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# Effects of Detrital Subsidy on Arthropod Communities in Louisiana Rice Fields and Predation on Rice Water Weevil (*Lissorhoptrus Oryzophilus*)

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EFFECTS OF DETRITAL SUBSIDY ON ARTHROPOD COMMUNITIES  
IN LOUISIANA RICE FIELDS AND PREDATION ON RICE WATER  
WEEVIL (*LISSORHOPTRUS ORYZOPHILUS*)

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
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Master of Science

in

The Department of Entomology

by  
Nathan Mercer  
B.S., University of Vermont, 2011  
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## ABSTRACT

The rice water weevil (RWW), *Lissorhoptus oryzophilus* (Kuschel), is the most important insect pest of rice in the United States. Integrated pest management strategies for RWW in Louisiana consist of cultural controls, resistant cultivars and chemical insecticides. The fourth component of IPM, biological control, is largely absent from the literature for RWW, making exploration of biological control a logical next step in developing a full set of IPM strategies. The three main types of biological control are augmentation, classical and conservation. With little known about RWW predators, conservation biological control makes the most sense as local natural enemy abundance is increased. Detrital subsidies have been shown to cause trophic cascades in agricultural system that can ultimately reduce herbivore populations via increased predator abundance.

During the summer of 2013 and 2014, field experiments were carried out to determine if compost-manure additions to rice fields would cause an increase in invertebrate diversity and translates to a reduction in RWW numbers. Surveying of treatment (compost-manure additions) and control plots (no additions) for differences in invertebrates used four different sampling methods: root/soil corer, Gee crayfish trap, aquatic netting and floating pitfall traps. Based on sampling from this experiment, *Notonecta* sp., immature *Pantala* sp. and *T. lateralis* were chosen to be used in aquaria experiments to test for predator effects on RWW.

Detrital subsidies in both years failed to increase diversity of invertebrates or reduce RWW numbers. Plotting of feeding guilds over the course of both years showed predator populations paralleling prey populations. RWW oviposition and larval emergence was unaltered whether *Notonecta* sp. or *Pantala* sp. were present or not. *Tropisternus lateralis*, a herbivore/scavenger, also failed to alter RWW fecundity, suggesting that RWW may either not alter their behavior when other organisms are present or they may be able to differentiate between dangerous and non-dangerous arthropods. These experiments failed to cause a trophic cascade or identify predators of RWW. They did however



demonstrate that a large prey population is present and that is utilized by a diverse predator assemblage still with the potential to be increased by detrital subsidies.

## CHAPTER 1. LITERATURE REVIEW

### 1.1 Rice Cultivation and Pests

Rice domestication is believed to have begun ~10,000 years ago in China and rice is now produced in countries around the world with 682.8 million tonnes produced in 2009 (Zheng et al. 2007, FAO 2011). Methods for growing rice vary depending on location and water supply. In many tropical regions, rice fields are flooded and young rice is then transplanted into the field (Fernando 1993). In subtropical regions, such as Louisiana, rice is usually planted as seeds (direct-seeded), either in a dry or flooded field (Blanche et al. 2012). Water for rice cultivation can be supplied by rain water, nearby surface water or well water (Fernando 1993, Blanche et al. 2012). Once fields in tropical regions are flooded, they generally are not drained until shortly before harvest (Fernando 1993, Settle et al. 1996). In southern Louisiana rice planting dates range from early March to May with wells being the main source of water (Blanche et al. 2012). Harvesting takes place in early August, and depending on favorable conditions for the next two months, a ratoon crop can be grown (Blanche et al. 2012).

Several different insect are pests on rice in Louisiana, with varying damage potential. The most damaging insect pest is the rice water weevil (RWW), *Lissorhoptus oryzophilus* (Kuschel), a native to Louisiana, with infested fields producing 20% less than uninfested fields (Zou et al. 2004a, Hummel et al. 2012, O'Brien and Haseeb 2014). RWW was first noticed on rice in Savannah, Georgia U.S.A. in 1880 and by 1914 was considered an economically important pest on rice (LeConte 1880, Webb 1914). The native range of RWW is from the U.S.A.'s eastern coast to Texas and the Great Plains and are now found in all rice growing regions of the U.S.A (O'Brien and Haseeb 2014). RWWs have expanded their range as rice pests to include California in 1959, Japan in 1976 then China and Korea in 1988 and are now reported in Italy and India (Lange and Grigarick 1959, Takenouchi 1978, Hix et al. 2000, Chen et al. 2005, Lupi et al. 2007).

RWWs do not exclusively feed on rice and are found on a number of other aquatic grasses, many of which are considered weeds in rice fields (Tindall and Stout 2003, Lupi et al. 2009). Some of these weeds, such as barnyardgrass, *Echinochloa crus-galli* (Beauv.), yellow nutsedge, *Cyperus esculentus* (L.), broadleaf signalgrass, *Brachiaria platyphylla* (Nash.), and fall panicum, *Panicum dichotomiflorum* (Michx.), are preferred as hosts and oviposition sites by RWW over cultivated rice (Tindall and Stout 2003). Field trials evaluating the effect of *E. crus-galli* on RWW larvae populations on rice have found no difference between plots with just rice or rice and *E. crus-galli* plots 30 days after a permanent flood (Tindall et al. 2004).

Adult RWWs emerge from overwintering in early spring and fly to hosts plants where they feed on leaf tissue (Muda et al. 1981, Shang et al. 2004). Females generally wait until the rice is flooded before ovipositing their eggs in the leaf sheaths (Stout et al. 2002b). Eggs take up to eight days to hatch and about 18 days to complete four larval instars and another eight days to complete the pupal stage (Isely and Schwardt 1934, Cave and Smith 1983). After hatching larvae feed on rice plant tissue briefly in the leaf sheath then exit the sheath and move toward the roots where they feed (Isely and Schwardt 1934, Bowling 1972). Among the roots, larvae use their dorsal spiracle hooks to pierce and breathe through the host's tissue (Zhang et al. 2006). Larvae spend the remainder of their life among the roots where they also pupate (Zhang et al. 2006). The eclosed adult, depending on when they have emerged, will either migrate to overwintering sites or produce another generation, with two to three generations occurring per year (Isely and Schwardt 1934, Muda et al. 1981, Shang et al. 2004). A portion of the RWW population is univoltine, returning to overwintering sites, wooded areas and under leaf litter, in June, and another portion is bivoltine and possibly trivoltine, returning to overwintering sites in August through October (Shang et al. 2004). RWWs feed on rice during the adult and larval stages, however larval feeding is responsible for the most significant damage to the plant occurs (Isely and Schwardt 1934). Heavy infestation of larvae can severely reduce root mass which translates to a reduction in yield

(Stout et al. 2002a). Adult feeding on leaves typically does not cause significant damage to the plant unless under heavy infestation (Isely and Schwardt 1934).

## 1.2 Rice Water Weevil Management

Management tactics for RWWs in the southern U.S. consist of planting resistant cultivars, using cultural control practices, and insecticide application (Hummel et al. 2012). Resistant rice cultivar development in the U.S. began in the 1970s when several lines were developed with moderate resistance to RWW feeding (Smith and Robinson 1982). Currently the cultivar Jefferson is the most resistant to RWW feeding (Stout et al. 2001, Zou et al. 2004a). This cultivar however is not among the ones normally chosen by farmers, most likely because RWW damage mitigation is not high enough to be considered in comparison to other lines, such as Clearfield that are herbicide-tolerant (Stout et al. 2001, LSU AgCenter 2012, Blackman et al. 2014). Another emerging tactic of rice resistance under study is to induce resistance by foliar application of jasmonic acid (JA) (Hamm et al. 2010). JA is a plant hormone known to help regulate both direct plant defenses, changes in the plant that negatively affect the fitness of herbivores directly, and indirect plant defenses, plant secondary chemicals that attract predators and parasitoids (Thaler et al. 2002). Application of JA has been shown to reduce the number of eggs laid and number of RWW larvae found on rice (Hamm et al. 2010).

RWW damage can be mitigated with five different cultural control practices. The first form of cultural control developed, and still used, is proper water management. This consists of timing of field drainage, delayed flooding and water depth (Webb 1914, Rice et al. 1999, Stout et al. 2002b). The objective of draining is to dry out the soil when larvae numbers are peaking, creating a poor larval habitat, and reducing the likelihood of females ovipositing on the rice field (Stout et al. 2002b). The duration of the dry period is usually 3-4 weeks, however the longer this is extended the more problematic weeds will become which can lead to conflicts between weed and RWW management (Quisenberry et al. 1992, Stout et al. 2002b, 2002a). Field drainage can also be costly due to loss of

fertilizer and improper timing can prevent effective RWW control (Smith 1983). Delayed flooding, as the name implies, prolongs the time between rice planting and when a permanent flood is applied to a field. This method avoids the problems associated with increased fertilizer and herbicide cost that can come from draining and flooding a rice field, however this method can be problematic with dormant weed seeds, such as red rice (*Oryza sativa* L.) (Rice et al. 1999). Populations of RWW can also be influenced by managing the flooding depth, a relatively shallow flood can decrease RWW numbers significantly (Stout et al. 2002b). Even with these potential extra costs, studies have shown that water management can effectively control RWWs while being cost efficient in conjunction with insecticide (Rice et al. 1999, Zou et al. 2004b).

The second cultural practice of early planting aims to have rice plants older at the time of flooding so they will be less susceptible to RWW larvae feeding (Stout et al. 2011, 2012). In southern Louisiana early planting dates are early March to early April (Stout et al. 2011). The third tactic is weed removal from field edges, which can decrease the level of RWW infestation, although its economic value is questionable and RWW population may not always be significantly affected by weeds (Palrang et al. 1994, Tindall et al. 2004). A fourth cultural control practice is to avoid over fertilization, as an increase in nitrogen has been associated with a larger RWW population (Jiang and Cheng 2003, Way et al. 2006). The fifth practice is to avoid seeding rice at low densities, which can increase RWW numbers and reduce yield (Stout et al. 2009). However, even with these range of cultural control practices that are effective at managing RWW, insecticides are still the most common method of RWW management (Zou et al. 2004b, Way et al. 2006, Blackman et al. 2014).

Aldrin was the first insecticide that was effective at controlling RWWs, but less than ten years after its implementation, resistant populations were found (Newsom and Swanson 1962, Bowling 1968). Carbofuran was next used to control the RWWs, but in 1990 carbofuran was found to have toxic effects on non-target species and in 1998, was banned (Way 1990, Stout et al. 2000). Currently Karate

®, Mustang Max®, Prolex®, Trebon and Declare®, pyrethroids and pyrethroid like chemicals, and Dimilin®, an insect growth regulator, are used as foliar insecticides for management of adults (LSU AgCenter 2012). Larvae are controlled by seed treatments using either Cruiser Maxx® or Dermacor X-100 (LSU AgCenter 2012). In 2013 Nipsit Inside® seed treatment and Belay® foliar spray were made available (Way et al. 2012). With insecticides being the most prevalent RWW management tactic, concerns have been raised about negative effects on non-target organisms and a continuing buildup of resistance by RWW to these insecticides (Zou et al. 2004b, Blackman et al. 2014). With so much dependence on a single tactic, exploration of other management methods is important to create a stronger IPM (Lewis et al. 1997, Bueno et al. 2011).

### 1.3 Past Experimentation of Biological Control of Rice Water Weevil

Insecticide application, resistant cultivars and cultural practices are all part of IPM and when used together can effectively manage RWW damage (Zou et al. 2004b). An aspect of IPM that is rare in the literature on RWW is the use of biological control, most of which focuses on microbial control methods (Puissegur 1976, Bunyarat et al. 1977, Carbonell 1983, Godfrey et al. 1993, Urtz and Rice 1997, Aghaee and Godfrey 2014, Kim et al. 2014). Bunyarat et al. (1977) looked at the effects of an unknown species of parasitic Mermithid nematode on RWW, finding mortality ranging from 43% to 26% of females found in the field. A study done on the effects of biopesticides on a different rice water weevil, *Lissorhoptrus brevitrostris*, in Cuba found moderate effectiveness, although their methods were not well explained (Carbonell 1983). A recent revision of *Lissorhoptrus*, however, makes no mention of this species and claims all RWW of North American are *L. oryzaophilus* (O'Brien and Haseeb 2014). If true, Carbonell's (1983) study could provide support for further biocontrol studies.

Godfrey et al. (1993) found that while *Beauveria bassiana*, a fungus, was effective at controlling RWW adults, but had little effect on larvae, the economically damaging life stage. Urtz and Rice (1997) looked at the genetics of *B. bassiana* found in infected RWW collected from Louisiana; however, no

mention of mortality or infection rate was made and no further work is known of *B. bassiana* on RWW in the U.S.A. A recent study in Korea found that application of *B. bassiana* granules to nursery rice, before transplanting into rice fields, was effective at managing RWW larvae once transplanted to the field (Kim et al. 2014). A recent study by Aghaee and Godfrey (2015) compared granular application of *Bacillus thuringiensis* spp. *galleriae* in California rice to the common insecticide  $\lambda$ -cyhalothrin. *Bt.galleriae* was found to be, in some green house and field experiments, as effective as  $\lambda$ -cyhalothrin at managing RWW larvae.

Puissegur (1976) tested the ability of predators found in rice fields to feed on RWW and analyzed gut contents of frogs for RWW remains. No-choice predation experiments were performed in tanks containing water, a predator and a number of RWW larvae. Out of the 19 predators tested, 4 did not consume RWW larvae. Field cage studies found that *Pantala* sp. (Odonata: Libellulidae) significantly reduced RWW numbers compared to no predator controls, although only four replications were performed. No-choice experiments were also performed on predation of RWW adults by Orthoptera, with mixed results. Gut content analysis of frogs revealed that RWW adults made up a small percentage of their diet, 4.5-9.3%. Field cage studies found that at least one predator is capable of influencing RWW numbers, however with only four replications, further studies are needed to determine *Pantala* sp. and other predator effectiveness in RWW control.

A study in California rice fields found that *Pardosa ramulosa* (Araneae: Lycosidae), a generalist predator, populations in rice paddies peaked in June (Oraze et al. 1989). *P.ramulosa*'s population peak overlaps when RWW populations are also high, indicating biological control is a possibility, although RWW was not mentioned in the study. High densities of *P. ramulosa* however led to cannibalism (Oraze and Grigarick 1989). If non-microbial biological control is to be used to help manage RWW, more studies are needed to better understand the population dynamics of predators in rice fields.

#### 1.4 RWW Biological Control Methods

Biological control methods generally are grouped into importation (or classical), augmentation, and conservation biological control (Lewis et al. 1997). Importation, as the name implies, is a method in which an exotic natural enemy of the pest is screened for its performance as a control agent, released and hopefully establishes a self-sustaining population. This is a common biological control method for introduced pests, RWW is native to Louisiana (Gilstrap 1997, O'Brien and Haseeb 2014).

Augmentative biological control involves rearing natural enemies, exotic or native, and then releasing them in mass (van Lenteren 2012). The third method, conservation biological control, uses natural enemies already present in the ecosystem to help manage pests. For this method the habitat is usually manipulated in some way, in combination with reduced pesticide applications to attract, retain and enhance the effectiveness and diversity of local natural enemies. Examples of manipulations are beetle banks, food sprays or flower strips (Landis et al. 2000, Wade et al. 2008, Skirvin et al. 2011).

The RWW is native to Louisiana, and therefore likely that local natural enemies exist capable of reducing RWW populations in rice fields. The major predators of RWW have not been identified and as such all aquatic and semi aquatic predators in rice fields should be considered a potential predator (Bellotti et al. 1987, Rutledge et al. 2004, Fritz et al. 2011). Targeting a specific natural enemy for importation or augmentation would be impractical since no evidence to support any specific predators or parasitoids is known (Puissegur 1976, Gilstrap 1997, van Lenteren 2012). Conservation biological control is therefore the best method to explore the potential impact of natural enemies on RWW and has been shown to help manage pests in crop systems including rice (Way and Heong 1994, Settle et al. 1996, Jiang and Cheng 2004, Schmidt et al. 2004, Lou et al. 2013). This approach also has the advantage of potentially promoting natural enemy abundance and diversity, which would allow for a larger pool of natural enemies to be identified (Landis et al. 2000, Gurr et al. 2003, Rutledge et al. 2004).



To find biological control methods that are feasible in ephemeral aquatic rice ecosystems, an examination of methods used in Asian rice would be prudent. Biological control methods in Asia have been developed during the past 40 years to combat secondary resurgence of pests, such as the brown planthopper, *Nilaparvata lugens* (Stal), due to the heavy reliance on chemical insecticides (Heinrichs and Mochida 1984, Settle et al. 1996). As has been described in Lou et al. (2013) numerous methods and control organisms used for conservation and augmentative control on rice in Asia have been successful in reducing pest pressure.

Settle et al. (1996) observed that on rice in Java, a predator population is present early in the season and likely feeds on the large detritivore population when relatively few herbivores are present. They hypothesized that detrital subsidies, in the form of composted cow manure, would cause a trophic cascade increasing the local predator populations due to an increase in detritivores and this larger predator population could negatively affect herbivore population.

A difficulty in using biological control in many crop systems is that they are usually an ephemeral habitat. This is especially true in rice systems rapidly changing from dry to flooded conditions (Settle et al. 1996, Gilstrap 1997). To try and overcome this, Settle et al. (1996) flooded the rice field three months before planting and found that detrital subsidies had a positive effect on predator abundance. This increase in predator numbers was followed by a significant decrease in herbivore abundance. No species description was given of feeding guild composition.

Jiang and Cheng (2004) replicated Settle et al. (1996) in China, finding a similar, although weaker, response by the predator community to detrital additions in their ability to control whitebacked planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae). However they indicated that predators may prefer Collembola over *S. furcifera* as prey, which would lower their ability to act as a control agent. The reason for the stronger response of predators in Settle et al. (1996) may be due to either lower levels of detritus (0.83 kg/m<sup>2</sup> vs. 0.53 kg/m<sup>2</sup>) or that Settle et al. (1996) measured the response of predators

and herbivores as a whole guild where as Jiang and Cheng (2004) measured the response of predators and a specific herbivore *S. furcifera*.

Conservation biological control can be accomplished by a number of methods, both at the landscape and field level (Landis et al. 2000, Gurr et al. 2003, Bianchi et al. 2006). Landscape level management involves diversifying and increasing the non-crop habitat around the crop field (Bianchi et al. 2006). This is not the best approach for an initial investigation because large scale manipulation of a system is required for a method of control that has not yet been demonstrated as effective. Field level manipulations can be done by several different methods: planting grass or flower strips on the edge or within a field, application of food sprays to a field, cover crops, or detrital subsidies (Wise et al. 1999, Wade et al. 2008, Skirvin et al. 2011, Lundgren et al. 2013, Huallacháin et al. 2014).

As stated before, many of the grasses that grow in and around rice fields are suitable hosts for RWWs and other rice pests, so attempting to use them to attract natural enemies would likely be counterproductive (Palrang et al. 1994, Tindall and Stout 2003, Tindall et al. 2004). Application of artificial food sprays are generally aimed at increasing arthropod parasitoids; currently no record of RWW parasitism by arthropods on RWW are known (Wade et al. 2008). While the effectiveness of detrital subsidies may be inconsistent among crops and pests, as discussed previously, trophic cascades are feasible in rice systems (Settle et al. 1996, Wise et al. 1999, Jiang and Cheng 2004, Schmidt et al. 2004). This, coupled with studies demonstrating detrital additions in natural aquatic habitats greatly increasing predator abundance, indicate this to be a good method for an exploratory study of conservation biological control (Ogren and King 2008, Hagen et al. 2012).

## **1.5 Predator-Prey Interactions**

Predators can greatly influence their environment through trophic cascades. One mechanism causing cascades is consumptive, or direct, effects, in which the predator eats the prey reducing the amount of plant material consumed by prey (Schmitz et al. 1997). The other type, called non-

consumptive, or indirect, effects, have been shown to dramatically alter the behavior and development of prey (Thaler et al. 2012, Brown et al. 2012). Indirect effects are an important aspect of predator prey interactions, affecting host and oviposition choice, physiology and resource uptake by prey (Schmitz et al. 1997, Thaler et al. 2012, Brown et al. 2012, Wasserberg et al. 2013). Classically, direct effects were studied and indirect effects were largely ignored (Brown et al. 1999). Indirect effects have demonstrated the ability to cause just as strong of a cascade as direct effects (Schmitz et al. 1997, Snyder and Wise 2000, Preisser et al. 2005). Direct and indirect interactions are important to understanding and more successfully implementing biological control as well as contributing to a more comprehensive understanding of ecology (Schmitz 1998, Wise et al. 1999).

Puissegur's (1976) exploratory survey of predators should be expanded upon to better assess their propensity to consume RWW. Considering RWWs are native to Louisiana, they should be “knowledgeable” of potential predators, such as *Pantala* sp., and may potentially alter their behavior in the presence of predators (Brown et al. 1999, O’Brien and Haseeb 2014). An introductory examination into whether or not RWW is “knowledgeable” of potential predators would test for any change in adult or larval RWW behavior. A common behavior to measure is feeding, however larval feeding takes place in the benthos and would be difficult to monitor (Stout et al. 2002a). Changes in oviposition is a potential indicator of indirect effects and is a feasible metric for measuring changes in RWW (Tindall and Stout 2003, Wasserberg et al. 2013, Hirayama and Kasuya 2013). Testing the effects of predators on RWW oviposition and larval emergence would give insight into any potential predators RWW recognize and therefore potential control agents.

RWW is a serious pest of rice in the U.S.A. and continues to spread to other rice growing regions (Lupi et al. 2007). Developing biological control methods that can be integrated into current IPM tactics is critical to sustainably managing RWW. In this thesis, two different studies are reported. The first was measuring the effect of detrital subsidies to rice fields, monitoring for a trophic cascades in which

generalist predator abundance and diversity is increased leading to a decrease in RWW abundance. This study was also an opportunity to survey arthropods present in rice fields, allowing for identification of possible RWW predators. The second study tested the effects of aquatic predators, found during the surveys, on RWW oviposition in aquaria.

## CHAPTER 2. DETRITAL SUBSIDES AND THEIR EFFECTS ON INVERTEBRATE DIVERSITY AND RICE WATER WEEVIL CONTROL

### 2.1 Introduction

#### 2.1.1 Rice Cultivation and Pests

Rice is produced around the world with 682.8 million tonnes produced in 2009, using a large diverse array of methods for cultivation (Fernando 1993, FAO 2011). In Louisiana U.S.A., rice is usually drill seeded using well water to flush and flood the field (Blanche et al. 2012). This rice is usually planted in early spring with harvesting taking place in early August, and possibly a ratoon crop in October depending on weather conditions (Blanche et al. 2012).

Several different insect pests of rice in Louisiana exist with varying damage potential. The most damaging pest is the rice water weevil (RWW), *Lissorhoptrus oryzophilus*, (Kuschel), a native to Louisiana, with infested fields having up to 20% less yield than untreated (Zou et al. 2004a, Hummel et al. 2012, O'Brien and Haseeb 2014). RWW was first noticed on rice in Savannah, Georgia in 1880 and by 1914 was considered an economically important pest on rice (LeConte 1880, Webb 1914). RWWs have since successfully established in rice growing regions across the U.S.A., Asia and Europe (Lange and Grigarick 1959, Chen et al. 2005, Lupi et al. 2007).

RWWs do not exclusively feed on rice and are found on a number of other aquatic grasses, many of which are considered weeds in rice fields (Tindall and Stout 2003). Several of these weeds are preferred over rice as hosts and can support similar populations of RWW as cultivated rice (Tindall and Stout 2003, Tindall et al. 2004, Lupi et al. 2009). Adult RWWs emerge in early spring and fly to host plants where they feed on leaf tissue (Muda et al. 1981, Shang et al. 2004). Feeding by adults generally does not cause economic loss except under very heavy infestation (Isely and Schwardt 1934). Females generally wait until the rice is flooded before ovipositing their eggs in the leaf sheaths (Stout et al. 2002b). RWW larval emerge from the leaf sheath and migrate towards the roots where they feed causing the most significant damage to the plant by reducing the root mass (Isely and Schwardt 1934, Stout et al.

2002a, Zhang et al. 2006). A complete generation takes about 35d, with two to three generations per year (Shang et al. 2004).

### 2.1.2 Rice Water Weevil Management

Integrated pest management (IPM) tactics for RWW consist of resistant cultivars, cultural control practices and chemical controls (Hummel et al. 2012). The most resistant cultivar, Jefferson, is not among the ones normally chosen by farmers, most likely because of low levels of resistance and more appealing traits such as higher yields and herbicide-tolerance can be found in other lines (Stout et al. 2001, Zou et al. 2004a, Blackman et al. 2014). The five main cultural control methods for RWW are proper water management, early planting, avoidance of over fertilization and low seeding rates and clearing weeds from the edges of fields (Webb 1914, Palrang et al. 1994, Stout et al. 2002b, 2009, Jiang and Cheng 2003, Way et al. 2006). Cultural control practices however are being used less frequently in favor of chemical practices, specifically seed treatments (Blackman et al. 2014). This has led to some concern of negative effects on non-target organisms and continued buildup of resistance by RWW to insecticides (Zou et al. 2004b, Blackman et al. 2014).

Information on biological control of RWW, the fourth aspect of IPM, is rare in the literature, with a few studies published on microbial controls and a thesis on predation of RWW (Puissegur 1976, Bunyarat et al. 1977, Carbonell 1983, Godfrey et al. 1993, Aghaee and Godfrey 2014, 2015). Bunyarat et al. (1977) observed the mortality rate of RWW due to parasitism by an unidentified species of Mermithidae, finding the rate to range from 26%-46% in females. Godfrey et al. (1993) looked at mortality rates of *Beauveria bassiana* on adults and larval RWW and while effective at controlling adults, no significant effect was found on the larval stage.

Studies have also been done on biopesticide effectiveness on a different rice water weevil, *Lissorhoptrus brevitrostris*, in Cuba finding moderate effectiveness, although their methods were not well explained (Carbonell 1983). A recent revision of *Lissorhoptrus*, however, makes no mention of this

species and states RWW of North American are all *Lissorhoptrus oryzophilus* making the Carbonell (1983) study more relevant to RWW management (O'Brien and Haseeb 2014). Examination of microbial control methods was done in Korea, applications of *B. bassiana* granules to nursery rice, before transplanting rice into fields, was found to be effective at managing RWW larvae once transplanted to the field (Kim et al. 2014). A recent study by Aghaee and Godfrey (2015) compared granular application of *Bacillus thuringiensis* spp. *galleriae* in California rice to the common insecticide  $\lambda$ -cyhalothrin. *Bt.galleriae* was found to be, in some green house and field experiments, as effective as  $\lambda$ -cyhalothrin at managing RWW larvae.

Puissegur's (1976) thesis examined the ability of predators found in rice fields to feed on RWW. No-choice predation experiments were performed in tanks of water with no shelter or barriers present; 15 out of 19 predators tested readily consumed RWW larvae. No choice experiments were also performed on Orthoptera predation of RWW adults, with mixed results. Gut content analysis of frogs collected in the field had low rates of RWW adult remains. Field cage studies found that immature *Pantala* sp. (Odonata: Libellulidae) significantly reduced RWW numbers compared to no predator controls, although only four replications were performed. These were basic tests and only demonstrated predator recognition of larvae as prey.

A study in California rice found that *Pardosa ramulosa* (Araneae: Lycosidae), a generalist predator, populations in rice paddies peaked in June (Oraze et al. 1989). *P. ramulosa*'s population peak coincided when RWW populations are also high, indicating the possibility of predation and biological control, although RWW was not mentioned in the study. However, high densities of *P. ramulosa* were found to led to cannibalism (Oraze and Grigarick 1989). If non-microbial biological control is to be used to help manage RWW, more studies are needed to better understand the population dynamics of predators in rice fields.

### 2.1.3 RWW Biological Control Methods

Biological control methods generally are grouped into importation (also known as classical), the release and establishment of foreign predators, augmentation, rearing predators or parasitoids (exotic or native) and then releasing them in mass, and conservation biological control, which manipulates agricultural environments to increase natural enemy abundance to manage pests (Gilstrap 1997, Landis et al. 2000, van Lenteren 2012). Importation and augmentation are not ideal methods for RWW management as they both require knowledge of specific natural enemies of the pest, and currently no favorable candidates are known (Gilstrap 1997, van Lenteren 2012). Conservation biological control has the advantage of promoting natural enemy abundance and diversity, increasing the likelihood of identifying RWW natural enemies (Landis et al. 2000, Gurr et al. 2003, Bianchi et al. 2006). Conservation biological control can be implemented in several different ways weed strips would contain alternate hosts for RWW and likely be counterproductive and no parasitoids or predators are known to target with food sprays (Palrang et al. 1994, Landis et al. 2000, Wade et al. 2008).

Conservation biological control has been performed in Asian rice fields (Settle et al. 1996, Jiang and Cheng 2004, Lou et al. 2013). Settle et al. (1996) hypothesized that detrital subsidies (composted cow manure) would cause a trophic cascade increasing local predator populations early in the season. The cascade would be caused by an increase in detritivores from an increase in resources leading to larger predator populations which could exert a negative influence on herbivores once they established later in the season. To try and mitigate the difficulty of biological control in ephemeral habitats, the authors allowed the field to be flooded for three months prior to planting. The authors found that detrital subsidies had a positive effect on the abundance of generalist predators and was followed by a significant decrease in the abundance of herbivores. No details were given on the composition of any of the feeding guilds.



Jiang and Cheng (2004) replicated Settle et al. (1996) in China, finding a similar, although weaker, response by predators to detrital additions in their ability to control whitebacked planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae). However they indicated that predators may prefer Collembola over *S. furcifera* as prey, which may have lowered their ability to act as a control agent. The reason for the weaker response of predators compared to Settle et al. (1996) may be due to either lower levels of detritus (0.83 kg/m<sup>2</sup> vs. 0.53 kg/m<sup>2</sup>) or that Settle et al. (1996) measured and compared predators and herbivores as a whole guild where as Jiang and Cheng (2004) measured the response of predators and a specific herbivore.

While studies have found the effectiveness of detrital subsidies to be inconsistent among different crops and pests, as discussed previously they can be effective in rice systems (Settle et al. 1996, Wise et al. 1999, Halaj and Wise 2002, Jiang and Cheng 2004, Oelbermann et al. 2008). This, coupled with studies demonstrating detrital additions in natural aquatic habitats greatly increasing predator abundance as well, indicates this to be a good method for an exploratory study of conservation biological control effectiveness for managing RWW (Ogren and King 2008, Hagen et al. 2012).

This study had three main objectives. The first was to test the ability of detrital subsidies to increase the species abundance, diversity and richness of natural enemies in rice fields. Detrital additions are expected to increase detritivores (alternate prey) leading to an increasing in predators. The second was to determine if this increase has an impact on RWW populations, with the expected result of the larger predator population having a negative effect on RWW abundance. The third was to thoroughly survey invertebrates present in rice fields during sampling of detrital effects, allowing for identification of possible RWW predators as well as an overall assessment of arthropod diversity in rice fields in southern Louisiana.

## 2.2 Materials and Methods

### 2.2.1 2013 Field Season

#### 2.2.1.1 Field Sites

Detrital subsidies were investigated at the LSU AgCenter Rice Research Station, Crowley, Louisiana. Treatment and control status was randomly assigned between 16 24.2m x 4.9m plots, eight controls and eight treatments. Plots were separated by a 1m levee. Due to space constraints a buffer region between plots was not possible. The levees between plots however provided a physical barrier that prevented water and detrital transfer between plots (Wise et al. 1999).

Detrital subsidies were applied to treatment plots in the form of Hopi-Gro Cow Manure and Compost Mix (Hopi-Gro, Hope Agri Products Inc., Hope Arkansas U.S.A.). Detritus was hand cast into unflooded treatment plots on March 26<sup>th</sup> and again into flooded plots on April 16<sup>th</sup> at a rate of 18.5kg per plot on both dates. All plots were flooded two days later, March 28<sup>th</sup>, with well water and floods were maintained for the duration of the experiment. While not ideal for growing rice in southern Louisiana, a continuous flood is likely more beneficial than standard water management practices for establishing and maintaining a relatively higher aquatic and semi-aquatic predator population (Settle et al. 1996). On April 25<sup>th</sup>, all plots were water seeded with 1.4kg of Cheniere which had been soaked for 24hr and allowed to dry another 24hr before planting (Blanche et al. 2012). A delay of 29d between flooding and planting was to allow aquatic predators to become established before the rice plants emerged and attracted RWW (Settle et al. 1996, Stout et al. 2002b). Rice plants began to emerge above the water's surface on May 8<sup>th</sup>. No pesticides were used in this experiment as they can have a range of effects on arthropod communities (Cohen et al. 1994, Geiger et al. 2010, Evans et al. 2010, Wrinn et al. 2012, Rittman et al. 2013). Sampling began on April 4<sup>th</sup> and was done every other week thereafter.

### 2.2.1.2 Sampling

In order to assess detrital effects on invertebrate populations, plots were sampled for aquatic fauna every two weeks using four different methods: floating pitfall traps, sweeps with D-nets, crayfish Gee traps and root/soil corers. Each of these methods was meant to sample a slightly different microhabitat in the rice field. Floating pitfall traps caught invertebrates on or near the water surface and those that fell from the canopy, D-nets sampled those in the water column and water surface, crayfish traps caught larger aquatic invertebrates and cores sampled the benthos. Sampling began two weeks after flooding on April 4<sup>th</sup> and continued until July 16<sup>th</sup> for a total of eight sampling dates.

A 42cm x 23cm 1.27cm mesh cylinder Gee crayfish trap with 2.5cm indented coned openings at each end was baited with a piece of commercial crayfish pellet bait and placed in a random location within the plot 48hrs before sampling. This trap was removed during sampling and all invertebrates were collected. Aquatic netting was performed with a standard 30cm, 500 micron mesh, aquatic D-net with a 2m sweep in each plot. Invertebrates collected by these two methods were placed in 80% ethanol and taken back to the lab where they were sorted under a dissecting scope. Identification was done to the lowest taxonomic level feasible using relevant keys and voucher specimens in the Louisiana State University Arthropod Museum (Arnett and Thomas 2000, Arnett et al. 2000, Triplehorn and Johnson 2005, Merritt et al. 2008).

Floating pitfall traps (see Parys and Johnson (2011) for trap design) were deployed at the time of flooding every 6m along the midline of the plot and tethered to a 0.6m landscape stake that was hammered into the sediment for a total of three traps per plot. Traps were left out in experimental plots for the duration of the experiment and only removed for servicing. Each pitfall trap contained 2.5cm of Prestone® green anti-freeze. Floating pitfall traps were serviced by pouring their contents into Whirl-Pak® (Nasco©) bags, refilling them with anti-freeze and attaching them back to their anchor stake. Whirl-Pak® contents were identified and stored using the same methods used for Gee traps and D-

netting. Coring was done by taking a single root/soil core sample at a random location within each plot with a 9.2cm x 7.6cm metal corer.

Cored samples were washed through a 40 mesh screen sieve bucket, and the remaining material was collected, taken back to the lab, and stored at -20 C until later sorting. Sorting sediment samples for invertebrates was done in a 5cm deep pan filled with 2cm of water and invertebrates were identified using the same methods as described previously. All these methods led to a total of 6 samples per plot for a total of 96 samples per sampling date. Labeled voucher specimens collected from all four sampling methods were deposited in both the Louisiana State Arthropod Museum and the Rice Entomology Lab at Louisiana State University.

Starting June 6, monitoring of RWW populations began by taking an additional core during the biweekly sampling. Each core contained at least one rice plant. Both cores were washed through a 40 mesh screen sieve bucket, and the remaining material was placed in a salt water basin and the larvae and pupa were counted as they float to the surface (Stout et al. 2001). One of these cores from each plot was randomly chosen and recollected from the sieve and sorted for invertebrates using the previously mentioned methods.

### **2.2.1.3 Statistical Analysis**

In order to determine detrital effects on aquatic invertebrate populations, several different methods were employed. Invertebrate species abundance was compared between compost-manure and controls using a paired t-test which would allow for a basic comparison of effects that detritus has on the community as a whole. Species diversity and richness, Shannon-Weiner Index (H) and abundance based coverage estimate (ACE) respectively, were computed as another metric of comparing compost-manure and control plot invertebrate communities. H and ACE indexes were quantified for each plot across all sampling dates with EstimateS v. 9.1.0 (Colwell 2013). EstimateS was performed with 100 randomizations of species orderings without replacement and 10 individuals as the cutoff for rare

species. Treatment and control scores were then compared using the nonparametric Wilcoxon sign rank test (Crawley 2007). Rarefaction curves for compost-manure and control and their 95% CI of species richness were obtained in EstimateS using the same parameters and compared for similarity using the conservative criteria of Colwell et al. (2012) and Colwell (2013).

Another metric of comparing species diversity is with ordination procedures using principle component analysis (PCA), detrended correspondence analysis (DCA), and non-metric multidimensional scaling (NMDS). To select the ordination most appropriate for the data, axes length and STRESS from these tests were assessed based on criteria described in ter Braak (1995), Leps and Smilauer (2003) and Hirst and Jackson (2007). Multi-response permutation procedure (MRPP) was then performed to determine differences between treatment and controls plots.

To assess predator and prey population changes and their relationships over time species counts were combined, regardless of treatment, broken into their respective groups (Merritt et al. 2008), log transformed [ $\log(x+1)$ ] and plotted by date. Relationships were assessed over time using analysis of covariance (ANCOVA). Treatment effects on RWW larvae were assessed using a t-test. Unless otherwise stated, all statistical analyses were performed in R v 3.0.3 (R Core Team 2014).

## **2.2.2 2014 Field Season**

### **2.2.2.1 Field Sites**

The 2014 field experiment was conducted in a manner similar to the previous year with some exceptions. The experiment was performed at a different location within the same station. A total of eight 62.5m x 5.8m plots were broken up into two blocks, with four plots on the north side of the central lateral and four plots on the south side with a minimum of 24.4m between each plot. Plots in each block were randomly assigned to control or compost-manure treatments, i.e. two compost-manure plots and two control plots in each block. All plots were flooded permanently on April 22.

The same detrital subsidy and application methods were used. However the rate of compost-manure was increased from 18.5kg to 72.6kg per plot per treatment application reflecting the increase in plot size, roughly 3x larger, as well as to increase ratio of detritus to plot area. The number of applications was also increased from two to four starting on April 22<sup>nd</sup> (day of flooding), then on May 16<sup>th</sup> and 30<sup>th</sup>, and June 12<sup>th</sup>. On May 23 plots were water seeded using the same methods as the previous year except 3.47kg per plot was planted to keep plant density similar (Thompson et al. 1994). A single application of the herbicide Basagran was applied to each plot on June 17<sup>th</sup> due to heavy weed infestation. The herbicide mixture consisted of 0.0167ml Basagran and 0.0079ml Voyager per liter of water with a total rate of 6.3L per plot. No other pesticides were applied.

#### 2.2.2.2 Sampling

Sampling in 2014 began May 7<sup>th</sup> and continued every other week until July 16 (n=6). The methods used were the same as the previous year with four exceptions, mostly to account for the larger plot size. The number of floating pitfall traps per plot was increased from three to four and traps were spaced 12.2m apart along the midline of the plot. A second Gee trap was added as well as an additional sweep with a D-net for a total of eight samples per plot per sampling date (72 samples per sampling date). Coring was increased to three per plot and was added to the sampling regimen on June 18<sup>th</sup> for RWW larvae monitoring, no other invertebrates were collected from cores for reasons discussed later.

Two additional sampling methods were added to the regimen. First, chlorophyll a was measured as it is another component of detrital food webs and a measure of water quality (Fabricius et al. 2012, Cochard et al. 2014). Second, dissolved oxygen was measured to determine if detrital additions were limiting dissolved oxygen, potentially impacting invertebrates (King and Brazner 1999). Chlorophyll a concentration was measured by taking a 1L water sample from each plot in the early afternoon of the sample day. Sample filtration was performed by the Freshwater Ecology Lab, School of Renewable Natural Resources, LSU Baton Rouge, LA 70803. These filtered samples were then analyzed for their

chlorophyll a content by the Wetland Biogeochemistry Institute Analytical Services, LSU Baton Rouge, LA 70803. Measurement of dissolved oxygen was taken using a HACH Dissolved Oxygen Test Kit Model OX-2P made by the HACH Company.

### **2.2.2.3 Statistical Analysis**

Statistical analysis was the same as in 2013 with the exception of ACE and H comparisons. Since the eight plots were broken into two blocks, a blocked ANOVA was done on H and ACE scores instead of Wilcoxon signed rank test. Chlorophyll a and dissolved oxygen were compared between treatment and controls using ANOVA.

### **2.2.3 Cataloging Rice Field Diversity**

To assess the invertebrate richness of rice plots, counts from treatment and controls for both years were combined and totaled. A sample-based rarefaction curve was plotted from this combined data set in EstimateS using the same settings as stated previously (Colwell 2013). This curve was used to estimate how much of the invertebrate richness had been sampled. When the estimated richness line becomes completely or nearly asymptotic, the interpretation is that all species have been sampled, and the difference between the curve and the theoretical maximum richness is a measure of any additional sampling needed (Colwell et al. 2012).

## **2.3 Results**

### **2.3.1 2013 Field Season**

#### **2.3.1.1 Invertebrate Abundance**

During the 2013 season, samples were taken on April 4<sup>th</sup> and 16<sup>th</sup>, May 8<sup>th</sup> and 23<sup>rd</sup>, June 6<sup>th</sup> and 20<sup>th</sup> and July 5<sup>th</sup> and 16<sup>th</sup> for a total of 687 samples from pitfall traps, Gee traps, D-nets and sediment corers. From these samples 22,873 invertebrates were collected comprising 101 species in 15 orders (Table 1). Of these individuals, 71% were Collembola (45%) and Chironomidae (26%). Due to the large number of these two taxa found in samples, some with over 400 individuals, counts of both taxa

Table 1. Number of individual insects sampled and number of species found in manure-compost and control plots for 2013 and 2014 for all four sampling methods. P is from a paired t-test comparison of species abundance found between compost-manure and controls.

	2013				2014			
	Species	Number of Individuals	t	p	Species	Number of Individuals	t	p
Compost-Manure	87	11,943	1.20	0.23	113	14,086	-0.83	0.41
Control	91	10,930			115	13,343		
Total	101	22,873			134	27,429		

were limited to 100 individuals per sample. This meant that if a sample contained 400 Collembola, only the first 100 were counted. The next highest number of individuals found in any one sample was 61 Lycosidae. No other counts of an individual species from a single sample exceeded 30. Species that were found in plots of one treatment but not the other did not exceed two representatives (i.e. two *Haliphus* sp. individuals were found in compost-manure plots but not in control plots) with the exception of Ichneumonidae of which only three representatives were found in control plots.

To compare the abundance of species between treatments, taxonomic resolution had to be consistent between adults and immatures. In cases that they were not, higher resolved taxons were downgraded to match the other life stage for the purposes of analysis (i.e. Adult *Tropisternus* sp. were identified to *T. lateralis* and *T. collaris*, whereas immature *Tropisternus* sp. were not identified past genus and therefore all *Tropisternus* counts were combined to the genus level for analyses). This led to a decrease from 101 unique taxa to 95 for comparison. A paired t-test found no significant differences in species abundance between compost-manure and control plots ( $t = 1.2$ ,  $P = 0.23$ ) (Table 1).

### 2.3.1.2 Detrital Subsidy Effects on Diversity

The same taxonomic reduction that was done for abundance comparison, as mentioned previously, was done for count data used in EstimateS. No significant difference was found between compost-manure and controls for either H ( $Z = 0.16$ ,  $P = 0.9$ ) or ACE ( $Z = -0.84$ ,  $P = 0.44$ ) (Table 2). Comparisons of sample based rarefaction curves indicated no interpretable differences between



Table 2. Species richness (abundance coverage estimator, ACE) and species diversity (Shannon-Wiener index, H) comparisons between compost-manure and control plots for 2013 (n = 16) and 2014 (n = 8). A Wilcoxon signed rank test was used in 2013 and a blocked ANOVA in 2014.

	2013				2014			
	ACE		H		ACE		H	
	Compost-Manure	Control	Compost-Manure	Control	Compost-Manure	Control	Compost-Manure	Control
Mean	65.29	70.91	1.55	1.60	83.45	94.11	1.95	1.91
SE	4.92	4.88	0.05	0.05	6.30	10.14	0.05	0.06
Statistics	Z = -0.84		Z = 0.16		F = 5.72		F = 0.65	
p	0.44		0.9		0.06		0.45	

treatments. While compost-manure's curve reached the controls upper 95% confidence interval (CI), the control curve was well within compost-manure's 95% CI (Fig. 1). Neither curved reached an asymptote, suggesting that more samples are needed to fully assess species richness in rice fields.

Ordinal procedures look at relationships between points in a matrix and do not require consistent taxonomic resolution between life stages, which was necessary for H and ACE computation. Ordination was performed with the R package *vegan* (Oksanen et al. 2013, R Core Team 2014). Initial data testing by PCA found curvilinear distortions in the data (i.e., data points distributed largely along the edge of the graph axes), signaling the data was likely unimodal (Hirst and Jackson 2007). To correct for unimodality, DCA, which breaks the data into segments and realigns the data to better fit a linear model, was performed (ter Braak 1995). As DCA employs chi-squared based distance, all samples that failed to capture invertebrates had to be removed from the data set, reducing the number of samples tested from 687 to 580. The DCA axes lengths were found to be greater than four (the data was being greatly stretched) indicating that a distance based method would work best (Leps and Smilauer 2003).

NMDS, which arranges points by their relationships with each other with as little stress as possible, found the stress to be nearly zero indicating that too many rare species were present (stress = 0.07) (Kruskal and Wish 1978, Leps and Smilauer 2003). To correct for this, rare species, less than ten individuals sampled during the season, were removed from the counts, reducing the number of species from 101 to 66 species and 577 samples. PCA and DCA were performed on this modified data to

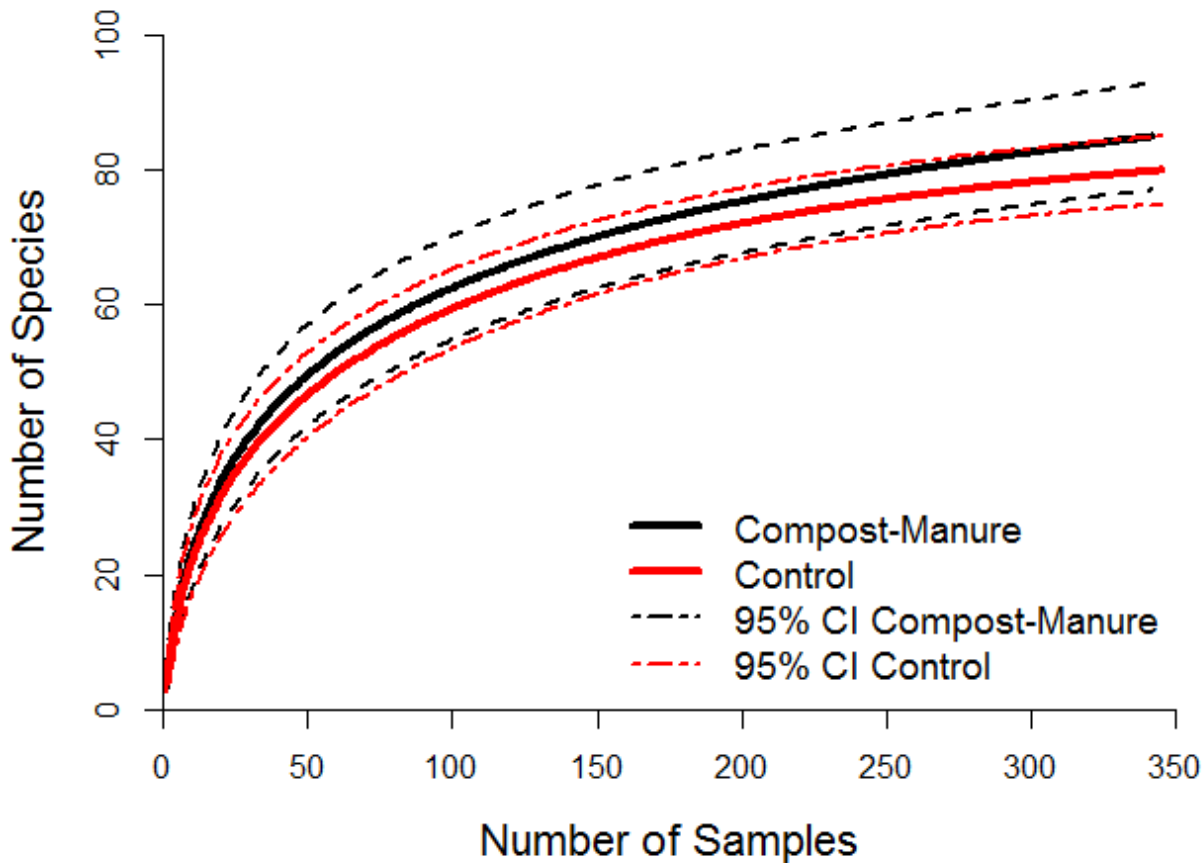


Fig 1. 2013 Sample based Rarefaction curves, number of species expected to be found for a given number of samples in compost-manure and control plots. Comparing Species richness between control and compost-manure plots.

determine how much alteration had occurred, and found to be largely unchanged, indicating NMDS was still the best method of assessing the data. NMDS found no separation between compost-manure and control plots (stress = 0.11). Date and plot (regardless of treatment), however, were found to significantly explain the grouping of samples and species ( $P = 0.001$ ,  $P=0.042$  respectively (Table 3, Fig. 2)). This indicates clustering of species and samples were best explained by differences over time and individual plots. Regression analysis was performed on distance between species and sites using Bray-Curtis and Euclidean methods, which found no relationship between species and treatments ( $R^2 = 0.009$ ) (Krebs 1999). MRPP, which is used in place of multivariate analysis of variance because of its fewer assumptions, also failed to find any relationships among species ( $F = 0.69$ ,  $R^2 = 0.001$ ,  $P = 0.80$ ) (Table 4) (Zimmerman et al. 1985). RWW larvae monitoring counts were taken on the last four

Table 3. Non-metric multidimensional scaling (NMDS) from 2013 and 2014 correlation ( $R^2$ ) and significance (p) for different explanatory factors of species and sample relationships.

Factors	2013		2014		
	$R^2$	p	$R^2$	p	
Date	0.08	0.001	0.06	0.001	**
Plot <sup>1</sup>	0.01	0.042	0.02	0.027	.
Compost-Manure	0.004	0.35	0.0003	0.96	
Control	0.004	0.35	0.0003	0.96	

Significance codes: 0.0001 = ‘\*\*\*’ 0.001 = ‘\*\*’ 0.01 = ‘\*’ 0.05 = ‘.’

<sup>1</sup>Plot is comparing individual plots to one another, not by control and compos-manure

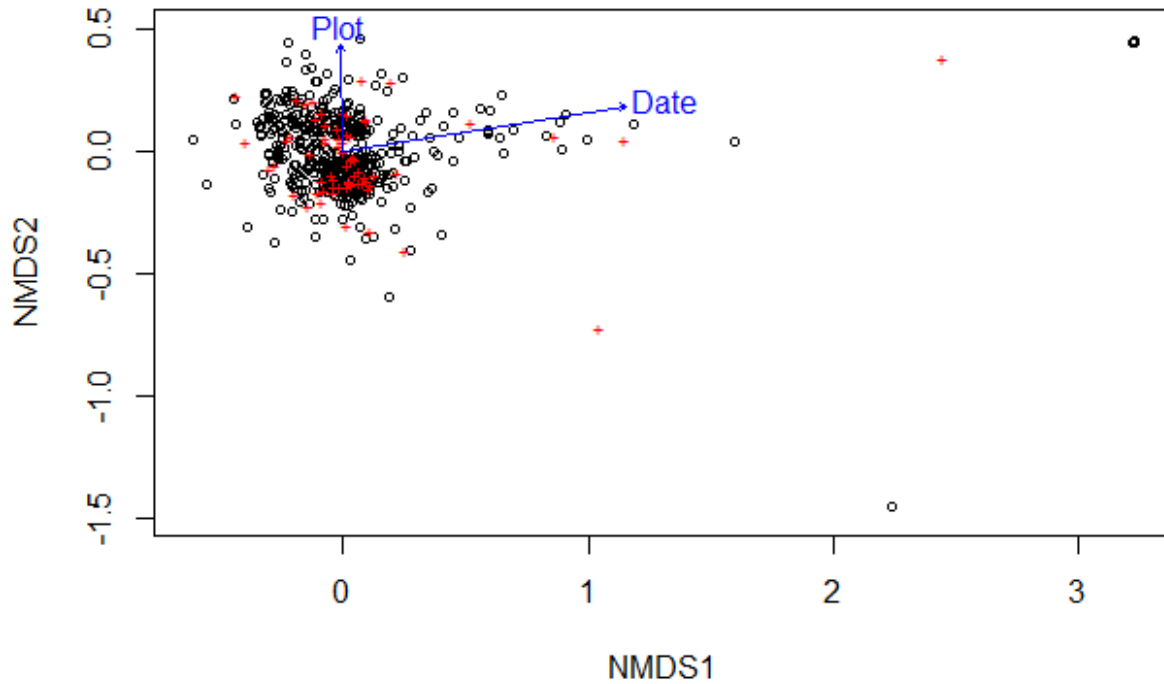


Fig. 2: Non-metric multidimensional scaling (NMDS) relative similarity plot of species (+) and samples (o) for 2013. Labeled arrows indicated relative strength of significant factors explaining grouping.

sampling dates, June 6<sup>th</sup> to July 16<sup>th</sup>. T-test found that RWW larvae numbers did not significantly vary among compost-manure and control plots on any sample date (Table 5).

### 2.3.1.3 Predator Prey Populations

Comparison of predator and prey populations over time indicated that they roughly paralleled each other during the course of the experiment (Fig. 3). Initial populations were significantly different from one another, prey being more abundant than predators (Type,  $F = 85.11$ ,  $p < 0.0001$ ) as well as

Table 4. Multi-response permutation procedure (MRPP) of species counts for 2013 field season between compost-manure and control plots

	df	SS	Mean sqs	F	R <sup>2</sup>	p
Compost-Manure	1	0.29	0.29	0.69	0.001	0.8
Residuals	575	246.66	0.43		0.999	
Total	576	237.96			1	

Table 5. Comparison of rice water weevil larval counts from compost-manure and control plots. P-values and F statistics for paired t-test between control and compost-manure counts.

Sample Date	Compost-manure		Control		F	p
	Mean	SE	Mean	SE		
6-Jun	5.4	0.84	7.5	1.1	2.37	0.15
20-Jun	15.69	1.65	15.38	2.26	0.01	0.92
5-Jul	12.38	1.08	9.82	0.98	2.24	0.16
18-Jul	5.44	1.19	4.81	0.87	0.17	0.69

exhibiting a significant linear trend over time (Date,  $F = 55.16$ ,  $p < 0.0001$ ) (Table 6). The sharp decrease in prey populations on June 20<sup>th</sup> was due to a large decline in Chironomidae adults (Fig. 3). Non-significant interactions of predator and prey population growth (Date:Type)(whether testing for linear, squared or cubed slopes) indicates that both populations paralleled each other over the course of sampling (Table 6).

### 2.3.2 2014 Field Season

#### 2.3.2.1 Invertebrate Abundance

Over the 2014 season, 384 samples were taken over six sampling dates: May 7<sup>th</sup> and 22<sup>nd</sup>, June 4<sup>th</sup> and 18<sup>th</sup> and July 3<sup>rd</sup> and 16<sup>th</sup>. A total of 27,429 individuals were caught spanning 134 species in 13 orders. The difference in orders from the previous year is due to differences in sampling. Corers were the only sampling method that found Annelids and Gastropods in 2013, neither of which are likely to have an impact on RWW. For this reason as well as time management, coring was not performed for invertebrate sampling, only for RWW monitoring and subsequently no Annelids or Gastropods were

Table 6. Analysis of covariance (ANCOVA) for 2013 between predator and prey populations. Date represents the slope of the lines, with linear, squared and cubed slopes respectively. These indicate if a population shows a linear, squared or cubed trend over the season. Type represents differences in intercepts between the two populations and Date:Type are possible interactions between predator and prey slopes.

	df	SS	Mean Sq	F	p	
Date	1	4.87	4.87	85.11	<0.0001	***
Date <sup>2</sup>	1	0.22	0.22	3.86	0.08	
Date <sup>3</sup>	1	0.04	0.04	0.67	0.44	
Type	1	3.16	3.16	55.16	<0.0001	***
Date:Type	1	0.17	0.17	2.98	0.12	
Date <sup>2</sup> :Type	1	0.03	0.03	0.46	0.52	
Date <sup>3</sup> :Type	1	0.0001	0.0001	0.002	0.97	
Residuals	8	0.46	0.06			

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Significance codes: 0.0001 = '\*\*\*' 0.001 = '\*\*' 0.01 = '\*' 0.05 = '.'

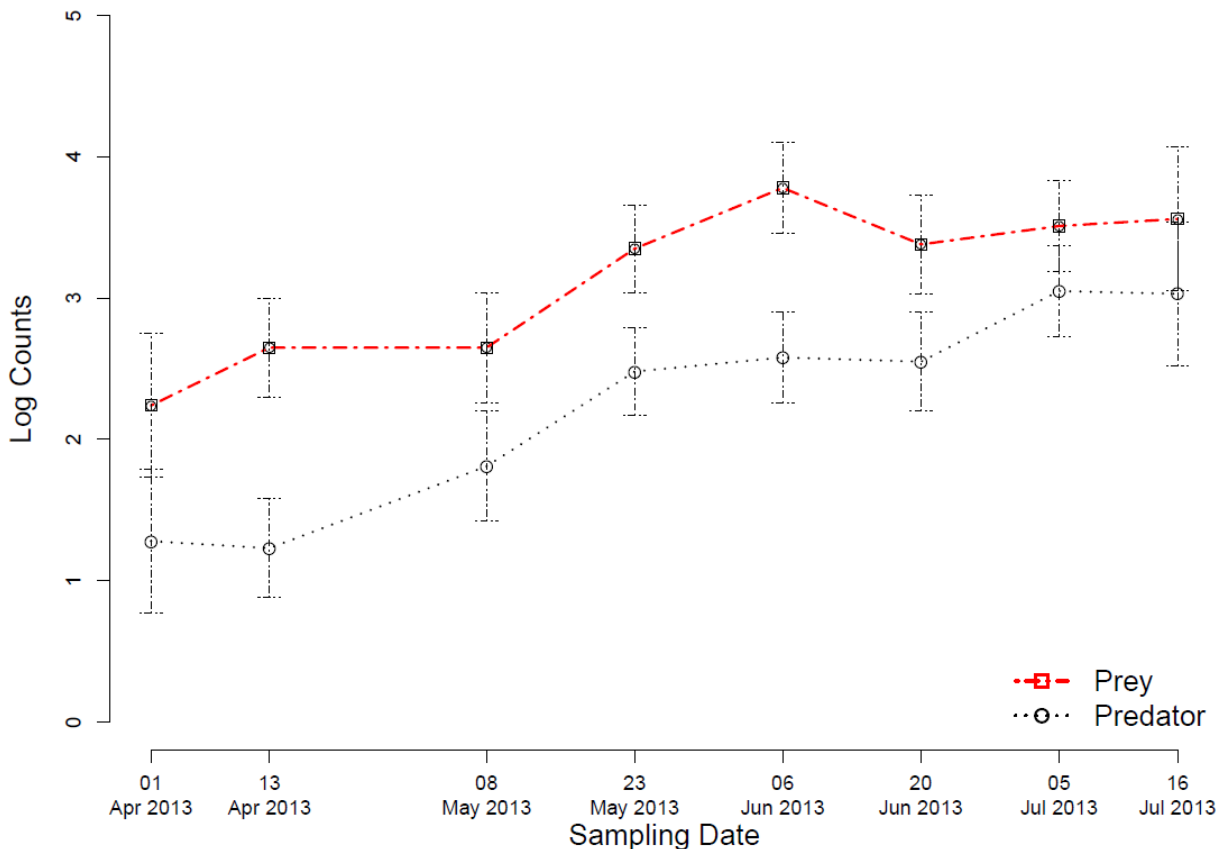


Fig. 3. Log transformation of predator and prey counts of individuals from all plots and sample methods plotted by date for 2013. Error bars represent 95% CI.

found in 2014. As in the previous year, the bulk of the individuals caught, 80%, were either Sminthuridae, 41%, Isotomidae, 23%, (both Collembolans) or Chironomidae, 16%. Counts were limited to 100 individuals per taxa for the same reason as in 2013. The next highest count from a single sample was 46 unidentified Lycosidae, most of which appeared to be from a recently hatched egg sac. Similar compression of species number as in 2013 was done and analysis found no significant difference in the abundance of species between compost-manure and controls ( $t = -0.83$ ,  $P = 0.41$ ) (Table 1).

### 2.3.2.2 Detrital Subsidy Effect on Diversity

Plot sizes were increased from 2013 in order to reduce the ratio of plot edge to area, 0.49 to 0.38, and were spaced out from each other to further reduce the likelihood of invertebrates moving from one plot to another (Gomez 1972, Sutcliffe et al. 1997). The rate of compost-manure used in 2013 failed to elicit a response, so the quantity and number of applications of compost-manure was increased to reflect the increase in plot size as well as to increase rates of detrital subsidies.

The same settings and data input methods for EstimateS were used as in the previous year to produce H and ACE scores. No significant difference was found between compost-manure and control plots for H ( $F = 0.65$ ,  $P = 0.45$ ) or ACE ( $F = 5.72$ ,  $P = 0.06$ ) (Table 2). The marginally non-significant difference in ACE score is in favor of higher richness in control plots. Wilcoxon signed rank test found a significantly higher species richness in the southern block compared to the northern block regardless of plot treatment ( $Z = -1.73$ ,  $P = 0.03$ ); however, diversity was not found to be significantly different between north and south blocks ( $Z = -1.73$ ,  $P = 0.11$ ) (Table 7). A sample-based rarefaction curve was plotted using the same methods as the 2013 season. A comparison of compost-manure and control curves found no interpretable difference (Fig. 4).

Initial testing of count data by PCA found evidence of curvilinear distortion. DCA failed initially because of sample zeros, which were removed, reducing the number of samples being tested to 354. Axes created by DCA with the modified count data were found to be greater than four indicating a

Table 7. Wilcoxon rank sum test comparing species richness (ACE) and species diversity (H) of invertebrates between north and south blocks in 2014

	Z	p
ACE	-2.31	0.03 .
H	-1.73	0.11

Significance codes: 0.0001 = '\*\*\*\*' 0.001 = '\*\*' 0.01 = '\*' 0.05 = '.'

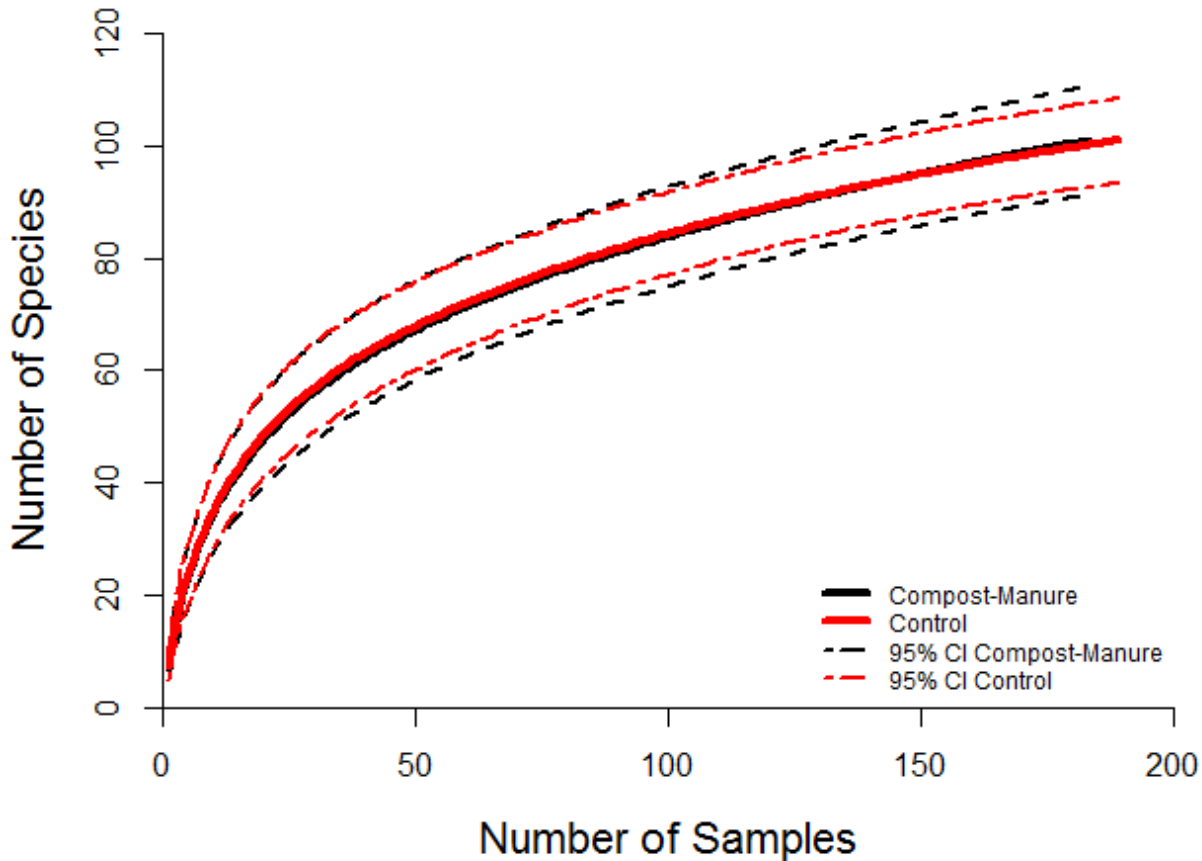


Fig. 4. 2014 Sample based rarefaction curves, comparing number of species expected to be found for a given number of samples in compost-manure and control plots.

distance method should be used (Leps and Smilauer 2003). NMDS found the stress to be nearly zero indicating that too many zeros were still present. Rare species were then removed, reducing the number of species to 70 from 353 samples. PCA was performed on the further altered data, plotted and found to have a similar shape as the original indicating that the removal of species and samples had not greatly altered the data. DCA again produced axes greater than four.

Table 8. Multi-response permutation procedure (MRPP) of species counts for 2014 field season between compost-manure and control plots

	df	SS	Mean Sqs	F	R <sup>2</sup>	p
Treatment	1	0.33	0.33	0.83	0.002	0.58
Residuals	351	140.6	0.4		0.99	
Total	352	140.89			1	

Two dimensional NMDS of the modified data found no separation between compost-manure and control plots (stress = 0.14) (Fig. 5). Regression of distances for species and sites using Bray-Curtis and Euclidean methods found no relationship between species and sites ( $R^2 = 0.03$ ) (Krebs 1999). MRPP also found no correlation between compost-manure and control plots ( $F = 0.83$ ,  $R^2 = 0.002$ ,  $P = 0.58$ ) (Table 8).

Chlorophyll a concentrations did not differ significantly between control and compost-manure plots ( $F = 0.97$ ,  $P = 0.33$ ). Monitoring chlorophyll a concentrations was stopped after June 18 because compost-manure was no longer being added. Dissolved oxygen was not measured on the first sampling date due to faulty equipment but was taken on the next three sampling dates. Titration for dissolved oxygen levels were consistently 7mg/L across all plots for the for all three sampling dates, after which no further testing of dissolved oxygen occurred for the same reason as Chlorophyll a.

Due to heavy weed pressure during the 2014 season, even after herbicide application, rice plants did not grow well and RWW larvae monitoring found only a few scattered larvae. Comparison of RWW larvae was not performed on 2014 data.

### 2.3.2.3 Predator Prey Populations

Predator and prey populations paralleled each other over the course of the 2014 season (Fig. 6). ANCOVA found the populations to exhibit a significant linear trend in growth over the course of sampling ( $F = 33.84$ ,  $p = 0.004$ ) (Table 9). Initial prey populations were also significantly higher than predator populations ( $F = 296.98$ ,  $p < 0.0001$ ) (Table 9). Non-significant interactions of predator and



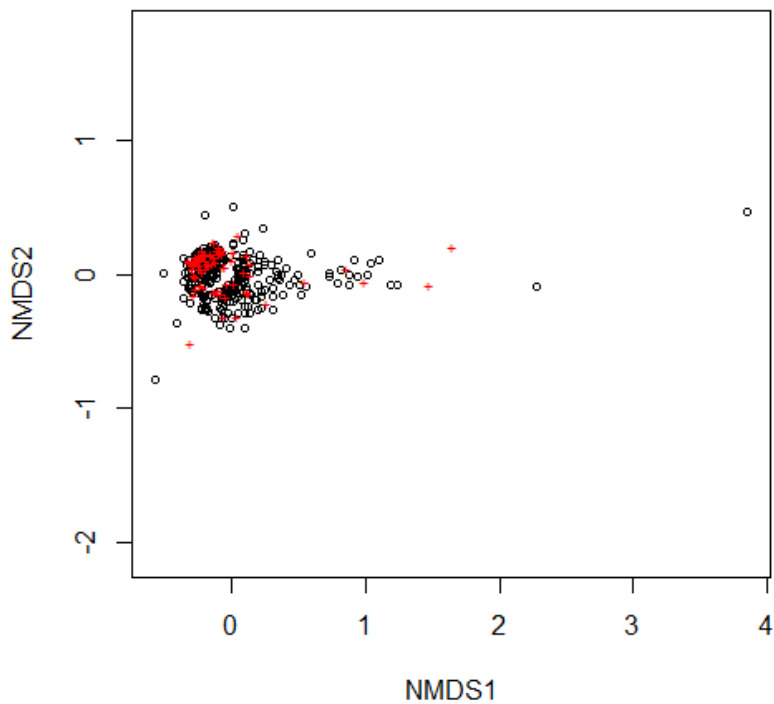


Fig. 5. Non-metric multidimensional scaling (NMDS) relative similarity plot of species (+) and samples (o) for 2014.

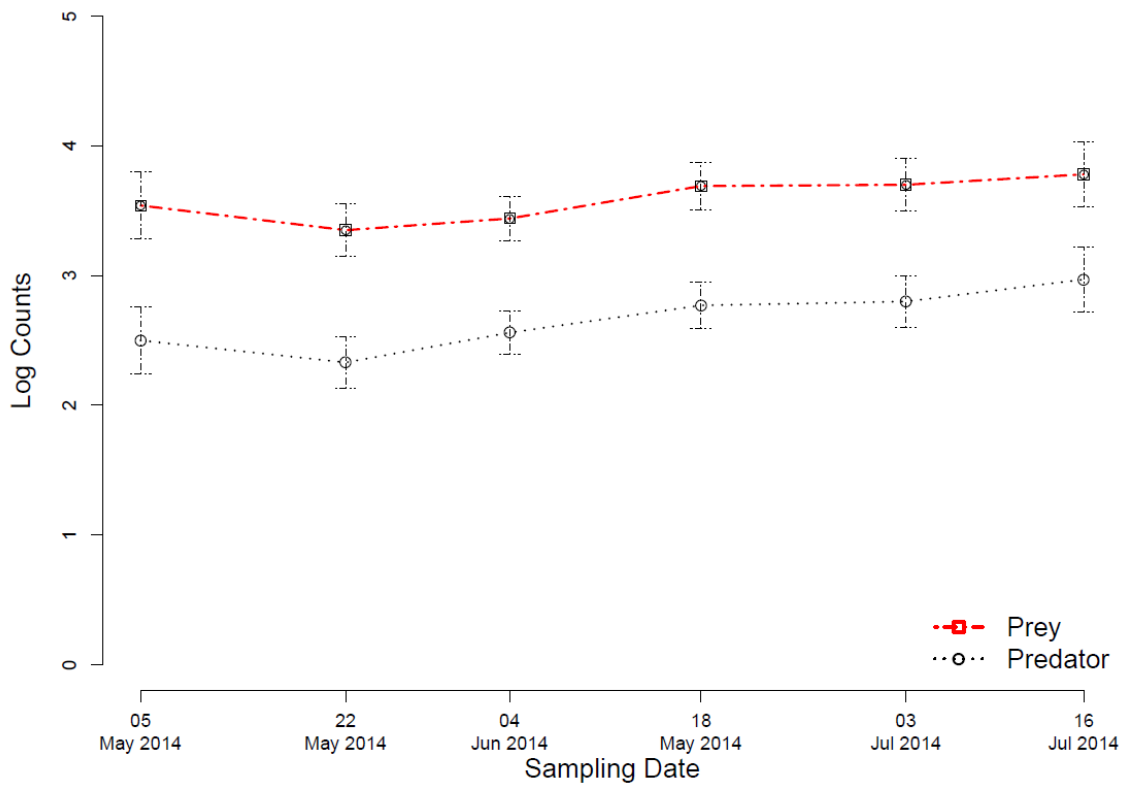


Fig 6. Log transformation of predator and prey counts of individuals from all plots and sample methods plotted by date for 2014. Error bars represent 95% CI.

prey population growth (Date:Type)(whether testing for linear, squared or cubed slopes) indicates that both populations paralleled each other over the course of sampling (Table 9).

### 2.3.3 Catalog of Species

Over the course of the two seasons, 1,152 samples were taken using four sample methods, yielding 146 different species spanning 15 orders for a total of 50,302 individuals. Of the 15 orders Collembola represented 56% (27,616 individuals) of the individuals collected with Diptera being the next closest order at 24% (12,049). Of the Diptera collected 87.5% (10,403) were either adult or larvae Chironomidae. These counts are likely greatly underestimating the true values because counts of both were capped at 100. Araneae (5.8%, 3,746) and Coleoptera (5.7%, 2,841) were the next largest orders (Appendix).

Coleoptera was the most species-rich order, comprising 54 different species. Hemiptera and Diptera were the next two most species-rich orders with 28 and 23 species respectively (Appendix). Of the 146 species found over both years, 76 (52.1%) were rare (10 or less individuals found). Of these rare species, 20 (13.7%) were singletons. Counts from all samples for both seasons were combined to create a sample-based rarefaction curve and was then extrapolated for an additional 500.

Table 9. Analysis of covariance (ANCOVA) for 2014 between predator and prey populations. Date represents the slope of the lines, with squared and cubed slopes respectively. These indicate to see if a population changes over the season. Type represents differences in intercepts between the two populations and Date:Type are the differences between predator and prey slopes.

	df	SS	Mean Sq	F	p	
Date	1	0.29	0.29	33.84	0.004	.
Date <sup>2</sup>	1	0.02	0.02	2.72	0.17	
Date <sup>3</sup>	1	0.04	0.04	5.03	0.09	
Type	1	2.59	2.59	296.98	<0.0001	***
Date:Type	1	0.02	0.02	1.74	0.26	
Date <sup>2</sup> :Type	1	0.0001	0.0001	0.01	0.92	
Date <sup>3</sup> :Type	1	0.001	0.001	0.07	0.81	
Residuals	4	0.03	0.01			

Significance codes: 0.0001 = '\*\*\*' 0.001 = '\*\*' 0.01 = '\*' 0.05 = '.'

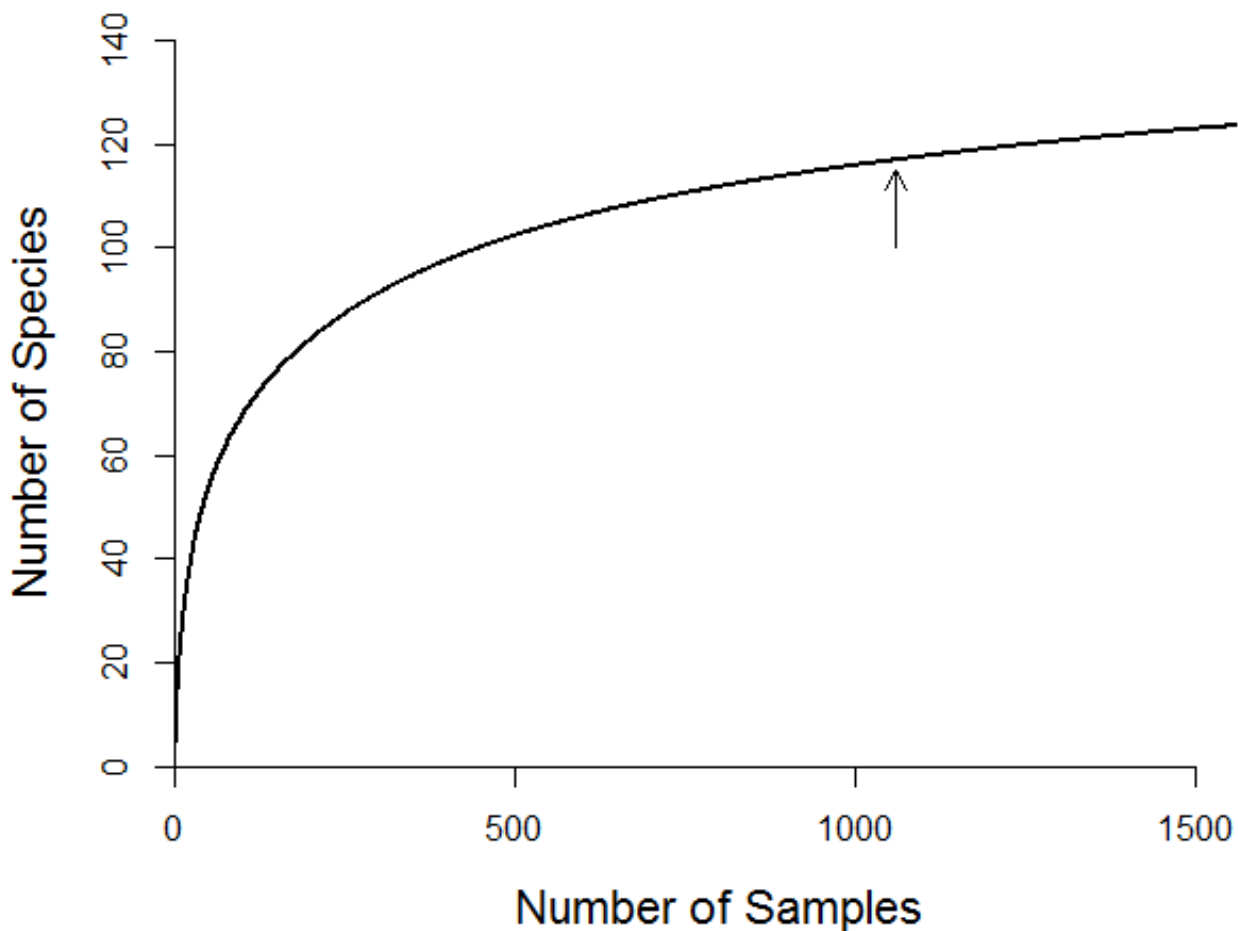


Fig. 7. Sample based rarefaction curve of all samples from 2013 and 2014 with a 500 sample extrapolation. Arrow indicates beginning of extrapolation

samples. The same settings in EstimateS were used as in 2013 and 2014 analysis. The number of taxa used for computation by EstimateS was reduced from 146 sampled species to 114 for the previously mentioned reasons and Annelids and Gastropods were excluded as well due to the difference in sampling between years. Neither the actual number of samples nor the extrapolated curve reached an asymptote (Fig.7).

## 2.4 DISCUSSION

### 2.4.1 Detrital Subsidy Experiment

Compost-manure additions failed to cause a significant increase in the invertebrate abundance or diversity for either season. This is unexpected as nutrient additions to aquatic systems have been shown

to cause an effect, positive or negative to aquatic systems (Cross et al. 2006, Ogren and King 2008, Zhou et al. 2008, Shaftel et al. 2011, Hagen et al. 2012). This is possibly due to: (1) an insufficient amount of detrital subsidy was applied to elicit a response from the arthropod community. Increasing application quantity, more frequent applications, or both may have been needed to cause a trophic cascade (Settle et al. 1996, Halaj and Wise 2002, Jiang and Cheng 2004, Schmidt et al. 2004). (2) Compost-manure is not a form of detritus that is useable or beneficial to arthropod detritivores in southern Louisiana rice fields. Detritus in a different form, such as mulch, may be more beneficial to detritivores, supplying a limiting factor(s) that compost-manure does not (Halaj and Wise 2002, Mathews et al. 2004, Schmidt et al. 2004). (3) Detritus is not what is limiting detritivore abundance, rather, another unknown factor(s) is limiting them (Halaj and Wise 2002). (4) Finally, experimental plots may be too small of a scale for detritus to noticeably affect diversity, and scaling plots up to a whole field may be required to elicit a trophic cascade (Garratt et al. 2011).

The most likely of the aforementioned reasons is not enough compost-manure was applied. The quantity of manure applied in Settle et al. (1996),  $0.83\text{kg/m}^2$ , and Jiang and Cheng (2004),  $0.52\text{kg/m}^2$ , where both higher than the amount used in this study,  $0.23\text{kg/m}^2$ , as this was an exploratory study. Increasing detrital levels closer to those used by either of the studies may be more successful in causing a trophic cascade. However after four detrital additions, total detritus applied was greater than Settle's et al. (1996) experiment,  $0.83\text{kg/m}^2$  vs.  $0.92\text{kg/m}^2$ .

Both predator and prey populations increased over the course of the season, more notably in 2013. As water for Louisiana rice fields comes from wells which are likely to lack invertebrates, establishing an aquatic environment early may allow for the predator populations to increase enough before RWW begin to migrate to rice fields.

During the month of April, which was only sampled in 2013, only 34 predators were found across all samples (Fig. 3). This is opposed to the 616 non-predacious invertebrates found during the

same month. Although we were unable to begin sampling in April in 2014, May 5<sup>th</sup> of 2014 found 314 predators and 3451 non-predacious invertebrates, much higher than May 8<sup>th</sup> 2013 sample date which found 63 and 442 predacious and non-predacious invertebrates respectively. Whether this is due to the difference in yearly variation, increase in compost-manure, or other factors is unknown. Understanding the responsible factor(s) for the difference in initial populations between years can aid in increasing predator abundance earlier in the season (Settle et al. 1996, Jiang and Cheng 2004).

Herbicide application was avoided to prevent any potential effects they might have on arthropods (Fowlkes et al. 2003, Sanchez et al. 2006, Evans et al. 2010, Wrinn et al. 2012, Rittman et al. 2013). Weeds, while present in 2013, did not appear to greatly hinder rice establishment or growth. The opposite was true for 2014, where weed densities were much higher, this site was at a different location at the same research station. Even with the application of Basagran, weeds still appeared to hinder rice establishment. The reason for such high weed numbers in 2014, more so in the southern block than then northern, is unknown (personal observation). Quantifying the density of weeds was attempted, however many of the weeds were false pimpernel (*Lindernia* spp.) and red ludwigia (*Ludwigia palustris*) which grow in dense mats and is extremely difficult to identify individual weeds to calculate densities. No significant difference in rice abundance between north and south plots was found (Table 10) (rice was measured by three randomly placed 1m x 1m squares in each plot).

Greater weed abundance in the southern block also correlated with a greater species richness (Table 7). A positive relationship between plant diversity and abundance with arthropod diversity has been shown in other wetland habitats (Meyer et al. 2011). RWW adults were also far more numerous in the southern block as well, although rice density was not significantly different between north and south blocks (Table 10). This is likely due to the higher abundance of alternate hosts present, although it is unknown if this corresponds to an increase in RWW larvae (Tindall and Stout 2003, Tindall et al. 2004).

Table 10. Comparison between North and South blocks for RWW adults found during 2014 sampling of invertebrates and the density of rice per m<sup>2</sup>

	SE	df	t	p	
RWW adults	0.99	41	3.54	0.001	**
Rice	27.99	6	2.07	0.08	

Significance codes: 0.0001 = '\*\*\*' 0.001 = '\*\*' 0.01 = '\*' 0.05 = '.'

While RWW larvae monitoring was not feasible for 2014, a significant difference between compost-manure and control plots is unlikely because the arthropod community was similar in both.

While the exact reason for a lack of a response to compost-manure additions is unknown, increasing rates would likely have some effect on the community, positive or negative. A positive increase in diversity would be the most desirable result, from a biological control point of view, as conservation biological control for RWW management could then be tested (Settle et al. 1996, Jiang and Cheng 2004). A negative result, a decrease in invertebrate diversity, would also provide a method of comparing different levels of diversity, and their effects on RWW. Detrital additions to non-crop aquatic systems have been shown to increase diversity including predators and should still be considered as a potential means of conservation biological control (Cross et al. 2006, Ogren and King 2008, Hagen et al. 2012).

#### 2.4.2 Catalog of Invertebrates

The combination of four different sampling methods allowed for the occurrence of a wide range of species (King and Porter 2005, Meyer et al. 2011). The small to large species on the water's surface and in the canopy were caught in floating pitfall traps, aquatic netting caught those in the water column, benthic organisms were sampled by the corer, and the larger aquatic species were caught by the Gee traps. Of the 146 species collected 52% of them were rare ( $\leq 10$  representatives) and 14% of them were singletons. The number of singletons found here is lower than expected for arthropods in southern Louisiana, which, being a sub-tropical region, is expected to be closer to 32% (Coddington et al. 2009,

Parys et al. 2013). If families, such as Chironomidae or Muscidae, were identified to the genus or species level the number of singletons would likely increase and approach 32%.

Over 5000 more individuals were caught in 2014 than in 2013. This difference, however, is mostly due to differences in taxonomic resolution of Collembola in 2013 and 2014 (order level identification versus family level) (Appendix). In 2013 Collembola counts were stopped at 100 in a given sample. This is opposed to 2014 where Collembola were identified to family level and counts for a family were stopped at 100. This means that in 2014, total Collembola could reach 200 in a sample, as opposed to the 100 in 2013. Even ignoring Collembola, 2013 had over 3000 more individuals, most likely because twice as many samples were taken in 2013. As mentioned in the result section, the number of Collembola and Chironomidae are greatly underestimated by this study.

During the second field season 20 more species were found than 2013 (ignoring Collembola, Carabidae and Staphylinidae due to taxonomic resolution differences), however much like differences between control and compost-manure plots within a season, the species that were found in one season but not in another had low representation, less than 10, most of which were represented by either one or two individuals. The exception to this was *Lysanthia ludoviciana* (Coleoptera: Chrysomelidae) which was found 153 times in 2014.

Collembola and Chironomidae, both likely to be detritivores were by far the most abundant, representing 63% of the individuals sampled over both years. This is underestimating the true abundance, as discussed in the results section, meaning that in southern Louisiana rice fields, detritivores constituted 2/3 of all arthropods. This differs from other arthropod surveys in rice system where herbivores are the most common (Heong et al. 1991, Catling and Islam 2013, Chen et al. 2013, Zhang et al. 2013). This is quite likely due to sampling method and geographic differences. Most rice surveys of arthropods in Asia use suction sampling as opposed to floating pitfall traps, which caught the majority of individuals in this study (Heong et al. 1991, Schoenly et al. 1995, Settle et al. 1996, Wilby et

al. 2006, Fritz et al. 2011, Catling and Islam 2013, Chen et al. 2013, Zhang et al. 2013, Cochard et al. 2014). Geographically more similar surveys such as Puissegur's (1976) survey of invertebrates in rice fields of Louisiana was performed with sweep and aquatic netting and found similar taxa to those in this study, although quantities for aquatic species found were not given. Bolduc's (2002) thesis, while only identified to order, survey of southern Louisiana wetlands found similar abundances of insects, with Diptera being the largest, followed by Coleoptera and Hemiptera. Parys's et al. (2013) survey of wetlands in Louisiana used floating pitfall traps, but found a far larger species richness of Hemiptera and Coleoptera (Diptera were not quantified).

This study does agree with Asian rice surveys and local surveys that Coleoptera is the most diverse order (Bolduc 2002, Catling and Islam 2013, Zhang et al. 2013). However, other studies of rice fields have found that Diptera is the most speciose order (Chen et al. 2013, Lupi et al. 2013). If the Diptera found in this study were to be identified down to genus or species, they may become the most speciose order sampled instead.



## CHAPTER 3. PREDATION EFFECTS ON RICE WATER WEEVIL OVIPOSITION

### 3.1 Introduction

To gauge the impact of predator-prey interactions on communities, ecologists have developed and tested models to help understand and predict the dynamics of these interactions and the effects these trophic interactions might have on the surrounding ecosystem (Brown et al. 1999). Classically, ecologists have used consumption of prey, or direct effects, to measure the impact of predators and subsequent trophic cascades (Schmitz et al. 1997). Models, theories and experiments based on this, however, miss an important aspect of predator-prey systems; the effect predators have on prey in their environment without direct interaction. Non-consumptive, or indirect effects, have been shown to dramatically alter the behavior and development of prey (Thaler et al. 2012, Brown et al. 2012).

The presence of predators can alter what host an immature may choose, where a female oviposits, as well as immature feeding and growth rate of prey (Schmitz et al. 1997, Thaler et al. 2012, Brown et al. 2012, Wasserberg et al. 2013). Trophic cascades can result from indirect effects of predator presence, from reduction in feeding rate by a herbivore increasing plant growth or herbivores altering their host selection in response to predators (Beckerman et al. 1997, Schmitz et al. 1997). In laboratory studies, indirect effects have been demonstrated to cause cascades as strong as those caused by direct effects making it an important consideration for biological control agents as measures of direct effects may be underestimating the effect that predators have on herbivores (Schmitz et al. 1997, Snyder and Wise 2000). Direct and indirect interactions are important to a more comprehensive understanding of ecology and a more successful implementation of pest management practices (Schmitz 1998, Wise et al. 1999).

#### 3.1.1 Rice Water Weevil

The most damaging insect pest of rice in Louisiana is the rice water weevil (RWW), *Lissorhoptus oryzophilus* (Kuschel), a native to Louisiana (Isely and Schwardt 1934, Hummel et al.

2012, O'Brien and Haseeb 2014). RWWs emerge in the spring and lay their eggs in the leaf sheath generally only after rice fields are flooded (Stout et al. 2002b). After hatching, larvae exit the leaf sheath and move toward the roots where they feed and use their dorsal spiracle hooks to pierce and breathe through the host's tissue (Zhang et al. 2006). While both life stages feed on rice, the larval stage that causes the most damage, with heavy infestation severely reducing root mass and yield whereas the damage caused by adults feeding generally has an insignificant effect on yield (Isely and Schwardt 1934, Stout et al. 2002a).

Puissegur (1976) tested the ability of predators found in rice fields to feed on RWW and analyzed gut contents of frogs for RWW remains. No-choice predation experiments were performed in tanks containing water, a predator and a number of RWW larvae. Out of the 19 predators tested, 4 did not consume RWW larvae. Field cage studies found that *Pantala* sp. (Odonata: Libellulidae) significantly reduced RWW numbers compared to no predator controls, although only four replications were performed. No-choice experiments were also performed on predation of RWW adults by Orthoptera, with mixed results. Gut content analysis of frogs revealed that RWW adults made up a small percentage of their diet, 4.5-9.3%. Field cage studies found that at least one predator is capable of influencing RWW numbers, however with only four replications, further studies are needed to determine *Pantala* sp. and other predator effectiveness in RWW control. Considering RWWs are native to Louisiana, they should be "knowledgeable" of predators that pose a risk and may potentially alter their behavior when dangerous predators are present (Brown et al. 1999, O'Brien and Haseeb 2014).

A study in California rice fields found that *Pardosa ramulosa* (Araneae: Lycosidae), a generalist predator, populations in rice paddies peaked in June (Oraze et al. 1989). *P. ramulosa*'s population peak is during the same time that RWW populations are also high, indicating a possibility for biological control, although RWW were not mentioned in the study. However high densities of *P. ramulosa* led to cannibalism (Oraze and Grigarick 1989).

With such limited knowledge on RWWs and their predators, a better understanding of not only a predator's inclination to consume RWW, but also of any indirect effects by predators on RWW behavior is important to better managing this pest. Several potential methods for measuring impacts of predators on prey exist, such as physiological and behavioral changes (Schmitz 1998, Thaler and Griffin 2008, Thaler et al. 2012, Wasserberg et al. 2013). An introductory look into whether or not RWW is "knowledgeable" of potential predators would test for a change in behavior of adults or larvae. Larvae are well hidden in the roots and monitoring any change in their behavior would be difficult (Webb 1914). The two common methods of monitoring RWW in greenhouse conditions, are to count the number of eggs that have been oviposited, done by destructively removing a rice plant, bleaching the whole plant in ethanol and counting the number of eggs in the leaf sheaths (Tindall and Stout 2003). The second method involves transferring the rice plant from the pot to a test tube of water and counting the number of larvae that emerge (Stout et al. 2002b, Tindall and Stout 2003, Cosme et al. 2011). In the context of predation both of these methods measure direct as well as indirect predation effects (Preisser et al. 2005, Thaler and Griffin 2008, Wasserberg et al. 2013, Hirayama and Kasuya 2013). Directly, predators can consume RWW adults, thus reducing the number of eggs that are laid (Preisser et al. 2005, Thaler and Griffin 2008). Indirectly, RWW can reduce the number of eggs they lay due to predator presence reducing the quality of the habitat in terms of foraging safety (Wasserberg et al. 2013, Hirayama and Kasuya 2013). Testing potential predators of RWW and effects they may have would give insight into predators that may be targeted for use in biological control.

In this experiment we aimed to assess the direct and indirect interactions of commonly found predators in rice fields with RWW and quantify predator effects on RWW. This was done by greenhouse studies that expose adults to potential predators and measure the resulting fecundity of RWW on rice. This would test the hypothesis that detection of predators by RWW will negatively affect their behavior and fitness.

### 3.2 Materials and Methods

To assess predation effects on RWW no-choice trials measuring adult oviposition were conducted in aquariums. These experiments were designed to measure indirectly adult leaf consumption, ovipositional choice (whether to lay or not), egg and adult mortality. These trials took place at the LSU AgCenter Rice Research Station greenhouse in, Crowley, LA U.S.A., during the summer of 2014. Rice was planted in 9cm diameter pots with a 2:1:1 mixture of soil, sand and peat moss and grown in the LSU Campus greenhouse until needed for experiments. Only ambient light was used, and temperatures ranged from 25°C to 35°C in the greenhouse. RWW adults were collected from rice plots at the LSU AgCenter Rice Research Station in Crowley, LA U.S.A. within 24hrs of being used for trials and kept in jars with fresh rice leaves and ~1cm water. Monitoring of rice field invertebrates (Chapter 2) revealed two abundant predators that could potentially feed on RWW (personal observation), adult *Notonecta* sp. (Hemiptera: Notonectidae) and immature *Pantala* sp. (Odonata: Libellulidae) due to gape size and previous work by Puissegur (1976). A third insect, adult *Tropisternus lateralis* (Coleoptera: Hydrophilidae), a common herbivore/scavenger, was used as a treatment to test if RWW behavior is altered by the presence of invertebrates that pose no risk (Snyder and Wise 2000). All three of these species were collected from the same location as RWW adults, but were collected on the same day as the trial they were being used for began. None of the treatment invertebrates were starved or satiated before being used.

Each treatment and control replicate consisted of three pots with three rice plants per pot in 109.78L glass aquariums, 76.2cm x 30.48cm x 45.72cm. Aquaria were filled to a depth of 12.7 cm with water. Twenty five RWW adults were then added and the tank was covered with a mesh screen. Depending on the treatment, three *Pantala* sp., three *Notonecta* sp., or three *T. lateralis* were then added to aquaria. Control aquaria contained only rice plants and RWW. This produced a total of three different no-choice treatments and one control treatment. Invertebrates were allowed to move freely in

their tanks with no barriers between RWW and treatment invertebrates. This allowed for potential consumption of RWW by predators (direct effects) as well as the possibility of predator induced RWW behavioral changes (indirect effects). For each set of treatment replicates, *Notoenecta* spp., *T. lateralis*, or *Pantala* spp., an equal number of controls were run in tandem for that trial date. For example, if four *T. lateralis* replicates were being run, then four control replicates were run as well.

Replicates were left to run for four days and then terminated. All remaining invertebrates were removed and not used in any further replicates. Mortality rate for RWW was not measured due to difficulty in recapturing adults. Pots were removed and two plants per pot were destructively removed with roots still attached and placed in either a test tube filled with water or bleached in 50% ethanol. The pot with the remaining rice plant was placed in a wooden basins lined with plastic pool liner and flooded to 12.7cm and kept for three weeks. Soil and rice plants were then removed and washed through a 40 mesh screen sieve bucket, the remaining material was placed in a salt water basin and the larvae and pupa were counted as they float to the surface (Stout et al. 2001).

Rice plants in test tubes were kept in an incubator at 25°C with 16:12 L:D cycle and used to measure the number of larvae emerging from rice plants. Every other day test tubes were emptied into petri dishes and the number of larvae present were counted. Rice plants in these test tubes were vigorously shaken before the water was poured into the petri dishes to dislodge any larvae attached to the roots (Tindall and Stout 2003). This was done until no larvae were found in the test tubes for six continuous days. Plants bleached in ethanol were used to measure the number of eggs oviposited in the leaf sheaths. After a minimum of 21d of bleaching the plants were dissected under a dissecting scope and the number of eggs present were counted (Tindall and Stout 2003). Monitoring 1<sup>st</sup> instar emergence and egg abundance allows for the measurement of, indirectly, adult mortality, feeding, and oviposition and directly on egg mortality. Comparison of RWW larvae counts from both test tube counts and

washing down of rice pots and egg counts were compared between treatments using ANOVA (PROC GLIMMIX) ,SAS 9.4, SAS Institute Inc 2014.

### 3.3 Results

A total of 11 *Notonecta* sp., 10 *T. lateralis*, six *Pantala* sp. and 27 control replicates were conducted. The lower number of *Pantala* sp. replicates was due to the short appearance of immatures, end of June to early July. Due to extremely low numbers of RWW larvae found from washing down rice pots, counts from wash downs were not analyzed. Some of the bleached sample trials contained little to no eggs for both treatment and their paired control plants , fully hatched 1<sup>st</sup> instar RWW were also found in the leaf sheath (although the latter were rare). First instar larvae counts found this way were pooled with egg counts and analyzed together. Lack of larvae found meant only three replicates of *Pantala* spp., nine of *Notonecta* spp. and six *T. lateralis* and their respective controls were being used for analysis of oviposition. Treatments and their paired controls were bleached in the same container, hence the same number of analyzable of treatments and controls were obtained.

Larvae counted from test tubes had a similar problem in which some trial dates produced almost no larvae although the reason for this is unknown. Therefore only eleven replicates of *Notonecta* sp. and ten *T. lateralis* and their respective controls were used for analysis of emerged larvae. No significant difference between any of the treatments and controls was found in either larvae counts (*Notonecta* sp. and *T. lateralis*) (F = 0.62, P = 0.59) (Table 11) or egg counts (*Notonecta* sp., *T. lateralis* and *Pantala* sp.) (F = 0.49, P = 0.74) (Table 12).

Table 11. Generalized mixed model comparing 1<sup>st</sup> instar RWW emergence with or without *Notonecta* spp. or *Tropisternus lateralis* from plants removed from aquaria (experimental arenas) in 2014 greenhouse studies.

Effect	df	F	p
Date	6, 105	4.06	0.0011 *
Treatment	2, 4	0.62	0.5867
Date*Treatment	8, 106	1.06	0.3977

Significance codes: 0.0001 = '\*\*\*\*' 0.001 = '\*\*\*' 0.01 = '\*\*' 0.05 = '.'

Table 12. Generalized mixed model comparing RWW eggs counted in bleached rice leaf sheaths from aquaria (experimental arenas) either with or without *Notonecta* spp. or *Tropisternus lateralis* from plants in 2014 greenhouse studies.

Effect	df	F	p
Date	5, 93	2.05	0.0784
Treatment	4, 93	0.49	0.74
Date*Treatment	5, 93	1.19	0.322

### 3.4 Discussion

Counts of eggs laid by RWW and counts of emerged first-instar RWW showed no difference between RWW exposed to predators, insect herbivores or controls. The reason that some trials failed to have any larvae emerge from rice in test tubes is unknown. A possible reason for the low egg counts could be that the ethanol used for bleaching was too weak and a higher concentration would correct the issue (Tindall et al. 2004, Stout et al. 2012). A low ethanol percentage may have allowed for eggs to fully develop, hatch and emerge from the leaf sheath. This hypothesis is supported by finding 1<sup>st</sup> instar RWW and potential exit holes in the leaf sheath of some plants, however this was not consistent with replicates that contained higher levels of eggs and the same ethanol concentration.

Insect oviposition has been documented to be influenced by the presence of predators (Bond et al. 2005, Vonesh and Blaustein 2010, Hirayama and Kasuya 2013). Since RWW are native to Louisiana, predators that RWW recognize as dangerous are likely to be present in rice fields (Heads 1986, Brown and Kotler 2004). This study measured prey response to predators by counting eggs laid and larval emergence. A possibility is that RWW, adult or larval, physiology change in the presence of predators or a choice experiment might have elicited a response. The life history of RWW however makes monitoring larvae development and behavior difficult (Isely and Schwardt 1934, Zhang et al. 2006). Future experiments might test for any physiological differences in adults.

Predators were chosen based on their size and abundance. Both *Notonecta* sp. and *Pantala* sp. are common aquatic predators in rice fields and are of a size that should be able to consume RWW adults or larvae. Due to only three analyzable replications, *Pantala* sp. should be tested again as it has

been shown to negatively affect RWW numbers. Other potential predators are adult Lycosidae and *Anax* sp. (Odonata: Aeshnidae), both large generalist predators (Oraze et al. 1989, Halaj and Wise 2002, Merritt et al. 2008), although plentiful late in the season were less common in the early to mid-season when control of RWW would be critical. RWW larvae are unlikely to face high levels of predation as they spend a brief amount of time in the water column and no predators were found among the roots they develop on in surveys (Webb 1914, Isely and Schwardt 1934, Chapter 2).

The most likely reason is that RWW were not exposed to appropriate natural enemies. If RWW were exposed to a dangerous natural enemy they would likely alter their behavior in a detectable manor (Brown et al. 1999). Another possibility is that their natural enemies are not present in rice fields. This could be because either resources required by the predators are not present in rice fields or some deterrent in rice fields is preventing colonization. If this is the case then RWW have locally escaped their predators and no top down controlling influences are present in rice fields (Keane and Crawley 2002). A survey of aquatic grasses in unmanaged areas would be necessary to identify potential predators for testing. The study performed here was a no-choice experiment and if repeated as a preference test, a discernible difference in oviposition depending on predator presence and absence may be found (Bond et al. 2005, Wasserberg et al. 2013). However from a biological control point of view this means predators provide no additional benefit because RWW lay their eggs the same if not given a choice. The final possibility is that RWW are not controlled by top down forces and are only limited in a system by bottom up forces, i.e. host availability.

While predator treatments failed to cause a change in RWW behavior, the presence of *T. lateralis* did not alter RWW oviposition. This means that either RWW, regardless of the identity of other organisms in their environment, will not alter their behavior, or RWW is capable of differentiating between potential predators and non-lethal organisms (Snyder and Wise 2000).



## CHAPTER 4. SUMMARY AND CONCLUSIONS

The RWW is the most damaging pest on rice in the U.S., with feeding by the insect reducing yield of rice plants up to 20% (Way 1990, Zou et al. 2004a). IPM practices for RWW management consist of both chemical (seed and foliar), resistant cultivars and cultural controls (Thompson et al. 1994, Stout et al. 2000, Hummel et al. 2012). Cultural control methods are effective at managing RWW, however drainage is being used less frequently (Stout et al. 2002b, Blackman et al. 2014). While resistant cultivars do exist for RWW they are more often than not, not chosen by growers as the level of resistance provided is less preferred when compared to other traits such as herbicide-resistant (Stout et al. 2001, Blackman et al. 2014). This leaves insecticide treatments as the primary means of RWW management, particularly seed treatments (Blackman et al. 2014). Such heavy reliance on insecticides has led to some concern about increased resistance of RWW and negative non-target effects (Zou et al. 2004b). Using prophylactics, such as seed treatments, while effective at controlling pests, is not as cost effective as using IPM. Researching methods to add to and make non-chemical IPM tactics more appealing to growers should be a high priority (Johnson et al. 2009, Bueno et al. 2011).

With a trend of more and more reliance on insecticides which can cause an increase in insecticide resistance and negative effects on non-target organisms, exploring biological control methods for RWW is important (Settle et al. 1996). In these experiments, detrital subsidies, compost-manure, were added to rice plots in an attempt to elicit a trophic cascade which would increase predators and decrease herbivore populations specifically RWW. Surveys from those manipulations were used to select potential predators which were then tested for effects on RWW. Even though compost-manure additions failed to cause a trophic cascade, conservation biological control is still a likely candidate for biological control of RWW.

Increasing the amount of compost-manure has the potential to cause a trophic cascade via detritivores. Sampling revealed that detritivores (Collembola and Chironomidae) represent a large

component of the arthropod community in rice plots. Detritivores likely represent a large portion of prey eaten by generalist predators (Settle et al. 1996, Jiang and Cheng 2004). Increasing the quantity of detrital supplements may cause an increase in detritivores and subsequently increase the number of predators present as had been found in similar studies (Settle et al. 1996, Jiang and Cheng 2004, Mathews et al. 2004, Schmidt et al. 2004, Bell et al. 2008, Navarro-Campos et al. 2012). Over the course of both seasons predator numbers grew from spring to mid-summer, if this increase can be started early enough before RWWs oviposit generalist predators can possibly have a negative effect on herbivores (Settle et al. 1996, Jiang and Cheng 2004).

In order for biological control to be successful, control agents need to be present in sufficient numbers during a key life phase of the pests (Settle et al. 1996, Rutledge et al. 2004). RWW begin to emerge from overwintering sites in late March and then quickly oviposit on rice plants once they are inundated, generally mid-April to mid-May, and are present at crop damaging levels until mid-July (Stout et al. 2002b, Shang et al. 2004). This means that in order for predators to significantly impact RWW populations, they should be present in fields in sufficient numbers during and following field flooding. While the two predators tested here are not present immediately after flooding, they were present from late May through July, a time when RWW are present at damaging levels and ovipositing (Chapter 2, Shang et al. 2004).

The heavily weed infested plots during the second season indicated a correlation between high plant diversity and increased arthropod species richness in rice fields. These plots also had higher numbers of RWW adults, although the effect this had on the economically important larval stage is unknown. Future experiments could look into varying the amount of weed variety and density with a constant level of rice, looking for interactions between species richness and RWW. While this would unlikely be practical in RWW IPM, testing varying levels of weeds and species richness effects on RWW could further reveal information on factors that influence RWW population densities.

Arthropod surveys are important assessments of ecosystems and their diversity. Specimens deposited in the Louisiana State Arthropod Museum will provide a valuable record of species ranges, habitat and abundance for future research. Screening of predators failed to identify species that could be targeted in augmentation or importation biological control. The survey of rice fields was able to identify several potential predators and added upon Puissegur's (1976) survey by diversifying the sampling methods used to identify potential predators as well as to assess the community composition of rice fields.

Further screening of predators is necessary to identify those capable of negatively affecting RWW fitness. While this study presents evidence that *Notonecta* sp. is unlikely to be a major predator of RWW, further tests are needed to rule out *Pantala* sp., as it is a possible natural enemy of RWW (Puissegur 1976) *T. lateralis* testing showed that either RWW are capable of identifying other organisms in their environment and assessing their potential lethality or do not alter their behavior regardless of other organisms present. Surveys of other habitat similar to rice fields, wetlands or small ponds, may be able to identify other candidates for screening as major predators of RWW are not present or in abundance in rice fields.

Identification of a biological control agent for RWW is an important step in changing the trend of an increasing reliance on pesticides as a management tactic. Using biological control, especially conservation biological control can help to reduce the negative impact that agriculture is having on our environment.

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**APPENDIX. LIST OF SPECIES COUNTS FROM 2013 AND 2014**

Order or Higher	Family	Genus	Species	2103 Totals	2014 Totals
Collembola				10272	0
	Sminthuridae			0	11170
	Isotomidae			0	6280
Ephemeroptera	Baetidae	<i>Callibaetis</i>		23	14
				0	221
				493	137
	Caenidae	<i>Amercaenis</i> or <i>Caenis</i>		5	4
Odonata	Aeshnidae			0	3
		<i>Anax</i>		45	62
	Coenagrionidae			81	62
		<i>Nehalinna</i>		151	30
	Corduliidae			0	8
	Lestidae	<i>Lestes</i>		0	4
	Libellulidae			0	2
		<i>Erythemis</i>		0	2
		<i>Orthemis</i>		0	2
		<i>Pantala</i>		9	68
Orthoptera	Gryllidae	<i>Nemobiinae</i>		1	2
	Gryllotalpidae	<i>Neocurtila</i>	<i>hexadactyla</i>	14	2
Hemiptera	Aphididae			19	20
	Belostomatidae			105	37
		<i>Belostoma</i>	<i>lutarium</i>	44	31
			<i>fusciventre</i>	58	28
		<i>Lethocerus</i>	<i>uhleri</i>	18	31
	Cicadellidae			31	20
	Corixidae	<i>Immature</i>		81	28
		<i>Ramphocorixa</i>		45	331
		<i>Trichocorixa</i>		153	24
	Delphacidae			5	1

Appendix con. Order or Higher	Family	Genus	Species	2103 Totals	2014 Totals
	Gerridae	<i>Neogerris</i>	<i>hesioine</i>	2	0
	Hebridae	<i>Hebrus</i>	<i>consolidus</i>	2	4
		<i>Lipogomphus</i>		0	1
		<i>Merragata</i>	<i>brunnea</i>	0	1
			<i>hebroides</i>	0	2
	Hydrometridae	<i>Hydrometra</i>		7	0
	Largidae			2	4
	Lygaeidae			0	1
	Mesoveliidae	<i>Mesovelia</i>	<i>mulsanti</i>	3	12
	Nepidae	<i>Ranatra</i>	<i>australia</i>	11	7
	Notonectidae	<i>Buenoa</i>		267	172
		<i>Notonecta</i>		230	157
	Pentatomidae			5	0
	Reduviidae			0	1
	Saldidae	<i>Micracanthia</i>	<i>humilis</i>	8	7
		<i>Pentacora</i>		0	1
	Scutelleridae			0	2
	Tingidae			0	1
	Veliidae	<i>Microvelia</i>		15	21
		<i>Platyvelia</i>	<i>brachialis</i>	2	3
Thysanoptera				55	74
Pscocoptera				12	2
Coleoptera	Carabidae			60	0
		<i>Acupalpus</i> or <i>Stemolophus</i>		0	26
		<i>Acupalpus</i>	<i>indistinctus</i>	0	47
		<i>Aspidaglossa</i>	<i>angulata</i>	0	2
		<i>Bembidion</i>		0	95
		<i>Chlaenius</i>	<i>perplexus</i>	0	3
		<i>Clivinia</i>		0	5
		CMorph1		0	2
		CMorph2		0	1



Appendix con. Order or Higher	Family	Genus	Species	2103 Totals	2014 Totals
		<i>Elaphropus</i>		0	2
		<i>Semiardistomis</i>	<i>viridis</i>	0	3
		Small brown/red		0	6
	Chrysomelidae			19	9
		<i>Lysanthia</i>	<i>ludoviciana</i>	0	153
		ChMorp1		0	2
	Curculionidae			11	23
		<i>Lissorhoptrus</i>	<i>oryzophilus</i>	488	168
		<i>Scolytinae</i>		0	2
	Dytiscidae	<i>Bidessonatus</i>		3	6
		<i>Copelatus</i>		3	6
		<i>Cybister</i>		10	0
			<i>fimbriolatus</i>	20	25
		<i>Desmopachria</i>		1	2
		<i>Hydroporus</i>		5	0
		<i>Laccophilus</i>		73	34
		<i>Liodessus</i>		1	0
		<i>Neoporus</i>		4	1
		<i>Thermonectus</i>	Larvae	14	15
			<i>basillaris</i>	8	43
			<i>nigrofasciatus</i>		
			<i>ornaticollis</i>	3	27
	Elateridae			1	2
	Endomychidae			0	1
	Haliplidae	<i>Halipus</i>		0	1
	Heteroceridae	<i>Tropicus</i>	<i>pusillus</i>	6	15
	Hydrophilidae	<i>Berosus</i>	Adult	38	96
			Larvae	23	8
		<i>Enochrus</i>	Adult	6	26
			Larvae	0	14
		<i>Helophorus</i>		102	84

Appendix con.				2103	2014
Order or Higher	Family	Genus	Species	Totals	Totals
		<i>Hydrobius</i>	<i>tumidus</i>	0	1
		<i>Hydrophilus</i>		1	1
			<i>triangularis</i>	45	28
		<i>Paracymus</i>		1	4
		<i>Phaenonotum</i>		5	0
		<i>Tropisternus</i>	<i>Immature</i>	177	78
			<i>blatchleyi</i>	0	1
			<i>collaris</i>	50	95
			<i>lateralis</i>	142	253
	Lampyridae			0	1
	Latridiidae			2	3
	Nitidulidae			2	0
	Noteridae	<i>Hydrocanthus</i>	<i>oblongus</i>	12	6
	Scarabaeidae			3	8
	Silvanidae			0	3
	Staphylinidae			56	0
		<i>Athetini</i>		0	3
		<i>Bisnius</i>		0	11
		<i>Carpelimus</i>		0	100
		SMorph1		0	3
		<i>Philonthus</i>		0	4
	Tenebrionidae			1	5
Neuroptera	Chrysopidae			1	0
Hymenoptera	Argidae			1	0
	HMorph1			11	1
	Braconidae			10	6
	Diapriidae			9	8
	Encyrtidae			2	1
	Eulophidae			8	4
	Figitidae			2	2
	Formicidae			21	50

Appendix con.			2103	2014
Order or Higher	Family	Genus	Totals	Totals
	Ichneumonidae		3	1
	Mymaridae		31	3
	Platygastridae		19	17
		<i>Baeus</i>	0	17
	Tiphiidae		2	0
	Vespidae		0	1
Trichoptera	Hydroptilidae		19	44
Lepodoptera			0	20
Diptera	Unknown 1		0	2
	Calliphoridae		2	4
	Ceciomyiidae		0	1
	Ceratopogonidae	Adults	28	34
		Larvae	37	0
		<i>Alluaudomyia</i>	0	2
		<i>Palpomyia/Bezzia</i>	20	9
		<i>Serromyia</i>	0	1
	Chaoboridae	<i>Chaoborus</i>	1	8
	Chironomidae	Larvae	2187	380
		Adult	3764	4199
	Culicidae		27	14
	Dolichopodidae		14	27
	Muscidae		36	100
	Mycetophilidae		0	1
	Phoridae		6	6
	Psychodidae		3	19
	Scatopsidae		0	1
	Sciaridae		3	0
	Simuliidae		4	1
	Stratiomyidae	<i>Odontomyia</i>	454	230
	Syrphidae		1	1
	Tabanidae		248	107

Appendix con. Order or Higher	Family	Genus	Species	2103 Totals	2014 Totals
	Tachinidae			3	2
	Thaumaleidae			1	0
	Tipulidae			107	10
		<i>Antoch</i>		53	24
Araneae				336	402
	Dictynidae			0	1
	Lycosidae			1126	540
		<i>Pirata</i>	<i>insularis</i>	0	201
			<i>piraticus</i>	0	100
	Salticidae			0	3
	Tetraganathidae	<i>Glenognatha</i>	<i>foxi</i>	0	120
		<i>Tetragnatha</i>	<i>laboriosa</i>	0	44
Gastropod				212	0
Annelid				391	0

## VITA

Nathan Mercer was born and raised in eastern Massachusetts. He attended the University of Vermont from August 2007 to May 2011 earning his bachelors of science in Zoology and a minor in Wildlife Biology. In August 2012 he enrolled at Louisiana State University in the Entomology Department in order to pursue his master's in entomology under Dr. Michael Stout. During the course of his master's, he studied the diversity of aquatic invertebrates in rice fields and possible predators of rice water weevils. He will be graduating in May 2015 and plans on obtaining his PhD in entomology afterwards.