# Success of Slimy Sculpin Reintroductions in Minnesota Trout Streams: Influence of Feeding and Diets

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ABSTRACT.—Slimy sculpin *Cottus cognatus* are being reintroduced into coldwater streams in southeastern Minnesota to restore native biotic diversity and provide forage for large trout, but success has been variable. We examined slimy sculpin diets and prey consumption in a series of field and laboratory experiments to assess the potential role of invertebrate prey in affecting reintroduction success. Sculpin consumed 35 different types of prey in the field, but frequency of occurrence was highest and preferences (Ivlev's selectivity index) strongest for Diptera larvae and Amphipoda. Benthic samples indicated that preferred prey items were abundant in most streams. In 24-h feeding experiments, sculpin typically consumed 5 to 15 prey/day, and exhibited selective feeding for Amphipoda (*Gammarus*), Isopoda (*Asellus*), and Ephemeroptera (*Baetis*) while rejecting Trichoptera (*Brachycentrus*) and Gastropoda (*Physella*). Sculpin are euryphagous and flexible to varying prey availability in different streams, demonstrating both mixed diets and multiple prey preferences that allow fish to maximize their consumption when confronted with differing prey assemblages. Our data indicate that preferred prey taxa are not limiting and that lack of suitable prey is not a factor in the limited success of sculpin reintroductions in some streams.

#### INTRODUCTION

Slimy sculpin *Cottus cognatus* Richardson are being reintroduced to coldwater streams in southeastern Minnesota (MN DNR, 2003). These streams are naturally species-poor (often <5 species of fish per stream; Lyons *et al.*, 1996; Mundahl and Simon, 1998), and many have lacked sculpin since extensive land use changes (conversion to row-crop agriculture and intensive livestock grazing) caused severe stream degradation (burying of coarse substrates by fines, creating wider and shallower channels, stream warming) a century ago (Thorn *et al.*, 1997). Now that stream conditions have improved dramatically because of improved watershed management, channel and instream habitat restorations and protection of riparian buffers (Thorn *et al.*, 1997), sculpin reintroductions are attempting to reestablish a native, ecologically important nongame species (Dineen, 1951; MN DNR, 2003; Adams and Schmetterling, 2007), coinciding with reintroductions of native brook trout *Salvelinus fontinalis* Mitchill and expanding management for wild brown trout *Salmo trutta* Linnaeus (Thorn *et al.*, 1997).

Success of sculpin reintroductions to date has been mixed in the 10 streams where they were stocked beginning in 2003. Stocked sculpin survived and reproduced in all 10 streams, but only in five streams have sculpin populations increased significantly in number (population estimates ranging from 895–3147 fish) beyond initial 150 fish founder populations and expanded away from initial stocking sites (Huff, 2010; Vaughn Snook, MN

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DNR-Lanesboro, unpub. data). Genetic problems with reintroduced sculpin (Huff *et al.*, 2010, 2011), recent 1000-y stream flooding (Keillor, 2010), predation by brown trout (Dineen, 1951) and variations in amount of preferred sculpin habitats (Anderson, 1985; Mundahl *et al.*, 2012) have been speculated as reasons for reduced success of sculpin in some systems. To date, only outbreeding depression resulting from the mixed-source reintroductions (reintroduced sculpin obtained from three separate stream populations in three distinct watersheds) has been implicated in reducing the relative fitness of sculpin offspring at reintroduction sites (Huff *et al.*, 2011).

A growing body of evidence suggests that availability of benthic prey is considerably more important to the success of sculpin populations than are other physical and biotic factors (*e.g.*, Brocksen *et al.*, 1968; Petty and Grossman, 1996; Ruetz *et al.*, 2004; Grossman *et al.*, 2006; Zimmerman and Vondracek, 2006, 2007a, 2007b; Petty and Grossman, 2007, 2010). Sculpin may select habitat patches on the basis of high macroinvertebrate abundance, irrespective of the patches' physical attributes (Petty and Grossman, 1996), competing directly with conspecifics for habitats with the most invertebrate prey (Grossman *et al.*, 2006; Petty and Grossman, 2007, 2010). Low prey densities, especially of larger prey, can result in reduced rates of food consumption and poorer growth in sculpin (Brocksen *et al.*, 1968; Zimmerman and Vondracek, 2006), ultimately influencing individual fitness and population success.

Many invertebrate taxa are preyed on by sculpin in Minnesota streams, with diets often differing among streams and seasons (Dineen, 1951; Zimmerman and Vondracek, 2007a, 2007b). Amphipods, caddisfly larvae, mayfly nymphs, blackfly and midge larvae, and snails typically comprise large proportions of sculpin diets, with mayflies, amphipods, and blackflies preferentially selected by sculpin in one stream (Zimmerman and Vondracek, 2007a, 2007b). Meanwhile, streams in southeastern Minnesota display wide variation in production and diversity of benthic invertebrates (Troelstrup and Perry, 1989; Waters, 2000), with variation frequently correlated with subsurface geology (via effects on nutrients and alkalinity in spring water, water temperature, and flow stability) and riparian land-use (*e.g.*, row-crop agriculture, pasture, urban landscape, forest, protected buffer) practices (Troelstrup and Perry, 1989; Muck and Newman, 1992).

The present study was initiated to examine the possible influence of benthic invertebrate availability (both abundance and type) on sculpin diets and the success of sculpin reintroductions. We assessed benthos availability, sculpin diets and prey selectivity in streams with native and introduced populations of slimy sculpin, as well as benthos availability in streams where sculpin may be reintroduced in the future. We also conducted 24-h feeding experiments in the laboratory to assess selective feeding and maximum rates of food consumption by sculpin at various temperatures for comparison to field-fed fish.

#### METHODS

#### STUDY AREA

Our study area included 16 coldwater streams in the counties of Olmsted, Wabasha, and Winona (Fig. 1). Seven of these streams support healthy populations of slimy sculpin, and three of them (Garvin Brook, Beaver Creek, Cold Spring Brook) have been used as sources of fish for reintroduction to other streams. Five streams presently are lacking sculpin, and four others recently had sculpin reintroduced to them. Slimy sculpin were reintroduced into Latsch Creek and Sugarloaf Creek in 2004 and Big Trout Creek and Little Trout Creek in 2005. The Latsch Creek and Little Trout Creek reintroductions have been termed successful, the Big Trout Creek reintroduction unsuccessful, and the Sugarloaf Creek reintroduction undetermined (V. Snook, MN DNR, pers. comm.).



FIG. 1.—Coldwater streams tributary to the Mississippi River in southeastern Minnesota, USA, where slimy sculpin and benthic macroinvertebrate communities were assessed. Solid dots represent sample sites. 1 – Garvin Brook, 2 – Gilmore Creek, 3 – Cold Spring Brook, 4 – Mid. Br. Whitewater River, 5 – S. Br. Whitewater River, 6 – Beaver Creek, 7 – Trout Run, 8 – Latsch Creek, 9 – Big Trout Creek, 10 – Little Trout Creek, 11 – Sugarloaf Creek, 12 – Snake Creek, 13 – East Indian Creek, 14 – West Burns Valley Creek, 15 – East Burns Valley Creek, 16 – Trout Valley Creek

#### SCULPIN DIETS

Feeding habits of slimy sculpin were examined in fish collected from eight streams in southeastern Minnesota during three time periods: winter 1992–1993 (Gilmore, Garvin), summer 2006 (Gilmore, Garvin, Beaver, Cold Spring, S. Br. Whitewater, Trout Run), and summer 2007 (Gilmore, Garvin, Latsch, Big Trout). We collected approximately 20 fish at each site/date, spanning the complete size range of fish present. We specifically chose to examine the diets of fish from two reintroduction streams in 2007: Latsch Creek (a successful reintroduction) and Big Trout Creek (least successful reintroduction). Fish were collected with a Smith-Root backpack electrofisher, over-anesthetized immediately with MS-222, fixed in 10% formalin for at least 24 h and then preserved in 70% ethanol. No regurgitation of food items was observed with this procedure.

Fish were weighed, measured, and then dissected to remove preserved stomachs. An incision was made down the side of the stomach and stomach contents were flushed into a watch glass with water. Prey items in the stomach contents were viewed, identified, and counted under a dissecting microscope ( $8-40 \times$  magnification). We quantified diet items by frequency of occurrence (presence-absence in individual fish) and as percent composition by number (Bowen, 1996). Ingested foods were then transferred to a pre-weighed plastic dish, dried for 24 h in a drying oven (35 C) and weighed (nearest 0.1 mg) to determine the

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total dry weight of foods (2006 and 2007 samples only). Dry weights of foods were standardized for fish size (mg food dry weight/g fish wet weight) and compared among streams for each year with single-factor analysis of variance. No attempt was made to determine separate dry weights for different prey taxa.

We examined patterns among fish diets in 2006 (six streams, all with native populations of sculpin) using non-metric dimensional scaling (NMS) with the statistical software R (available online at http://www.r-project.org/). All analyses used Bray-Curtis dissimilarity indices to characterize differences in diet composition between individual sculpin. We first examined a scree plot to determine the number of dimensions (using a stress value of 0.2 as the criterion), and then used the "metaMDS" function in R to conduct the NMS analysis. We also used the "ordiellipse" function to determine 95% confidence limits for the diet data originating from each stream. Additionally, we examined whether fish size (total length or wet weight) influenced diet composition, using the "envfit" function in R with 1000 permutations. For each environmental variable (*i.e.*, total length or wet weight) the "envfit" function of points in the NMS ordination.

#### BENTHIC INVERTEBRATE AVAILABILITY

We assessed the availability of sculpin prey during 2006 and 2007 at each of the same eight stream sites where sculpin diets were assessed during those 2 y (six streams with native sculpin populations and two with reintroduced populations). Benthic invertebrates were collected using triplicate Hess or D-frame kick net samples (0.1 m<sup>2</sup>) from erosional habitats with coarse (gravel: 2-64 mm, rubble: 64-250 mm, boulder: >250 mm) substrates (Rabeni, 1996). In addition, similar samples were collected from five streams where sculpin may be introduced in the future (non-sculpin streams), two additional streams where sculpin were recently reintroduced, and one more stream that supported large populations of sculpin. Samples were preserved in 70% ethanol and returned to the lab for analyses. Each invertebrate sample was sorted and specimens were identified (most insects to genus or family, most non-insects to order or class) and counted. Triplicate samples from each site were individually assessed for total invertebrate abundance, taxa richness, EPT (Ephemeroptera-Plecoptera-Trichoptera) taxa richness, and benthic IBI (Wittman and Mundahl, 2003) score and rating. Assessments were averaged to produce single values for each stream site, and comparisons were made among native sculpin streams (n = 7), non-sculpin streams (n = 5) and reintroduction streams (n = 4) with four single-factor ANOVAs (total invertebrate abundance, taxa richness, EPT taxa richness, and benthic IBI as response variables). To assess benthos quality in reintroduction streams in more detail, total invertebrate abundance, taxa richness, EPT taxa richness, and benthic IBI values for individual triplicate benthos samples at each site were compared among reintroduction sites with four, single-factor ANOVAs.

### PREY SELECTIVITY

Using the benthic invertebrate samples and sculpin diet data gathered in 2006 and 2007, we compared prey availability with prey consumption by sculpin in six streams (Gilmore, Garvin, Beaver, Cold Spring, S. Br. Whitewater, Trout Run) with native sculpin populations and two streams (Latsch, Big Trout) with reintroduced populations. Invertebrates were grouped into major taxonomic categories (*e.g.*, insect orders), and proportional abundances of the various categories were compared between benthic samples and sculpin diets (stream by stream) with the Ivlev selectivity index (Krebs, 1989) to determine whether various invertebrates were preferentially selected or rejected by slimy sculpin (Bowen, 1996).

The use of the term "rejected" here only means that prey were consumed in proportions less than they were present in the environment, regardless of what mechanism(s) or prey attribute(s) may have caused that under-consumption.

#### 24-HOUR FEEDING EXPERIMENTS: CONSUMPTION RATES

We assessed food consumption rates of slimy sculpin at different water temperatures with a series of laboratory experiments. Sculpin of all sizes available were collected from Garvin Brook and Gilmore Creek in Jun. and Jul. 2006 and 2007 (water temperatures = 12 to 16 C) with a Smith-Root backpack electrofisher and dip nets. Fish were transported immediately to the lab and acclimated to a constant water temperature of 7 (2007 only), 12, 17, or 22 C for 24 h prior to testing. This was done because these temperatures span the range of summer water temperatures in streams inhabited by sculpin in this region (N. Mundahl, unpub. data), and we wanted to determine if temperature differences may result in different rates of prey consumption. Because of equipment limitations, testing could be done at only one temperature at a time, and new fish were used at each temperature. A refrigerated, recirculating acclimation aquarium (150 L) was aerated and filtered, and water was changed between experiments. Sculpin received natural lighting and a photoperiod of 15 h light : 9 h dark via large laboratory windows. No food was provided to sculpin during the 24-h acclimation period.

Fish were tested individually in rectangular, 1-L translucent plastic testing chambers. Openings (approximately  $75 \times 100$  mm) were cut in two opposite sides of each container and covered with screening (1.4-mm mesh) to allow for water circulation. Each container was closed with a translucent, snap-on lid.

Sculpin were placed into testing chambers along with amphipods Gammarus pseudolimnaeus Bousfiel numbering 10–30 depending on fish size. Amphipods were collected from a vegetated habitat in Burns Valley Creek (a local coldwater stream lacking sculpin) for use as a natural, live food source during feeding experiments. Preliminary diet studies had indicated that sculpin often fed heavily on amphipods when they were available and abundant. Sculpin in individual testing chambers (12–19 per experiment) were placed into the acclimation aquarium at 7, 12, 17, or 22 C and fish were allowed to feed for 24 h. After each experiment, the number of amphipods consumed was determined and wet weight and total length of the fish were measured. Representative samples (10–15 individuals) of amphipods were weighed individually (nearest mg dry weight after 24 h in a drying oven at 35 C) each year to estimate prey biomass consumed by sculpin in the lab. As in field-caught fish, consumption was standardized by fish weight (mg food dry weight/g fish wet weight).

Comparisons of sculpin food consumption were made among fish feeding at different temperatures in the laboratory, and between laboratory-fed and stream-caught fish. Standardized amphipod consumption rates in the laboratory were compared among test temperatures each year with single-factor analysis of variance. Standardized food consumption rates and a 2-factor analysis of variance (lab vs. field, 2006 vs. 2007) were used to compare consumption between lab-fed and stream-caught sculpin.

### 24-HOUR FEEDING EXPERIMENTS: PREY SELECTION

Prey selection experiments were conducted in the laboratory during Oct. 2008 and 2009. Sculpin were electrofished from Garvin Brook and acclimated to laboratory conditions (12 h light : 12 h dark photoperiod, 12 C refrigerated, 400-L recirculating stream tank) for 48–72 h prior to feeding trials. Fish were not fed during acclimation.

Sculpin were tested individually in the 1-L test chambers described above with varying combinations of five different types of prey (amphipods Gammarus pseudolimnaeus, isopods

Asellus sp., caddisflies Brachycentrus occidentalis Banks, snails Physella sp., and mayflies Baetis sp.). Test chambers were submerged in the stream tank and fish were allowed to feed for 24 h. Six different trials were conducted, with each fish offered 10 individual prey of each of two, three, or four different prey types (20–40 individual prey/fish). Amphipods and caddisflies were used in all trials, isopods in five trials, and snails and mayflies in one trial each.

Possible selective feeding was assessed for each trial separately by comparing prey numbers offered versus those consumed with chi-square contingency table tests. Ivlev selectivity values (Krebs, 1989) also were calculated for each prey type in each trial to quantify selected versus rejected prey types.

#### RESULTS

### SCULPIN DIETS

Sculpin collected for diet examination ranged in size from 0.1 to 30.0 g in weight, and from 20 to 116 mm in length (Table 1). Fish average sizes and size ranges differed among sites and years, largely the result of differences in sampling season (average fish larger during winter 1992–1993) and availability and abundance of different age classes of fish [average fish smaller in Jul. 2007 because of presence of many year-of-young (YOY) fish]. All comparisons of sculpin wet weights and total lengths among sites and among years indicated significant (all ANOVA P < 0.05) differences.

The 272 sculpin examined contained 2674 individual prey items, and only 10 (3.7%) fish had empty stomachs. Numbers of prey in stomachs varied widely within and among stream sites and seasons (Table 1). The mean ( $\pm$ sD) number of prey per fish at the stream sites ranged from 4 ( $\pm$ 2) to 31 ( $\pm$ 21), with an overall mean of 10 ( $\pm$ 9) diet items per fish. None of the fish from the reintroduction streams (Latsch, Big Trout Creek) in 2007 had empty stomachs (Table 1), and fish from reintroduction streams contained numbers of prey (mean  $\pm$  sD: 14  $\pm$  16) similar ( $t_{(73)} = 0.01$ , P = 0.99) to fish from native sculpin streams (15  $\pm$  16).

Standardized dry weights of prey contained in sculpin stomachs displayed considerable variation among sites and between the 2 y of sampling, with site means ( $\pm$ sD) ranging from 0.6 ( $\pm$ 6) to 10.1 ( $\pm$ 5.0) mg prey dry weight/g fish wet weight (Fig. 2). Standardized prey weights were significantly different (ANOVA F<sub>(3,50)</sub> = 4.06, P = 0.01) among sites in 2007, but not (ANOVA F<sub>(6,120)</sub> = 1.65, P = 0.14) in 2006. Standardized weights of prey from sculpin collected during afternoon hours were frequently reduced (Fig. 2), but not significantly ( $t_{(125)} = 1.87$ , P = 0.12) less than those from sculpin collected during morning hours during 2006. Similarly, standardized weights of prey were slightly, but not significantly ( $t_{(179)} = 2.42$ , P = 0.07), higher in 2007 than in 2006 (Fig. 2). The standardized weights of prey from sculpin in the two reintroduction streams differed significantly between reintroduction sites but were similar (Big Trout) to those from fish from the native sculpin streams or significantly higher than those at one native site (Latsch > Garvin) (Tukey HSD tests, Fig. 2).

Throughout all the sites and years, there were 35 different types of prey consumed by sculpin (Table 2). Aquatic organisms dominated sculpin diets in all streams, with terrestrial prey items (*e.g.*, Arachnida, Lepidoptera larvae) rarely consumed. Sculpin diets were least diverse in Cold Spring Brook, with only eight different types of organisms consumed, and most diverse in Beaver Creek, with 21 different prey types consumed. The average number of different prey types consumed for all sites was 13. However, most (94%) individual sculpin stomachs examined contained four or fewer different types of prey.

The most common types of prey found in stomachs were fly larvae (Diptera) and Amphipoda (Fig. 3). Fly larvae, especially Chironomidae and Simuliidae, were important TABLE 1.—Mean ( $\pm$ SD) wet weights (g), total lengths (mm), and prey consumption (number per stomach) of slimy sculpin collected from streams in southeastern Minnesota in 1992–1993, 2006, and 2007

Season/Year/Stream	Number of fish	Wet weight (range)	Total length (range)	Number of prey (range)	Number of empty stomachs	Total prey
Winter 1992–1993	171					
Gilmore Creek (Dec.)	18	$8.5 \pm 5.3$ (1.8-30.0)	$67 \pm 14$ (52–116)	$5 \pm 4$ (1-17)	0	87
Garvin Brook (Dec.)	11	$12.1 \pm 5.0$ (0.7–18.5)	$92 \pm 21$ (38–111)	$7 \pm 6$ (0-18)	1	73
Garvin Brook (Mar.)	42	$6.3 \pm 6.3$ (0.5-23.2)	$71 \pm 22$ (39–116)	$10 \pm 13$ (0-69)	- 4	423
Summer 2006						
Garvin Brook (Jun.)	37	$4.6 \pm 3.0$ (1.6-12.9)	$67 \pm 12$ (50–97)	$7 \pm 5$ (0-21)	1	270
Beaver Creek (Jun.)	18	$5.6 \pm 3.7$ (2.6-17.3)	$73 \pm 12$ (60-102)	$11 \pm 15$ (2-62)	0	198
Cold Spring Brook (Jul.)		$5.3 \pm 2.9$ (1.9-9.3)	$73 \pm 13$ (56–90)	$8 \pm 8$ (0-30)	1	148
S.Br. Whitewater (Jul.)		$9.6 \pm 3.9$ (3.6-15.9)	$85 \pm 11$ (66-100)	$4 \pm 2$ (2-10)	0	79
Gilmore Creek (Jul.)	18	$5.1 \pm 4.3$ (1.4-19.4)	$69 \pm 16$ (48-112)	$9 \pm 7$ (1-24)	0	165
Trout Run (Jul.)	18	$5.3 \pm 2.7$ (2.0-10.0)	71 ± 11 (57–88)	$7 \pm 7$ (0-28)	3	123
Summer 2007						
Garvin Brook (Jun.)	7	$8.3 \pm 3.8$ (2.8–12.6)	$77 \pm 13$ (55–90)	$31 \pm 21$ (1-56)	0	219
Gilmore Creek (Jul.)	18	$1.3 \pm 2.0$ (0.2-6.9)	$40 \pm 15$ (27-76)	$8 \pm 7$ (1-25)	0	142
Latsch Creek (Jul.)	26	$1.6 \pm 2.9$ (0.1-8.9)	$36 \pm 23$ (20-85)	$19 \pm 21$ (1-81)	0	480
Big Trout Creek (Jul.)	23	$5.3 \pm 8.4$ (0.7-24.0)	$57 \pm 27$ (38–112)	$10 \pm 5$ (2-23)	0	267

components of the diet in nearly all streams (especially in 2007), whereas amphipods were more variable in their importance. These two taxa had the highest frequency of occurrence of any prey consumed (Table 3). Other organisms widely consumed, both as a percentage of the diet and as frequency of occurrence, included mayflies (Ephemeroptera), caddisflies (Trichoptera), and snails (Gastropoda) (Fig. 3, Table 3). Organisms that were relatively rare in stomach contents included beetles (Coleoptera), horsehair worms (Nematomorpha), segmented worms (Oligochaeta), terrestrial arthropods, and fish. Isopoda seldom comprised a large percentage of the diet (Fig. 3) but were consumed by a significant proportion of sculpin in some streams (Table 3). Sculpin in the two reintroduction streams (Latsch, Big Trout) had diets dominated by Diptera, with fish from Big Trout Creek consuming more Gastopoda, Emphemeroptera, and Trichoptera than fish from Latsch Creek (Fig. 3, Table 3).

NMS revealed significant differences in sculpin diets among the six streams examined in 2006 (Fig. 4). NMS was conducted using three dimensions (based on the scree plot); stress

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FIG. 2.—Standardized weights (mean + sD) of prey removed from stomachs of slimy sculpin captured from streams in southeastern Minnesota during 2006 and 2007. Sculpin sample sizes, lengths and weights and prey numbers are in Table 1. Letters above bars for 2007 are results from Tukey HSD tests; sites sharing a letter are not significantly different from one another

of the final solution was 0.164. The 95% confidence ellipses indicated that diet composition at two streams (Gilmore Creek and Cold Spring Brook) were both different from two other streams (Trout Run and Beaver Creek) and that no other significant differences existed among streams. Diet composition at Gilmore Creek and Cold Spring Brook included more Isopoda, Amphipoda, and Coleoptera, whereas composition at Trout Run included more Oligochaeta and Diptera and at Beaver Creek included more Ephemeroptera and Trichoptera. Fish size (length or weight) had no significant effect on diet composition (both P > 0.2).

#### BENTHIC AVAILABILITY

In benthic invertebrate samples collected from the 16 streams, Amphipoda, Diptera, Trichoptera, and Ephemeroptera were the most common organisms present (Fig. 5). Every stream site contained Amphipoda and one or more types of Diptera larvae. The least common organisms were Plecoptera, Gastropoda, Ostracoda, Oligochaeta, and Nematomorpha. Benthic community composition, although highly variable from stream to stream, was similar among streams with and without sculpin, and those where sculpin were reintroduced (Fig. 5). Benthic composition in sculpin reintroduction streams was highly variable among streams, especially for Amphipoda, Ephemeroptera, and Trichoptera (Fig. 6). In addition, invertebrate community measures (abundance, taxa richness, EPT richness, benthic IBI score) differed significantly among reintroduction streams (Table 4). However, differences in these benthic community parameters did not clearly separate the two successful reintroduction streams from the two unsuccessful streams (Table 4). When

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TABLE 2.—Taxa of prey consumed by slimy sculpin in streams of southeastern Minnesota

Phylum	Class	Order	Family (Genus)
Chordata			· · · · ·
(slimy sculpin, fish	n eggs)		
Mollusca			
	Gastropoda (Physella,		
	snail eggs)		
Nematomorpha			
Annelida			
	Oligochaeta		
	Hirudinea		
Arthropoda			
	Ostracoda		
	Isopoda (Asellus)		
	Amphipoda (Gammarus)		
	Arachnida		
	Acari		
	Insecta	Lanidoptora	
		Plecontera	
		Megaloptera	
		megaloptera	Sialidae (Siglis)
		Diptera	Shahade (Shaha)
		1	Chironomidae
			Simuliidae (Simulium)
			Ceratopogonidae
			Empididae
			Tipulidae ( <i>Tipula, Limnophora</i> Antocha, Hexatoma)
		Ephemeroptera	
			Ephemerellidae (Ephemerella)
			Baetidae (Baetis)
			Leptohyphidae (Tricorythodes)
			Heptageniidae (Stenonema)
		Trichoptera	
			Hydropsychidae (Hydropsyche, Cheumatopsyche)
			Brachycentridae
			(Brachycentrus, Micrasema)
			Limnephilidae (Limnephilus)
			Glossosomatidae (Glossosoma)
		<u> </u>	Helicopsychidae (Helicopsyche)
		Coleoptera	
			Elmidae (Optioservus, Stenelmis)
			Dytiscidae (Agabus)

these same community measures were compared among the reintroduction streams, native sculpin streams, and streams lacking sculpin, no significant differences were observed (Table 5). Among the streams assessed, invertebrate densities ranged from 93 to 668 organisms/ $0.1 \text{ m}^2$ , taxa richness ranged from six to 16, EPT richness from one to seven and benthic IBI score from 8 (very poor) to 52 (good).



FIG. 3.—Diet composition (percent numerical abundance) of slimy sculpin captured from streams in southeastern Minnesota during three time periods

Year/stream	Amphipoda Gammarus	Isopoda Asellus	Gastropoda Physella	Ephemeroptera Baetis	Trichoptera Hydropsyche	Diptera Chironomidae
1992–1993						
Garvin Brook ('92)	27	0	73	9	18	55
Garvin Brook ('93)	12	2	7	29	26	74
Gilmore Creek ('92)	94	61	6	0	22	33
2006						
Garvin Brook (AM)	78	0	6	6	17	72
Garvin Brook (PM)	37	5	5	0	47	84
Beaver Creek	6	0	17	22	17	44
Cold Spring Brook	83	0	11	17	0	61
S. Br. Whitewater River	44	0	22	33	17	67
Gilmore Creek	89	50	6	0	6	39
Trout Run	22	0	17	17	11	78
2007						
Garvin Brook	29	0	0	0	0	86
Gilmore Creek	22	11	0	6	50	72
Latsch Creek	12	30	0	12	23	96
Big Trout Creek	22	35	9	48	61	83
Median	28	1	7	11	18	72
Average	41	14	13	14	23	67

TABLE 3.—Frequency of occurrence (percent of stomachs examined) of major prey taxa of slimy sculpin from streams in southeastern Minnesota, 1992, 1993, 2006, and 2007

#### PREY SELECTIVITY

Slimy sculpin demonstrated diet preferences by selecting for some organisms and rejecting others (Fig. 7). Fly larvae represented the only group in both years that always comprised more than 5% of the diet and/or benthos and was preferred by sculpin in every stream examined. Nematomorpha were always preferred prey, but seldom were a major proportion of the diet or benthos. Amphipoda, Isopoda, Gastropoda, and Ephemeroptera were important taxa that were selected for in some streams and selected against in others. Trichoptera and Coleoptera always were rejected by sculpin, except in one stream in 2006. Sculpin in both reintroduction streams (Latsch, Big Trout) selected for Diptera and against Amphipoda and Trichoptera, but differed in selectivity of Isopoda (Fig. 7).

#### 24-HOUR FEEDING EXPERIMENTS: CONSUMPTION RATES

Slimy sculpin used for the 24-h consumption rate feeding experiments ranged in size from 1.6 to 22.7 g wet weight, and from 24 to 115 mm total length (Table 6). Fish representative of the entire size range were used in feeding trials at each temperature.

Feeding trials conducted in the two years produced generally similar results. In the first trial, sculpin consumed slightly, but not significantly (ANOVA F = 2.97, P = 0.06), more Amphipoda (mg amphipods/g fish) at 17 C than at either 12 C or 22 C (Table 6). In the second trial, consumption rates also did not differ (ANOVA F = 0.11, P = 0.95) among the four temperatures tested, but fish in the second trial consumed significantly (t = 3.44, P < 0.001) more amphipods than did fish in the first trial (trial 1 overall mean = 6.6 mg amphipod dry weight/g sculpin wet weight; trial 2 overall mean = 10.4 mg amphipod dry weight/g sculpin wet weight).



NMDS1

FIG. 4.—Non-metric multidimensional scaling ordination plot of diets of slimy sculpin from six streams in southeastern Minnesota during 2006, based on Bray-Curtis dissimilarity. Only two of the three dimensions used in the ordination are shown for the sake of clarity. Plain letters represent the diets of individual fish, bold letters label the 95% confidence ellipses for the diets of all fish from a single stream and taxa names indicate the ends of scaling vectors originating from the diagram center (0.0, 0.0). B – Beaver Creek, T – Trout Run, S – S. Br. Whitewater River, Ga – Garvin Brook, Gi – Gilmore Creek, C – Cold Spring Brook

Consumption rates were higher for sculpin in laboratory feeding trials than in fieldcaught fish (two-factor ANOVA, lab vs. field F = 29.49, P < 0.001; Fig. 8). Higher rates during 2007 for both lab-fed and field-caught fish (two-factor ANOVA, 2006 vs. 2007 F =25.86, P < 0.001) did not alter this relationship.

#### 24-HOUR FEEDING EXPERIMENTS: PREY SELECTION

Slimy sculpin (n = 72) used for the 24-h prey selection feeding experiments ranged in size from 0.8 to 15.4 g wet weight, and from 40 to 102 mm total length. Both juvenile and adult fish were used in each of the six feeding trials.

Fish consumed 954 prey items during the feeding trials, averaging 13 prey/fish (range six-24 prey/fish). In none of the trials did sculpin consume the different prey types in the same



FIG. 5.—Percent composition (mean + s<sub>D</sub>) of benthic macroinvertebrate communities in 16 coldwater streams in southeastern Minnesota where slimy sculpin are native (n = 7), where sculpin recently have been introduced (n = 4) and where no sculpin occur (n = 5)

proportions that they were offered, instead exhibiting selective feeding behavior (all contingency table P values <<0.001). Amphipoda were favored (selected for) in five of the six trials, Trichoptera were rejected (selected against) in all six trials and Isopoda were favored in four of five trials (Fig. 9). Gastropoda were rejected in the single trial tested, whereas Ephemeroptera were favored in the single trial (also the same trial where Amphipoda and Isopoda were selected against; Fig. 9).

### DISCUSSION

This study highlighted several aspects of slimy sculpin diets, sculpin feeding selectivity, and benthic invertebrate assemblages in coldwater streams in southeastern Minnesota. First, sculpin had flexible diets, consuming a variety of aquatic invertebrate prey, with diets varying significantly among streams. Secondly, sculpin consistently fed selectively on specific taxa in the field and laboratory, typically consuming  $\sim 10$  prey/sculpin/day. Third, potential invertebrate assemblages varied among streams, but all streams contained preferred prey in quantities sufficient to support sculpin populations in densities typical for the region. Taken together, these aspects largely eliminate food resources as a factor contributing to failures of recent sculpin reintroductions in southeastern Minnesota.

Despite the important role of invertebrate prey in habitat patch selection, rates of food consumption and growth, and survival of sculpin (e.g., Brocksen et al., 1968; Petty and

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FIG. 6.—Variation in percent composition of benthic macroinvertebrate communities in four coldwater streams in southeastern Minnesota where slimy sculpin have been introduced. Values represent means of triplicate samples from each stream

Grossman, 1996, 2007, 2010; Ruetz *et al.*, 2004; Grossman *et al.*, 2006; Zimmerman and Vondracek, 2006, 2007a, 2007b), sculpin populations can be successful in streams that vary greatly in their invertebrate communities (Dineen, 1951; Zimmerman and Vondracek, 2007a, 2007b, present study). Slimy sculpin in the present study were euryphagous, consuming an average of 13 different taxa of prey at each stream site. Sculpin in most of the

TABLE 4.—Assessments of benthic invertebrate communities in four streams where sculpin have been reintroduced: two streams where reintroductions are considered successful, two where introductions have been unsuccessful. Values are means, with sp in parentheses. Abundance = number/0.1 m<sup>2</sup>. Within each row, values followed by different letters are significantly different from one another (Tukey HSD tests)

	Successful		Unsuccessful		ANOVA	
Parameter	Little Trout	Latsch	Big Trout	Sugarloaf	F	Р
Abundance	612 (46) A	266 (22) B	294 (97) B	164 (142) B	11.8	< 0.001
Taxa richness	13.3 (2.3) A	12.3 (1.5) A	13.5 (1.8) A	6.7 (2.1) B	25.4	< 0.001
EPT richness	6.0 (0.0) A	4.7 (0.6) A	4.7 (0.5) A	2.1 (1.1) B	27.5	< 0.001
BIBI score	26.7 (2.9) A	31.7 (11.5) A	30.0 (7.1) A	12.5 (6.5) B	14.9	< 0.001
BIBI rating	poor/fair	poor/fair	poor/fair	very poor/poor		

	Stream type			ANOVA	
Parameter	Native	Introduced	Non-sculpin	F	Р
Abundance	344 (151)	284 (165)	311 (237)	0.23	0.80
Taxa richness	11.7 (2.7)	11.2 (3.0)	12.3 (3.9)	0.17	0.84
EPT richness	4.9 (1.4)	4.2(1.5)	4.1 (1.4)	0.70	0.51
BIBI score	28.3 (12.9)	24.3 (8.2)	28.0 (14.6)	0.24	0.79
BIBI rating	poor/fair	poor/fair	poor/fair		

TABLE 5.—Assessments of benthic invertebrate communities in streams with native populations of slimy sculpin (n = 7), streams where sculpin have been reintroduced (n = 4), and non-sculpin streams (n = 5). Values are means, with sp in parentheses. Abundance = number/ $0.1 \text{ m}^2$ 

study streams consumed primarily Diptera larvae and Amphipoda, but Ephemeroptera, Trichoptera, and Gastropoda also contributed significantly to the diet in some streams. Previous studies have reported similar, mixed diets for sculpin in Minnesota (Dineen, 1951; Petrosky and Waters, 1975; Ruetz *et al.*, 2004; Zimmerman and Vondracek, 2007b), Alaska (Hershey, 1985; Cuker *et al.*, 1992), Oklahoma (Tumlison and Cline, 2002), and Lake Ontario (Owens and Dittman, 2003), although various methods for reporting diets (frequency of occurrence, number, biomass) complicate direct comparisons among study streams.

Variation in sculpin diets among nearby streams, or even within the same stream during different seasons or years, was likely the result of differences in prey availability and accessibility among streams (Cuker et al., 1992; Tumlison and Cline, 2002). Sculpin also may exhibit ontogenetic changes in diets (Brandt, 1986; Tabor et al., 2007), at least in part due to habitat shifts (Koczaja et al., 2005; Petty and Grossman, 2010) that result from intraspecific competition between juvenile and adult fish (Petty and Grossman, 2007). Sculpin generally are insensitive to even major changes in prey assemblages, shifting diets to compensate for declines in important prey items (Owens and Dittmann, 2003; Hondorp et al., 2005) with no observable effect on sculpin population parameters (O'Brien et al., 2005). Fish with such mixed, flexible diets are well equipped to cope with changes in prey availability resulting from environmental change (Pratchett et al., 2004), species invasions (Pothoven et al., 2001; Truemper and Lauer, 2005; Pothoven and Nalepa, 2006), or disturbance (Wilson et al., 2006; Emslie et al., 2011), and consequently can thrive in a variety of systems where fishes with more specialized diets may be affected disproportionately (Schoener, 1971; Dill, 1983; Munday, 2004; Berumen and Pratchett, 2008). Because of their diet flexibility, slimy sculpin can be successful in reintroduction streams in southeastern Minnesota even if these streams vary considerably in their invertebrate prey assemblages (Troelstrup and Perry, 1989).

Slimy sculpin selected for certain types of prey while rejecting others in both field and laboratory studies. Selective predation has been documented previously for several species of sculpins under both field and laboratory conditions, with selection for or against prey items generally attributed to prey size, behavior, and/or accessibility (Newman and Waters, 1984; Hershey and Dodson, 1985; Kohler and McPeek, 1989; Tumlison and Cline, 2002; Miyasaka *et al.*, 2003; Fairchild and Holomuzki, 2005; Zimmerman and Vondracek, 2007b). Because of their relatively large gape (Foote and Brown, 1998), slimy sculpin can access successfully all but the largest individuals (*e.g.*, crayfishes, large stoneflies) in benthic invertebrate assemblages and can prey selectively on the larger, more energy-efficient sizes of food organisms (Newman and Waters, 1984; Sparkes, 1996). This suggests that sculpin might select for taxa of larger prey, as long as reduced attack success and longer handling



FIG. 7.—Prey selectivity of slimy sculpin in southeastern Minnesota streams in 2006 and 2007, based on Ivlev's selectivity index

TABLE 6Mean (±sD) wet weights (g), total lengths (mm) and consumption rates (mg amphipod
dry weight/g sculpin wet weight/24 h) of slimy sculpin in 24-h feeding experiments at constant
temperatures. Values in parentheses are ranges. N values are sample sizes

Temperature	· n	Wet weight	Total length	Consumption rate
Trial 1-2006				
12 C	19	$6.1 \pm 5.2$	$76 \pm 18$	$5.4 \pm 5.5$
		(1.6 - 22.7)	(52 - 115)	(0.0 - 17.3)
17 C	12	$6.8 \pm 2.6$	$80 \pm 9$	$9.2 \pm 2.9$
		(3.7 - 10.6)	(24 - 70)	(4.9 - 14.9)
22 C	14	$6.7 \pm 5.5$	$77 \pm 17$	$5.9 \pm 3.8$
		(2.0-20.1)	(57 - 110)	(1.3-12.1)
Trial 2–2007				
7 C	12	$8.2 \pm 6.3$	$79 \pm 17$	$9.8 \pm 4.2$
		(3.0 - 21.2)	(61 - 109)	(4.8 - 17.0)
12 C	6	$7.0 \pm 6.8$	$76 \pm 19$	$10.7 \pm 3.6$
		(2.9 - 20.6)	(61 - 111)	(7.4 - 17.6)
17 C	12	$8.8 \pm 7.1$	$81 \pm 22$	$10.5 \pm 6.7$
		(2.0 - 23.0)	(53 - 118)	(4.4 - 25.5)
22 C	6	$7.3 \pm 6.9$	$75 \pm 23$	$11.3 \pm 6.7$
		(2.1 - 20.6)	(53 - 115)	(5.4 - 24.3)



## Year

FIG. 8.—Prey consumption (mean + sp) by slimy sculpin in 24-h laboratory feeding experiments (amphipod prey only) and field-caught fish (mixed diets) from streams in southeastern Minnesota in 2006 and 2007



FIG. 9.—Prey selectivity of slimy sculpin in six, 24-h laboratory feeding trials, based on Ivlev's selectivity index

times do not negate the energy efficiencies that come with larger size prey (Kratz and Vinyard, 1981). However, slimy sculpin selected for the relatively small Diptera larvae (usually Chironomidae) in every stream examined. Because sculpin often rely on their mechanosensory lateral line to locate and orient to prey in low-light environments (Hoekstra and Janssen, 1985; Kanter and Coombs, 2003), selection by sculpin may be more related to prey movement than to other factors (Kratz and Vinyard, 1981). Consequently, what are generally interpreted as being prey preferences of sculpin based on diet analyses (Bowen, 1996; Begon *et al.*, 2006) may largely be a function of detection and encounter rates of foraging sculpin with moving prey (Kratz and Vinyard, 1981). "Selected" prey are those that are abundant enough to be encountered regularly by feeding sculpin and whose movements produce vibrations of the type that can be detected readily by a sculpin's lateral line system (Kantner and Coombs, 2003). "Rejected" prey are those that do not move (Kratz and Vinyard, 1981; Moore and Williams, 1990) or can otherwise escape detection and capture (Feltmate and Williams, 1989).

Rates of prey consumption (numbers and biomass of prey) by slimy sculpin observed in laboratory and field-caught fish were in agreement with those reported for sculpin in various systems in Minnesota, Alaska, Washington, and other locations in North America (Brocksen *et al.*, 1968; Hershey, 1985; Greenberg and Holtzman, 1987; Foote and Brown, 1998; Moss, 2001; Zimmerman and Vondracek, 2007a, b). Most field-caught and laboratory-fed sculpin averaged  $\sim 10$  prey items per fish, although individual fish may consume >50 smaller prey in a single day (Foote and Brown, 1998; present study). Under normal sculpin densities (<1 fish/m<sup>2</sup>; WI DNR, 1978; Anderson, 1985; Gibson *et al.*, 2004), these rates of prey consumption may produce an observable effect on the density of benthic invertebrates (Brocksen *et al.*, 1968; Cuker *et al.*, 1992; Gibson *et al.*, 2004), although this observation is not universal (Miyasaka *et al.*, 2003; Ruetz *et al.*, 2003; Zimmerman and Vondracek, 2007a, b). The presence of potential predators (*e.g.*, large trout; Chivers *et al.*, 2001) or competitors of sculpin may alter sculpin feeding behavior, lessening their effects on invertebrate assemblages (Freeman and Stouder, 1989; Grossman *et al.*, 1995; Chivers *et al.*, 2001). With benthic invertebrate densities ranging from 1500 to 6000 organisms/m<sup>2</sup>, and with no significant difference in invertebrate densities between streams with and without sculpin, streams in southeastern Minnesota appear to have adequate prey to support populations of slimy sculpin. Even after a 1000-y flood event in Aug. 2007 reduced benthic invertebrate densities in many streams in southeastern Minnesota by >95% (Mundahl and Hunt, 2011), slimy sculpin retained normal feeding rates, maintained or improved condition throughout fall and winter months, and spawned successfully the following spring (N. Mundahl, unpub. data).

In conclusion, slimy sculpin reintroductions into southeastern Minnesota streams have not been compromised by a lack of available suitable prey. Although benthic invertebrate communities varied among streams, all streams contained adequate abundances of prey that were selectively preferred by sculpin, and sculpin were highly flexible to varying prey assemblages. There were no consistent differences in either the diets of sculpin collected from successful and unsuccessful reintroduction streams, or in the prey communities available to sculpin in these streams. These streams also have adequate sculpin habitat (Mundahl et al., 2012) and recent, massive flooding had no observable effects on sculpin populations (N. Mundahl, unpub. data). The genetics of the reintroduced sculpin may be responsible for some reintroduction failures, as heterozygosity is lower than expected and some degree of outbreeding depression may be occurring at some sculpin reintroduction sites (Huff et al., 2010, 2011). Although few brown or brook trout consume sculpin in these small streams (<7% of 292 fish stomachs examined contained sculpin; Dineen, 1951), the possible effects of salmonid predation on reintroduced sculpin should be examined to determine if increasing and expanding brown trout populations may be suppressing sculpin populations and hindering reintroduction efforts.

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