ABSTRACT

Title of Document:	PREDATION BY EASTERN MUDMINNOWS (*UMBRA PYGMAEA*) ON MACROINVERTEBRATES OF TEMPORARY WETLANDS
	Susan Elizabeth Lombardi, Master of Science, 2009
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Fish play a substantial role in aquatic food webs, yet the effect of feeding activities of small stream fish that enter seasonally-flooded temporary wetlands during periods of hydrologic connectivity is not well understood. In this study, eastern mudminnows (*Umbra pygmaea*) were introduced to a fishless wetland in Caroline County, Maryland, and the aquatic macroinvertebrate community did not significantly change within two weeks. Gut contents of mudminnows collected from the wetland and a stream consisted primarily of dipteran larvae; ostracods were also a common food source for wetland mudminnows. Common prey not found in gut contents but present in the wetland were tested as food, and all taxa were consumed in a no-choice predation experiment. Mudminnows have the potential to directly affect multiple trophic levels and subsequent ecosystem functioning through predatory interactions with sustained hydrologic connectivity between fish sources and temporary wetlands.

PREDATION BY EASTERN MUDMINNOWS (*UMBRA PYGMAEA*) ON MACROINVERTEBRATES OF TEMPORARY WETLANDS

by

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Advisory Committee:

Associate Professor William O. Lamp, Chair
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To my mother

Acknowledgements

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Chapter 1: Introduction and Literature Review

Faunal organisms in wetlands make up a complex community in which biotic interactions influence food web dynamics. Studying food webs can shed light on trophic relationships, and the interactions that occur in wetland habitats can shape the community and alter its biodiversity, ecosystem processes, and functions (Mallory et al. 1994; Welborn et al. 1996; Giller et al. 2004). Macroinvertebrates, in particular, are a significant component of the food web of wetlands, as they are involved in nutrient cycling through primary and secondary consumption, and they are a critical food source for other organisms, ranging from fish and waterfowl to other insects (Euliss and Grodhaus 1987; Heck and Crowder 1991; Batzer and Wissinger 1996). Predators outside of the macroinvertebrate community that use them as a food resource could play a subsequent role in ecosystem functioning through keystone predation or related biotic interactions that can cascade across trophic levels (Frank et al. 2005). As macroinvertebrates are bioindicators of ecosystem health, surveys of these communities are useful when assessing the condition of aquatic habitats, and the presence or absence of an intolerant taxon can indicate the state of the ecosystem (Sharma and Rawat 2009). Therefore, macroinvertebrates are an important component of monitoring wetland conditions after a restoration or construction project, and changes to the composition and/or structure of these communities can influence wetland functions and services.

Temporary wetlands are habitats that change physically over a short time period as a result of a brief hydroperiod, in contrast to more permanent bodies of water. As wetlands generally occur where aquatic and terrestrial ecosystems intersect, the formation of this habitat is unique in its physical and biological character, and is typically very productive and diverse (Semlitsch and Bodie 1998; Gleason et al. 2004; Nicolet et al. 2004; Gibbons et al. 2006; Scheffer et al. 2006). Over 400 years ago, there were an estimated 220 million acres of wetlands in what is now the continental United States. Over half of the wetlands have since been lost due to drainage for agriculture and development, combined with sea-level rise (Dudgeon et al. 2006). Efforts to protect, conserve and create new wetlands have become a focus of many government agencies and other organizations in the late 20th and early 21st centuries as the ecosystem functions and services these habitats provide have garnered recognition for their importance in floodwater retention, water quality improvement, and as critical habitat for wildlife (Giller et al. 2004).

Although there are many different types of wetlands, they are all united under a few common characteristics. All wetlands have standing water, though the frequency and duration of flooding is variable (Welborn et al. 1996). Consequently, the hydrology of a wetland is said to be the determining factor for the physical, chemical and biological aspects of the habitat (Mitsch and Gosselink 2000). For example, a longer or shorter hydroperiod will affect the plant community in terms of flood-tolerant or flood-intolerant species dominance, and the saturation of soils affects anaerobic conditions and biogeochemical cycling of nutrients by the bacterial community (Van der Valk 2006). Wetland fauna, such as amphibians, turtles, insects, mammals, birds, and fish are adapted to the physical and chemical factors that shape their particular habitat (Heck and Crowder 1991; Euliss and Grodhaus 1987; Chase 2003; Porej and Hetherington 2005).

Water source also affects the physical and biological makeup of wetlands (Dietz-Brantley et al. 2002). All wetlands receive water from precipitation, but habitat variation occurs when wetlands obtain greater proportion of their water from either groundwater recharge or from inflow or overland flow from more permanent waterways during periods of hydrologic connectivity (Mitsch and Gosselink 2000). Water flowing into the basin of a wetland from the latter source transports nutrients, oxygen, organic matter, sediment, and biota such as fish and aquatic insects. Likewise, water discharging from the wetland into the permanent waterway will carry with it these products, with the abiotic components often transformed by processes that occur within the wetland (Van der Valk 2006). These biotic and abiotic fluxes will more frequently affect wetlands located near lakes and the floodplains of streams and rivers, which frequently receive inflow or overland flow. Geographically isolated wetlands will obtain a majority of their standing water from precipitation and groundwater recharge, while inflow or overland flow happens under more extreme conditions that induce hydrologic connectivity, such as spring thaw or heavy storms (Mitsch and Gosselink 2000).

Attention has focused on to fish populations and their role in structuring wetland macroinvertebrate communities that are closely associated with streams and lakes (Mitsch and Gosselink 2000). In contrast, fish in isolated wetlands have received less attention, as wetlands that become dry cannot support persistent fish populations, and are less likely to receive an influx of fish from inflow or overland flow (Schneider and Frost 1996; Figuerola and Green 2002; Humphries and Baldwin 2003; Langston and Kent 2007). Fish have been shown to play an important role in aquatic community structure by affecting certain aspects of the food web (Baxter et al. 2004), and permanent wetlands without fish tend to have greater insect diversity and biomass in comparison to fish-bearing wetlands (Hanson et al. 1995; Batzer and Wissinger 1996; Batzer et al. 2000; Zimmer et al. 2001; Hornung and Foote 2006; Dorn 2008). Generally, fish presence is an important factor in aquatic ecosystem dynamics.

Predaceous fish seasonally entering isolated wetlands as a result of hydrologic connectivity could have short-term and long-term consequences on their prey community (Batzer and Wissinger 1996; Langston and Kent 1997; Pierce and Hinrichs 1997; Snodgrass et al. 1996). This scenario occurred at a wetland restoration site in Maryland, USA. In 2003, The Nature Conservancy, U.S. Fish & Wildlife Service, Maryland Department of the Environment, and the Natural Resource Conservation Service partnered to create and restore approximately 30 seasonal depressional wetlands on a 300 acre site that was previously used for agriculture. Wetland restoration started in 2003 and included the plugging of drainage ditches and construction of earthen ditch plugs. Coarse-woody debris (e.g. tree stumps and logs) was placed in the wetlands to provide habitat heterogeneity. As the site was previously utilized as farmland, a series of agricultural ditches ran through the Restoration Site to drain water off of the fields. There are still some ditches on the property that did not become plugged during the wetland creation process, and they connected the normally isolated wetlands to nearby streams when hydrologic connectivity was present. Beginning in 2005, the wetlands were monitored for physical, chemical and biological characteristics to assess the success of the restoration, and fish were found to be present in many wetlands. Two species of fish were identified, the eastern mudminnow (*Umbra pygmaea* DeKay 1842), and the chain pickerel (*Esox niger* Lesueur 1818), and although densities were not assessed, the eastern mudminnow was more frequently observed. It was believed the fish were accessing the wetlands by swimming from their native streams into the drainage ditches, then continuing into the wetlands through that network if hydrologic connectivity was present. Assuming fish were unable to migrate back to the ditches or streams when the connection between the two still existed, they perished when the wetlands dried down completely. The presence of the fish in the wetland when standing water was present led to the question of what effect the fish have on the temporary wetland community.

In streams, eastern mudminnows are predators of macroinvertebrates, where they will bury themselves in the muddy substrate and feed on those organisms they can subdue (Panek 1981). This behavior could be related to foraging activity, predator escape, or some combination of the two. Typically 50 to 100 mm in length at reproductive age (Fig. 1.1), they live in the wild up to 4 years and have a geographic range that extends along the Atlantic coast (Rohde et al. 1994). In Maryland, they are the third most abundant stream fish, behind the blacknose dace (*Rhinichthys atratulus* Hermann 1804) and the creek chub (*Semotilus atromaculatus* Mitchill 1818) (Roth et al. 2001). In streams closest to the Jackson Lane Restoration Site, the eastern mudminnow was regularly the most abundant fish sampled, reaching upwards of 1,000 individuals within a 50-m stream reach (Roth et al. 2001). They are identified in the field by a dark vertical stripe near their rounded caudal fin, and brown to olive coloration and slight horizontal banding (Rohde et al. 1994). They are capable of surviving in anoxic conditions, partially through the ability to breathe atmospheric oxygen. This ability, combined with the preference for a benthic substrate, make wetlands a suitable secondary habitat for these fish as these two conditions are characteristic of temporary wetlands when standing water recedes (Panek 1981; Rohde et al. 1994; Cucherousset et al. 2007).

While the fish inhabit the wetland, the extent to which fish presence impacts the macroinvertebrate community and the wetland ecosystem is unknown. In the wetlands, the dietary breadth of the eastern mudminnow is unknown, as is whether or not they actively move around the water column or prefer to occupy the muddy substrate as they do in streams. Their foraging behavior would affect the type of prey they encounter and their diet choices, as most macroinvertebrates can be characterized by different "habits" or locomotive styles, as summarized in Table 1.1 (Merritt et al. 2008). These attributes affect how the organisms are interacting with each other and their environment, including other predators. Macroinvertebrates can also be classified by their trophic position in the food web, as herbivores, predators, detritivores, or omnivores (Sih et al. 1985). As a consequence of dietary preference based upon macroinvertebrate location, abundance, or other unrecognized factors, the eastern mudminnow may affect one trophic level more than another and as a result change food web structure and energy flow.

The overall goal of this study was to determine the dietary breadth of the eastern mudminnow and its potential impact in temporary wetland ecosystems. I hypothesized that the eastern mudminnow was consuming common wetland macroinvertebrates at the Jackson Lane Restoration Site, causing measureable changes to wetland macroinvertebrate communities as a result of predator/prey interactions. Through this study, I wanted to know what trophic relationships existed between the fish and macroinvertebrate taxa in temporary wetlands, if fish feeding preferences were similar between stream and wetland habitats, and if fish presence could affect wetland restoration efforts with their impacts on prey communities. To achieve this, I analyzed gut contents of eastern mudminnows collected from a wetland habitat and a stream habitat, I tested the potential of common wetland macroinvertebrate taxa as food for eastern mudminnows, and I experimentally introduced a population into a temporary wetland and compared changes in the macroinvertebrate community over time to a fishless wetland.

Table 1.1. Summary of primary habits and locomotive styles of macroinvertebrates. Adapted from Merritt, et al. (2008).

Classification	**Habit/locomotive style**	**Example representative in wetland habitat**
Burrowers	Burrow into fine sediment	Chironomidae: *Chironomini* (dipteran larvae)
Climbers	Move along stems of aquatic plants	Haliplidae: *Peltodytes* (beetle larvae)
Planktonic	Suspended in open water	Culicidae: *Culex* (dipteran larvae)
Skaters	Move along surface water	Veliidae: *Microvelia* (true bugs)
Sprawlers	Crawl along settled debris	Libellulidae: *Libellula* (dragonfly larvae)
Swimmers	Move actively in water column	Notonectidae *Notonecta* (backswimmers)

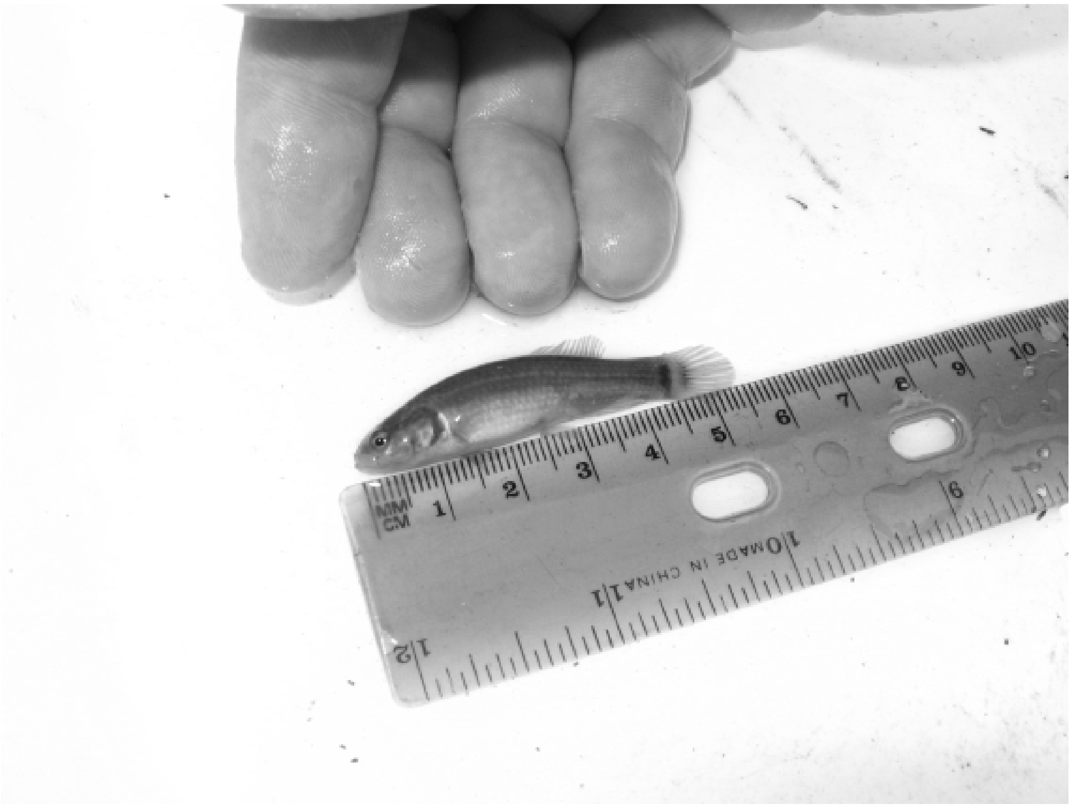


Figure 1.1. Photo of the eastern mudminnow (*Umbra pygmaea*).

Chapter 2: Methods

2.1: Field Site

Research was conducted during July and August 2008 at The Nature Conservancy’s Jackson Lane Restoration Site, located in the Choptank River watershed in Caroline County, Maryland (39°03’11.9’’N, 75°44’50.2’’W). The several dozen created wetlands on the site are seasonal-depressional freshwater marshes. A drought in 2007 resulted in no standing water in the wetlands from late June to December of that year (Lamp, unpub. data). As a result, wetlands in 2008 were fishless, probably due to severed hydrologic connectivity with the habitats and usual fish sources. When sampled with D-nets and a fish electroshocker, the fishless status was confirmed when no fish were found in the ditches and wetlands where fish had been found previously. Two wetland communities on the Restoration Site were chosen for study (reference site: (39°03’02.09”N, 75°44’47.17”W; test site: 39°03’05.04”N, 75°44’47.17”W) and a third was used for general macroinvertebrate collection for experiments. Water chemistry (measured with YSI probes) and physical measurements of standing water were taken for the reference and test wetlands at the start (8-July) and end (23-July) of the fish introduction experiment. I was given permission by The Nature Conservancy to introduce eastern mudminnows to the test wetland for purposes of this study.

2.2: Specimen Collection

All eastern mudminnows were collected with a fish electroshocker, borrowed from the Montgomery County Department of Environmental Protection, MD. The source population for experiments came from a tributary stream of Forge Branch in Caroline

County, MD (Maryland Biological Stream Survey Site UPCK-101-R) between 9-July and 6-August 2008. A scientific fish collection permit was obtained from the Maryland Department of Natural Resources (permit number SCP200886). The protocol for humane fish treatment was approved by the University of Maryland's Institute for Animal Care and Use Committee (IACUC) in July 2007 (R-07-54).

2.3: Fish Introduction and Macroinvertebrate Community Sampling

I used a BACI (before/after-control/impact) design to test how the addition of eastern mudminnows affected the food web of a wetland macroinvertebrate community over a two week time period between 8-July and 23-July 2008. I introduced fish to a fishless wetland and observed changes to the macroinvertebrate community over time in comparison to a macroinvertebrate community in a wetland that had remained fishless over the same time period. I recaptured 30 fish from the test wetland after at the two week mark, and examined their gut contents to compare them to the available macroinvertebrate community that I sampled concurrently. This allowed me to determine what the fish were feeding on in relation to what was available.

Twenty macroinvertebrate samples were collected each from the reference and test wetland both the day before and two weeks after eastern mudminnows were introduced into the test wetland. Samples were allocated by habitat composition, e.g. a wetland that I approximated to be 50% "open water," 30% "shallow edge," and 20% "course-woody debris" were designated to have 10, 6, and 4 samples, respectively, taken from each habitat type throughout the wetland. I took samples by using a 500-micron D-net to make two passes in the chosen area. The first pass disturbed the bottom of the microhabitat with three consecutive jabs. For the second pass I quickly returned to my initial position and I moved the net through the water, ending the pass by pulling the net up through and completely out of the water column. The sample collected in the net was immediately placed in a 500-micron sieve and drained, then transferred to a plastic container with 100% ethyl alcohol to kill and preserve organisms, and tightly screwed shut. The depth and microhabitat type from which the sample was taken were recorded. All the samples taken from each wetland were not combined and considered independent for the purposes of this study. In the lab, the samples were initially transferred to 80% ethyl alcohol. During for processing, the sample was placed on two stacked sieves, a 500-micron sieve placed under a 4.00 mm sieve. I picked up any debris that did not pass through the 4.00 mm sieve and placed it a white tray and sorted for macroinvertebrates with the naked eye. Materials that passed through the 4.00 mm sieve and were retained above the 500-micron sieve were examined under a microscope. All macroinvertebrates were removed from each sample with forceps and placed into vials containing 80% ethyl alcohol. Aquatic insects in the samples were identified to genera using Merritt, et al. (2008). Mollusks, annelids and roundworms were sorted but not counted and identified in this study. Microcrustaceans if found were also picked out of the sample and preserved, but most are believed to fall through the screen of a 500-micron sieve.

Macroinvertebrates from each sample were identified, counted, and categorized by trophic position and primary habit/locomotive style (Merritt et al. 2008). (Note: Primary locomotive style was determined by using the first habit/locomotive style listed next to each genera in the Merritt et al. reference.) Abundance of trophic positions per sample and locomotive style per sample were compared between the two habitats with a two-way analysis of variance for each variable, with time as a repeated measure. I looked for a significant interaction between treatment and time, which would indicate an effect of fish presence on a particular parameter.

After I sampled the test and reference wetlands for macroinvertebrates on 8-July, I collected the maximum number of eastern mudminnows as possible from the stream site on 9-July, and brought them to the Jackson Lane Restoration Site in a bucket. In total, I collected 254 fish to be introduced to the test wetland. To get an estimate of variation within the population, I measured all fish lengthwise (end of snout to the tip of caudal fin) before I released them altogether into the test wetland. The starting density of fish in the test wetland was approximately 7 fish per m^2, with an average fish length of 45 mm^2 ± 8 mm (Figure 2.1).

2.4: Gut Content Examination

I recaptured 30 of the 254 fish from the test wetland with a fish electroshocker two weeks after the fish were introduced so that I would have wetland mudminnows for gut content examination. In addition, 30 eastern mudminnows collected from the Forge Branch tributary stream on 9-July were examined for gut contents. All fish that were collected for gut content examination were euthanized with fish anesthetic tricaine methanesulfonate (MS-222), placed into individual plastic bags, and frozen for storage. For dissection, the fish were thawed and patted dry with a paper towel, and length measurements were recorded. In the laboratory, the digestive tract was removed with scissors. Under a microscope, the stomach was located, and contents were removed with forceps and preserved in 80% ethyl alcohol. Prey items were identified and counted using whole body remains or head capsules. Fish euthanasia and dissection methods were adapted from Gelwick and Matthews (2006).A *t*-test compared the average number of items consumed by fish from the wetland to those from the stream. Frequency of prey occurrence for wetland fish were calculated.

2.5: No-Choice Predation Experiment

In a completely randomized 4x2 factorial design, with four different prey types tested at each of two levels (fish present and fish absent), I ran a no-choice predation experiment, lasting 24 hours, at the Jackson Lane Restoration Site under a shade cover in early August 2008 (Figure 2.2). Each test was run in a 2-L opaque plastic container filled with water that I had collected from a wetland on the Restoration Site and had filtered with a 500-micron sieve, and each container included a 7-cm plastic plant. Fish-present treatments each contained one eastern mudminnow (starved for 24 hours) and five live prey items collected from a Jackson Lane wetland in one of the following families: backswimmers (family Notonectidae), dragonfly larvae (family Libellulidae), mayfly larvae (families Baetidae and Caenidae), or mosquito larvae (family Culicidae). Each prey item was regarded objectively to be an appropriate size for consumption by an eastern mudminnow; any items thought to potentially exceed the gape of the fish were not used in the experiment. Identical treatments with prey items but missing fish were set up as controls. Each container was covered with mesh fabric to avoid prey escape and to exclude outside interference. Each treatment combination had five replicates, so 40 containers total were used, with 20 containing a live fish. After 24 hours, prey items were recovered from each container. I removed the fish (if present) from the container with a small aquarium net and euthanized it with MS-222. I poured the remaining water into a 500-micron sieve, and examined the screen for surviving prey items. The lid, mesh-covering, and plastic plant were also thoroughly inspected for prey items.

Survivorship of taxa between fish-present and fish-absent treatments within each prey type were compared with one-tailed *t*-tests (alpha = 0.05).

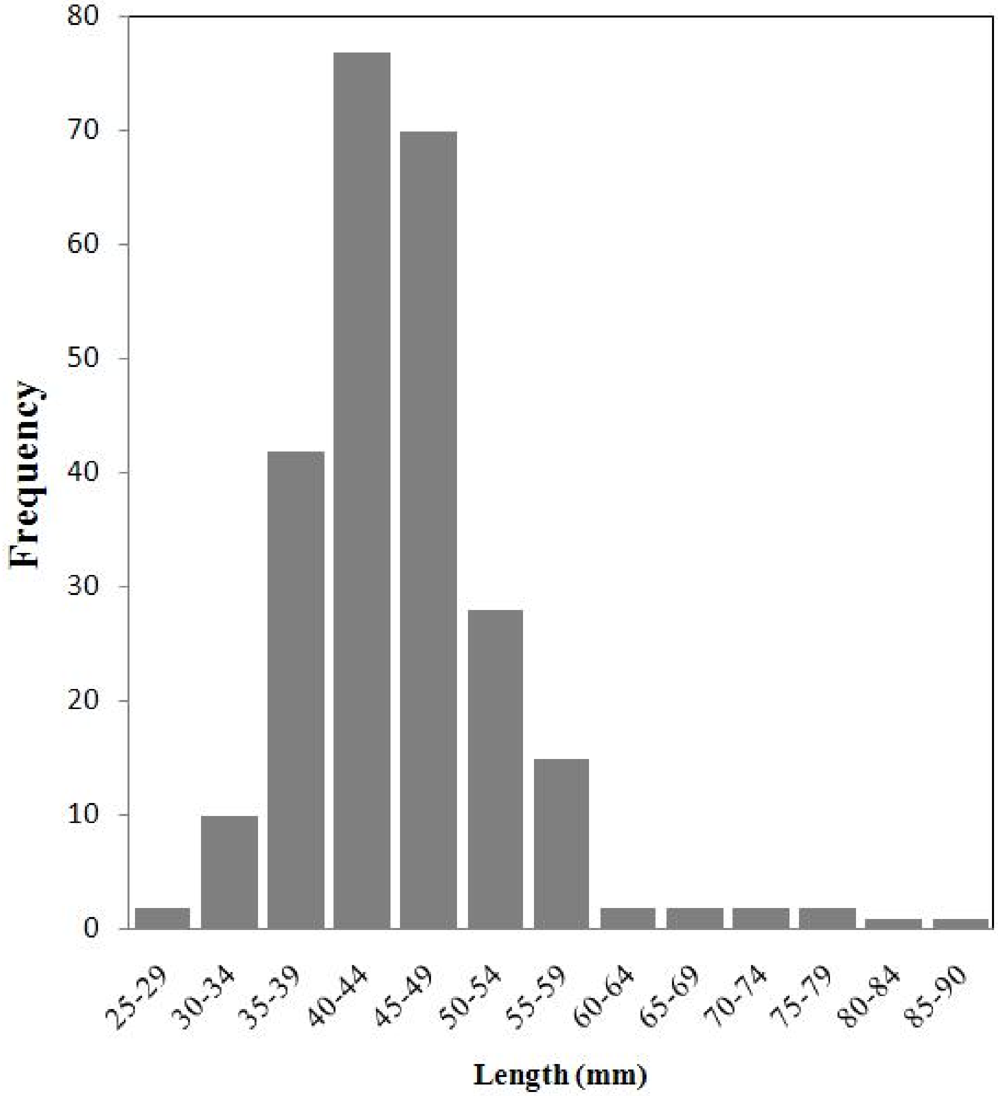


Figure 2.1. Frequency distribution by length of all eastern mudminnows introduced to the test wetland (n = 254).

Figure 2.2. Photo of the experimental set-up of the no-choice predation experiment under a shade cover the Jackson Lane restoration. Photo by Alan Leslie.

Chapter 3: Results

3.1: Fish Introduction and Macroinvertebrate Community Changes

There was no significant effect of fish on the macroinvertebrate community after the two-week introduction with respect to trophic position abundances. An increase in the average number of predators, detritivores, and herbivore/detritivores per sample over time occurred in both the test and reference wetland (Figs. 3.1, 3.2, 3.3). There were also significant differences with respect to the average number of predators and herbivores between the test wetland and the reference wetland, but not related to the fish addition; the reference wetland had higher averages in this regard from the beginning to the end of the study (Figs. 3.1 and 3.4). No significant time*treatment interactions, to indicate a change due to the fish treatment, were found regarding trophic positions. The omnivore class did not experience any change between over time or between treatments (Fig. 3.5).

Similar non-significant time*treatment interactions were found for most of the key habit/locomotive style groups. Average number of burrowers per sample increased over time, but no difference between treatments was found (Fig. 3.6). The number of climbers was significantly lower in the treatment wetland throughout the study, but no time effect or time*treatment interaction occurred (Fig. 3.7). The average number of planktonic macroinvertebrates per sample were different between the test and reference wetland throughout the study, and fish presence did not have an effect (Fig. 3.8). The average number of sprawlers per sample decreased in the reference wetland and increased in the test wetland after two weeks (Fig. 3.9). This is attributed to the change in abundance of chironomid larvae classified as sprawlers (subfamily Tanypodinae). For macroinvertebrate swimmers, differences existed between the two wetlands throughout the study, and there was an overall increase in the average number of swimmers per sample over time for both treatments. There was not a significant time*treatment interaction (Fig. 3.10; see Appendix for complete taxa list).

Habitat compositions with respect to percentage of open water, shallow-edge, and course-woody debris microhabitats of the reference and test wetlands before (8-July) and after (23-July) the fish introduction event were very similar, but there were some differences between test and reference wetlands in water chemistry, and initial and final areas measured with standing water. Notably, the pH of the water was the same for the wetlands on 8-July, but the pH was lower in the test wetland and higher in the reference wetland on 23-July. There were also fluxes in dissolved oxygen, but both wetlands experienced an increase with time. The area covered with standing water decreased considerably between the start and end of the experiment in both wetlands (Table 3.1).

3.2: Gut Content Examination

Of the 30 eastern mudminnows collected from the test wetland, 28 fish had prey items in their stomachs. Ostracod microcrustaceans were the most abundant prey item, contributing to 244 of the total 416 items recovered, and were found in 80% of the fish (Table 3.2). Dipteran larvae (family Chironomidae) were the second most abundant group at 31.6%, and shared the same frequency of occurrence in fish stomachs as the ostracods (Table 3.2). Thirty-five adult beetles were found (Coleoptera: families Dytiscidae, Hydrophilidae, and Noteridae), but one eastern mudminnow had eaten 30 of the beetles, while only three other fish had recently consumed the beetles (Table 3.2).

Mollusks showed the same frequency of occurrence in fish stomachs as beetles, but the total abundance of mollusks was much lower at four found in total.

Of the 30 eastern mudminnows collected from the stream site, 27 had at least one prey item in their stomachs. The mean number of prey items was significantly lower in the stream fish compared to the wetland fish (*t* Stat = 3.48, df = 58, alpha = 0.05; Table 3.2). The most common prey items in the stream mudminnows were dipteran larvae (family Chironomidae), which made up 86.4% of the 147 items recovered from the 30 fish samples. Present in the stomachs of mudminnows from the stream but absent in the fish in the wetland were trichopterans, hydracarinids, and isopods. In contrast, beetles were found only in the guts of mudminnows collected from the wetland. Prey items eaten by fish in both habitats were ostracods, dipteran larvae, and mollusks. Unlike the wetland, macroinvertebrate samples were not taken from the stream, so it is unknown how the stream prey items compared to the macroinvertebrate community in the stream habitat as a whole.

3.3: No-Choice Predation Experiment

There was a significant loss of prey in all fish-present treatments, presumably due to consumption (Fig. 3.11). On average, the mudminnows consumed 55-100% of the taxa tested when given no alternative choice, while survivorship of prey items in the controls was essentially 100% in all treatments and replicates (except for the loss of one backswimmer). In the fish-present trials, 24% of notonectids, 56% of dragonfly larvae, 15% of mayfly larvae, and 0% of mosquito larvae were recovered. For each prey type, recovery rates of prey items in fish-absent treatments were significantly higher than in fish-present treatments ($P<0.05$).

3.4: Comparison of Gut Contents and No-Choice Predation Experiment Results to Available Prey Community in the Test Wetland

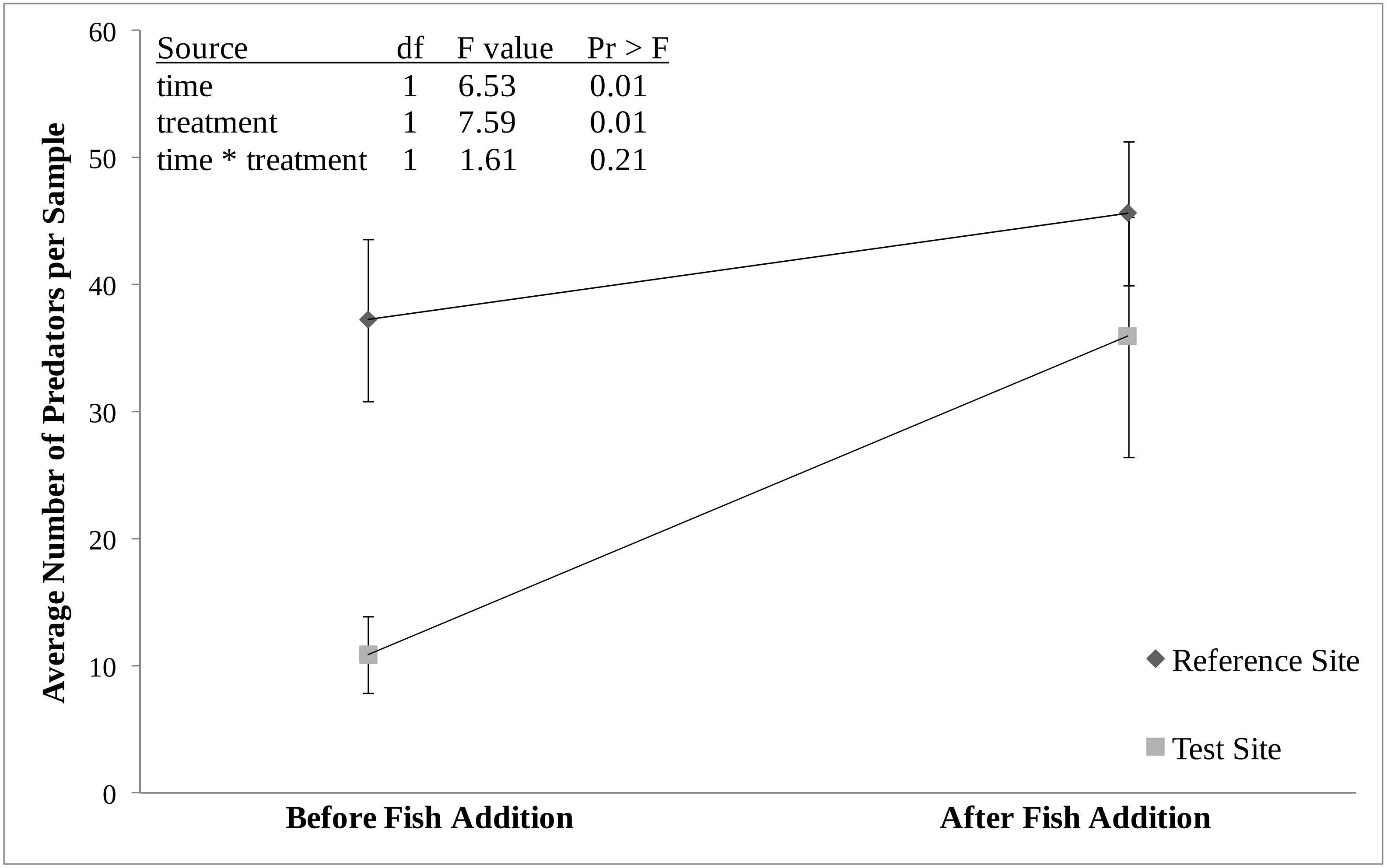
The no-choice predation experiment indicated that mudminnows could consume notonectids (backswimmers), libellulids (dragonfly larvae), baetids and caenids (mayfly larvae), and culicids (mosquito larvae), but no remains of these individuals, or other closely related taxa, were found in the stomach contents of the fish collected from the test wetland or the stream. All of the tested prey items from the no-choice predation experiment were present in the wetland community at the time the fish were collected for gut content examination. Notably, however, the dipteran larvae that were abundant in the gut contents of the wetland fish were also the most abundant aquatic insect in the wetland at the time. Chironomid larvae were ubiquitous in the samples compared to all other aquatic insect taxa. Of the 1,236 aquatic insects collected from the test wetland after two weeks with fish, chironomids made up 67% of the community. Other prey items were found at considerably lower frequencies (see Appendix).

Table 3.1. Water chemistry and habitat composition of reference and test wetlands, before (8-July) and after (23-July) fish introduction. Abbreviations: D.O. = dissolved oxygen; SE = shallow-edge, CWD = course-woody debris, and OW = open water. Water chemistry was taken at maximum depth and at mid-day.

Date	**Site**	**Area with Standing Water (m^2)**	**Maximum Depth (cm)**	**Temperature (°C)**	**pH**	**D.O. (mg/L)**	**D.O. (%)**	**Microhabitat (%)**
8-July	Reference	209	47	29.4	6.26	4.21	44.3	SE: 40
								CWD: 20
								OW: 20
8-July	Test	38	60	25.9	6.26	5.25	64.6	SE: 30
								CWD: 30
								OW: 40
23-July	Reference	121	20	30.2	7.12	17.9	185.5	SE: 35
								CWD: 25
								OW: 40
23-July	Test	27	35	25.8	5.84	7.51	92.8	SE: 30
								CWD: 30
								OW: 40

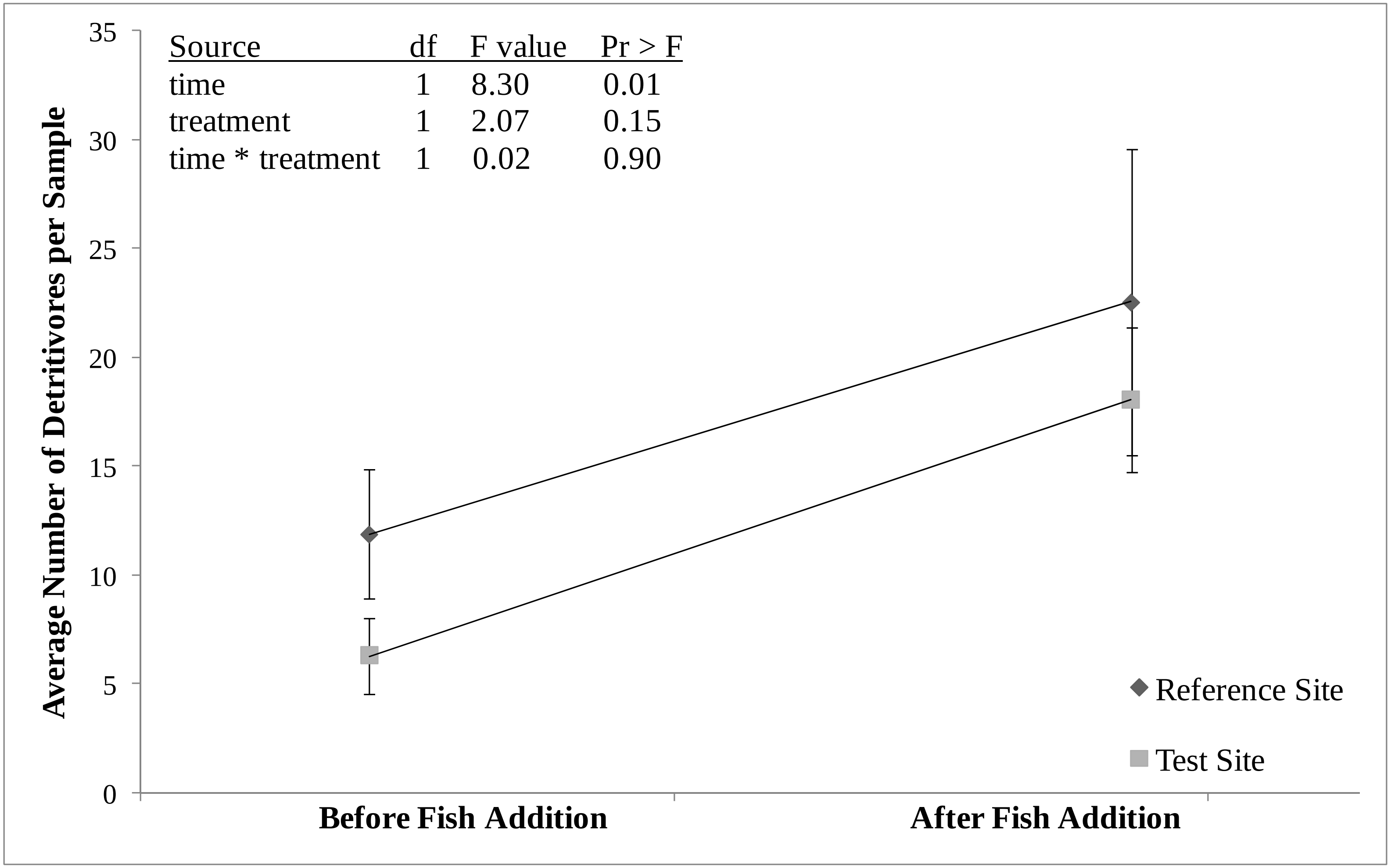
Table 3.2. Summary of gut contents of mudminnows collected from the stream site and the wetland site. Labels as follows: A = average number per fish ± standard error; B = percentage of total prey items; C = percentage of fish with prey items. Note: N/A means there were no taxon of this type found in gut contents.

Taxon	**Stream Habitat**			**Wetland Habitat**		
	A	**B**	**C**	**A**	**B**	**C**
Coleoptera	0	0	0	1.2 ± 1.0	8.5	13.3
Diptera: Chironomidae	4.5 ± 0.9	87.2	80.0	4.4 ± 0.7	31.6	80.0
Hydracarinidae	0.1 ± <0.0	1.3	0.1	N/A	0.0	0.0
Isopoda	<0.0 ± <0.0	0.6	<0.0	N/A	0.0	0.0
Mollusca	0.2 ± 0.1	3.2	0.1	0.1 ± 0.1	1.0	13.3
Ostracoda	<0.0 ± <0.0	0.6	<0.0	8.1 ± 3.3	58.9	80.0
Trichoptera: Hydropsychidae	0.4 ± 0.1	7.1	30.0	N/A	0.0	0.0



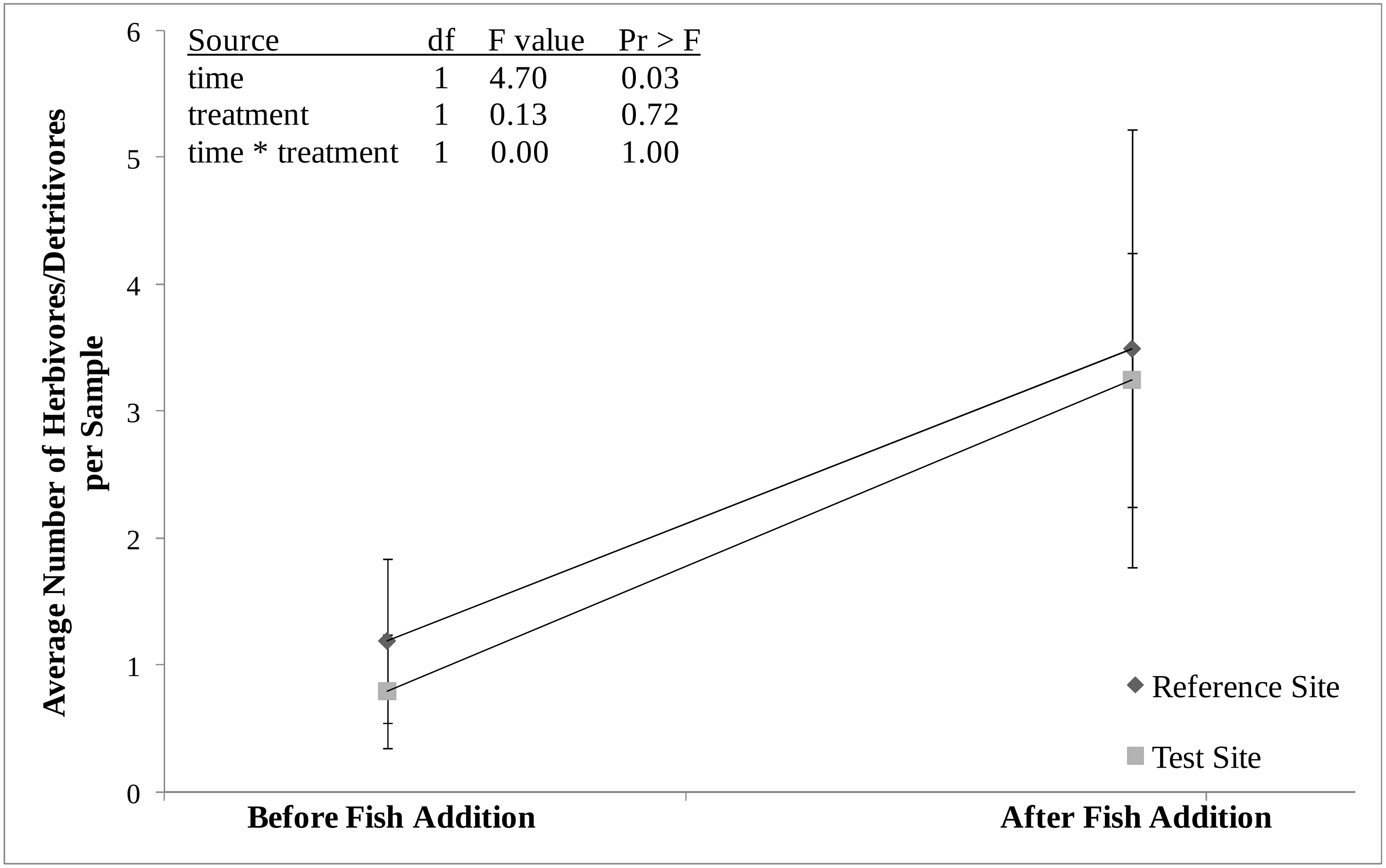
Source	df	F value	Pr > F
time	1	6.53	0.01
treatment	1	7.59	0.01
time * treatment	1	1.61	0.21

Figure 3.1. Average number of macroinvertebrate predators in the reference and test wetland samples (n=20) before and after fish introduction, and two-way analysis of variance table with time as a repeated measure. Standard error is shown.



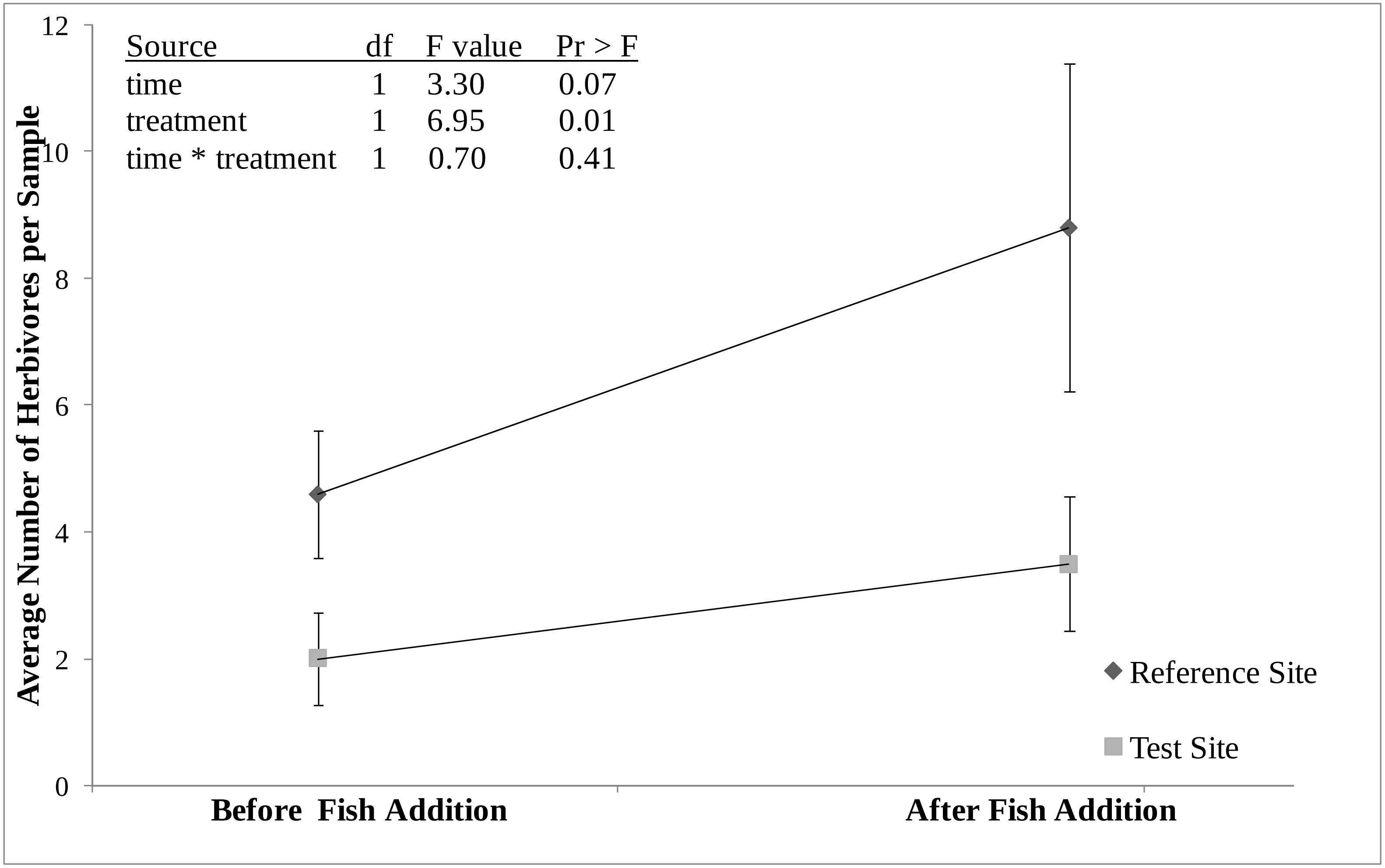
Source	df	F value	Pr > F
time	1	8.30	0.01
treatment	1	2.07	0.15
time * treatment	1	0.02	0.90

Figure 3.2. Average number of macroinvertebrate detritivores in the reference and test wetland samples (n=20) before and after fish introduction, and two-way analysis of variance table with time as a repeated measure. Standard error is shown.



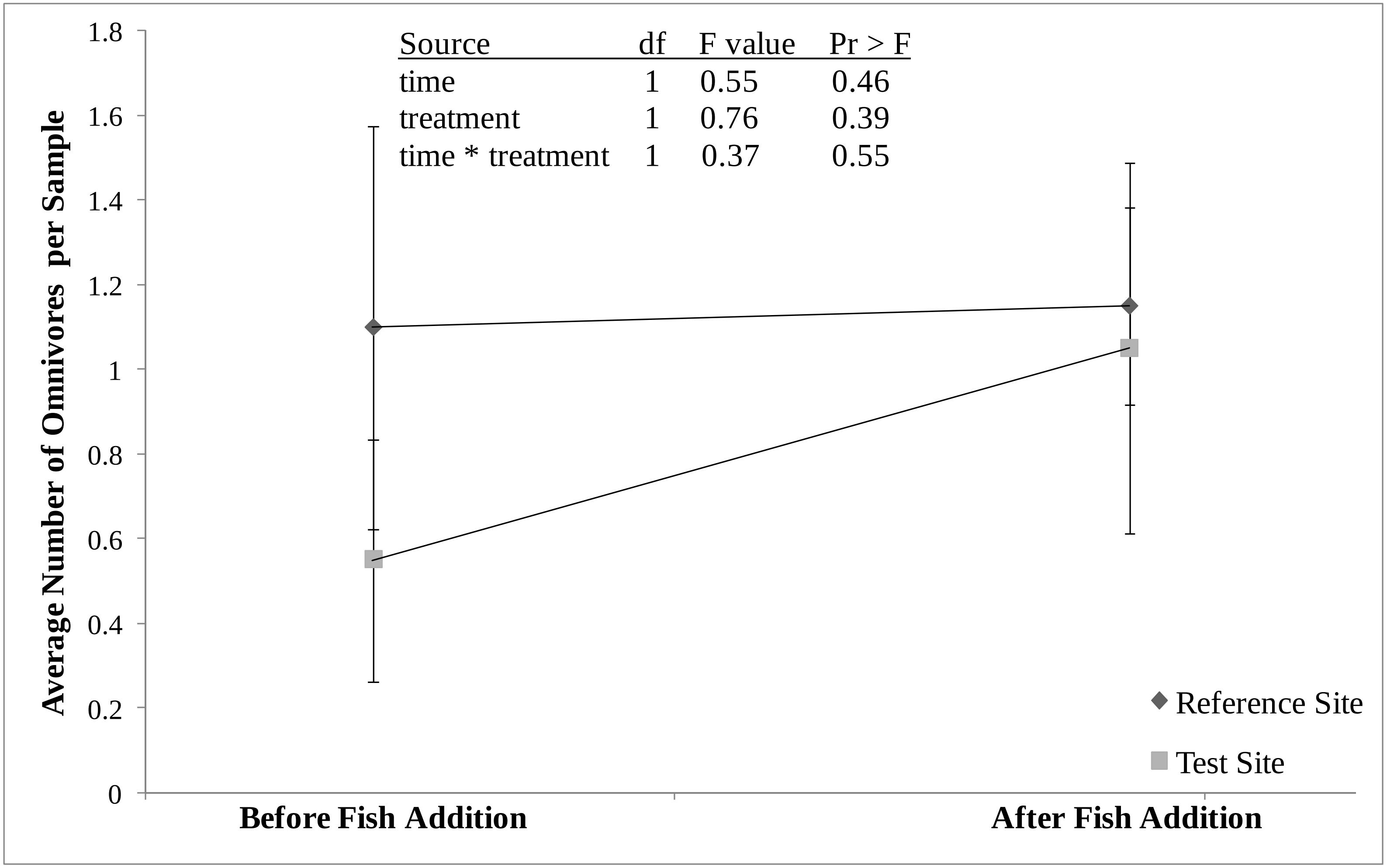
Source	df	F value	Pr > F
time	1	4.70	0.03
treatment	1	0.13	0.72
time * treatment	1	0.00	1.00

Figure 3.3. Average number of macroinvertebrate herbivore/detritivores in the reference and test wetland samples (n=20) before and after fish introduction, and two-way analysis of variance table with time as a repeated measure. Standard error is shown.



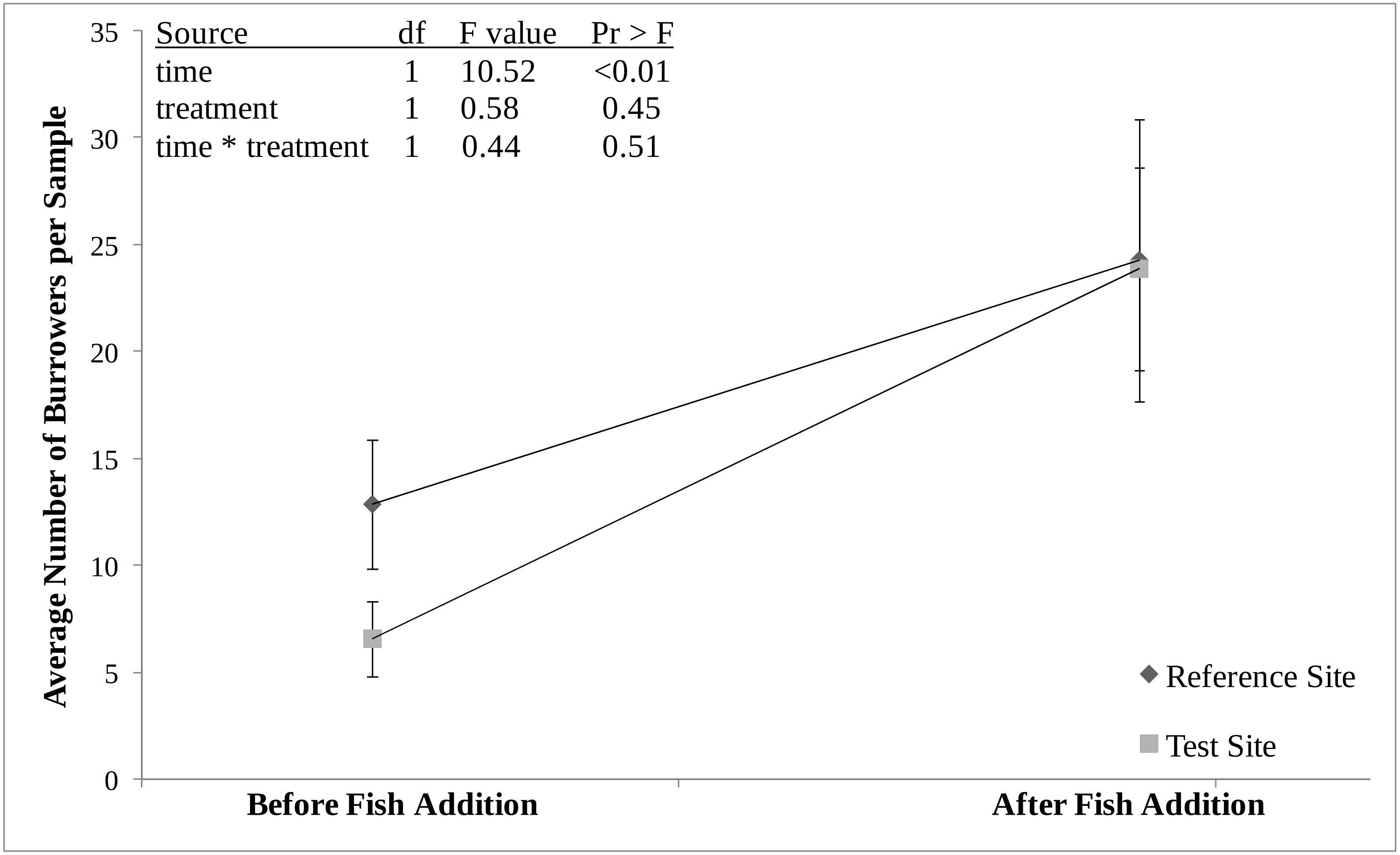
Source	df	F value	Pr > F
time	1	3.30	0.07
treatment	1	6.95	0.01
time * treatment	1	0.70	0.41

Figure 3.4. Average number of macroinvertebrate herbivores in the reference and test wetland samples (n=20) before and after fish introduction, and two-way analysis of variance table with time as a repeated measure. Standard error is shown.



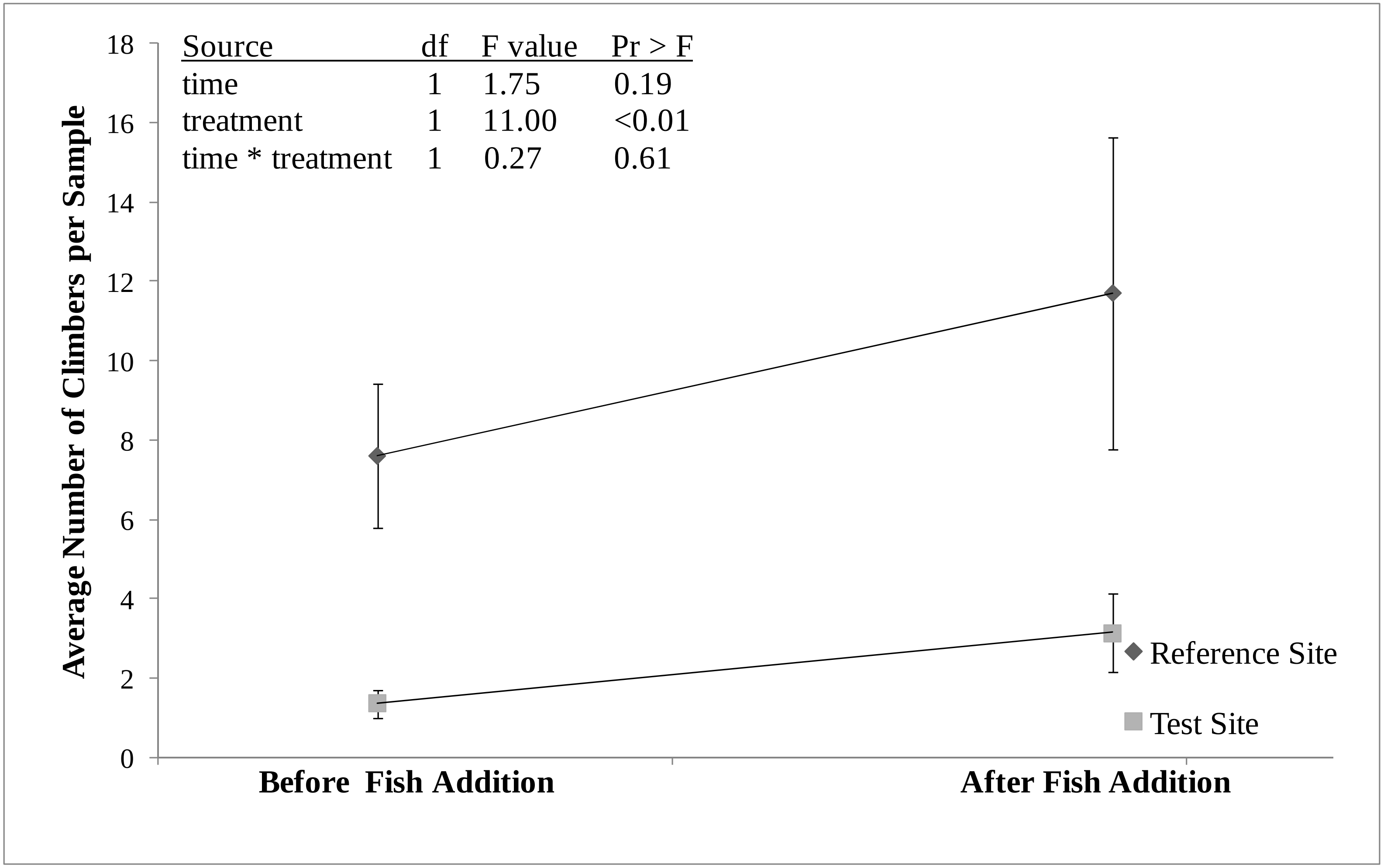
Source	df	F value	Pr > F
time	1	0.55	0.46
treatment	1	0.76	0.39
time * treatment	1	0.37	0.55

Figure 3.5. Average number of macroinvertebrate omnivores in the reference and test wetland samples (n=20) before and after fish introduction, and two-way analysis of variance table with time as a repeated measure. Standard error is shown.



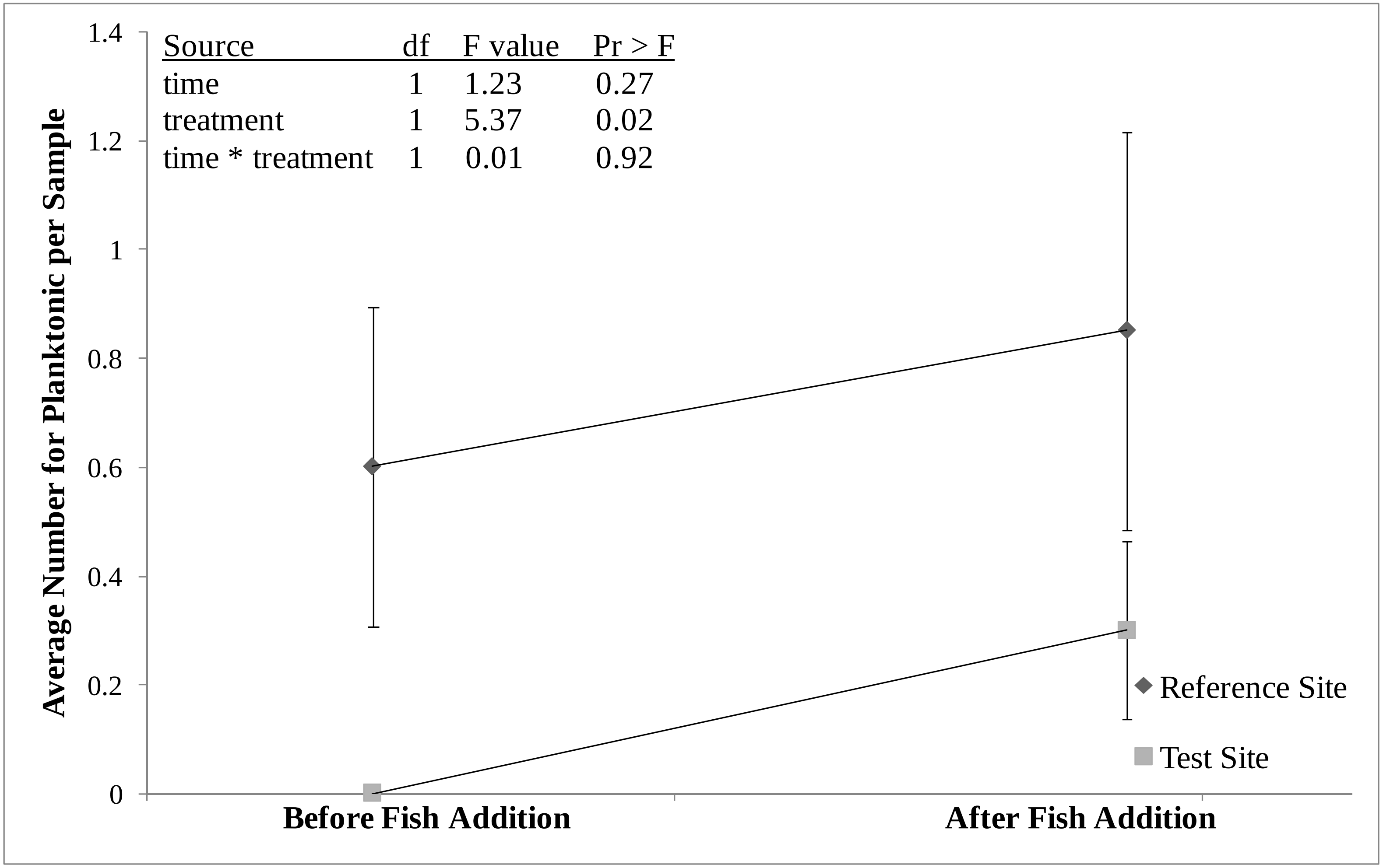
Source	df	F value	Pr > F
time	1	10.52	<0.01
treatment	1	0.58	0.45
time * treatment	1	0.44	0.51

Figure 3.6. Average number of macroinvertebrate burrowers in the reference and test wetland samples (n=20) before and after fish introduction, and two-way analysis of variance table with time as a repeated measure. Standard error is shown.



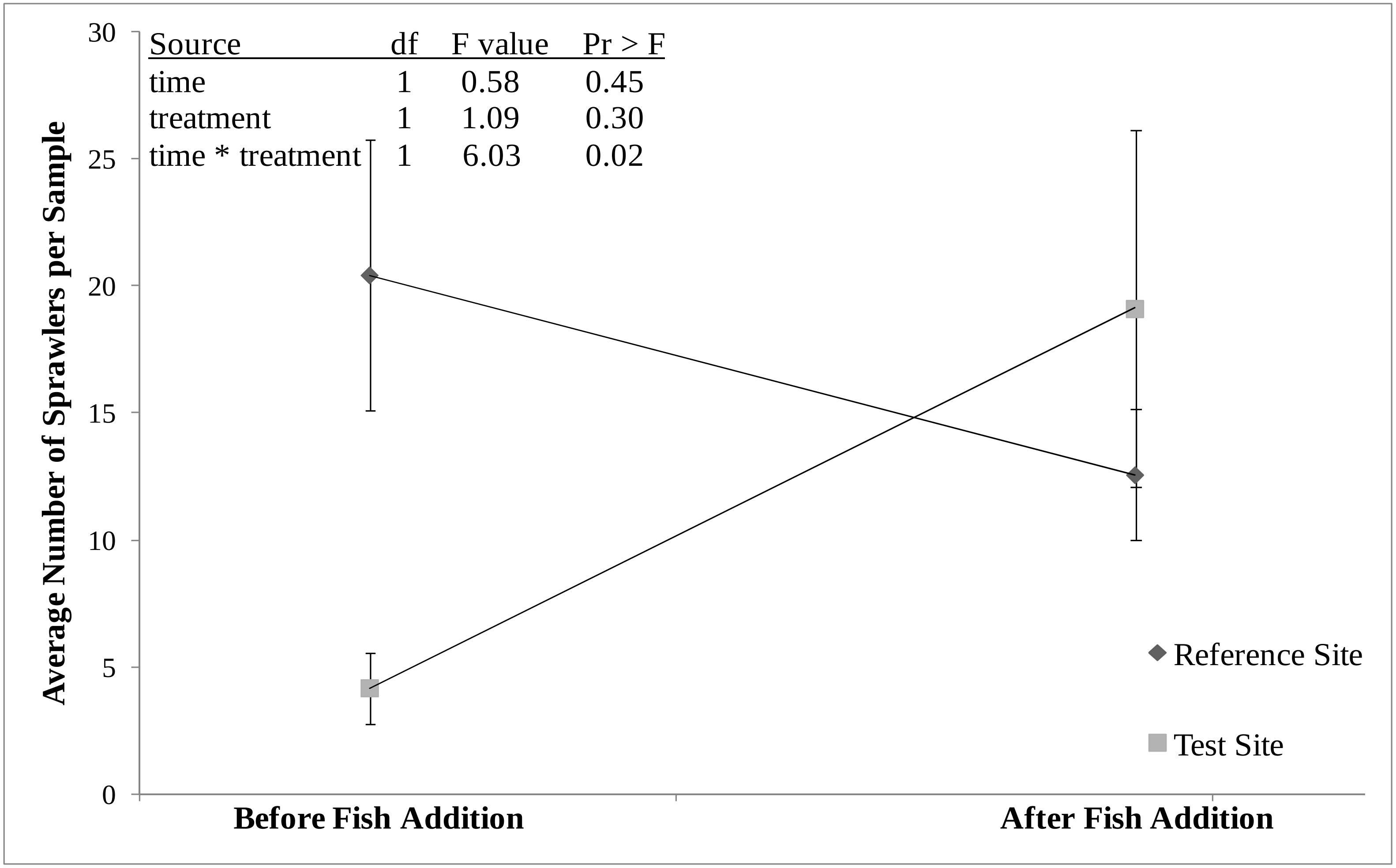
Source	df	F value	Pr > F
time	1	1.75	0.19
treatment	1	11.00	<0.01
time * treatment	1	0.27	0.61

Figure 3.7. Average number of macroinvertebrate climbers in the reference and test wetland samples (n=20) before and after fish introduction, and two-way analysis of variance table with time as a repeated measure. Standard error is shown.



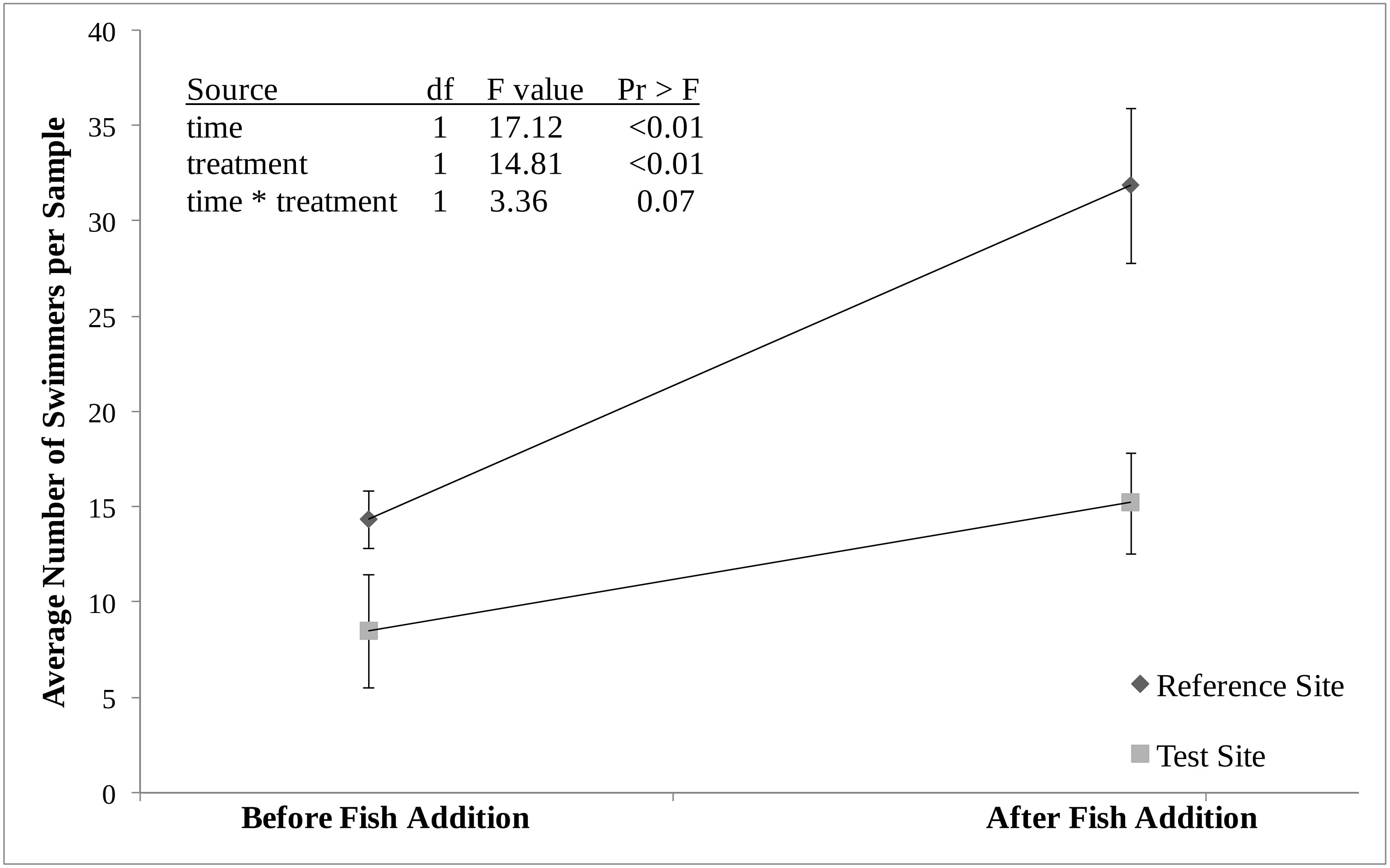
Source	df	F value	Pr > F
time	1	1.23	0.27
treatment	1	5.37	0.02
time * treatment	1	0.01	0.92

Figure 3.8. Average number of planktonic macroinvertebrate in the reference and test wetland samples (n=20) before and after fish introduction, and two-way analysis of variance table with time as a repeated measure. Standard error is shown.



Source	df	F value	Pr > F
time	1	0.58	0.45
treatment	1	1.09	0.30
time * treatment	1	6.03	0.02

Figure 3.9. Average number of macroinvertebrate sprawlers in the reference and test wetland samples (n=20) before and after fish introduction, and two-way analysis of variance table with time as a repeated measure. Standard error is shown.



Source	df	F value	Pr > F
time	1	17.12	<0.01
treatment	1	14.81	<0.01
time * treatment	1	3.36	0.07

Figure 3.10. Average number of macroinvertebrate swimmers in the reference and test wetland samples (n=20) before and after fish introduction, and two-way analysis of variance table with time as a repeated measure. Standard error is shown

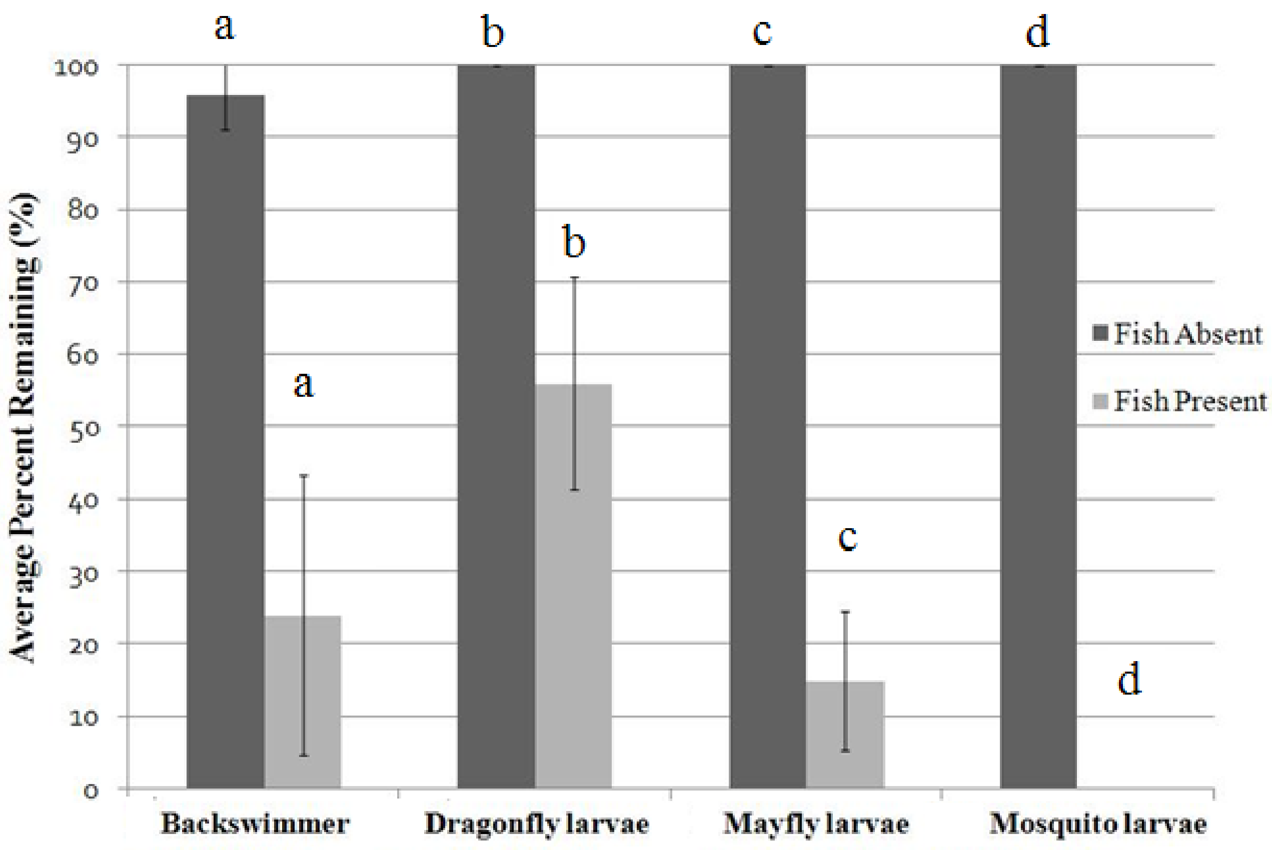


Figure 3.11. Survivorship of prey items in fish-present and fish-absent treatments after 24 hours. Standard error is shown. Paired letters indicate significant differences (*P*<0.01).

Chapter 4: Discussion and Conclusions

It is well established that fish affect macroinvertebrate communities in permanent waters (Hanson et al. 1995; Batzer and Wissinger 1996; Hornung and Foote 2006). The question of whether or not fish might also be affecting macroinvertebrate communities in temporary wetland habitats arose after eastern mudminnows were observed to be abundant in many of the seasonal depressional wetlands at the Jackson Lane Restoration Site in Caroline County, MD. Mudminnows were apparently periodically colonizing created ponds at this site by moving from nearby streams through agricultural drainage ditches during periods of hydrologic connectivity. The dietary breadth or feeding preference of the predaceous fish in wetlands was not known at the beginning of this study.

Panek (1981) showed that mudminnows are predators of macroinvertebrates in streams, and the gut content examination of fish collected from a stream in this study confirmed this. By combining results of the gut content examinations and the no-choice predation experiment, this study provided evidence of the mudminnow's ability to consume dipteran larvae (including mosquitoes), beetles, snails, ostracod microcrustaceans, isopods, dragonfly larvae, backswimmers, mayfly larvae, and water mites. (Note that this list does not include any taxa that might have been digested too rapidly to be discovered in the gut contents of the fish). When mudminnows were removed from their natural habitat and were tested with one prey type, they showed their potential as generalist feeders, able to prey on multiple different types of aquatic organisms that live in seasonal wetlands. They were able to eat macroinvertebrates that swim (mayflies and backswimmers), sprawl (mayflies and dragonfly larvae), climb (the dragonfly larvae) along with those that are planktonic (mosquito larvae). The presence of these fish may also have significant ecological consequences on macroinvertebrate community trophic structure and function, as they consumed both primary consumers (mayfly larvae and mosquito larvae) and secondary consumers (backswimmers and dragonfly larvae) (Merritt et al. 2008).

Comparing the feeding activities between the stream mudminnows and wetland mudminnows showed that the fish ate similar prey when it was available in both habitats, like dipteran larvae and snails. Of interest was the significantly higher number of prey items that were found in the wetland fish compared to the stream fish. Temporary wetlands can contain high abundances of insects (Nicolet et al. 2004), so this may play a role in making temporary wetlands attractive to mudminnows. Further studies would be needed to determine whether or not macroinvertebrate abundances differ in streams/ditches and adjacent seasonal wetlands at the time of peak fish movement (usually early spring, when hydrologic connectivity is highest, due to water level rise from snow melt and low rates of evapotranspiration).

A surprising result of this study was that there were no major effects by fish on the aquatic insect community in wetlands in the field, at least at the tested density and over the time scale used in the experiment. Changes were seen in community composition between both fishless and fish-bearing wetlands over time as the wetlands decreased in size, making it difficult to discern patterns. Previous studies involving community comparisons in wetlands with and without fish often show significant negative effects on the biodiversity and abundance of macroinvertebrates (Zimmer et al.

2001; Dorn 2008). Those studies were done over a longer period of time, with community sampling spanning multiple ponds and multiple months. Since I looked at only short-term changes, it is not known if similar effects would have taken place if the wetlands had been monitored over an extended duration. However, the temporary wetlands typically become dry during the year, so long-term studies would usually be restricted by this hydroperiod. The physical changes that occurred to the wetlands over time could have contributed to general pattern of an increase in macroinvertebrates of different classifications over time; the area of standing water had decreased, so the macroinvertebrates might have increased in density, not total abundance. It is unknown if sampling in temporary wetlands as water level recedes over time has a confounding effect on biomonitoring.

The high frequency of occurrence of microcrustaceans and dipteran larvae in the wetland mudminnow diet could be due to: 1) specific preferences for those types of prey items; 2) relative prey abundance, or; 3) selection of foraging microhabitat. Disparity in diet based upon fish size was not a factor, since all fish examined were similar in size, and could presumably manage to consume the same types of prey. No changes to any particular habit/locomotive style besides the sprawlers after two weeks of fish presence may indicate that mobility of macroinvertebrates is not related to consumption by eastern mudminnows; the ease with which the fish were able to consume prey with differing locomotive styles based upon results from the no-choice predation experiment confirmed this. It is possible that backswimmers and dragonfly larvae were only consumed in the no-choice predation experiment because the fish had no alternative prey, and that they would generally not co-occur in the same microhabitat within the wetland on a frequent basis. It is unknown if the fish might also prefer to consume higher numbers of lower-quality items than lower numbers of higher-quality items, as this question was raised by the high frequency and abundance of microcrustaceans in the gut contents of the mudminnows.

My results suggest that management decisions involving wetland restoration projects should consider potential of fish colonization as an important factor in the design and construction process. As the Jackson Lane Restoration Site matures, the wetlands may continue to alternate between fishless and fish-present states, depending upon precipitation and hydrological patterns, and when the fish are present, they may affect the macroinvertebrate food web through predation. During long periods when fish are absent, the macroinvertebrate community may be characterized by a different structure; macroinvertebrate predators may act differently without the threat of intraguild predation by fish, and the primary consumers (herbivores and detritivores) that were more directly affected by fish predation could experience population growth (Gilinsky 1984).

Though not studied here, fish presence in created wetlands could even be important for supporting higher trophic levels, such as migratory birds, resident/breeding birds, small mammals, and reptiles that frequent temporary wetlands and depend upon fish as a food source (Erikkson 1985; Ford and Lancaster 2007; Wingate et al. 2009). Current restoration practices for seasonal wetlands do not necessarily allow the periodic hydrologic connectivity that would allow stream fish like eastern mudminnows to naturally colonize the created habitats, as was the case at the Jackson Lane Restoration Site. At the Jackson Lane wetlands, fish may periodically be able to enter wetland habitats to feed, but then become trapped as the hydrologic connectivity recedes and the wetland becomes dry, rather than being able to migrate back to their stream habitat. Management plans for created wetlands should consider fish presence as a potential factor influencing the ecosystem, since fish movement into temporary wetlands is possible if the position of the wetland on the landscape is close to permanent fish sources, and hydrological connectivity periodically occurs. Long-term effects of this predation factor need to be studied to better assure wetland restoration success.

Appendix

Aquatic insect community composition and assignments of trophic position and habit of all individuals collected one day prior to fish introduction (8-July), and two weeks after fish introduction (23-July). Percentages of each taxon are based upon total abundance of aquatic insects found in combined 20 samples for each site/date combination, as follows: 1,121 organisms (8-July: reference), 1,633 organisms (23-July: reference), 412 organisms (8-July: test), and 1,236 organisms (23-July: test).

Order	**Family**	**Genus**	**Trophic Position**	**Key Habit**	**Site**			
					Fishless Reference		Test Wetland	
					8-July	*23-July*	*8 -July*	*23-July*
Ephemeroptera	Baetidae	*Callibaetis*	Herbivore	Swimmer	4.6 %	9.7 %	1.5 %	2.9 %
Ephemeroptera	Caenidae	*Caenis*	Herbivore	Sprawler	0.8 %	0.2 %	0.2 %	0.0 %
Odonata	Aeshnidae	*Anax*	Predator	Climber	1.6 %	0.4 %	0.5 %	0.2 %
Odonata	Coenagrionidae	*Enallagma*	Predator	Climber	3.3 %	9.6 %	0.7 %	1.8 %
Odonata	Coenagrionidae	*Ischnura*	Predator	Climber	0.4 %	0.8 %	0.0 %	0.2 %
Odonata	Lestidae	*Lestes*	Predator	Climber	0.3 %	0.0 %	0.0 %	0.0 %

Appendix continued.

Order	**Family**	**Genus**	**Trophic Position**	**Key Habit**	**Site**			
					Fishless Reference		Test Wetland	
					8-July	*23-July*	*8-July*	*23-July*
Odonata	Libellulidae	*Erythemis*	Predator	Sprawler	0.0 %	0.1 %	0.0 %	0.1 %
Odonata	Libellulidae	*Leucorrhinia*	Predator	Climber	0.0 %	0.1 %	0.0 %	0.0 %
Odonata	Libellulidae	*Libellula*	Predator	Sprawler	0.3 %	1.6 %	0.0 %	2.3 %
Odonata	Libellulidae	*Pachydiplax*	Predator	Sprawler	2.6 %	1.0 %	0.2 %	0.2 %
Odonata	Libellulidae	*Tramea*	Predator	Sprawler	1.2 %	0.4 %	0.0 %	0.1 %
Hemiptera	Corixidae	*Hesperocorixa*	Herbivore	Swimmer	2.1 %	0.4 %	6.1 %	2.3 %
Hemiptera	Mesovellidae	*Mesovelia*	Predator	Skater	0.0 %	0.0 %	0.0 %	0.1 %
Hemiptera	Naucoridae	*Pelocoris*	Predator	Climber	0.8 %	0.9 %	0.2 %	0.2 %
Hemiptera	Notonectidae	*Buenoa*	Predator	Swimmer	9.7 %	19.0 %	4.9 %	6.2 %
Hemiptera	Notonectidae	*Notonecta*	Predator	Swimmer	3.4 %	3.5 %	4.9 %	0.8 %
Hemiptera	Veliidae	*Microvelia*	Predator	Skater	0.3 %	0.0 %	0.0 %	0.0 %

Appendix continued.

Order	**Family**	**Genus**	**Trophic Position**	**Key Habit**	**Site**			
					Fishless Reference		Test Wetland	
					8-July	*23-July*	*8-July*	*23-July*
Hemiptera	Veliidae	*Steinovelia*	Predator	Skater	0.0 %	0.2 %	0.0 %	0.1 %
Coleoptera	Dytiscidae	*Acilus*	Predator	Swimmer (adult)	0.0 %	0.0 %	0.0 %	0.1 %
Coleoptera	Dytiscidae	*Copototomus*	Predator	Climber (larvae), Swimmer (adult)	1.0 %	0.4 %	10.4 %	2.8 %
Coleoptera	Dytiscidae	*Cybister*	Predator	Climber (larvae)	0.1 %	0.1 %	0.0 %	0.0 %
Coleoptera	Dytiscidae	*Hydaticus*	Predator	Swimmer (adult)	0.1 %	0.1 %	1.2 %	0.3 %
Coleoptera	Dytiscidae	*Laccophilus*	Predator	Climber (larvae), Swimmer (adult)	5.6 %	1.2 %	8.0 %	1.9 %
Coleoptera	Elmidae	*Dubiraphia*	Detritivore/ Herbivore	Clinger (adult)	0.0 %	0.1 %	0.0 %	0.0 %
Coleoptera	Haliplidae	*Peltodytes*	Omnivore	Climber (larvae), Swimmer (adult)	1.1 %	1.0 %	1.2 %	1.2 %
Coleoptera	Hydrophilidae	*Berosus*	Omnivore	Swimmer (adult)	1.2 %	0.6 %	2.4 %	2.3 %
Coleoptera	Hydrophilidae	*Helophorus*	Herbivore	Climber (adult)	0.2 %	0.0 %	0.0 %	0.0 %

Appendix continued.

Order	**Family**	**Genus**	**Trophic Position**	**Key Habit**	**Site**			
					Fishless Reference		Test Wetland	
					8-July	*23-July*	*8-July*	*23-July*
Coleoptera	Hydrophilidae	*Helocombus*	Herbivore	Burrower (adult)	0.1 %	0.0 %	0.0 %	0.0 %
Coleoptera	Hydrophilidae	*Hydrochara*	Detritivore/ Herbivore	Swimmer (adult)	0.2 %	0.1 %	0.0 %	0.3 %
Coleoptera	Hydrophilidae	*Hydrochus*	Predator (larvae), Herbivore (adult)	Climber (larvae and adult)	0.6 %	0.4 %	1.9 %	0.4 %
Coleoptera	Hydrophilidae	*Tropisternus*	Predator (larvae), Detritivore/ Herbivore (adult)	Climber (larvae), Swimmer (adult)	2.3 %	5.1 %	3.9 %	5.5 %
Coleoptera	Noteridae	*Hydrocanthus*	Predator	Burrower (larvae), Climber (adult)	0.8 %	0.1 %	0.2 %	0.2 %
Diptera	Ceratopogonidae	*Bezzia*	Predator	Burrower	2.6 %	3.4 %	2.2 %	12.1 %

Appendix continued.

Order	**Family**	**Genus**	**Trophic Position**	**Key Habit**	**Site**			
					Fishless Reference		Test Wetland	
					8-July	*23-July*	*8-July*	*23-July*
Diptera	Chaoboridae	*Chaoborus*	Predator	Sprawler	4.9 %	1.2 %	2.4 %	1.1 %
Diptera	Chironomidae	*Chironomini*	Detritivore/ Herbivore	Burrower	19.5%	26.3 %	29.6 %	26.5 %
Diptera	Chironomidae	*Tanypodinae*	Predator	Sprawler	26.7%	11.0 %	17.0 %	27.1 %
Diptera	Culicidae	*Anopholes*	Detritivore/ Herbivore	Planktonic	0.7 %	0.4 %	0.0 %	0.4 %
Diptera	Culicidae	*Culex*	Detritivore/ Herbivore	Planktonic	0.4 %	0.7 %	0.0 %	0.1 %
Diptera	Tabanidae	*Chrysops*	Predator	Sprawler	0.0 %	0.0 %	0.2 %	0.0 %
Diptera	Tipulidae	sp.	Detritivore/ Herbivore	Burrower	0.1 %	0.0 %	0.0 %	0.0 %

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