we might have detected a stronger relationship if these data were available.

The boards provided a slightly cooler and moister microenvironment than the surrounding uncovered habitat. Temperature and moisture readings were taken under and adjacent to board B3 during each sampling

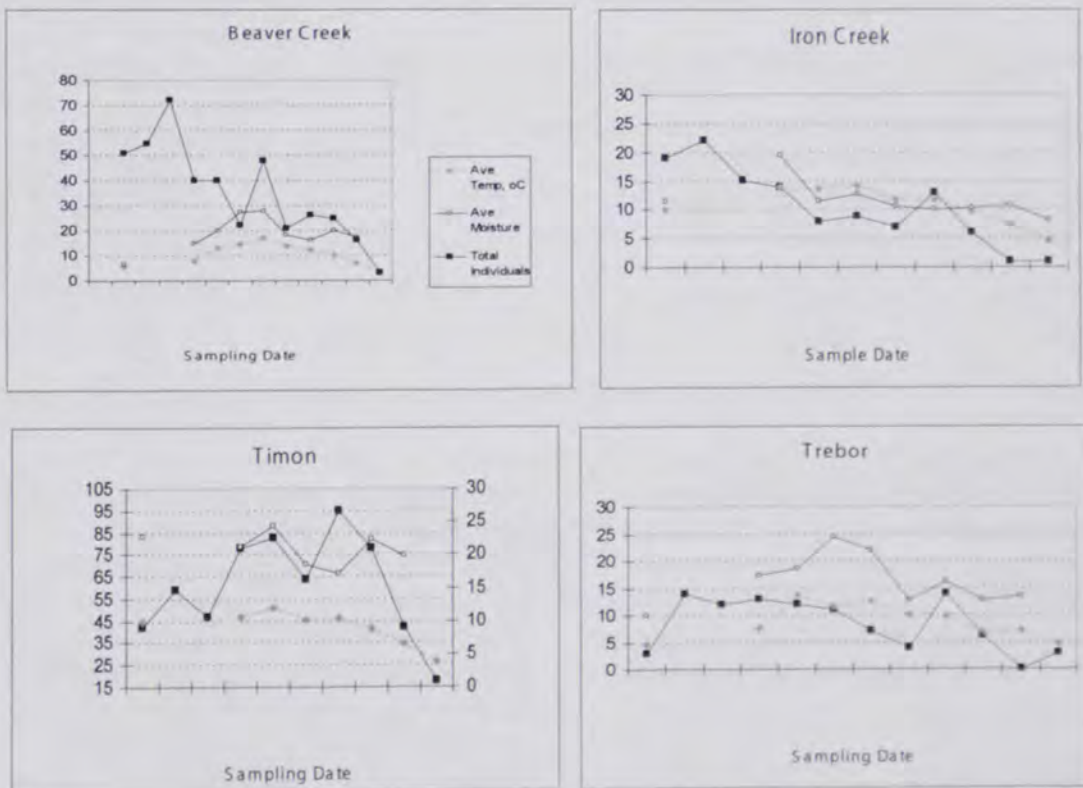


Figure 9. Average temperature and moisture measurements at each location. The plotted points are an average of the measurements across all cover boards on each particular sampling date.

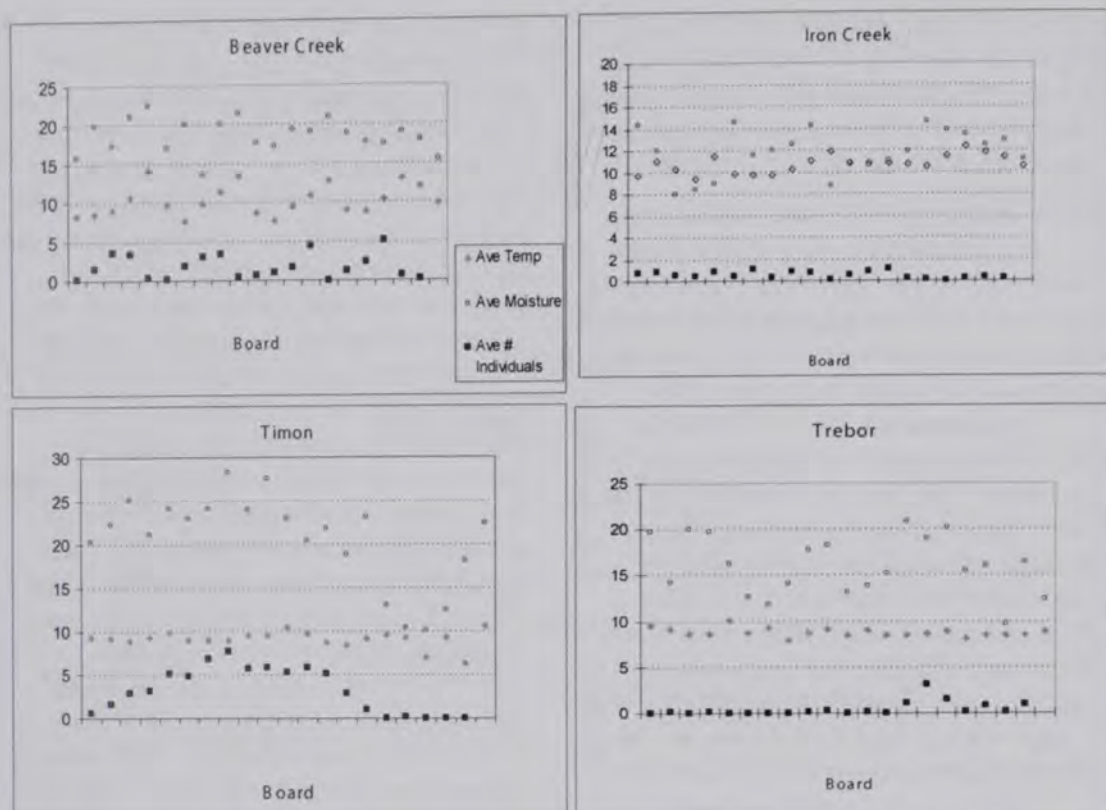


Figure 10. Variation in temperature and moisture within sites. Averages are taken from each board across all the sampling occasions during the summer.

Table 3. Ranges in temperature and moisture by site and date.

Site	Low Temp, °C	High Temp, °C	Moistest, in centibars	Driest, in centibars
Beaver Creek	3 (29 Sep)	17 (21 Jul)	7 (26 May)	28 (21 Jul)
Iron Creek	5 (28 Sep)	14 (8 & 22 Jul)	8 (28 Sep)	19 (23 Jun)
Timon	4 (28 Sep)	12 (22 Jul)	17 (21 Aug)	23 (27 Jun)
Trebor	5 (26 May, 28 Sep)	14 (23 Jun)	10 (26 May)	24 (7 Jul)

occasion. A matched pairs comparison detected a difference ($P < 0.05$) for both temperature and moisture. Temperature averaged 0.8°C higher outside the board than under the board. Moisture readings averaged 2 centibars lower outside the board than under the board.

DISCUSSION

Reliability of Density Estimates

The Cooper's Rocky Mountain snail populations technically violated some

assumptions for closed population modeling, i.e., no migration and no mortality. However, given that observed migration distances were low compared to the grid size and that observed mortality was low, closed models seemingly provided useful estimates (White et al. 1982). According to White et al. (1982), the coefficient of variation of population estimates ($\text{se}(N)/N$) should be < 20 percent for precise estimates. Two of four coefficients of variation for the estimates (Timon and Trebor) reported in this study fell below 20

percent, so those estimates were presumably realistic. Estimates for the other two sites yielded higher standard errors so may have been less precise. In addition, average estimates of probability of capture (p) at all four sites were low (0.07), which reduced reliability of our models.

It should be noted that these estimates only predicted the number of snails on the trapping grid itself and not the entire population. We did not measure the total area that snails actually inhabit at each site, which indeed was difficult to determine since snails apparently were not always visible; thus, the grid estimate was not extrapolated to a wider area.

Although some sites apparently had denser populations than others, direct comparison among sites from our limited data might be suspect. Sampling sessions were not conducted on the same days although grid sizes were equal at each site. As discussed below, snail activity varied across the summer, and this may have affected the number of snails observed at a particular time.

In addition, grids at all sites may not cover the same amount of "ideal" habitat. For example, grids at some sites, e.g., Trebor, may have been placed at the edge of the population rather than the center, which might have reduced number of snails observed. Spatial variation in snail activity was evident (Fig. 8), which shows how captures varied among boards at each

site. Although we attempted to place grids in areas where shells or live snails were observed, precisely predicting snail activity across the entire grid before sampling began was impractical.

Despite the complications surrounding the estimates, this study provided the only repeatable, mark-recapture density estimates for any land snails from the western United States as far as we could determine from searches of published literature. Our results demonstrated that a mark-recapture protocol with grids of cover-board traps can effectively serve as a useful "trap." The protocol described here is repeatable, and, if desired, estimates can be used for comparison in a long-term monitoring program. We suggest that the estimates be used only as density estimates or minimum estimates of populations on the grid and NOT extrapolated as full population estimates. A precedent for such monitoring exists in Iowa where a mark-recapture protocol is being used for monitoring of the federally-endangered Iowa Pleistocene snail, (*Discus macclintocki*; Henry et al. 2003).

Comparison with Previous Estimates

Comparison between these results and density estimates provided by Frest and Johannes (2002) are difficult to interpret (Table 4). Their estimates are based on averaging counts from 0.25-m² quadrats randomly placed around a site on one particular day.

Table 4. Comparison to density estimates by Frest and Johannes (2002).

Site	Frest's 1999 estimates of snails/m ² (nearest Frest location number)	Snails/m ² derived from the population estimates in this study	Low sample snails/m ² (date)	High sample snails/m ² (date)
Beaver Creek	5-10 (82) 2-10 (83)	4.8	0.6 (29 Sept)	14 (28 May)
Iron Creek	4 (11)	1.5	0.2 (16 & 28 Sept)	4 (6 June)
Timon	10-15 (19)	8.0	4 (28 Sept)	19 (21 Aug)
Trebor Draw	Up to 20 (87)	1.5	0 (16 Sept)	3 (27 May & 20 Aug)

For comparison with this study, two different calculations were made. First, we used grid population estimates (Table 1). For this purpose, we assumed that the area sampled included not just the area under the boards but also between boards and a slight buffer around the boards, for ~ 10 m x 10 m area. Thus, we divided population estimates by 100 for the number of snails/m². Note that density estimates were based on recapture probabilities and not just on numbers of snails from a sample. For perhaps a more direct comparison to Frest and Johannes' methods, numbers from each sampling occasion were examined individually and were divided by 20—number of 0.25-m² boards used as traps—then multiplied by four to obtain an estimate/m². The lowest and highest of these estimates also appear in the table to illustrate the range.

Exact reasons for differences between these estimates remain unknown, but several methodological possibilities are available. First, collection sites from Frest and Johannes (2002) are not an exact overlay of areas that grids were placed in this study. In fact, in some cases they did their sampling at quite a distance from the sites in this study, i.e., the Beaver Creek site from which Frest and Johannes (2002) had no samples near the campground but provided estimates from other sites along the creek. Since distribution of snails is patchy, it is difficult to make comparisons from different locations.

Second, as can be seen from the low/high estimates, date of sampling can heavily influence results when estimates are based on data from a single day. In addition, it is unknown if the cover boards used in this study would increase the probability of viewing snails across the summer over what would be seen in a quadrat without a cover-board; however, observed recapture rates of 26 to 33 percent suggested some "trap-happy" behavior. These issues illustrated the importance of using a repeatable protocol at a fixed location to monitor the population over time rather than base it on one day's sampling.

Compared to published studies of land snails using similar methods, we provide estimates at least as reliable. A mark-

recapture study of the Iowa Pleistocene snail, used a closed model, $M(th)$, to estimate population size (Anderson 2000). In that study, sample sizes were smaller (16-297/site) and using a maximum number of eight sampling sessions compared to 10-12 in this study. Probabilities of capture were low in that study (0.01 to 0.23) as well. Recapture rates for the Iowa Pleistocene snail were generally lower (0- 48%, with seven populations having recapture rates < 10%).

Another snail study of marked copse snails (*Arianta arbustorum*) in roadside areas in Sweden did not use cover-boards, but searched grids by hand (Baur and Baur 1990). Their recapture rates averaged 29.4 percent one month after marking.

Importance of Temperature and Moisture

Moisture apparently was more important than temperature to presence of Cooper's Rocky Mountain snail underneath a board. However, our temperature and moisture measurements were limited because they were only taken at specific times and might vary at other times of the day. Further examination of temperature and moisture across the summer using environmental recorders would be useful.

The range of conditions experienced at a site may be important and should be examined further. For example, we observed the moistest environment overall at Iron Creek where we found the largest individuals.

Movement

Movement rates and distances in this study were comparable to the rates found in other snail studies. Between 0 and 17 percent of Iowa Pleistocene snails migrated between cover boards on different cold-air slopes in Iowa (Anderson 2000). Furthest movement was 8 m. Average linear movement by copse snails along roadside areas ranged from 1.5 to 4.9 m at different sites in Sweden (Baur and Baur 1990). Average distances moved by copse snails in subalpine areas in Sweden ranged from 7 to 12 m/year (Baur 1986). Longer dispersals of ≤ 500 m in 6 months were known from an African giant snail (*Achatina fulica*),

which was fitted with a radio-transmitter (Tomiya and Nakane 1993).

More information is needed to understand dispersal and other movement in this species. Some Cooper's Rocky Mountain snails moved horizontally from underneath the boards, based on observations of marked individuals both on and off the grid that had previously been marked while under a board. Projecting whether active dispersal alone can account for the gene flow evident from genetic studies is difficult (Weaver et al. 2006). Passive movement resulting from human or animal activity or from rolling downhill is probably needed to explain some of the gene flow. Vertical movement was also likely. Snails were probably burrowing into the soil or retreating into crevices more readily than expected.

Although we observed some mortality (Table 1), yielding robust survival rates from our data was impractical. Longer-term monitoring that included some winter sampling would be helpful to better understand survival in this species.

Growth and Size

Individual snails apparently took advantage of microclimate conditions to add to their diameter in spurts during the season. Growth rates in this study appeared to be lower than those reported from a laboratory study of the related subalpine mountain snail (*Oreohelix subrudis*) where growth rates of ≤ 1.4 mm occurred over 2-month periods (Beetle 1987). Beetle noted that for subalpine mountain snail to reach its average adult diameter of 20 mm may require 3 years. Using our growth rates, Cooper's Rocky Mountain snail would take 10 years to reach maturity assuming an average four-whorl adult was ~ 8.5 mm in diameter and born at 2.25 whorls and 3.25 mm in diameter. This being unlikely, growth must continue during other parts of the year as was the case for the subalpine mountain snail.

The shift in whorl size to larger classes as summer progresses suggests growth in whorl number to adult size. Not enough data were available to determine if whorl growth reached a maximum number or if growth continued as conditions allowed. We would expect distinct

size classes for previous years' cohorts if there was continual, steady increase in whorls, but that did not seem readily apparent. Whorl number was used to identify species for many snails, so our results would be expected either due to die-off of older individuals or slowing of growth after maturity.

Our diameter data supported presence of more than one size morph as described in Anderson et al. (2007). We were unable to conclusively correlate a specific environmental factor to differences in diameter although Anderson et al. (2007) showed that temperature is likely a factor using long-range climate data.

Size data indicated that snails are not "born" at full size but are growing during the season. The range in average size across summer was much narrower in two populations (Timon and Beaver), which also show smaller-diameter individuals. This may suggest interplay between habitat conditions and periods of growth. For example, if conditions suitable for growth are present for shorter time periods at Timon and Beaver sites, they would have had a much narrower opportunity to increase in size.

A complicating factor involved juvenile size. Whether snails in the different populations are born at the same size was unclear since very few juveniles were observed. However, snails in populations of the larger size morph likely were already larger at birth based on findings by Anderson et al. (2007) that indicated larger adults produced larger offspring.

In a study of the rock-dwelling land snail (*Chondrina clienta*) in Sweden, Baur (1988) found shell size was related to density and the amount of plant cover. Although overall density estimates were not related to average mid-season shell diameter ($P > 0.05$) in our study, the difficulties in the precision of density estimates make this difficult to evaluate from this data alone. Anderson et al. (2007) found shell density was a significant factor in adult shell size for Cooper's Rocky Mountain snail. We did not measure thickness of the litter layer on the grids, which would provide a similar variable to plant cover for Cooper's Rocky Mountain snail.

CONCLUSIONS

Our results fill in several gaps in information identified in the species assessment document for the R2 Region of the USDA Forest Service (Anderson 2005) and illustrated the importance of field work in providing information for planning and management of rare invertebrates.

We demonstrated that a mark-recapture protocol is possible to monitor Cooper's Rocky Mountain snail densities. We provided conclusive evidence that size differences observed among populations were not the artifact of the time of season that populations were sampled. We also provided evidence that growth is not continuous but most likely occurs as conditions allow. We also demonstrated snail activity varies over the summer and management activities could be planned around such times. For example, disruptive activities should probably be avoided in May and June and after rainy periods in late July and August, but may have less impact in September. Growth may be occurring at times of the year not covered in this study.

Additional field seasons would greatly increase reliability of population estimates, provide a greater understanding of survival, and possibly increase understanding of movement of these snails. However, that is not possible with current funding and personnel limitations. Should additional field seasons be conducted, or for those setting up similar studies in different systems, a few recommendations follow. Automatic temperature and moisture recorders should be placed at each site to allow a better understanding of the range of conditions experienced by the snails and to more accurately compare conditions between years. Some soil cores should be taken to provide an understanding of whether (and how deep) snails are moving down into the soil and when (presumably when surface conditions are less favorable). Site boundaries should be defined to allow extrapolation of grid population estimates to actual location estimates. In sites that are especially large, secondary grids could be set up to monitor the variation in density across the site as well as allow for more information on movement.

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REPRODUCTIVE STATUS OF CYTOFORMS IN A BLACK FLY COMPLEX IN MONTANA

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ABSTRACT

We studied the reproductive status of cytospecies and cytotypes of the *Simulium arcticum* complex at four sites in Montana by comparing banding sequences of polytene chromosomes of the larval salivary glands to 1) identify cytospecies and cytotypes, 2) determine genotypes and frequencies of autosomal polymorphisms, and 3) assess the degree of reproductive isolation of taxa. We hypothesized that taxa within the complex that have large geographic distributions would be reproductively isolated in sympatry, while those that have very limited geographic distributions would not. Data from four separate collection sites support our hypothesis and possibly suggest a model for divergence within the group.

Key words: black flies, reproductive status, siblings, cytospecies, cytotypes, autosomal polymorphisms, hybrids

INTRODUCTION

In black flies (*Diptera: Simuliidae*) the morphospecies of classical taxonomy often reveals itself as any number of sibling species when polytene chromosomes of larval salivary glands are analyzed (Rothfels 1956). Taxa are described primarily on the basis of fixed-inversion sequences and by the presence of sex-linked chromosomal rearrangements and other features of their biology including the extent of shared autosomal polymorphisms, presence or absence of supernumerary or B chromosomes, the most advanced developmental stage of meiosis, and numbers of generations/year. We describe populations as cytotypes if they are cytologically distinct, whereas we designate sibling species status (cytospecies) if these cytologically defined taxa are reproductively isolated from other such forms in sympatry.

Shields and Procnier (1982) described five siblings of the *Simulium arcticum* complex in Alaska and western Canada (*S. arcticum* st, *S. arcticum* IIL-3·4, *S. arcticum* IIL-1, *S. arcticum* IIL-2, and *S. arcticum* IIL-3). The former two and the latter two

have now been designated *S. brevicercum*, *S. negativum*, *S. saxosum*, and *S. arcticum* sensu stricto, respectively (Adler et al. 2004). Two additional siblings, *S. arcticum* IIL8·9/IIS-10·11 (*S. vampirum*, Adler et al., 2004) and *S. arcticum* IIS-4, have been described from the Athabasca River drainage of Alberta, Canada, by Procnier and Shemanchuk (1983) and by Procnier (1984), respectively. Finally, two other siblings, *S. apricarium* and *S. chromatium*, have been recognized (Adler et al. 2004).

We have recently documented the presence of *S. brevicercum*, *S. negativum*, *S. arcticum* sensu stricto and *S. apricarium* in Montana along with eleven cytotypes of the complex (Shields, unpub.). Distribution of siblings in the *S. arcticum* complex is associated with elevation in Montana (Shields et al. 2006). The considerable diversity within a single morphospecies and the abundance of larvae here provide opportunity to investigate the extent of reproductive isolation of taxa in sympatry, the focus of this research. Specifically, tests of the extent of reproductive isolation in sympatry can be conducted because taxa can be differentiated by the presence of unique

sex-linked chromosomal rearrangements and individuals of each type can be scored for autosomal polymorphisms that can then be subjected to genetic equilibrium analyses. While fixation of alternative inversion sequences between two taxa in sympatry may suggest reproductive isolation, sharing of identical polymorphisms does not necessarily indicate random breeding. Black flies (Rothfels 1978), including cytospecies of the *S. arcticum* complex, share autosomal polymorphisms that are retained in respective populations after reproductive isolation has occurred (Shields and Procunier, 1982; Adler et al. 2004).

Cytospecies of the *arcticum* complex are broadly distributed across western North America (Adler et al. 2004), whereas 11 new cytotypes in Montana have restricted distributions, some of which occur only at a single site within a drainage (Shields, unpub.). Patterns of geographic distribution suggest that cytospecies may be evolutionarily old and genetically divergent from other members of the complex while cytotypes may be evolutionarily young and still in the process of divergence from other types with which they are sympatric. The combination of this diversity at the level of chromosomes and knowledge of the geographic distributions of the various types suggests the possibility of defining the extent of reproductive isolation for each taxon and may allow insight into the process of speciation. As Jerry Coyne and Allan Orr (2004:69) emphasize in their recent book, *Speciation*, “How can we tell which isolating barriers actually caused speciation instead of having evolved after speciation was complete? Comparative analysis of taxa at different stages of evolutionary divergence, ranging from populations through full species, may show which barriers persist throughout this transition.” In the present study, we determined distribution and frequency of autosomal polymorphisms among taxa of the *S. arcticum* complex in sympatry to estimate extent of reproductive isolation. We hypothesized that taxa with broad geographic distributions would be reproductively isolated in sympatry while those with limited distributions would not.

MATERIALS AND METHODS

Assessment of Reproductive Status

Tests of reproductive status of cytological entities can be conducted in a number of ways. Taxa that develop at different times (temporal isolation) cannot produce hybrid progeny. However, reproductive status of two cytologically distinct taxa in both sympatry and synchrony can be determined by analysis of the distribution and frequency of autosomal polymorphisms and other cytological criteria. For example, presence of alternative homozygotes, without heterozygotes, suggests reproductive isolation. Floating autosomal polymorphisms among types can be tested for adherence to equilibrium frequencies and thus indicate reproductive status.

Chromosome Nomenclature

Sex chromosomes in black flies are often associated with chromosomal rearrangements. Thus, for most cytospecies and cytotypes within the *S. arcticum* complex males are heterozygous for unique, sex-linked inversions whereas females generally possess the standard chromosome sequence. Consequently, we designated the sex chromosomes of females as X_0X_0 to indicate that they possess the standard (non-inverted) chromosome sequence for corresponding sex-linked inversions in males. Males are characterized as having one chromosome, the X with the standard sequence, X_0 , and a second chromosome Y with a paracentric inversion. Sex-linked inversions are numbered according to the sequence of their discovery. For example, *S. arcticum* IIL-1 males are designated X_0Y_{IIL-1} , and those in *S. arcticum* s. s. as X_0Y_{IIL-3} . Sex-chromosomes in *S. apricarium* are polymorphic for all classes; thus, females can be X_0X_0 , X_0X_{IIL-7} , or $X_{IIL-7}X_{IIL-7}$ whereas males can be X_0Y_0 , X_0Y_{IIL-7} , or $X_{IIL-7}Y_{IIL-7}$. The subscript indicates the specific inversion characterizing each sibling or cytotype.

Selection of Sites

Presence of autosomal polymorphisms in sufficient frequencies allows statistical analyses

at sites where two or more siblings or cytotypes are present. Our previous observations (Shields, unpub.) suggested that study of the extent of reproductive isolation among cytological entities of the *S. arcticum* complex was possible at four sites (Fig. 1, Table 1).

Sample Collection and Analysis

At each collection site we sampled larvae regardless of species richness. Larvae were removed from various substrata and fixed in cold Carnoy’s fixative. Upon return to the laboratory we sorted larvae of the *S. arcticum* complex to morphospecies (Currie 1986)

and selected penultimate and ultimate instar larvae for analysis because they possessed polytene chromosomes of the highest quality. We used the Feulgen method of Rothfels and Dunbar (1953) to stain polytene chromosomes and gonads and used standard chromosome maps of the *S. arcticum* complex (Shields and Procunier 1982) to differentiate cytospecies and cytotypes and to determine frequencies of autosomal polymorphisms. Hardy-Weinberg equilibrium statistics were calculated where cytospecies and cytotypes were present in geographic sympatry.

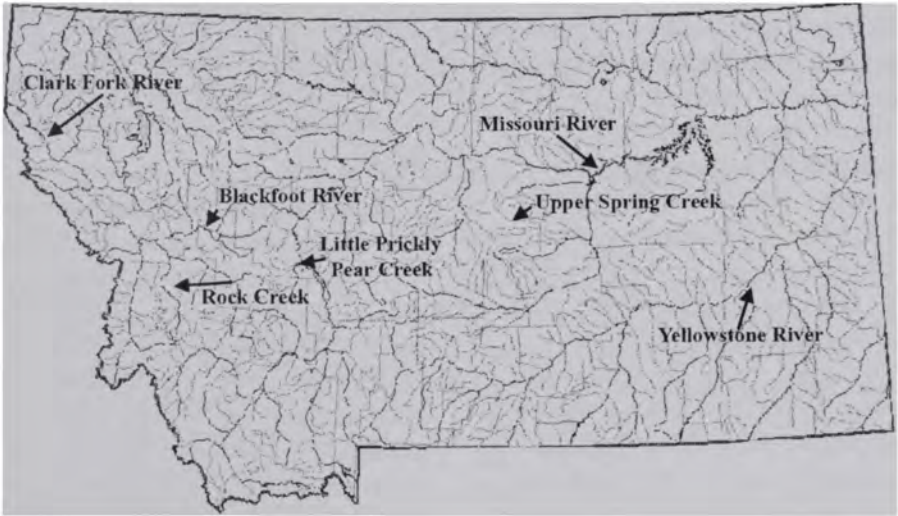


Figure 1. Map of Montana showing the three major drainage basins (Clark Fork, Missouri, and Yellowstone rivers), along with most other drainages, and the specific locations of collection sites studied here.

Table 1. Sites studied, taxa analyzed in detail, collection schemes, and autosomal polymorphisms used to determine reproductive status of various siblings and cytotypes of the *S. arcticum* complex in western Montana.

Location	Taxa Present	Collections Analyzed in Detail	Autosomal Polymorphisms
Blackfoot River	<i>S. negativum</i> and <i>S. arcticum</i> IIL-9	Eight dates, four summers	IS-1, IL-3.4, IIS-10-11, IIIL-4
Upper Spring Cr.	<i>S. arcticum</i> IIL-10	Five years	None
Little Prickly Pear Creek	<i>S. arcticum</i> s. s. and <i>S. apricarium</i>	Four summers	IIS- 10-11, IIL-20
Rock Creek	<i>S. arcticum</i> IIL-9 and IIL-19	Single date (3/14/06)	IS-1, IL-1, IL-3.4

De cription of Sites

Blackfoot River.— This site was chosen for study because previous analysis indicated the abundance of at least one sibling, *S. negativum* (IL-3-4, Fig. 2), and one cytotype, *S. arcticum* IIL-9 (Fig. 3). Other taxa of the complex were present but in low numbers. Also, certain autosomal polymorphisms appeared to be in sufficient abundance to perform equilibrium analysis. We sampled this site at three-week intervals from mid-March to mid-July in parts of four years.

Upper Spring Creek.— This site appeared to be unique among 40 sites analyzed (Shields, unpub.) in that only one cytotype was present year-round. This site

has been sampled for five year , with the composite sample representing each month of the year.

Little Prickly Pear Creek — Previous analyses indicated presence of two cytospecies, *S. arcticum* s. s. (IIL-3) and *S. apricarium* (IIL-7). The latter was said to be reproductively isolated from other known taxa of the complex (Adler et al., 2004), though study on the scale intended here (> 1000 larvae) had not yet been conducted. We used autosomal polymorphisms IIS-10-11 (Fig. 4) and IIL-20 to estimate reproductive status at this site.

Rock Creek. Previous analyses indicated the presence of at least two cytotypes, *S. arcticum* IIL-9 and *S. arcticum*

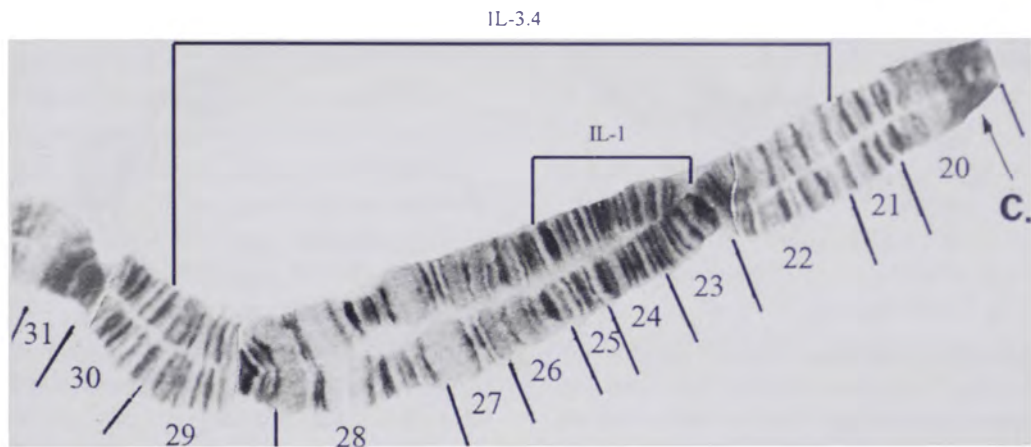


Figure 2. Chromosome map of a portion of the long arm of chromosome I of the *S. arcticum* complex (modified from figure 4 of Shields and Procunier, 1982). Brackets indicate breakpoints of the IL-3.4 and IL-1 inversions. Numbers below the chromosome indicate sections of the chromosome, as is the case in Figs. 2-6.

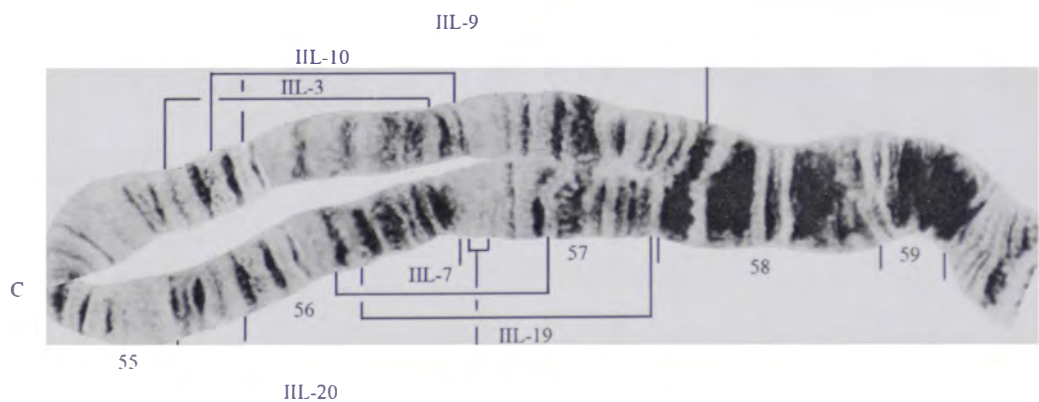


Figure 3. Chromosome map of a portion of the long arm of chromosome II of the *S. arcticum* complex (modified from figure 5 of Adler et al., 2004). Brackets indicate breakpoints of the IIL-3, IIL-7, IIL-9, IIL-10 and IIL19 sex-linked inversions and the IIL-20 autosomal inversion.

IIL-19, with chromosomes of excellent quality so that we could determine all three genotypes of the autosomal polymorphisms IS-1 (Fig. 5) and IL-1. Our investigation of the reproductive status of *S. arcticum* IIL-9 and IIL-19 was based on analysis of larvae collected only on 14 Mar 2006.

RESULTS AND DISCUSSION

Blackfoot River

Our analyses of seven collections from the Blackfoot River are shown in Table 2.

Because of high water at the Blackfoot in late March and April 2006; we were unable to determine presence of the *S. arcticum* complex but rather used samples collected at comparable dates in 2003 and 2004. The data suggested 1) an early presence of the cytotype *S. arcticum* IIL-9, 2) presence of the cytospecies *S. negativum* (*S. arcticum* IL-3-4) in May and June, and 3) a return of IIL-9 in July. We suggest temporal reproductive isolation because neither taxon appeared in the presence of the other though overlap of adults may have occurred. Genetic data suggested

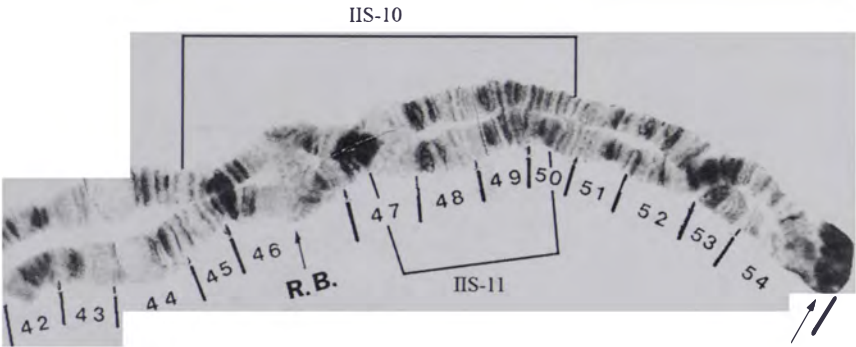


Figure 4. Chromosome map of the short arm of chromosome II of the *S. arcticum* complex indicating breakpoints for the IIS-10 and IIS-11 inversions (modified from figure 5 of Shields and Procunier, 1982).

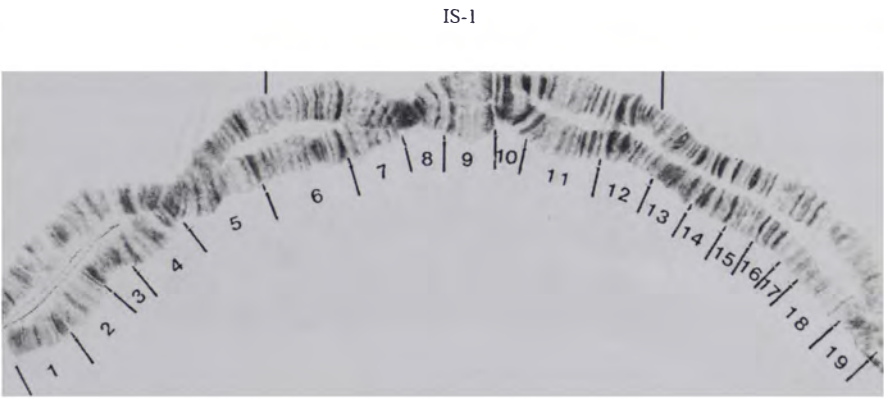


Figure 5. Chromosome map of the short arm of chromosome I of the *S. arcticum* complex (modified from figure 3 of Shields and Procunier, 1982). Brackets indicate breakpoints of the IS-1 inversion.

that if hybrids had been formed when and if the taxa were synchronous, they were formed in low numbers. Specifically, the entire population of IIL-9 was fixed for the standard homozygote of the autosomal inversion II - 10·11 in late March (Table 3). Alternatively, 125 of 129 individuals of *S. negativum* collected in May and June were inverted homozygotes for IIS -10·11, which suggested near total reproductive isolation. A similar pattern occurred for the transition from *S. negativum* in June to *S. arcticum* IIL-9 in July. Although 100 percent of males after March and prior to July were *S. negativum*, no *negativum* larvae occurred in July (Table 2), and among 103 larvae analyzed from July 10, none were IIS-10·11 inverted homozygotes (Table 3). Therefore, we observed little evidence for hybridization between *S. arcticum* IIL-9 and *S. negativum* at the Blackfoot River. Eleven IIL-19 males occurred in the 30 Mar 2003 collection of which all were IIS-10·11 st/st that also indicated potential temporal and genetic reproductive isolation from *S. negativum*.

Distribution and frequency of two other autosomal polymorphisms, IIIL-4 (Fig. 6) and IS-1, were of interest. IIIL-4 occurs in low frequency (% heterozygosity) in the IIL-9 population in March and increased dramatically to ~ 33 percent heterozygosity in the negativum population in May and June, but was not present in the July population of *arcticum* IIL-9 (Table 3). This

suggested additional support for reproductive isolation between *S. negativum* and the second generation of *S. arcticum* IIL-9 in July.

Early and late populations of IIL-9 had similar distributions of the IS-1 inversion, with 19.1 and 13.9 percent heterozygosities, respectively, and were in equilibrium (Table 4). However, within the *S. negativum* population, 81 percent of males were heterozygous for the IS-1 inversion (Table 4). Thus, there may be two Y chromosomes, II.-3·4 and II.-3·4 + IS-1, in the *S. negativum* population at the Blackfoot River

Two II.-3.4 males occurred in the 28 March 2004 collection, whereas three male and two female larvae were II.-3·4 in the 10 Jul 2006 collection. One of these male larvae was a III.-3 heterozygote and two other males were III -9 heterozygotes. All these larvae were chromocentric and possessed positive head patterns, unlike the achromocentric *S. negativum* that has negative head patterns in its females (Adler et al. 2004). This suggested that the II.-3.4 inversion is autosomal in the March and July populations of *S. arcticum* IIL-9 at the Blackfoot River, while being sex-linked in the *S. negativum* population in May and June. One individual in the March collection and two in the July collection were III.-9 inverted homozygous males (Table 2.). One male III.-3/III.-7 heterozygote was also observed.

Table 2. Cytological diversity of the *Simulium arcticum* complex at the Blackfoot River, Russell Gates Campground, Missoula County, Montana.

	Females		Males				
	X ₀ X ₀	X ₀ X _{IL-3.4}	X ₀ Y ₀	X ₀ Y _{3.4}	X ₀ Y _{L-3.4+IS-1}	X ₀ Y _{IIL-9}	X _{L-9} Y _{I-9}
Date							
3/13/06	no larvae present						
3/28/04	31	0	1	0	0	27	1
3/30/03	54	0	0	0	0	27	0
4/1/05	17	0	0	0	0	10	0
5/9/06 many small larvae							
5/18/03	7	0	0	6	6	0	0
5/23/06	71	6	0	8	60	0	0
6/15/06	25	2	0	2	6	0	0
7/10/06	54	0	5	0	0	42	2
Total	259	8	6	16	72	106	3

Table 3. Distribution of autosomal polymorphisms among members of the *Simulium arcticum* complex at the Blackfoot River, Russell Gates Campground, Missoula County, Montana.

Date	Autosomal Polymorphisms			
3/13/06	no larvae present			
<i>S. arcticum</i> IIL-9				
	st/st	IIS-10.11 st/i	i/i	IIIL-4 % st/i
3/28/04	60	0	0	9.7
3/30/03	81	0	0	not scored
4/1/05	10	0	0	0
<i>S. negativum</i>				
	st/st	IIS-10.11 st/i	i/i	IIIL-4 % st/i
5/9/06	many small larvae			
5/23/06	3	0	145	40.0
6/15/06	1	0	34	37.1
<i>S. arcticum</i> IIL-9				
	st/st	IIS-10.11 st/i	i/i	IIIL-4 % st/i
7/10/06	102	1	0	0
Total	247	1	125	



Figure 6. Chromosome map of the long arm of chromosome III of the *S. arcticum* complex (modified from figure 8 of Shields and Procunier, 1982). Brackets indicate breakpoints for the IIIL-4 autosomal inversion.

Table 4. Distribution of the IS-1 inversion among sexes and populations of the *S. arcticum* complex at the Blackfoot River, Russell Gates Campground, Missoula County, Montana and Chi-square Statistics Based on Equilibrium Frequencies of Genotypes.

<i>S. arcticum</i> IIL-9						
						
	st/st	st/i	i/i	st/st	st/i	i/i
3/28/04	25	6	0	30	7	0
3/30/03	54	7	0	27	2	0
4/1/05	12	4	0	12	0	0
						$X^2 = 1.06,$ d.f. ₂ , 0.60 < p < 0.70
<i>S. negativum</i>						
5/18/03	109	10	0	16	72	0
5/23/06						$X^2 = 12.6,$
6/15/06						d.f. ₂ , 0.01 < p < 0.02
<i>S. arcticum</i> IIL-9						
7/10/06	51	4	0	42	11	0
						$X^2 = 0.61,$ d.f. ₂ , 0.70 < p < 0.80

We did not observe inversion homozygotes for IS-1. The eight females heterozygous for the IS-1 inversion may represent sex-exceptional individuals. On the contrary, IS-1 was apparently autosomal in the IIL-9 populations because in both early and late populations the distributions occurred as expected: March, $X^2 = 0.904$, d.f.₂, $0.70 < P < 0.80$; July, $X^2 = 0.648$, d.f.₂, $0.70 < P < 0.80$. Although we observed no IS-1 homozygous inverted individuals for the IIL-9 cytotype in our samples, expected probabilities indicated that there should have been at least one in each population.

The single IIS-10·11 heterozygote in the 10 July population of *S. negativum* is of interest. No males of *S. negativum* occurred in our samples from 10 July. Since this IIL-10·11 heterozygote was also heterozygous for IIL-9, it might have been a hybrid between a IIL-9 male and a *S. negativum* female. However, tight pairing of the homologs of this individual suggested that it was not a hybrid. Alaskan populations of *S. negativum* (Shields and Procunier 1982), as well as populations at the Gallatin, Sun and Yellowstone rivers in Montana, were not polymorphic for either IS-1 or for IIL-4 (Shields, unpub.). Thus, these polymorphisms may be newly derived in the

populations at the Blackfoot River.

Eleven IIL-19 st/i males occurred in the 30 March 2003 sample at the Blackfoot River of which all were IIS-10·11 st/st homozygotes, suggesting that they were reproductively isolated from *S. negativum*. Presence of two IIL-3/IIL-7 heterozygotes is of interest because only one male *S. arcticum* s. s. larva (IIL-3) and no *S. apricarium* (IIL-7) larvae were found among nearly 400 larvae analyzed from this site. These IIL-3/IIL-7 heterozygotes were also heterozygotic for a large inversion near the end of the IIL-arm. Presence of two *S. arcticum* s. s./*S. apricarium* heterozygotes was difficult to explain because the apparent parental types were either rare or nonexistent. This may be a case of very rare polymorphisms. Whereas IIL-4 may be shared by local populations of *S. negativum* and *S. arcticum* IIL-9, our data suggested that the later population of IIL-9 may be derived from the early IIL-9 population.

Upper Spring Creek

Our analyses of larvae from Upper Spring Creek are shown in Table 5. Y-linkage of the IIL-10 inversion appears complete since all males and no females analyzed possess this inversion. Some IIL-10 larvae at this site possess as many as four B chromosomes in

their germ lines. All of these characteristics, particularly IIL-10's unique presence as larvae year-round, suggested that *S. arcticum* IIL-10 at Upper Spring Creek was reproductively isolated and may be a good biological species. The IIL-10 cytotype spends winter as larvae,

Table 5. Temporal Distribution of the *S. arcticum* IIL-10 Cytospecies at Upper Spring Creek, Fergus County, Montana.

Date	Females X ₀ X ₀	Males X ₀ Y ₁₀
1/22/05	4	11
1/26/03	11	25
2/7/04	3	2
2/17/02	3	1
2/27/04	12	8
3/25/06	5	10
4/24/04	37	25
5/21/05	8	1
7/16/05	1	6
10/5/03	9	3
Total	102	92

whereas many other taxa of black flies spend winter as eggs (Adler et al. 2004). Possibly as a consequence of this early maturation, male IIL-10 larvae at Upper Spring Creek possess mature sperm in February (Shields, unpub.). Documentation of species status for IIL-10 might require collection of pupae that could be reared to adults.

The unique presence of the IIL-10 cytotype at Upper Spring Creek may have been due to characteristics of the drainage. The creek originates from Big Spring, a natural freshwater spring with a relatively constant flow rate year-round. Moreover, water temperature at Upper Spring Creek was nearly constant yearlong having a range of temperatures of only 10 to 13 °C (Shields, unpub.). This restricted range in temperature at Upper Spring Creek occurs when other non-spring fed drainages nearby experience temperatures from 0 °C in December to 24 °C in August. Relatively constant flow rate and water temperatures at Upper Spring Creek possibly provide a unique environment for IIL-10 *arcticum*.

Little Prickly Pear Creek

We have monitored the presence, density, and number of potential hybrids of *S. arcticum* s. s. and *S. apricarium* at this site since 2002. Most populations of *S. arcticum* s. s. in allopatry are fixed for the standard form of the IIS-10·11 sequence, whereas most pure populations of *S. apricarium* are fixed for the inverted homozygote (Adler et al. 2004). Potential hybrids between the two taxa therefore, might be indicated by the presence of IIS-10·11 heterozygotes. Thirteen of the 1254 larvae analyzed (0.01 %) at this site were IIS- 10·11 heterozygotes (Table 6) and these may suggest rare hybridizations between *S. arcticum* s. s. and *S. apricarium*. However, this number could be misleading. Both IIS-10·11, and especially IIL-7, are polymorphic in some populations, especially westward in the distribution (Adler et al. 2004). This could explain six of the IIS- 10·11 st/i larvae that were IIL-st/st. The remaining seven IIS-10·11 heterozygotes may be evidence for hybridization between the taxa or they may be ancestral relics, i.e., IIL-3 types in *S. apricarium* or IIL-7 and IIS-11 types in *S. arcticum* s. s. Only one individual among 1254 larvae analyzed was a IIL-3i/IIL-7i type and this could be explained by hybridization between a *S. arcticum* s. s. male and a *S. apricarium* female. This male was IIS- 10·11 st/st, and had tight pairing of the homologues, suggesting that IIS-10·11 may be polymorphic in this population, as mentioned above.

Approximately 10 percent of *S. arcticum* s. s. larvae at Little Prickly Pear Creek were heterozygotes for the IIL-20 autosomal inversion during 2005 and 2006, and we have never seen this inversion among *S. apricarium* larvae (Shields, unpub.). We have also found the IIL-20 inversion in the nearby drainages of the Boulder River, Little Blackfoot River and Canyon Creek, but in frequencies < 1.0 percent (Shields, unpub.). Therefore, IIL-20 possibly arose at or near Little Prickly Pear Creek, and some unknown factor maintains its relatively high heterozygosity there. Taken as a whole, data from Little Prickly Pear Creek did not argue for hybridization between *S. arcticum* s. s. and *S. apricarium*. Little Prickly Pear Creek has the

Table 6. Temporal Distribution of *S. arcticum* s. s. and *S. apricarium* and Frequency of IIL-11 Heterozygotes at Little Prickly Pear Creek, Lewis and Clark County., Montana.

Date	<i>S. arcticum</i> s. s. 	% = 90.4 	<i>S. apricarium</i> 	% = 9.6 	IIS-11 st/i
3/15/02	1	22	3	7	0
3/16/03	3	7	1	15	0
3/30/06	131	323	4	5	5
3/31/05	109	116	8	20	3
4/1/06	3	10	1	0	0
4/4/02	3	26	2	0	0
4/10/03	30	24	4	9	0
4/18/02	17	29	2	2	0
4/30/02	6	4	0	0	0
5/6/05	53	63	5	14	1
5/6/06	65	76	0	0	1
5/26/05	5	0	8	11	3
Total	432	701	38	83	13 n = 1254

highest density of larvae among our collection sites with an estimated 50 larvae/cm².

Rock Creek

Among males at Rock Creek, *S. arcticum* IIL-19 (55.4 %) and *S. arcticum* IIL-9 (37.9 %) predominated (Table 7). Five X₀Y₀ males, ten male *S. arcticum* s. s., and four male and four female *S. apricarium* also occurred in the sample. Heterozygosities of the autosomal inversions IIL-1 (22.0 %) and IS-1 (15.2 %) were sufficiently high among *S. arcticum* IIL-19 and IIL-9, respectively (Table 8), so we subjected these data to tests of random mating. IIL-1 was in equilibrium among the IIL-9 and IIL-19 populations as was the IS-1 inversion (Table 8). Among 534 larvae analyzed from the 14 Mar 2006 collection from Rock Creek, 13 were IIS-11 heterozygotes (three st/st females, eight IIL-9 males and two IIL-19 males). o

larvae had both the IIL-7 inversion, which is polymorphic in *S. apricarium*, and any of the other sex-linked inversions characteristic of the other taxa of the arcticum complex (i. e., IIL-3, IIL-9 and IIL-19) at Rock Creek. Consequently, *S. arcticum* IIL-9 and IIL-19 form a single cytospecies/cytype at Rock Creek in March. Twelve of 534 individuals analyzed from Rock Creek were IIL-3.4 heterozygotes. Five were st/st females, five were IIL-19 males, one was a st/st male and the twelfth was a IIL-9 male. IIL-3.4 is the sex-linked inversion in *S. negativum* (Shields and Procunier 1982, Adler et al. 2004), but it was apparently an autosomal polymorphism among members of the *S. arcticum* complex at Rock Creek. This may be an example of the phenomenon of “one sibling’s sex-linked inversion being another sibling’s autosomal polymorphism” (Rothfels 1979).

Table 7. Distribution of Sex Chromosomes of the *S. arcticum* Complex at Rock Creek, Missoula County, Montana.*

Date	Females				Males					
	<i>S. apricarium</i>				<i>S. apricarium</i>					
	X ₀ X ₀	X ₀ X ₀	X ₀ X ₇	X ₇ X ₇	X ₀ Y ₀	X ₀ Y ₃	X ₀ Y ₀	X ₀ Y ₇	X ₇ Y ₇	X ₀ Y ₉ X ₀ Y ₁₉
3/14/06	243			2	5	10	0		3	106 155

* Seven other larvae of the *S. arcticum* complex, each having newly discovered inversions in the long arm of chromosome II, were also observed. Since their frequencies were low, we did not include them in these analyses.

Table 8. Genotypic distributions of the IL-1 and IS-1 autosomal polymorphisms among *S. arcticum* IIL-9 and *S. arcticum* IIL-19 at Rock Creek on 14 March 2006 and Chi-square analysis suggesting random mating of the two cytotypes.

Cytotype	Autosomal	Polymorphism -	IL-1	
	st/st	st/i	i/i	
IIL-9	66	35	5	$X^2 = 0.004$, d. f. ₂ , $0.90 < p < 1.0$
IIL-19	86	60	10	

Cytotype	Autosomal	Polymorphism-	IS-1	
	st/st	st/i	i/i	
IIL-9	92	14	0	$X^2 = 1.446$, d. f. ₂ , $0.50 < p < 0.60$
IIL-19	127	28	0	

Of the 13 males heterozygotic for IIS-14, 10 were IIL-9 st/i, two were IIL-st/st, and one was IIL-19 st/i., suggesting an additional rare sex chromosome in the 14 March 2006 population at Rock Creek. We hesitate to speculate on the reproductive status of other members of the *S. arcticum* complex at Rock Creek (*S. arcticum* s. s., *S. apricarium* and possibly *S. brevicercum*) because each occurred in low frequency. Finally, no data suggested reproductive isolation for the IIL-9 and IIL-19 cytotypes at Rock Creek.

Comparisons Among Sites

IIL-9 and IIL-19 and *S. negativum*.—Our results from the Blackfoot River and Rock Creek revealed several trends. *S. arcticum* IIL-9 and IIL-19 were present at both sites in March. *S. negativum* was present at the Blackfoot River in mid-summer and individuals having the IL-3.4 inversion were present in the 13 March 2006 sample from Rock Creek although that inversion appeared autosomal. Despite extensive sampling in western Montana, we found *S. arcticum* IIL-19 only at two other sites (Bitterroot River on 28 March 2004 and at the Clearwater River on 28 February 2003 and 14 March 2006, suggesting that IIL-19 has a very restricted geographic distribution and by inference, it may be evolutionarily young.

S. arcticum IIL-9 was also present at the Clearwater River on 14 March 2006, which is only 7 km from the Blackfoot River. Limited geographic distribution, early presence in spring, and sympatric presence at some sites

for these two cytotypes also suggested a close relationship. The three sites in question were close geographically (< 84 km.), and each flows into the Clark Fork River that drains northwestern Montana. Sampling of additional drainages flowing into the Clark Fork River may prove informative regarding the relationships of IIL-9 and IIL-19. Finally, we never observed *S. negativum* in early spring that suggested it has adapted to mid-summer development that may be temperature dependent.

IIL-10.—*S. arcticum* IIL-10 was the only cytotype at Upper Spring Creek year round. We found males with the IIL-10 inversion at five widely scattered sites in our study area but always in very low numbers ($n \leq 7$) on any date. Based on ~ 30 collections at Upper Spring Creek, we have never found abundant larvae of IIL-10. We have found it in small clusters of larvae at the tips of twigs in the swiftest flowing waters. Whether low densities of IIL-10 at Upper Spring Creek and its presence in low numbers elsewhere were correlated remains unknown.

General Summary.—We monitored four collection sites for sex-linked and autosomal inversions among members of the *S. arcticum* complex in western Montana to gain insight into their reproductive status. We found little evidence for random mating between *S. negativum* and *S. arcticum* IIL-9 at the Blackfoot River or between *S. arcticum* s. s. and *S. apricarium* at Little Prickly Pear Creek. *S. arcticum* IIL-10 is the only member of the *S. arcticum* complex at Upper Spring

Creek. Study of sites near Upper Spring Creek may result in a better understanding of the reproductive status of this cytotype. The cytotypes, *S. arcticum* III-9 and III-19 appear to be randomly mating at Rock Creek.

Our observations are an initial step in understanding relationships in this complex. Our current research includes 1) study of environmental factors that might influence dispersal of cytotypes, 2) study of the extent of reproductive isolation among taxa within the complex and 3) molecular analyses of larvae individually identified to cytospecies and cytotype to test hypotheses about the ages and relationships of members of the *S. arcticum* complex.

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A CASE STUDY OF MUSCULAR STRENGTH, ENDURANCE AND POWER RESPONSES TO A 6-WEEK HIGH INTENSITY TRAINING PROGRAM

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ABSTRACT

We evaluated the muscular strength, endurance, and power responses of 12 college students, ranging in age from 19-40 years, who participated in a 6-wk high-intensity training program commonly used to improve muscular endurance. Muscular strength was measured by a one repetition maximum (1RM) bench press test and a 1RM Hammer bench press test; muscular endurance was measured by administering a 70-percent 1RM test to failure on the Hammer bench press; and upper body power was measured by administering a medicine ball throw test. We observed a 4.8-percent improvement of 2.7 kg on the bench press, a 14.6-percent improvement of 10.5 kg on the Hammer bench press, a 45.5-percent improvement with an average increase of five repetitions on the submaximal test to failure and an average improvement of ~ 20 percent, 60 cm, for the medicine ball throw. For our subjects, a commonly used high-intensity training muscular endurance program resulted in improved performance on tests measuring muscular strength, endurance, and power, and resulted in zero reported injuries during training or assessment procedures.

Key words: high intensity training, muscular endurance, strength, power

INTRODUCTION

There are two philosophies concerning performance-related power enhancement. One is that muscular hypertrophy, endurance, strength, and power result from different methods of training (Fleck and Kraemer 1997). The other philosophy holds that muscular hypertrophy, endurance, strength, and power are all interrelated, and improvements in these areas are based simply on improvements in strength (Brzycki 1995, Mannie 1999). Additionally, debate exists over effectiveness of free weights versus resistance training machines. Many strength and conditioning professionals believe free weight training is superior to using resistance training machines (Fleck and Kraemer 1997, Shepard 2004). Finally, some researchers believe that training to the point of muscular failure will not enhance strength, power or hypertrophy, but will cause injury (Stone et al. 1996).

With these thoughts in mind, we observed the effects of participating in a

high-intensity muscular endurance weight lifting program over a 6-wk period in which each set of each exercise was taken to muscular failure, defined as that point when a repetition cannot be completed in good form due to physical exhaustion.

Research Questions

Did a high-intensity endurance strength training program have any effects on maximum strength or power as measured by a one repetition maximum bench press test on two implements (free weight and Hammer) and a medicine ball throw test of upper body power? Was there a transfer of strength from a machine bench press to a free weight bench press? Were there any reported injuries from participating in a weight lifting program that required completing each set to muscular failure? We consider the bench press exercise a "free weight" exercise in which participants support and balance the entire weight of the barbell throughout the full range of motion.

The Hammer bench press is considered a “machine” exercise in which participants do not have to balance the resistance. We explored these questions.

METHODS

Subject

This study was a non-experimental descriptive study that used a convenience sample of 13 college students ranging in age from 19 to 40 years. Experience level of each subject ranged from little to no experience in strength training to having over 28 years of experience lifting weights as a form of strength training. We analyzed data from a 6-wk high-intensity muscular endurance training program to determine effects on muscular strength, endurance, and power. The university approved all procedures, and each subject signed an informed consent document. All subjects volunteered for the project and completed a physical activity readiness questionnaire.

Procedures

All participants were assessed prior to the start of the program and again at the end after a 3-day rest from the final training day to ensure recovery, and followed procedures established by the American College of Sports Medicine (ACSM) (2006). Beginning with a warm-up and ending with a cool down, the initial and final assessments included (1) 1 repetition maximum (1RM) bench press test (BP) in which each individual performed a one-repetition maximum on the bench press; we allowed 5 min rest between 1 RM attempts; and weight was added in 5- to 10-percent increments until participants could not complete the repetition, (2) following a 5-min rest from the BP test, participants completed a one-repetition maximum Hammer Incline Press Test (HBP) following the same procedures for the BP test, (3) following a 5-min rest, participants completed a set to momentary muscular failure with 70 percent of the 1RM of the Hammer test (SHBP), and (4) following a 5-min rest, participants completed the medicine ball throw test (MBT) to test upper body power.

The resistance training program used was a modified form of Kelso's (2000) muscular endurance training recommendation and included two options. Option one consisted of performing two sets of one exercise, the Hammer Incline Press, twice/week. The sets and repetitions varied according to the following schedule: (1) 2 wks of 2 sets of 15-17 repetitions, (2) 2 wks of 2 sets of 12-15 repetitions, and (3) 2 wks of 2 sets of 10-12 repetitions. If the subjects chose option two they performed the following exercises: (1) Abdominal Curls, (2) Bicep Curls, (3) Hammer Deadlift (optional), (4) Hyper-extensions, (5) Tricep Extension, (6) Leg Extension, (7) Leg Curl, (8) Lat Pull Down, and (9) Wrist Curls (optional).

Two subjects chose to participate in option one and the rest of the subjects chose to participate in option two. Option two required one set of each exercise during two sessions/week and followed the same repetition scheme for option one, i.e., a target repetition range of 15-17 for the first 2 wks. The target repetition range for weeks 3 and 4 was 12-15, and for weeks 5 and 6 the range was 10-12. Each subject was given personal instruction in the performance of each exercise and, during this time, the subject was directed to take each set to muscular failure, i.e., the point at which they could no longer complete a repetition without deviating from the posture required of the exercise. The teaching points that were emphasized for safety included (ACSM 2006) (1) slow movements, (2) full range of motion, (3) breathing, (4) posture, (5) proper warm-up, and (6) proper cool-down.

Each week subjects handed in performance sheets that included the number of repetitions and amount of weight used for each exercise performed. Subjects were also questioned about the development of any injuries. This ensured that communication about the program was maintained throughout the 6-week period in case there were any questions, comments or concerns.

All necessary measures were put into effect to ensure the safety of each participant. A health screen evaluation, the physical activity readiness questionnaire (PAR-Q), was

used for each subject to ensure that he/she had no adverse health risks that would affect the participant during activity. Also, to ensure the privacy of these participants, we assigned a random number to each subject to maintain confidentiality and used an informed consent form that stated the participant’s willingness to allow us to use their results in this case study.

RESULTS

Because we did not meet the assumption of the central limit theorem with only 13 subjects, we chose to describe results in a case study format. Out of 13 original participants, 12 completed requirements of the 6-wk study. Results of the 1RM bench press (BP) suggested a 4.8-percent improvement of 2.7 kg (Table 1), and the

Hammer bench press test (HBP) showed 14.6-percent improvement of 10.5 kg. The submaximal test to failure (SHBP) resulted in a 45.5-percent improvement with an average increase of five repetitions. Eleven of 12 subjects improved on the SHBP, whereas subject eight finished with a decrement of two repetitions. Nine subjects improved performance on the MBT test; average increase for the 12 subjects was 60 cm for ~ 20 percent improvement. There were no injuries reported during the training or assessment procedures.

DISCUSSION

The principle of overload states that for a system to improve its function, it must be exposed to a stimulus greater than it is

Table 1. Performance Results.

Subject	BP pre*	BP post*	HBP pre*	HBP post*	SHBP pre*	SHBP post*	MBT pre+	
	29.5	31.8	31.8	40.9	11	20	3	3.4
2	38.6	40.9	54.5	59.1	10	11	2.9	2.9
3	100	104.5	131.8	136.4	9	11	4.8	5.1
4	47.7	50	59.1	70.5	13	16	2.9	2.9
5	72.7	75	95.5	104.5	14	16	3.7	4.9
6	75	77.3	81.8	95.5	12	20	3.9	4.6
7	25	25	18.2	27.3	15	23	2	2.2
8	70.5	65.9	86.4	86.4	12	10	3	2.9
9	38.6	43.2	40.9	54.5	14	21	2.7	3.1
10	75	79.5	104.5	113.6	7	9	3.7	4
11	102.3	113.6	122.7	154.5	6	14	3.1	4
12	29.5	29.5	29.5	36.4	12	16	2.2	2.6
Mean	58.6	61.4	71.4	81.9	11	16	3.1	3.7
% change		4.8		14.6		45.5		20

* units in Kilograms (kg).
+ units in meters (m).

normally accustomed to. Some strength coaches prescribe strength and power programs with a range of three to five sets for multi-joint exercises, and prescribe two to six repetitions, while other strength coaches adhere to a single set training protocol with higher repetitions, usually anywhere from 6-20 repetitions. Though they are employing different acute program variables, each is attempting to progressively overload the musculoskeletal system to improve muscular strength, endurance, and power. There has been much debate over the effectiveness of these various strength and conditioning programs.

This particular protocol, commonly thought of as a muscular endurance training program, effectively improved one repetition maximum bench press strength on two different implements for all but two of the subjects; subjects eight and twelve. Subject eight actually decreased performance on the BP, SHBP and the MBT. Though we instructed all subjects to maintain normal activity, it was not until after the 6-week study that the subject told us that she began a rigorous cardiovascular endurance-training program that involved running over 6 mi/day, 6 days/week during the study. This may have been a factor in her performance decrement.

There were limitations in our study design. One limitation was the small non-representative sample with no control group, and the other is the age range of 19-40 years, which is quite large. Another limitation was a range of little to no previous training experience to an advanced level of previous training. This may possibly have had some effect on the physiological response to this training program and/or their performance on the tests. Also, the 6-wk duration of the study is a relatively short period of time.

Any conditioning in unfit individuals will usually produce changes in physiological variables up to a certain level of performance competency. Some strength and conditioning specialists believe that beyond moderate performance competency, usually only more specialized conditioning including higher volume training and power training, will produce further performance improvements.

This was not the case in our study. Subjects three and eleven had the most previous training experience of all the subjects. Based on common perceptions described above, this kind of low volume program would have less of an effect on subjects with more previous experience. These two subjects, however, improved performance on all measurements. Likewise, subjects one, two, four and seven had the least amount of previous training; yet their performance improvements were relatively modest.

Another limitation of this study was the fact that we were unable to supervise every training session the subjects participated in. Often, motivation in the form of personal supervision can have an influence on how far individuals will push themselves. When a "coach" is present and expects improvement from one training session to the next, subjects may push themselves further than they would during a training session with no supervision. Though training sheets were turned in on a weekly basis and verbal encouragement was given to ensure each subject was training to a point of complete muscular failure, some people were more or less able to withstand the discomfort that accompanies this type of training.

Subject number six was almost eliminated prior to the beginning of the study because of a pre-existing non-specific shoulder condition that was identified during the pre-participation screening. Because the subject was enthusiastic about participating in the project we allowed him to continue and monitored him closely. His performance during the six weeks showed steady improvement, and the post program assessment was impressive with improvements in each test. Though we did not conduct post study interviews, subject number six emailed us with subjective perceptions of his experience and we felt it was appropriate to include this qualitative aspect within this manuscript. "Before the strength program," he wrote, "I worked for the beer distributor and stocked shelves. I could not lift a single 6 pack above my head without pain (7-8 on a scale of 1-10). It also hurt to do the bench press. bad enough that

I hit my max on the first try. The Hammer press didn't hurt though. After the [final] strength tests, I have had ZERO pain in my shoulder. Not even when I lay on my side on it in bed. Also, doing the bench press for the second time, we started with my old max and I pushed it up, and moved on to the next weight, with very little pain. I have had no pain since the strength training and am loving it. Thanks!"

This subject's statement, combined with the fact that there were zero injuries reported, indicated that training to muscular failure may not cause injury, as some strength and conditioning specialists suggest.

We expected muscular endurance to improve, but the researchers were unaware of the effects this training program would have on strength and power, or whether there would be transfer of strength from one implement to another. Strength and conditioning coaches should be aware that higher repetition ranges may be effective in improving strength and power.

Additionally, higher repetitions require lower weights and reduce orthopedic stress and risk of injury; this may be an important consideration to coaches who want their athletes to remain healthy and injury free. For future research we recommend use of matched control groups using various higher volume programs, and using participants who are all experienced weight lifters.

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CONTENTS

ARTICLES

Biological Sciences - Aquatic

- Toxicity of Fintrol® (Antimycin) and Prenfish® (Rotenone) to Three Amphibian Species 1

Grant G. Grisak, Donald R. Skaar, Gary L. Michael, Mark E. Schnee and Brian L. Marotz

- Toxicity of Fintrol® (Antimycin) and Prenfish® (Rotenone) to Slimy Sculpin..... 9

Grant G. Grisak, Mark E. Schnee, Gary L. Michael and Donald R. Skaar

Biological Sciences - Terrestrial

- Population Demography of a Land Snail Species of Conservation Concern in the Black Hills.....13

Tamara K. Anderson and Christina Schmidt

- Reproductive Status of Cytoforms in a Black Fly Complex in Montana32

Gerald F. Shields, Judith A. Pickens, Gregory M. Clausen and Lindee M. Strizich

Health and Human Development

- A Case Study of Muscular Strength, Endurance and Power Responses to a 6-Week High Intensity Training Program.....44

John Amtmann, Kelly Amtmann, Jack Kukay and William K. Spath