

**Research article****Experimental evaluation of the impacts of the invasive catfish *Hoplosternum littorale* (Hancock, 1828) on aquatic macroinvertebrates**Craig Duxbury<sup>1\*</sup>, Jeff Holland<sup>2</sup> and Marianne Pluchino<sup>3</sup><sup>1</sup>Walt Disney Imagineering, Research and Development, 1365 Ave. of The Stars, Lake Buena Vista, Florida, USA<sup>2</sup>Reedy Creek Improvement District, 2191 South Service Ln., Lake Buena Vista, Florida, USA<sup>3</sup>Seminole County, Surface Water Quality, 1101 East First St., Sanford, FL, 32711, USAE-mail: [craig.v.duxbury@disney.com](mailto:craig.v.duxbury@disney.com) (CD), [jholland@rcid.fl.us](mailto:jholland@rcid.fl.us) (JH), [mpluchino@seminolecountyfl.gov](mailto:mpluchino@seminolecountyfl.gov) (MP)

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**Abstract**

The hoplo catfish [*Hoplosternum littorale* (Hancock, 1828)] is a callichthyid catfish native to South America. It was first recorded in Florida in 1995. It has now dispersed throughout much of Florida. It is thought that this fish has had little or no impacts to native fish. However, it is unknown if the introduction of this fish can cause other ecological impacts, such as alteration of aquatic invertebrates assemblages. We conducted a cage experiment to evaluate the effects of the hoplo catfish on macroinvertebrates. Results showed that macroinvertebrate abundance and taxa on artificial substrates (MAS) were reduced by 31 and 50% in the fish treatments, respectively. The entire macroinvertebrate assemblage structure was significantly different between fish and no-fish treatments. This difference was driven primarily by reductions in amphipods, and chironomids. Macroinvertebrates were also identified from fish stomachs and these were compared to assemblages on the MAS. We found a smaller subset of taxa in the stomachs, as compared to the MAS. These results suggest that this fish could alter the macroinvertebrate assemblage structure. This could have implications for environmental monitoring programs that use macroinvertebrates to assess water quality.

**Key words:** aquatic invasive species, cage experiments, biomonitoring, Florida**Introduction**

Invasive species are considered to be a significant threat to biodiversity (Levine 2008). Non-native, invasive fish have also contributed to the worldwide loss of biodiversity (Clavero and Garcia-Berthou 2005). Introduced and invasive fishes are now present in all states and all major aquatic ecosystems in the United States. A moderate climate, extensive waterbodies, and a large human population means that Florida has 127 nonindigenous fish species (Fuller et al. 1999). In 1995, the hoplo catfish [*Hoplosternum littorale* (Hancock, 1828)], a callichthyid catfish native to South America, was reported in Florida (Nico et al. 1996), and has rapidly spread throughout the state (Fuller et al. 1999; Pearlstine et al. 2007; Gestring et al. 2009; Emerson 2007). This fish exhibits a number of characteristics typical of successful invaders, including tolerance to a wide range in temperature and dissolved oxygen (Brauner et al. 1995), a wide dietary breadth (Mol 1995), and high fecundity (Hostache and Mol 1998; Nico and Muench 2004).

Many experimental and observational studies have shown that fish can have significant top-down and bottom-up impacts on primary and secondary production (Matsuzaki et al. 2008). Furthermore, introductions of fish can lead to ecological cascades (Kaufman 1992) and alteration of nutrient cycles (Schindler et al. 2001), and changes in aquatic (Power 1990) and terrestrial food webs (Baxter et al. 2004). Fish predation has been found to be a strong influence on the structure of macroinvertebrate assemblages (Williams and Taylor 2003; Cheever and Simon 2008; Flecker 1992a; Allan 1982; Dudgeon 1991; Miller and Crowl 2005). In addition, introduced fish can also impact macroinvertebrate assemblages (Flecker and Townsend 1994; Knapp et al. 2001); however, this result is not always certain (Harig and Bain 1998; Englund and Polhemus 2001).

Macroinvertebrates are a diverse group of organisms, that are important component of freshwater ecosystems (Wallace and Webster 1996). Because of their high abundance and a wide range in environmental tolerances, these organisms are frequently used in biomonitoring

programs to assess water quality (Rosenberg and Resh 1993). In these programs, changes in macroinvertebrate assemblages are assumed to be due to changes in water quality. However, biotic interactions are not often assessed, especially for a novel invader. Thus, our objective was to assess the effects of this fish on the macroinvertebrate assemblage of central Florida.

## Materials and methods

The study site was located on Reedy Creek, a blackwater stream (Meyer 1990) in the Kissimmee River watershed. The stream is characterized by relatively low nutrients, low ionic strength, and high concentrations of organic acids (Hampson 1993). This low-gradient stream flows through extensive, mixed hardwood swamps, dominated by cypress [*Taxodium distichum* (L.) L.C. Rich.].

We used enclosure cages ( $n = 10$ ) to test the effects of fish on macroinvertebrates. The experimental design included two treatments: fish and a control with no fish, with five replicates per treatment. Cages were 1m (H) $\times$ 0.82m (L) $\times$ 0.65m (W) composed of polyvinyl pipes forming the frame and then completely covered with 14 mm mesh size aquaculture screen. Cages were secured to the stream bottom with metal stakes. Cages were placed in pairs, the two located next to each other in areas of similar depth and velocity; one cage with fish, and the control treatment. Water depth in cages ranged from 0.52 to 0.88 m. Water velocity averaged 0.4 and 0.2m sec<sup>-1</sup> outside and inside the cages, respectively. Debris was cleared from the front of the cages once per week.

Prior to the start of the experiment (October 2005), we caught the hoplo catfish from the study stream using a cast net. We then placed two catfish into each fish-treatment cage. We chose two fish per cage for several reasons. The first is that there are no published reports of typical brown hoplo densities. Second, it appears that these fish exhibit schooling behavior based on our observations (unpublished data) and synchronized breathing behavior has been reported (Sloman et al. 2009). The ten fish averaged 205 mm (range: 176 to 246 mm) in total length, and 159 g in average weight (range: 113 to 227 g).

## Macroinvertebrate analyses

Stream invertebrates were quantified using a modification of the 14 plate artificial substrates (APHA et al. 1989), hereafter referred to as macroinvertebrate artificial substrates (MAS). We modified the MAS by reducing the number of hardboard plates from 14 to five per artificial substrate. Three MAS were placed in each treatment cage, attached with monofilament line in the center of the cage. After 28 days, the MAS and fish were removed from the cages. At the laboratory, the MAS were disassembled, and the macroinvertebrates were carefully dislodged using soft brushes. Macroinvertebrates were sorted alive. The macroinvertebrates were then identified to the family or order. The total number of individuals and total number of taxa were calculated separately for each MAS. Since cages were the experimental units, we averaged the numbers of individuals and taxa from the three MAS for each cage to obtain a mean number per cage.

## Stomach analyses

At the end of the experiment, fish were sacrificed, weight and length were measured, and stomachs were removed. The macroinvertebrate data were pooled from the two fish in each cage. Invertebrates in the stomachs were identified and enumerated to the lowest practicable level, as noted above.

## Water quality

At the beginning and end of the experiment temperature, conductivity, dissolved oxygen, and pH was measured with a Hydrolab Quanta meter. Measurements were made mid-depth, approximately 0.25 m.

## Analyses

Differences in macroinvertebrates (total individuals and taxa) between fish and no-fish treatments were compared with t-tests after checking for homogeneity of variances. Since we found no evidence of heterokedasticity, we used the untransformed data. The relationship between fish length and number of organisms found in the stomachs were assessed using simple linear regression. Univariate statistics were calculated with Statistica Ver. 7.1 (StatSoft 2006).

We also conducted several multivariate analyses to examine the impacts of fish on macroinvertebrates. First, we used nonmetric multidimensional scaling (MDS) to visualize patterns in the entire macroinvertebrate assemblage structure between fish and no-fish MAS (Clarke 1993; Clarke and Ainsworth 1993). This nonparametric technique was based on the Bray-Curtis similarity matrix of the fourth-root transformed macroinvertebrate assemblage data. Organisms were averaged from the three MAS per cage for this analyses. A 4<sup>th</sup> root transformation was applied to downweight the strong dominance from several taxa. An analyses of similarity (ANOSIM) was then used to test for significant differences between the treatments. ANOSIM is a nonparametric permutation procedure applied to the rank similarity matrix underlying the MDS ordination that compares the degree of differences among treatment groups, and calculates an R statistic. The significance level of the R statistic is calculated by comparing to a permutation distribution (Clarke 1999). We used 9,999 permutations on the Bray-Curtis distance of the 4th root transformed data. SIMPER was used to calculate the contributions of each taxon to differences. The multivariate analyses were conducted using the PRIMER-E software package 6.1.11 (Clarke and Warwick 2001).

## Results

### Water quality

Temperature and dissolved oxygen decreased from the beginning to the end of the experiment (Table 1). Conductivity and pH did not change between the time periods.

### Macroinvertebrates

A total of 1,998 macroinvertebrates were identified from the MAS. On average, there were about 30% fewer organisms found on the MAS in the fish treatments ( $p = 0.08$ , 2 sided  $t$ -test) (Figure 1A). There were a total of 11 taxa, and a 50% reduction in taxa richness on the fish treatment MAS (Figure 1B) ( $p=0.05$ ). Chironomids and oligochaetes dominated, accounting for greater than 90% of the total number of organisms. Chironomids comprised 46 and 63%, and Oligochaeta comprised about 50 and 31%, of the organisms on the MAS in the fish and no-fish treatments, respectively.

**Table 1.** Physiochemical water quality data in stream at start and end of experiment, measured with a hand-held meter.

Parameter	Value at Beginning of Experiment	Value at End of Experiment
Temperature (°C)	25.1	18.5
Dissolved Oxygen (mg/L)	3.9	0.96
Conductivity (µS/cm)	139	139
pH	6.2	6.2

**Table 2.** A comparison of the relative abundance (within treatments) of taxa (Family or Order level) found on the artificial substrates (MAS) in fish and no-fish treatments ( $n=10$ , each), and in the stomach contents of caged fish ( $n=10$ ).

Family or Order	Fish MAS	No Fish MAS	Caged Fish Stomach
Chironomidae	46.3	63.2	87.9
Oligochaeta	50.3	30.6	0.0
Gastropoda	2.30	4.00	1.7
Amphipoda	0.13	0.80	5.5
Odonata	0.63	0.50	0.0
Ephemeroptera	0.00	0.10	0.0
Clitellata	0.00	0.10	0.0
Collembola	0.38	0.08	0.0
Coleoptera	0.00	0.10	0.0
Acarifomres	0.00	0.00	1.7
Bivalva	0.00	0.20	1.0
Decapoda	0.00	0.00	0.3
Ceratopogonidae	0.13	0.33	1.7

**Table 3.** Results from SIMPER analyses indicating the five dominant taxa that contributed the greatest influence in differences in macroinvertebrate assemblage structure between fish and no-fish MAS. Average abundance based on 4th root transformed data.

Taxa	Average Abundance Fish Treatment	Average Abundance No-Fish Treatment	Contribution (%)
Amphipoda	0.15	0.73	17.1
Odonata	0.50	0.40	13.3
Chironomidae	2.20	2.64	12.3
Ceratopogonidae	0.15	0.48	11.8
Oligochaeta	2.24	2.14	9.7

### Stomach analyses

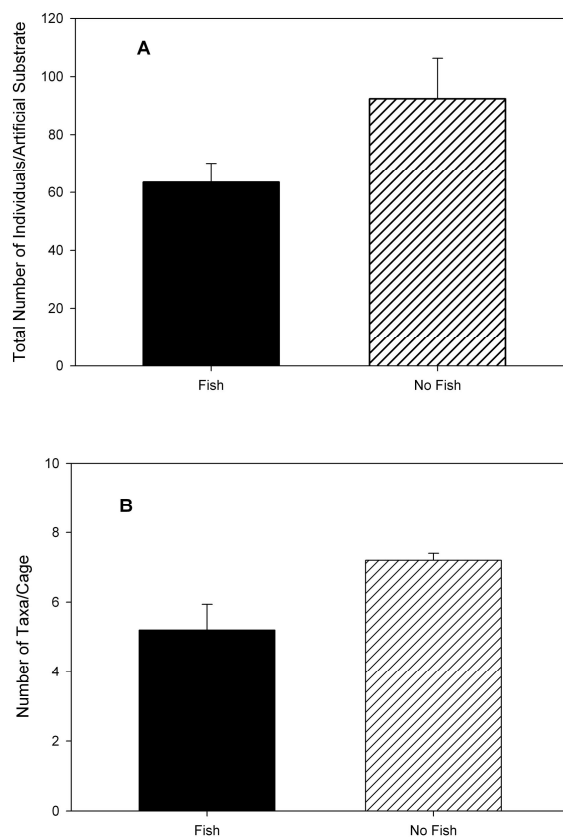
A total of 290 organisms were identified from the fish stomachs collected from the caged fish. There were nine to 65 organisms per stomach. There was no relationship between length of fish and the number of organisms found in the stomachs ( $p > 0.05$ ). Seven taxa were found in the stomachs of the caged fish (Table 2). Chironomids were the most abundant taxa, accounting for almost 90% of the total number of organisms found in the stomachs of the caged fish. Amphipods were the second most abundant taxon, accounting for almost 6% of the total number of organisms.

### Multivariate analyses

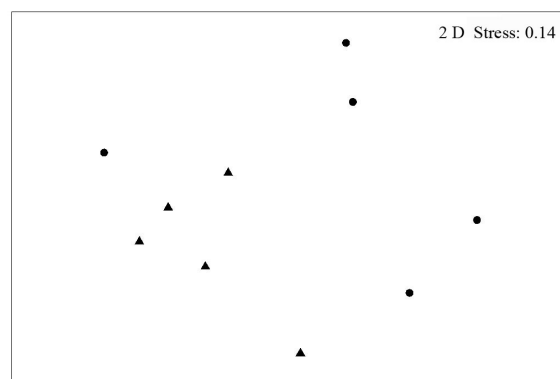
The MDS plot shows a clear separation in macroinvertebrate assemblages between fish and no-fish treatments (Figure 2). The relatively low stress (0.14) indicates that these data can be effectively described in two-dimensional space (Clarke and Warwick 2001). The ANOSIM test showed that there were significant ( $R = 0.424$ ,  $P = 0.032$ ) differences in macroinvertebrate assemblages between treatments. The SIMPER analyses indicated that the difference in assemblage structure was influenced by five taxa which accounted for a cumulative contribution of 64% (Table 3). The difference in assemblage structure was primarily influenced by larger numbers of dragonflies (Odonata) and fewer numbers of amphipods, chironomids, and the biting chironomids (Ceratopogonidae) on the fish treatment MAS.

### Discussion

Our results suggest that hoplo catfish impact aquatic macroinvertebrate assemblages on artificial substrates. We found that macroinvertebrate abundances and taxa richness on MAS in cages with fish were significantly reduced by about 30 and 50%, respectively (Figures 1A, B). While there are many examples in the literature documenting strong impacts of fish on macroinvertebrates in freshwater systems (Flecker 1992b; Dudgeon 1991; Williams and Taylor 2003; Englund and Polhemus 2001; Gido 2003; Ruetz et al. 2006; Baxter et al. 2004; Miller and Crowl 2005) the results are sometimes equivocal (Allan 1982; Flecker and Allan 1984; Culp 2009; Reice 1991; Englund and Polhemus 2001).



**Figure 1.** (A) Mean ( $\pm 1$  SE) number of organisms collected from the modified artificial substrates ( $p = 0.08$ ). (B) Mean ( $\pm 1$  SE) number of taxa from the MAS ( $p = 0.05$ ). Dark bars represent fish treatment and hatched bars represent no-fish treatments.



**Figure 2.** Nonmetric multidimensional scaling plot (MDS) showing macroinvertebrate assemblages (MAS) collected from artificial substrates in cages with fish ( $\blacktriangle$ ) and no-fish ( $\bullet$ ). Each symbol represents the average of three MAS.

We think that direct predation could be one explanation for the macroinvertebrate reductions on the fish treatment substrates. We base this conclusion on the fact that many of the macroinvertebrates found in fish stomachs were similar to macroinvertebrates found on MAS. Further, chironomids were the most abundant organism found in the stomachs, and these insects are a common food item for these fish (Mol 1995; Gestring et al. 2009). However, indirect influences could also explain the reduction in invertebrates. For example, we observed greater numbers of predatory dragonflies on the fish MAS. This may have been because the catfish may not eat these insects. A larger number of dragonflies, could have decreased the overall number of invertebrates. Another indirect influence may have been related to behavioral impacts. The cage mesh size did not preclude macroinvertebrates from leaving the cages. Therefore, fish may have caused macroinvertebrates to flee from predation, or may have simply been dislodged from the MAS. The presence of fish can result in a strong drift response from aquatic insects (Flecker 1992a).

Regardless of the mechanism, our results suggest that these catfish can alter the macroinvertebrate assemblage structure (Figure 2). Other investigators have found that introduced fish result in changes in macroinvertebrate assemblages (Englund and Polhemus 2001; Miller and Crowl 2005). Alterations of the macroinvertebrate assemblage and changes in relative abundances, similar to what we observed can have important implications for water quality monitoring programs. Many water quality monitoring programs incorporate biomonitoring as part of an environmental assessment. Macroinvertebrates are considered to integrate water quality, and changes in macroinvertebrate assemblages are largely assumed to be in direct response to changes in water chemistry. Thus, a novel, invasive predator such as the hoplo catfish could alter the macroinvertebrate assemblage, leading to incorrect conclusions about water quality.

While our results suggest that this invasive catfish could impact macroinvertebrates, clearly there are uncertainties. For example, a mechanistic explanation would help clarify these impacts. Also, environmentally realistic fish densities, especially, as compared to native fish, should be estimated. To our knowledge, there are no published data on the densities of these fish.

Finally, the impacts of the hoplo must be compared to native fish. This knowledge would lead to a better understanding of the ecological impacts of this invasive fish.

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