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Mesocosm experiments revealed variable effects of non-native crayfish on native crayfish and aquatic ecosystems

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**ABSTRACT**

***Keywords:***

**INTRODUCTION**

Freshwater crayfish are one of the most imperiled groups of aquatic invertebrates in North America ( ) with about 50% of them threatened with extinction (Taylor et al. 2007). Despite their very restricted distribution coupled with habitat loss, non-native crayfish impacts cause a serious threat to the existence of native crayfish (Taylor et al. 2007; Richmon et al. 2015). Non-native crayfish are typically much aggressive than native counterparts and may outcompete native species for limited food and shelter ( ). Additionally, non-native crayfish may degrade the quality of aquatic habitats, mainly due to removal of aquatic macrophytes and macroinvertebrates ( ), and also act as vectors for diseases such as crayfish plague ( ).

Paragraph elaborating population/community level impacts- Sujan.

Paragraph elaborating ecosystem level impacts, heavy on leaf litter decomposition and water quality

Crayfish are common, long-lived, omnivorous macroconsumers in temperate aquatic ecosystems (Momot 1995) and therefore have the potential to substantially alter ecosystem function where they occur. Crayfish feeding has been shown to directly and indirectly alter algal abundance (Creed 1994) and detrital processing (Huryn and Wallace 1987, Parkyn et al. 1997, Usio 2000, Schofield et al. 2001, Creed and Reed 2004). Although highly omnivorous (Momot et al. 1978, Parkyn et al. 2001), crayfish species do differ in their feeding preferences (Momot 1995, Adams et al 2003) and thus, crayfish species invasions have the potential to alter ecosystem functioning (Carvalho et al. 2016). However, not all crayfish invasions have had a significant effect on ecosystem functioning, especially with respect to leaf litter processing rates (Lagrue et al. 2014). In many aquatic ecosystems, leaf litter decomposition is a dominant energy processing pathway (Wetzel 2001). Therefore, it is essential to test the way that potential crayfish invaders may differ from native species in their effect on leaf litter processing if we are to fully understand the impact of invasions.

Previous laboratory experiments showed that non-native red-swamp crayfish (*Procambarus clarkii*) and virile crayfish (*Orconectes virilis*) could outcompete native Piedmont crayfish (*Cambarus sp. C*) due to aggressive interactions and cause a significant reduction of survival of the native species under sympatry (Hale et al. 2016).

Although laboratory experiments generate limited information due to simplicity of the experimental systems and conditions under the results were generated, they may provide important first insights to understand the potential impacts and may help designing experiments to gather more realistic data. One approach would be to employ field mesocosm experiments which allow native and invasive species to interact under semi-natural conditions, allow experimenters to include natural variation of environmental conditions, adjust animal densities according to natural conditions and also to include other potential prey and predators (Henkanaththegedara and Stockwell 2014, 2015). Hill and Lodge successfully used large mesocosms to assess crayfish competition for habitats (1994) and effects of predation on crayfish growth and mortality (1999).

**METHODS**

*Collection, acclimation and preparation of crayfish*

Invasive virile crayfish (*O. virilis*) were collected from Clinch River at Dungannon in Smith County, Virginia using seines and dip nets. The native Piedmont crayfish (*Cambarus sp. C*) were collected from a first order tributary of Buffalo Creek in Prince Edward County, Virginia using dip nets. All crayfish were collected in fourth week of May 2016 and kept with stream water in coolers during transportation. Subsequently, crayfish were acclimated in 1250 L plastic mesocosms for 24 hours before stocking.

*Mesocosm setup and Experimental design*

We conducted a mesocosm experiment using thirty 1250 L circular plastic tanks as experimental units. Tanks were setup in an outside field at Longwood University, Farmville, Virginia allowing us to include the effects of natural variation of environmental conditions (e.g. temperature and precipitation) and natural colonizers of water bodies. Mesocosms were filled with treated tap water up to 30cm (XXX L) and aged for two weeks before stocking crayfish. Each mesocosm was provided with 8 pieces of 7.5 cm PVC tubes as shelter for crayfish and covered with poultry fence to exclude predators. About 70% of the tank surface was covered with shade cloth to mimic the average shade of a typical crayfish habitat and to avoid excess direct sunlight (Figure 1). Our experimental design included two controls and three treatments with 6 replicates for each group. We stocked crayfish according to following scheme allowing us to assess density-dependent invasive crayfish effects on native crayfish.

Table 1: Experimental design

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Crayfish species** | **Native control** | **Invasive low-density** | **Equal density** | **Invasive high-density** | **Invasive control** |
| Native *Cambarus sp. C* | 4 | 4 | 4 | 4 | 0 |
| Invasive *Orconectes virilis* | 0 | 2 | 4 | 6 | 4 |
| Total crayfish | 4 | 6 | 8 | 10 | 4 |

All crayfish were weighed for wet biomass (to the nearest 0.1 g) using a digital scale, and carapace length was measured (to the nearest 0.1 mm) using dial calipers before stocking. We attempted to stock each mesocosm with at least one male and one female reproductively mature crayfish of each species (carapace length > XX mm). We selected additional crayfish to represent a range of sizes and sexes depending on the availability of crayfish. This allowed us to track individual crayfish growth assuming equal growth rates of individuals within the same species. Additionally, we stocked only intermolt individuals and attempted to match average body size of invasive crayfish with native crayfish to eliminate any biased results due to extremely large crayfish.

*Crayfish sampling*

Crayfish were sampled using dip nets at day 18 (midpoint) and at the conclusion of the experiment at day 35. Individual crayfish were weighed for wet biomass (to the nearest 0.1 g) using a digital scale, and carapace length was measured (to the nearest 0.1 mm) using dial calipers.

*Leaf Mass Loss*

The effect of crayfish community composition and density on leaf litter decomposition was evaluated using leaf packs made from senescent tulip poplar leaves (*Liriodendron tulipifera*). The leaves were collected in the fall of 2013 by gently tugging senescent leaves still attached to the tree. Only leaves that came off without resistance were collected. After collection, the leaves were air dried until use. Leaf packs were constructed by softening 1.5 g of leaves in deionized water for 5 minutes and then binding them at the petioles with a 19 mm metal binder clip. Prior to adding to the experimental mesocosms, the leaf packs were leached in deionized water for approximately 72 h. On June 3, 2016, after the leaching period, 3 leaf packs were randomly added to each of the experimental mesocosms, taking care to distribute them evenly around the tank.

To determine the initial mass of the leaf packs after leaching and handling, a leaf pack was added to 11 randomly selected mesocosms and then immediately removed by gently lifting the leaf pack as a 1 mm mesh sieve was placed under the leaf pack and used to lift it from the water. We collected any leaf material retained by the sieve even if it was not attached to the binder clip. After removal from the mesocosm, the binder clip was removed and the collected leaves were placed into a paper bag, returned to the lab and dried at 50o C for 48 hours. The dried leaves were then homogenized with a mortar and pestle and ash free dry mass (AFDM) was determined by loss on ignition at 550o C for 4 h.

Leaf pack mass loss over time was determined by harvesting and processing a single haphazardly selected leaf pack from each mesocosm on days 3, 10, and 24 using the same methods described above. During collection each leaf pack was carefully scanned in the field for macroinvertebrate colonization, although none were ever found.

On June 2, June 16, June 30, July 22, and August 5, we measured the temperature, dissolved oxygen, percent oxygen saturation, and conductivity in each tank using a YSI SONDE NAME. On the same dates we collected a 500 ml water sample from approximately mid-depth in each tank by submerging an open wide-mouth plastic bottle. These water samples were returned to the lab where we measured pH using a PH METER and filtered (GFF) 10 and 30 ml samples for spectral absorbance profile and dissolved organic carbon and total nitrogen, respectively. We measured spectral absorbance using a 1 cm cuvette in a NanoDrop 2000 UV-Vis spectrophotometer. Dissolved organic carbon and total nitrogen on acidified samples was determined using a Shimadzu TOC – L total carbon analyzer with a TNM – L module. After filtering the above samples, we clogged the filter with additional sample if necessary and extracted the filter in 90% acetone a -20o C for 24 hours. Chlorophyll a, corrected for phaeophyton by acidification with 0.1N HCl, was measured with a Turner Trilogy Fluorometer.

On August 6 we measured the Secchi depth of each tank.

*Data analysis*

The effect of crayfish community composition and density on leaf mass loss was assessed by calculating the decay coefficient (k) of the mass loss using a negative exponential model for each of the tanks (Webster and Benfield 1986, Benfield 2006). The decay coefficient (k) is calculated as the slope of the regression of the natural log of the percent AFDM of the leaf material remaining relative to the mass on day 0 (i.e., the initial mass after calculating handling loss) by days of incubation (Benfield 2006). Since 2 of the tanks had 0 AFDM remaining on the final day of the incubation, 1 was added to the percent AFDM remaining prior to taking the natural log. We used a 1-way ANOVA with k as the response variable and treatment as the effect to test the effect of crayfish community composition and density on the rate of leaf mass loss.

The effect of crayfish community composition and density on chlorophyll concentration was evaluated using a repeated measures ANCOVA of chlorophyll concentration by treatment and days elapsed with an interaction term and tank as the random effect. The analysis was conducted with the lmerTest package (Kuznetsova et al. 2015) in R (R Core Team 2014).

**Results**

*Leaf Mass Loss*

The initial mass of the nominally 1.5 g leaf packs placed into the tanks, following leaching and handling losses ranged from 0.92 to 1.12 g with a mean mass (± 1 SD) of 1.02 (± 0.07) g. After 24 days of incubation a mean (± 1 SD) of 31.65 (± 20.91) % of the initial mass of the leaves remained across all the tanks (Figure #). The decay coefficient (k) ranged between -0.2321 to -0.0146 day-1 with a mean (± 1 SD) of -0.0688 (± 0.0555) day-1. There was no effect of our manipulation of invasive crayfish density on the leaf mass loss rate (Table #), nor did the native and invasive crayfish differ in the rate of leaf mass loss when they were alone (t = 0.9603, p = 0.3775).

*Algal Density*

The chlorophyll content of the tanks increased significantly (p < 0.0001) from a mean (± SD) of 4.88 (± 3.89) μg L-1 on June 3 2016 to 27.72 (± 14.21) μg L-1 on July 22 2016. There was a significant effect of the crayfish community composition or density on the chlorophyll concentration of the tanks (p = 0.119).

**Discussion**

**Acknowledgements**

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**TABLES**

**Figure Legends**

Figure 1: Mesocosm setup utilized for current experiments.