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Predation of Threespine Stickleback by Dragonfly Naiads

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Abstract

Threespine stickleback (*Gasterosteus aculeatus*) populations that have evolved pelvic girdle reduction are most commonly found in lakes with low dissolved ion concentration, a lack of piscivorous fishes, and abundant macroinvertebrate predators. Researchers have speculated that macroinvertebrates have a propensity to consume prey with pelvic spines. If this is true, perhaps macroinvertebrates use the stickleback's spines to facilitate capture and manipulation. This study tested whether dragonfly naiads differentially prey upon stickleback possessing either a complete or reduced pelvis and documented naiad hunting and capturing behavior. Results from an arena experiment suggest that naiads do not prey more heavily upon individuals with one pelvic phenotype over the other. However, results from trials where the naiads were presented with one stickleback with pelvic spines and another without suggest that naiads prey more heavily upon small stickleback with pelvic spines and large stickleback without pelvic spines and that they adjust their predatory behavior based upon the pelvic phenotype of the prey.

Introduction

Stickleback have a bilateral pelvic girdle comprised of four elements: the anterior process, ascending branch, posterior process, and pelvic spine (Nelson 1971; Bell 1988). This structure protects stickleback from vertebrate predators by increasing diameter and resisting compression from the predator's jaws (Hoogland et al. 1957; Hagen & Gilbertson 1972; Moodie 1972; Gross 1978; Reimchen 1983, 1991, 1994; Bell 1988; Lescak & von Hippel 2011). Oceanic (marine and anadromous) threespine stickleback (*Gasterosteus aculeatus*) have robust pelvic girdles containing all four elements on each side. Pelvic girdle reduction (decrease in size or number of elements) has evolved rapidly and independently in many resident freshwater populations found in recently deglaciated regions (Bell 1987, 1988). Populations that have evolved pelvic reduction are typically found in habitats with low dissolved ion concentration, a lack of piscivorous fishes, and abundant macroinvertebrate predators (Giles 1983; Bell 1988). The relative contributions of water chemistry and macroinvertebrate predation to the evolution of pelvic reduction in lakes lacking piscivorous fishes are unknown. The association between pelvic reduction and presence of

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macroinvertebrates has led to speculation about whether possession of a complete pelvic girdle may be disadvantageous in avoiding macroinvertebrate predation.

Reimchen (1980) conducted laboratory experiments to determine effects of stickleback size and pelvic spine length on susceptibility to predation by dragonfly naiads (genus *Aeshna*). Results demonstrated that naiads have a strong tendency to prey upon juvenile stickleback over adults. He suggested that the presence of spines could be disadvantageous to fish because they provide extensions upon which naiads can grasp to facilitate capture and manipulation. However, he found no significant differences in mortality rates between long-spined versus short-spined phenotypes. Marchinko (2009) also conducted predation experiments with juvenile stickleback using several types of macroinvertebrate predators, including dragonfly naiads, and found juveniles with robust pelvic development to be most susceptible to predation.

Morphological analyses have also been used to determine which pelvic phenotypes may be most susceptible to macroinvertebrate predators. Reimchen and Nosil (2002) found that juvenile cohorts tend to exhibit the full complement of spines during winter months when predation by vertebrates predominates, while they have fewer spines, on average, during summer months when macroinvertebrate predators are abundant. Reduction in spine number during summer months was attributed to predation by macroinvertebrates.

Similar experiments have been conducted using brook stickleback (*Culaea inconstans*) and ninespine stickleback (*Pungitius pungitius*). In Reist's (1980) experiment using brook stickleback with and without pelvic girdles, naiads demonstrated significantly higher rates of predation on fish lacking pelvic girdles in one set of trials, but no phenotype-dependent differential consumption in another set of trials. Ziuganov and Zotin (1995) found that macroinvertebrates, including dragonfly naiads, had a propensity to prey upon ninespine stickleback with complete pelvic girdles in an experimental pond.

Overall, experimental results seem inconclusive as to whether macroinvertebrate predators demonstrate differential consumption of stickleback with a particular pelvic phenotype. In addition, Reimchen's (1980) work was the only study to address behavior of naiads. The purposes of this study are to determine whether dragonfly naiads demonstrate differential consumption of threespine stickleback with highly reduced or complete pelvic girdles and to investigate potential differences in capture and manipulation strategies when naiads prey upon individuals with divergent pelvic phenotypes.

Materials and Methods

Study Populations

Stickleback were collected from Wallace Lake (61°34'27.9"N, 149°34'27.3"W), located in the Matanuska-Susitna Valley in south-central Alaska, in April–September, 2009 and May–July, 2010. Fish were trapped in unbaited 0.32 cm and 0.64 cm mesh minnow traps set near shore and left overnight. Fish were brought to the University of Alaska Anchorage, where they were grouped according to pelvic score (PS). Pelvic armor structure was quantified using the following scoring system developed by Bell et al. (1993): 4, all pelvic elements

present; 3, only pelvic spine and fin ray absent; 2, pelvic spine and posterior process absent (anterior process and ascending branch remain); 1, only anterior process present; 0, all elements absent. Each side of the pelvic structure was scored separately and the two scores were added together to obtain the individual's PS (possible scores range from 0 to 8). Stickleback were housed in 379 L covered, outdoor tanks in dechlorinated tap water. Stickleback were fed brine shrimp daily before being used in experiments. The amount of time spent in captivity before use in a predation trial varied from one day to several months.

Dragonfly naiads, *Aeshna* spp., were collected as by-catch in minnow traps set out for stickleback in Wallace Lake and various other lakes and wetlands in the Matanuska-Susitna Valley during the summers of 2009 and 2010. Naiads were housed individually in plastic tanks (17.8 cm × 10.2 cm × 12.7 cm) with lids. The bottom of each tank was lined with gravel to provide traction for movement. Each tank was filled halfway with dechlorinated tap water.

Arena Trials

Nine arena trials were conducted in outdoor pools, from 6 June – 5 August, 2009. Two additional trials were conducted in June, 2010. Each trial took place in a wading pool (140.8 cm in diameter), lined with gravel, filled to a depth of 4–5 cm with dechlorinated tap water (approximately 60 L), and covered with mesh. Each trial contained twenty stickleback of known standard length (SL; anterior tip of upper jaw to posterior end of hypural plate) ranging from 23–57 mm. Ten stickleback per trial had highly reduced pelvic girdles (PS 2) and ten had fully-formed pelvic girdles (PS 8). Six naiads were introduced to each pool and pools were monitored daily. Dead fish that had been preyed upon by naiads were removed. Fish that died of natural causes (evidenced by lack of wounds; three per trial on average) were replaced with fish of the same pelvic score and similar SL. The goal was to terminate trials when ten stickleback had been consumed, but for five trials, the naiads consumed between 11 and 13 fish. Surviving fish were scored for pelvic phenotype and SL.

A chi-square test was used to compare the frequencies of surviving PS 2 versus PS 8 individuals per trial. Number of PS 8 survivors was correlated with trial length using the Spearman-rank correlation (due to small sample size) to determine whether a relationship existed between length of trial and phenotype of survivors. The mean SL of PS 2 versus PS 8 survivors of each trial was compared using Wilcoxon Sign-Rank tests.

Video Trials

Ninety-six video trials were conducted using 32 dragonfly naiads. Fifty-eight of these resulted in successful capture of prey, while the naiad did not capture prey in the remaining 38 trials. Twenty-three naiads had one successful capture of a fish filmed and the other nine had multiple captures filmed (three to four, to look for consistency in prey). Trials took place 9 June – 8 December, 2009 and 14 July – 3 September, 2010. All trials were conducted indoors at room temperature.

Each trial consisted of two stickleback of similar SL, one with a PS of two and one with a PS of eight. The two fish were introduced to a tank (17.8 cm × 10.2 cm × 12.7 cm) filled to

a depth of 4–5 cm with dechlorinated tap water and lined with gravel. Once the fish had acclimated (3–5 minutes, as evidenced by normal swimming behavior), a naiad was introduced. Interactions between the naiad and fish were filmed for one hour using a SONY Digital 8 video recorder set to slow play. After each trial, the length of the naiad (L_N) was measured, and the pelvic scores of the captured and non-captured fish were noted. If no fish was captured after one hour, the trial was terminated and not included in analysis.

Films were analyzed by measuring parameters associated with predation events. We recorded the region of the stickleback's body grasped by the naiad during capture and whether the fish was able to escape post-capture. The latencies (amount of time that lapsed from the beginning of the trial) to strike and capture, the hunting strategy (categorized as sit and wait versus stalk), and whether the naiad used its legs to grapple with the fish as a means of controlling it were also recorded. Capture events were those in which the naiad struck at the fish and was able to hold it in its jaws.

Chi-square tests were performed to compare the number of PS 2 and PS 8 individuals that were preyed upon. Paired t-tests were used to compare the SL of introduced (SL_I) and captured PS 2 and PS 8 individuals. Independent samples t-tests were used to compare latencies to strike and capture and length of time between initial strike and capture for each pelvic phenotype. The difference between L_N and length of captured prey (SL_C) was calculated to determine upper and lower limits of prey size. The difference in L_N and SL_C for PS 2 and PS 8 stickleback were compared using independent samples t-tests to determine whether size of the prey relative to the predator differed significantly due to PS. L_N was regressed against SL_C separately for PS 2 and PS 8 stickleback as well as for both phenotypes combined to determine whether prey size increased with predator size. For naiads that were filmed multiple times, only data from the first trial were used in the above analyses. Additionally, for these naiads, latencies to strike and capture were regressed against trial number to determine if the naiads became more efficient hunters over time (determined by decreased latencies to strike and capture and decreased time between initial strike and capture). All tests were two-tailed.

Results

Arena Trials

In eight out of 11 arena trials, more PS 8 stickleback survived than PS 2 stickleback. However, this result was not significant, likely due to a small sample size ($\chi^2_1 = 3.37$, $P = 0.07$; Fig. 1). Introduced PS 2 and PS 8 stickleback did not significantly differ in SL ($x_{PS\ 2} = 36.1$ mm, $x_{PS\ 8} = 35.0$ mm; paired t-test: $t_{10} = 1.21$; $P = 0.25$). Captured PS 2 and PS 8 individuals also did not significantly differ in SL ($x_{PS\ 2} = 36.1$ mm, $x_{PS\ 8} = 34.4$ mm; paired t-test: $t_{10} = 1.71$; $P = 0.12$). The correlation between trial length and number of PS 8 survivors was significant (Spearman rank correlation: $\rho = 0.69$, $P = 0.018$; Fig. 2). The mean size of the naiads introduced to each trial was not significantly correlated with trial length (Spearman rank correlation: $\rho = 0.40$, $P = 0.226$).

Video Trials

There was no significant difference in the number of PS 2 and PS 8 individuals captured in video trials ($\chi^2_1 = 1.13$, $P = 0.29$). The mean SL of the PS 2 and PS 8 individuals that were introduced to the tanks did not differ significantly (paired t-test, $t_{28} = 0.80$, $P = 0.433$; Table 1). The difference in L_N and the SL of each of the two introduced fish also did not differ significantly (paired t-test: $t_{28} = 0.8$, $P = 0.433$; Table 1).

Captured PS 2 individuals were significantly larger than captured PS 8 individuals ($x_{PS2} = 37.4$ mm, $x_{PS8} = 31.0$ mm; independent samples t-test: $t_{28} = 3.39$, $P = 0.002$; Table 1). Mean difference in L_N and SL_C varied due to pelvic score ($x_{PS2} = -1.9$ mm, $x_{PS8} = 6.8$ mm; independent sample t-test: $t_{27} = -3.37$, $P = 0.002$; Table 1). On average, captured PS 2 individuals were larger than the naiad and captured PS 8 individuals were smaller than the naiad (Table 1, Fig. 3). The overall upper limit of prey size (difference in size between the naiad and largest captured fish) was +10 mm for PS 2 prey and +5 mm for PS 8 prey. The lower limit (difference in size between the naiad and smallest captured fish) was -18 mm (both phenotypes; Table 1). There was no significant relationship between L_N and SL_C for PS 2 fish (Spearman rank correlation: $\rho = 0.322$, $P = 0.193$), PS 8 fish (Spearman rank correlation: $\rho = 0.187$, $P = 0.562$), or PS 2 and PS 8 individuals pooled (Pearson product-moment correlation: $r = -0.063$, $P = 0.739$).

Latencies to strike ($x_{PS2} = 878$ seconds, $x_{PS8} = 509$ seconds; independent samples t-test: $t_{28} = 1.16$, $P = 0.254$) and capture ($x_{PS2} = 1001$ seconds, $x_{PS8} = 613$ seconds; independent samples t-test: $t_{28} = 1.12$, $P = 0.271$) were not significantly different between pelvic phenotypes. Latency between initial strike and capture also did not significantly differ between pelvic phenotypes ($x_{PS2} = 123$ seconds, $x_{PS8} = 98$ seconds; independent samples t-test: $t_{28} = -0.18$, $P = 0.861$).

Differences in other naiad behaviors could not be analyzed statistically due to small sample sizes. However, some behavioral patterns emerged (Table 2). Naiads appeared more likely to stalk and wrap their legs around (grapple with) PS 2 than PS 8 individuals. About half of the naiads that preyed upon PS 8 fish latched onto the pelvis and almost half that preyed upon PS 2 fish latched onto the head. The highest frequencies of escape for both phenotypes occurred when naiads held the fish by the tail.

Nine naiads were observed in multiple trials in which they captured prey (Table 3). Five of these individuals captured stickleback of the same phenotype in every trial. Of these, four captured PS 2 fish in every trial. The latency between strike and capture on the first versus the second trial did not vary significantly ($W = 49$, $P = 0.393$).

Discussion

The purpose of this study was to determine whether dragonfly naiads demonstrate differential consumption of stickleback with reduced or complete pelvic girdles. Results of arena trials suggest a tendency towards predation of PS 2 fish ($P = 0.07$, Fig. 1), contrary to the prediction (Reimchen 1980) that naiads can more easily capture stickleback with pelvic spines. However, the number of PS 8 survivors increased with increasing trial length (Fig.

2), indicating that the trend of predation of PS 2 fish could be an artifact of trial length. Future studies that standardize trial length could test this hypothesis. L_N was not significantly correlated with trial length, which implies that predator size does not factor into length of time required to capture and consume prey.

Results from video trials reveal that naiads preyed more heavily upon PS 2 individuals that were larger than themselves and PS 8 individuals smaller than themselves (Table 1, Fig. 3). This finding supports previous research that juvenile stickleback with complete pelvic girdles are at heightened susceptibility to macroinvertebrate predators (Reimchen & Nosil 2002, Marchinko 2009). Our study shows that predation by naiads continues into the adult size class, especially for PS 2 fish, which were up to 15 mm longer than the naiad. Differential predation on smaller stickleback with pelvic spines and larger stickleback without pelvic spines may have demographic consequences, particularly if the smaller fish with spines are likely to be eaten before they reproduce. This may select for faster maturation in juveniles with complete pelvic girdles, or pelvic reduction, since survival is greater for stickleback with reduced pelvises prior to reproduction.

Reimchen (1980) suggested that stickleback with pelvic spines may be at a disadvantage when faced with macroinvertebrate predators because the spines could be used to facilitate capture and manipulation. If this is the case, then it implies that one of two scenarios is taking place: either (1) macroinvertebrates are able to discern visually which prey have spines and which do not and select accordingly, or (2) that equal numbers of stickleback with and without pelvic spines are captured but stickleback without spines have greater escape success.

Aeschnids are primarily visual predators (Pritchard 1965), but the results from these experiments do not suggest that naiads, on average, are visually selecting stickleback with pelvic spines over those without. Arena results suggest a tendency toward the opposite, with more PS 8 fish surviving than PS 2 fish (Fig. 1). Results from multiple video trials conducted with the same individual naiads suggest that naiads repeatedly prey upon stickleback with the same pelvic phenotype, which would be facilitated by visual identification of PS. If these results are confirmed by additional trials, then it suggests that differential consumption of prey with a particular pelvic phenotype varies among individuals.

Naiads were observed grappling with PS 2 individuals more often than with PS 8 individuals (Table 2), so it appears that the spines are not often used by the legs of the naiad to facilitate control. Conversely, grappling may be more important with PS 2 fish of large size. Stickleback had the greatest likelihood of escape when captured at the tail, but there did not appear to be differential success at escaping due to PS (Table 2). Only six instances of escape by the stickleback were recorded in the video trials, three of each phenotype, out of 58 capture events. Similarly, Reimchen's (1980) work revealed only two escapes out of 80 capture events. As a result, we are limited in our interpretation of capture and manipulation behavior. Vermeij (1982) posited that it is the failed attempts at capture that drive the evolution of prey defenses. Since Odonates appear to be highly efficient predators, an

exceptionally large number of trials would be required to statistically verify differential predation of stickleback with varying pelvic phenotypes.

Naiads may be adjusting their hunting strategies based on prey phenotype, since they appeared more likely to stalk a PS 2 individual than a PS 8 individual (Table 2). Naiads in this study may have preyed more heavily upon large PS 2 stickleback if the pelvic girdle acts as a deterrent to predation, rather than an asset to the predator. The pelvic girdle may deter naiads when it is large enough to be a hindrance and may make the fish appear too large to prey upon.

Susceptibility to predation may also be an effect of prey behavior if stickleback with complete pelvic girdles exhibit different behavioral strategies than those with pelvic reduction. For example, Grand (2000) found that stickleback with complete pelvic girdles from Paxton Lake, British Columbia, were more willing to forage in the vicinity of a fish predator than individuals without pelvic girdles. Future research could employ naiad predators, rather than fish predators, in shy-bold experiments to test this possibility. It was not possible to track the behavior of the fish in the current study because they were not individually marked.

Stickleback with different pelvic phenotypes occupy different foraging microhabitats in Boulton Lake, British Columbia; pelvic-reduced individuals are more benthic in their foraging behavior (Reimchen 1980, 1997; Reimchen & Nosil 2001, 2002). It is currently unknown whether Alaskan populations are similar in this regard, but if they are, then higher attack rates on pelvic-reduced stickleback may be a result of spatial susceptibility. This factor was controlled for in our experiments since water depth in the arena and video trials was shallow (4–5 cm), which confined stickleback to the substrate.

Availability of refuge (Leinonen et al. 2011) and lateral plate number (Moodie & Reimchen 1976; Reimchen 2000) may also influence susceptibility to predation. Refuge was not included as a factor in the present study because we wanted to focus on the role of the pelvic girdle in predation and did not want our view of predator-prey interactions to be obscured by objects in the tank. Lateral plate number was not considered because the Wallace Lake population is uniformly low-plated (Lescak 2010) and lateral plate number did not differ between PS 2 versus PS 8 fish in a previous study (Lescak & von Hippel 2011).

Differential predation by macroinvertebrate predators on various size classes of different pelvic phenotypes may interact with energetic expenses associated with ion availability in lakes (Giles 1983) to result in pelvic girdle reduction. Additional studies with other macroinvertebrate predators (e.g., leeches [*Hirudinea*], predaceous diving beetles [*Coleoptera*]) and growth rate experiments of different pelvic phenotypes in water with low and high ion availability would help to resolve the relative importance of these factors in the evolution of pelvic reduction.

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Fig. 1.

Effect of pelvic phenotype on naiad predation in arena trials. Bars show the number of survivors of each pelvic phenotype. There were 20 fish in each trial, 10 with fully developed pelvic girdles (PS 8) and 10 with highly reduced pelvic girdles (PS 2). Seven to ten fish survived each trial.

Fig. 2.
Relationship between length of arena trial, in days, and the number of PS 8 survivors. N = 11 trials total.

Fig. 3.

Frequency distribution for standard length (SL) of captured pelvic score (PS) 2 and PS 8 individuals in video trials (one fish of each phenotype presented to naiad).

Table 1

Mean SL of introduced (SL_I) and captured (SL_C) stickleback, mean difference in stickleback SL relative to naiad length (L_N), and upper and lower limits of SL for PS 2 and PS 8 individuals relative to L_N. All values are in mm. SL was not measured for two trials. The upper limit is the greatest positive difference in size between the captured stickleback and naiad. The lower limit is the greatest negative difference in size between the captured stickleback and naiad. Numbers in parentheses are sample sizes.

PS	SL _I	SL _C	L _N - SL _I	L _N - SL _C	Upper Limit	Lower Limit
2	35.0 (30)	37.4 (18)	1.3 (30)	-2.1 (18)	10	-18
8	35.9 (30)	31.0 (12)	0.5 (30)	6.8 (12)	5	-18

Table 2

Differences in hunting and capture strategies and frequencies of escape. Hunting strategy, capture location, and escapes could have multiple values per trial if the naiad used both hunting strategies or captured a fish multiple times due to escapes. Behavioral assays were from video trials in which a naiad was given a choice of one PS 2 and one PS 8 prey item.

PS	Hunting strategy		Grappling		Capture location			Escapes		
	Stalk	Sit and wait			Pelvis	Tail	Head	Pelvis	Tail	Head
2	6	10		12	4	5	7	0	3	0
8	2	7		5	5	3	3	0	2	1

Table 3

Pelvic phenotypes (PS 2 or PS 8) of stickleback captured by naiads and difference in length between predator and prey (in mm) for naiads that were observed over multiple trials. $L_N - SL_C$ represents the difference in length between the naiad and captured prey. Fish SL was not recorded for two trials.

Naiad	Trial 1		Trial 2		Trial 3		Trial 4	
	PS	$L_N - SL_C$	PS	$L_N - SL_C$	PS	$L_N - SL_C$	PS	$L_N - SL_C$
1	2	-3	2	UNK	2	1		
2	8	18	8	17	8	5		
3	8	14	2	4	2	-2		
4	2	-1	2	-7	2	-3	2	-15
5	2	-4	2	-7	2	-7	2	-6
6	2	-5	2	-13	8	-10	8	-5
7	8	UNK	2	-11	2	0	8	-1
8	2	-4	2	-6	2	-10	2	-8
9	2	-6	8	-3	2	-6	2	-9