The Effects of Predation on Milfoil Weevils (*Euhrychiopsis lecontei*) and Impact on Eurasian Water Milfoil (*Myriophyllum spicatum*)

by

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Abstract

Eurasian watermilfoil *(EWM), Myriophyllum spicatum L,* is an invasive aquatic macrophyte in North America. The aquatic milfoil weevil, *Euhrychiopsis lecontei,* is a native herbivore on milfoils that has been used as a biological control agent for EWM. The objective of this study was to determine if predation by sunfishes (*Lepomis spp.)* and damselfly nymphs (*Lestidae spp.)* can suppress weevil populations below the density necessary to control EWM. In Lake Joanis, Wisconsin, where supplemental weevil stocking had not led to an increase in weevil density, 250 gallon (944 L) mesh exclusion cages stocked with weevils were used to manipulate densities of small bluegill (*Lepomis machrochirus*) to 0, 2, and 4 per cage. Results indicated an inverse relationship between bluegill and weevil densities. Mean densities of weevils were significantly different among treatments (ANOVA, p=0.005). The average density of weevils in cages with no bluegill was 0.31 w/s. Cages with two bluegill averaged 0.02 w/s. Cages with four bluegill averaged 0.01 w/s. These results indicate that sunfish, even at relatively low densities, substantially reduce weevil densities. Protecting stocked weevils in cages may allow them to establish higher densities where they may serve as a control for milfoil in some lakes. Variable success of weevils in controlling EWM in different lakes reported in the literature may be attributable to variable densities of sunfish populations.

A second study addressed the ability of damselflies (*Lestidae* spp.) to significantly suppress weevil densities. Ten gallon experimental tanks (37.5 L) were stocked with 8 adult weevils and 0, 3, 6, and 12 damselflies. Although damselflies have been observed feeding on weevils, differences between treatments were not significant (ANOVA, p=0.78). Further research into insect predation on weevils is warranted.

Acknowledgements

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I cannot express enough gratitude for the infinite patience shown by committee. There have been numerous personal challenges in the completion of this thesis and without their support, I would not have finished. Through all the health problems and extensions, Dr. Crunkilton was always caring and understanding.

Thank you to Paul Skawinski for taking me on my first kayak voyage and teaching me all about weevils and milfoil. His instruction and expertise laid the foundation for this thesis. Amy Thorstenson also provided a wealth of knowledge on all things weevil. Her advice and encouragement is greatly appreciated.

The enthusiasm and dedication of my field assistants Michael Regan, Sam Johnson, Steve Clark, and Jack Bushman was crucial to the success of this project. From almost getting caught moving metal cages in a lightning storm, to long hours searching for weevils in the lab, they all exceeded my expectations. Go team weevil!

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Introduction

Eurasian watermilfoil (*Myriophyllum spicatum)*, (EWM), since its introduction to the U. S. in the 1940s, is one of the most problematic species of invasive aquatic plant (Couch and Nelson 1985). EWM can have significant impacts on native plant species composition. It often outcompetes native species because it grows quickly and begins growing earlier in the spring than most native plants (Aiken et al. 1979). Because it grows in a tangled, crowded canopy, it can inhibit water circulation and alter fish community composition (Diehl 1988). EWM can spread quickly because it has adventitious roots, produces seeds that can establish new plants, and spreads through fragmentation. Fragments that remain on boats and boat trailers can spread EWM to other lakes (Kimbel 1982).

Euhrychiopsis lecontei (hereafter weevil) is a specialist herbivore on milfoils (*Myriophyllum* spp.) (Solarz and Newman 1995). It is native to North America, but prefers EWM where it is present (Newman et al. 1997). Adult weevils (Figure 1) live on the upper portion of the plant and eat the stems and leaves. The females lay eggs on the tips of EWM (Figure 2). The larvae burrow into the stem (Figure 3) and eat vascular tissue (Newman et al. 1996). They pupate inside the stem (Figure 4), further blocking nutrient transport to the tips. The damage caused by immature weevils (Figure 5) often causes EWM to lose buoyancy (Creed et al. 1992). Because damage to EWM is the intended result of weevil stocking, it is perhaps more important than the number of weevils found in assessing the impact of bluegill (Havel et al. 2017). Although adults are fully aquatic, they overwinter on shore under leaf litter (Newman et al. 2001) and may be affected by shoreline disturbance.

It is generally accepted that when weevils are present in sufficient densities, they will control the impact of EWM (Creed et al. 1992, Creed and Sheldon 1993, 1995, Sheldon and Creed 1995, Newman et al. 1996). The density necessary for some level of control varies from 1.5 weevils/stem (w/s) (Newman and Biesboer 2000) to 0.25 w/s. (Jester et al. 2000).

Complete collapses of EWM beds associated with natural weevil densities of 3-4 w/s have been observed in Vermont (Creed and Sheldon 1995). Newman and Biesboer (2000) documented a decline of EWM in Cenaiko Lake in Minnesota due to a natural weevil density of about 1.6 w/s. In both cases, native macrophytes regrew in open areas after the decline of EWM. Long-term studies suggest that weevil density and milfoil density may follow a predator-prey cycle as well as a seasonal cycle (Lillie 2000).

Several attempts at weevil stocking to control EWM in North America have been made. In Michigan's Les Cheneaux Islands on Lake Huron, 15,000 weevils were stocked into 3 (1.2 ha) acre plots and an 80 – 90% reduction in EWM was achieved with densities under 0.3 w/s (Smith 2010). The sampling effort to determine weevil density was minimal and was not subject to statistical analysis, but the results suggest that effective densities sufficient to control EWM may vary among lakes.

Weevils are widely distributed across Wisconsin (Jester et al. 2000), but only reach densities above 0.5 w/s in a few lakes. Jester et al. stocked as many as 10,000 weevils into study plots to try to achieve densities of 1, 2 and 4 weevils/stem. They found that 100% of plots stocked with 4 w/s and 60% with 1 w/s exhibited significant declines in EWM. The density of weevils stocked declined within 5 weeks. Their study did not make use of enclosures, so the weevils may have simply moved out of the plots. The authors also speculated that sunfish predation could have been a factor, reducing weevil densities.

Thorstenson (2011), reared and then stocked 13,000 weevils in August, 2008 and 9,000 in August, 2009 into Lake Joanis, a 9.3 Ha lake on the University of Wisconsin – Stevens Point campus which harbored nuisance levels of EWM. This stocking was estimated to be sufficient to bring the natural density of weevils from 0.01 - 0.03 w/s up to over 2 w/s and represented the highest stocking rate achieved per unit area of a whole lake treatment reported in the literature. The post-stocking density in September, 2008 was 0.03 w/s. Weevils migrate to the shore in September and October, so it is possible that they had already left the lake. The density of weevils the following June, however, was 0.06 w/s and continued to fall over the summer. Because Lake Joanis is part of a protected reserve, its shoreline is almost 100% natural (Thorstenson 2011) and should provide excellent overwintering habitat for weevils (Newman et al. 2001). The density 23 days after stocking in August, 2009 was 0.03 w/s. Subsequent surveys conducted through August 2015 indicated that weevils were present in the lake, but only at very low (<0.03 w/s) densities.

Paul Skawinski (2014) reported on the relationship amoung a large number of water quality, geographic, and land use variables in relation to weevil density on 14 Wisconsin lakes over two years. None of the water quality, geographic or land use variables were found to be significantly correlated with weevil density. Coarseness of substrate was negatively correlated to weevil density and the only variable to show significant correlation.

The question as to why weevil populations remained at low densities in a lake with suitable shoreline habitat and dense beds of EWM was addressed in this study. The answer to this question is paramount for assessing the viability of weevils as a biocontrol agent of EWM. The objective of this study was to determine if bluegill (*Lepomis macrochirus*) predation had a significant impact on weevil abundance in predator exclusion cages in Lake Joanis, a 23 acre (9.3 ha) lake in central Wisconsin.

A second study investigated the potential of predation by damselfly nymphs (*Lestidae spp.)* to limit weevil densities under indoor laboratory conditions in experimental tanks.

Figure 1. Adult *E. lecontei* climbing on *M. spicatum.* Photo by Dan Miller

Figure 2. *E. lecontei* eggs attached at the tip of an apical meristem of *M. spicatum*. Photo by Dan Miller.

Figure 3. *E lecontei* larva entering an *M. spicatum* stem. Photo by Dan Miller.

Figure 4. *E. lecontei* pupation site inside an *M. spicatum* stem. Photo by Dan Miller.

Figure 5. Damage to *M. spicatum* stem caused by adult *E. lecontei* exiting after pupation, Photo by Dan Miller.

Methods 2013, Bluegill Predation

Lake Joanis is a human-made lake on the University of Wisconsin – Stevens Point campus built in 1976. It has a maximum depth of 25 feet (7.6 m) and a surface area of 23 acres (9.3 ha). It is a seepage lake with inputs from groundwater and precipitation (Portage County lake study 2005). The average secchi depth is 14 feet (4.27 m). It has a very large population of small sunfish including pumpkinseed (*Lepomis gibbosus)* and green sunfish (*Lepomis cyanellus)* (Rocke 2016), but is almost entirely bluegill (*Lepomis macrochirus)*. The lake is surrounded by a nature reserve, thus its shoreline is mostly natural with no development. The bottom slopes sharply after a few feet from shore. The milfoil beds on Lake Joanis occur in 1 to 15 feet (0.3 to 4.6 m) of water. Because of its shallow depth and clear water, milfoil occurred in approximately 90% of the lake at the time of the study.

The exclusion cages consisted of perforated, galvanized angle iron bolted together to form a 3ft (0.91m) by 3ft cube 4ft (1.22m) tall with an open bottom. The approximate volume of water in the cages was 250 gallons (944L). At the bottom of the cage, additional pieces of angle iron were bolted upside-down to secure the cages in the sediment over the EWM. One eighth inch (3 mm) plastic aquaculture mesh was attached to the outside walls of the cage with zip ties. The mesh was also sewn on all sides with 30lb (13.6 kg) - test braided fishing line. The lids of the cages were created by folding mesh and zip-tying it at the corners. They were fastened to the cages with custom made bungee cords.

The cages were placed in Lake Joanis on July 3, 2013. No additional weevil were stocked in the cages. The existing weevil density in Lake Joanis was 0.03 w/s in 2011.

Weevils were not detected in a 2012 survey of EWM in the lake (Skawinski 2014). The open bottom cages placed on the milfoil beds were pushed firmly into the sediment. Cages were searched to remove any fish accidentally captured during placement. A seine was used to collect bluegills from an area adjacent to the cages. The bluegills were selected for a size of 2-3 inches (5.0 -8.0 cm) and placed in the cages at 0, 2, 4 or 8 per cage, with four replications of each treatment. The lids were fixed in place to prevent escape or intrusion by jumping and protect against temporary elevated water levels. The cages were visually inspected weekly and two bluegills found dead in separate cages were replaced immediately.

On August 25, the exclusion cages were assessed for EWM and fish after an exposure duration of 53 days. All milfoil to a depth of 20 inches (51 cm) was collected, sealed in gallon size freezer bags, and refrigerated. A small seine was used to extract and preserve all fish. The gut contents of these fish were later searched for weevils (Appendix B, Table 1). The milfoil was searched under magnification on a light table to count the number of all life stages of weevils present. Every attempt was made to sort the samples fresh. Because of the large number of samples, some were preserved in 80% isopropyl alcohol before sorting at a later date.

Methods 2014, Bluegill Experiment

Because the results from 2013 (Appendix C) showed very few weevils in most cages, reflecting their rarity in Lake Joanis, supplemental stocking with weevils occurred in 2014. Twenty four exclusion cages were placed in Lake Joanis on July 3 (Figure 6). There were eight replications of three treatments. The number of bluegill in each treatment was 0, 2 or 4. Placement of the cages was dependent on water levels and accessibility. Because the number of weevils present at the time of placement was unknown, the cages were placed in blocks instead of randomly to avoid a situation where all replications of a treatment were grouped in the highest or lowest density of starting weevils. As the cages were placed on the milfoil beds, they were pushed firmly into the sediment. Cages were searched to remove any fish accidentally captured during placement.

The weevils stocked into the cages were collected from Springville pond, a local impoundment with an abundance of weevils, on June 4, 2014 and bred in tanks to achieve the numbers required for stocking. From a kayak, milfoil was hand-pulled and placed in gallon-sized freezer bags for transport to the lab in an un-iced cooler. The milfoil was searched immediately and all weevils at every life stage were retained on the stems where they were found. Any macroinvertabrate predators on the milfoil were removed. Ten gallon (37.8 liter) tanks in the University of Wisconsin – Stevens Point greenhouse were used to raise weevils to stock into the cages. Eight weevils were stocked into each of 10 tanks on June 5. Additional milfoil stems from Lake Joanis were added to the tanks to bring the total number per tank to 15. All stems were affixed to a small rock with rubber bands. Each week, an additional bunch of 15 weevil free stems from Lake Joanis was added as food and egg laying sites for the weevils. During periods of temperatures above 90 degrees F, bamboo screens were placed on top of the tanks as shade to control water temperature. After 30 days approximately 400 weevils were removed from the tanks and placed in 24 plastic bags with EWM to be stocked in the cages.

Five adult weevils and 10 eggs attached to EWM were stocked into each of the 24 cages on July 6. On July 7, a seine was used to collect bluegills from an area adjacent to the cages. The bluegills were selected for a size range of 2-3 inches (5-8) cm and placed in the cages at the designated densities. The lids were fixed in place to prevent escape or intrusion by jumping and protect against temporary elevated water levels. The cages were visually inspected weekly using an underwater camera to verify the number of bluegill present.

On August 18, after an exposure period of 42 days, all milfoil (1604 stems) to a depth of 20 inches (50.8 cm) from the surface found in the cages were collected, sealed in gallon size freezer bags, and refrigerated. A small seine was used to extract and preserve all fish. All milfoil stems were counted and searched under magnification on a light table to count the number of all life stages of weevils (eggs, larvae and adults) present. The presence of damage caused by larval weevils was also recorded. In order to count as a damaged stem, a small entry hole and a larger exit hole with a hollow tunnel between them needed to be present (Figures 3 and 5). Every attempt was made to sort the samples fresh. Because of the large number of samples, some were preserved in 80% isopropyl alcohol before sorting.

Analysis of variance including Tukey's test and linear regression were performed on the number of weevils per stem and percent damaged stems in each treatment to assess the effect of the presence of bluegills with the null hypothesis of no difference between treatments. Because the data from damaged stems was expressed as a percentage, it was arc sin square root transformed before analysis. Two sets of replications (6 cages) were excluded from analyses because of a lack of evidence of weevils or weevil activity. The milfoil in these cages had very thin (< 7mm diameter) stems. Previous studies have suggested that stems with this small of a diameter may not be accessible as weevil habitat (Sheldon and Obryan 1996). Stems in cages included in the analysis had an average diameter greater than 1.2 mm. All statistical processes were performed in both the publically available R (www.r-project.org), using the RCMDR module, and Microsoft Excel to insure accuracy.

Figure 6. Location of experimental cages in Lake Joanis, Portage County, WI.

Methods, Damselfly Experiment

Damselfly predation experiments were conducted using aquaria inside a University of Wisconsin - Stevens Point, WI greenhouse open to natural sunlight. The air temperature was maintained at a minimum of 80 degrees F (26.7C) during day and a minimum of 60 degrees F (15.6 C) at night. There was no means of controlling the upper limits of temperature except by using overhead shade cloth when temperatures exceeded 90 F. Because space available was split equally between partial shade and sunlight, tanks were placed in blocks to avoid temperature effects.

The EWM, weevils and damselflies (*Lestidae* spp.) used were collected from Springville pond in Plover, Wisconsin. Milfoil was hand-pulled from a kayak and placed in gallon-sized freezer bags along with a small amount of water for immediate transport to the lab. The milfoil was searched immediately and all weevils at every life stage were counted and retained on the stems where they were found. Damselflies found in the EWM samples were removed, identified to Family and counted. An effort was made to remove any other macroinvertebrates that were predators, but other small macroinvertebrates associated with the EWM were retained.

Twenty 10 gallon (37.8 L) glass fish tanks were used in five replications of four treatment densities. All tanks were initially stocked with 15 Eurasian water milfoil (EWM) stems approximately 10 inches (25 cm) and eight adult weevils on August 1. Each week, an additional 15 stems were rinsed thoroughly to remove any additional invertebrates and added as food and egg laying sites for the weevils. All stems were affixed to a small rock with rubber bands to keep them upright in their natural growth position with new apical growth near the water surface where egg deposition by *E. lecontei* occurs (Thorstenson 2011). After 14 days damselfly nymphs between 0.6 -1 inch (1.5 - 2.5 cm) were added at densities of 3, 6, and 12 individuals in each of the five replicates along with an equal number of control replicates with no damselflies. After 35 days the milfoil was removed, and the remaining water passed through a fine mesh screen. All material was sorted under magnification on a light table to count all life stages of weevils and damselflies present as well as abundance of other invertebrates.

Both linear regression and ANOVA were used to analyze the number of weevils and damselflies present to test the null hypothesis that the presence of damselflies did not impact the number of weevil eggs, larva and adults. All statistical processes were performed in both the publically available R (www.r-project.org), using the RCMDR module, and Microsoft Excel to insure accuracy.

Results, Bluegill Predation

Density of *E. lecontei* per Eurasian water milfoil stem (w/s) was reduced 15-30 fold compared to controls in cage enclosures with two and four bluegill, respectively (Figure 7). The difference in mean density values between treatments was significant (ANOVA: df=2,15, F=7.6, p=0.005; Appendix A Table 1). Linear regression indicated a decrease in weevils per stem as the number of bluegill present increased from 0 to four per cage (Figure 8), (R^2 = 0.35, F (1,16) = 8.80, p = 0.009). The mean number of w/s ranged from 0.31 in the controls to 0.01 in the cages with four bluegill. The highest number of weevils was 58 in a single control cage. Nine out of twelve cages with two or more bluegill had two or fewer weevils. Five cages total had no weevils; three cages with two bluegill, and two cages with four bluegill.

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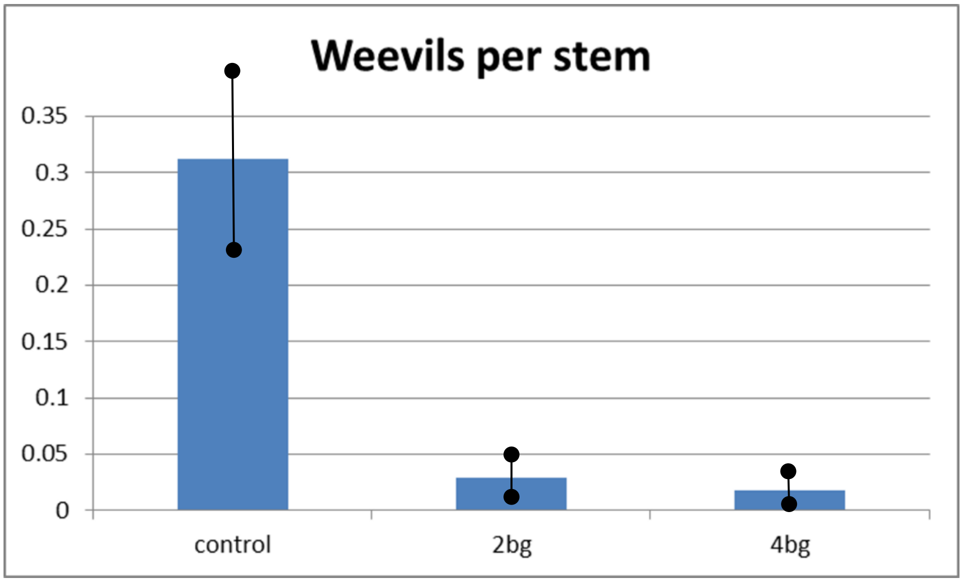

Figure 7. Mean number of *E. lecontei* weevils per Eurasian water milfoil stem from in-situ exclosure cages in Lake Joanis with 0 (control), 2 and 4 bluegill (bg). Error bars represent standard error.

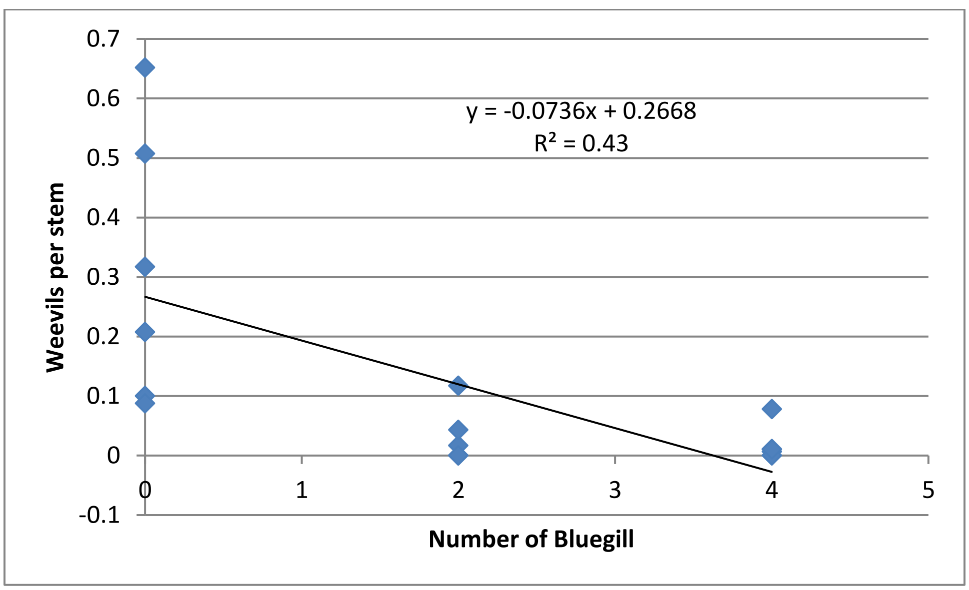

Figure 8. Linear regression of *E. lecontei* weevils per stem by number of bluegill from in-situ exclosure cages in Lake Joanis.

There were 471 stems found to have at least one set of the entry and exit holes associated with larval weevil use indicating stem damage. Density treatments were significantly different from the control (ANOVA: df = 2,15, F = 13.5, p = 0.0004; Appendix A Table 2). The mean percentage of damaged stems ranged from 60.4% in the control treatment without bluegill to 13.3% in the treatment replicates with four bluegill (Figure 9). Linear regression indicated the mean percent of damaged stems decreased with an increase in the number of bluegill present (Figure 10; $R^2 = 0.57$, F (1,16) = 21.77, p=0.0002). The only single treatment replicate with no stem damage had four bluegill. The highest percentage of stem damage was found in a control cage replicate with no bluegill (84%) and the least damage in a replicate cage with four bluegill (0% damage).

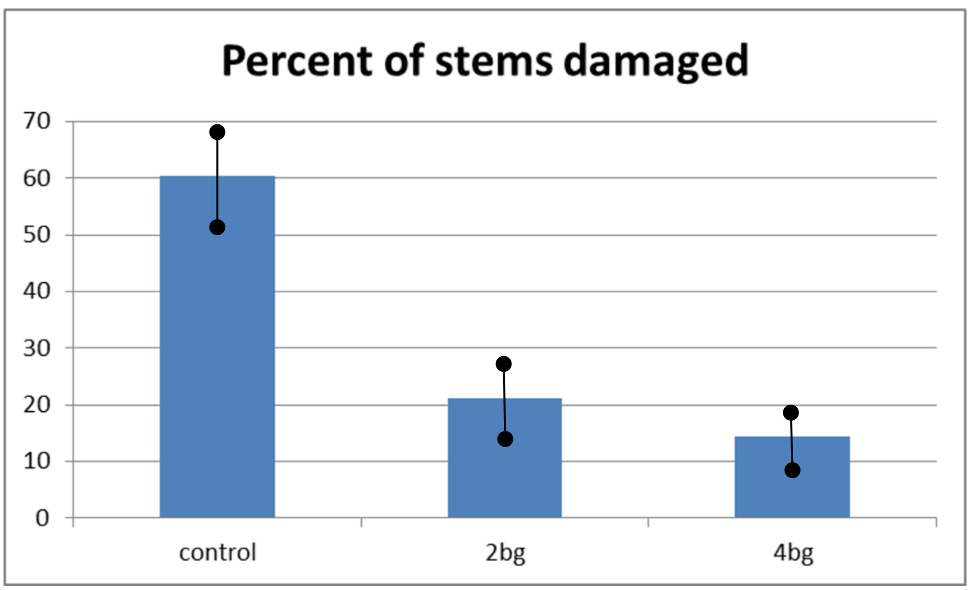

Figure 9. Mean percent of damaged Eurasian water milfoil stems from in-situ exclosure cages in Lake Joanis with 0 (control) 2, and 4 bluegill (bg). Error bars represent standard error.

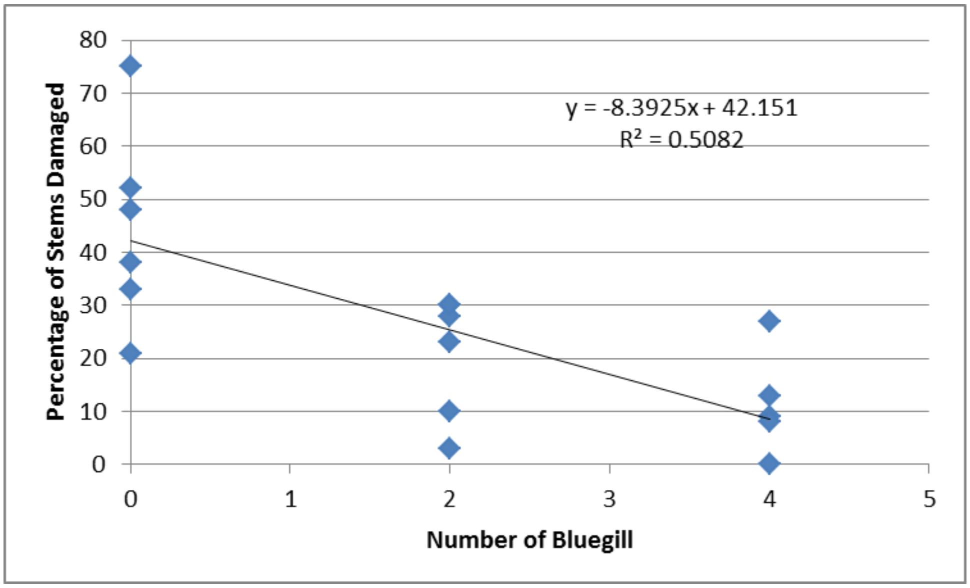

Figure 10. Linear regression of percentage of damaged Eurasian water milfoil stems by number of bluegill from in-situ exclosure cages.

Results, Damselfly Experiment

The mean number of weevils was greatest in control tanks, but differences with tanks containing damselflies were not significant (Figure 11; ANOVA: df = 3,19, F = 0.35, p = 0.78). The number of weevils in individual replicates ranged from 56 in a control tank to four in a tank with six damselflies (Appendix D, Table 1). Linear regression likewise did not show a significant correlation between damselfly numbers and weevil density (Figure 12; R^2 = 0.005, F (1,18) = 0.42, p = 0.52,)

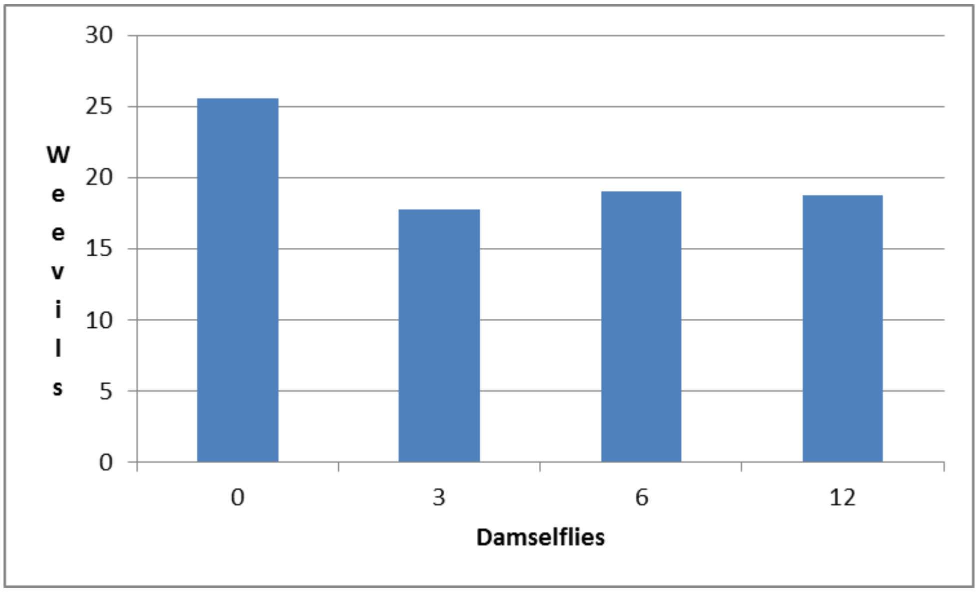

Figure 11. Mean number of *E. lecontei* weevils and number of damselflies in experimental tanks.

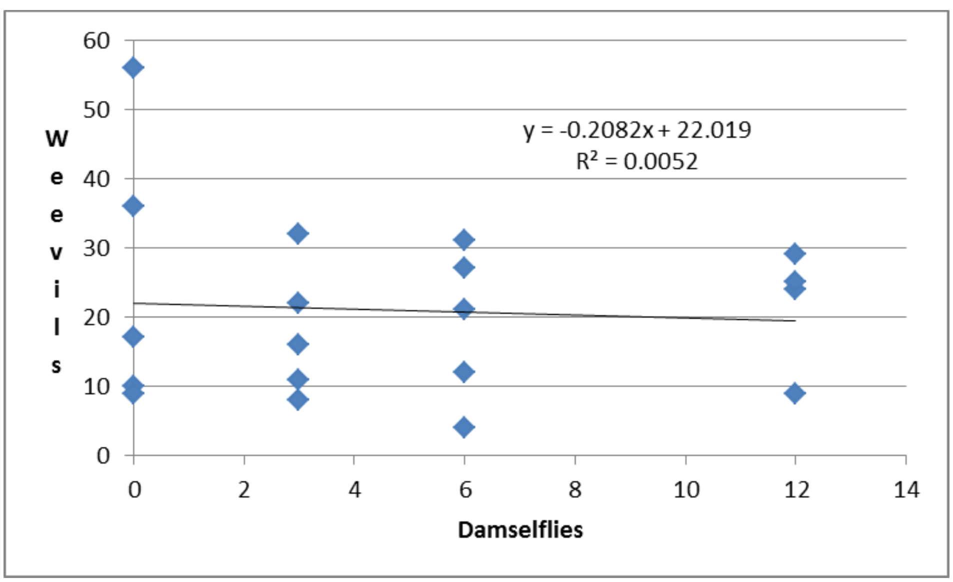

Figure 12. Linear regression of weevil density and number of damselflies in experimental tanks.

Discussion

Bluegill predation had a significant effect on weevil abundance in predator exclusion cages in Lake Joanis. Studies of bluegill diets have shown that they consume weevils (Newbrough 1993, Maxson 2016). Both adult and larval weevils were found in the stomachs of bluegill retrieved from the cages in this study (Appendix E, Table 1). Bluegill in the littoral zone feed on benthic invertebrates, molluscs, cladocerans and miscellaneous insects (Gerry et al. 2012). As bluegill grow from juveniles to adults, they select larger prey (Walton et al. 1992). In the presence of predators, they will often seek refuge in dense vegetation (Dewey et al. 1997). Bluegill in EWM stands often become stunted (Ward and Newman 2006) and remain at a size where small insects such as weevils are selected for prey. Stands of EWM support less diversity of invertebrates than native milfoils (Wilson and Ricciardi 2009). If bluegill, piscivourous predators, weevils and EWM are present in a lake, all of these factors can lead to a situation where bluegill may feed on weevils for an extended portion of their lives.

Previous experiments with exclusion cages have concluded that fish predation can have a significant effect on weevil populations. Ward and Newman (2006) constructed PVC cages with plastic mesh to exclude or include sunfish in two lakes in Minnesota. One had naturally high weevil density and low sunfish density, the other high sunfish density and low weevil density. They stocked weevils and sunfish into the cages where necessary. There were initially five sunfish in half of the cages and none in the other half, but fluctuating water levels allowed fish in and out. It appears that sunfish suppressed weevil density in both situations. Parsons et al. (2011) used a similar exclosure to stock weevils in a Washington state lake. They also stocked weevils outside the exclosure. The only location to retain weevils after stocking was in an exclosure.

Lake Joanis was previously stocked at one of the highest rates of weevils per acre without a long-term rise in weevil densities (Thorstenson 2011). Another study without exclosures saw weevil densities drop within weeks after stocking (Jester et al. 2000). In the present study, the presence of bluegill reduced the number of weevils found in cages. It also reduced the damage done to EWM by remaining weevils. The damage done in some control cages was almost complete, with collapse of most stems in the cage. The very high rates of damage inside the cages where bluegill were excluded in this study suggest that the use of weevils as a biocontrol agent for EWM should consider the potential impact of bluegill populations on weevil densities.

Management Implications

At this time there is no commercial source of weevils to be used in stocking in Wisconsin. Weevils are widely distributed throughout the state at varying densities (Skawinski 2014). Directly stocking weevils from other lakes is not permitted in Wisconsin because of potential transfer of invasive species. Volunteers have successfully captured and reared sufficient numbers of weevils to use in stocking programs within lakes in predator free tanks (Thorstenson 2015). Given the effectiveness of the control cages in this study at protecting weevils from predation, similar in-situ exclosures could be used as part of future stocking efforts to increase the chances of weevils establishing densities necessary for EWM control. In cases where there is a native population of weevils and a moderate density of bluegills, exclusion cages could be placed on EWM beds as a refuge for weevils. Thorstenson (2017) at Golden Sands Resource Conservation and Development used the 24 cages from this study to assess this possibility in Lake Joanis. She found that 68% of the stems in cages had weevil damage and the density of weevils in a point intercept survey of the whole lake rose to 0.25 w/s.

Mechanical harvesting of EWM negatively affects weevil populations by removing the apical meristems they use for egg laying, larval feeding, and pupation (Sheldon and Obryan 1996). It may be possible to integrate mechanical harvesting and biocontrol using weevils by limiting the harvesting to less than 15% of the total area (Newman and Inglis 2009). Harvesting in strips while leaving most of the shallow EWM beds intact with exclosures placed adjacent to the strips could potentially protect and concentrate weevils. The creation of more edge habitat in the beds caused by mowing in strips could also provide more opportunities for predation on bluegill by predators such as largemouth bass (*Micropterus salmoides)* (Trebitz et al. 1997).

Several other factors have been identified as necessary for maintaining sustainable weevil populations. Thorstenson et al. (2013) collected weevils from the shore of two lakes in central Wisconsin to assess overwintering habitat requirements. They found that weevils prefer dry sites close to shore. They also found that weevils likely require sites at a minimum of 50 cm above the water line. At least some duff is thought to be necessary for successful overwintering. They likely use only the top 2 in (5 cm) of the soil, so cover such as leaf litter is essential (Newman et al 2001). Skawinski (2014) found that weevil densities were negatively correlated with coarseness of substrate, perhaps because sandy shores tend to hold less duff. Water quality and local geographic factors were not found to be a factor in weevil density. Weevils prefer EWM with a stem diameter larger than 4 mm (Sheldon and Obryan 1996). In this experiment, 3 replications (6 cages) were placed on milfoil with an average stem diameter of less than 7 mm. No evidence of weevils or weevil damage was found in these cages despite stocking procedures identical to the other replications. Stem diameter may be an important factor limiting effectiveness of weevil success in some lakes and needs to be investigated further.

Damselfly larvae are also known predators on aquatic invertebrates, but there have been no studies specifically addressing the potential of Odonates to limit weevil densities. Some damselfly larvae prefer complex vegetation habitats (Verdonschot and Peeters 2012) as provided by EWM. They are known to prey on coleopteran larvae (Thompson 1978). They are also known to prey on other Odonates (Flynn and Moon 2011) and practice cannibalism (Block and Stoks 2004). They have been observed feeding on adult weevils from a Wisconsin population (Paul Skawinski, personal communication).

The results of the damselfly experiment did not show a significant reduction in weevil abundance with increased damselfly abundance. Damselflies cannibalized each other in tanks with weevils present (personal observation) indicating that weevils are not a preferred food source for this species. The damselflies (Lestidae spp.) used in the tanks were collected from Springville Pond in Portage County Wisconsin, which has an abundant weevil population at 1.6 w/s (unpublished data). It may be that the same experiment conducted with different Odonate taxa would produce different results. Further research into Odonate predation would be necessary to determine if they are capable of limiting weevil abundance.

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Appenix A

Table 1. R output of ANOVA testing for a difference in mean values of weevils per stem by treatment in the 2014 bluegill predation experiment.

```
> AnovaModel.3 <- avow(was ~ treatment, data=Dataset)

> summary(AnovaModel.3)

             Df   Sum Sq  Mean Sq  F value     Pr(>F)

treatment   2    0.3012    0.1506     7.644    0.00514 **

Residuals   15   0.2955    0.0197

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

           mean              sd data:n

control 0.31187857       0.22789874     6

2 bg      0.02951231       0.04607234     6

4bg       0.04620250       0.07099616     6

Multiple Comparisons of Means: Tukey Contrasts

Fit: aov(formula = ws ~ treatment, data = Dataset)

Linear Hypotheses:

                         Estimate Std.     Error    t value     Pr(>|t|)

2 bg - control == 0 -0.28237    0.08104  -3.484     0.00865 **

4bg - control == 0  -0.26568    0.08104  -3.279     0.01325 *

4bg - 2 bg == 0        0.01669    0.08104   0.206     0.97692

---

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Adjusted p values reported -- single-step method)
```

Table 2. Excel output of an ANOVA testing for a difference in the arcsin square root transformed mean percentage of damaged stems in the 2014 bluegill experiment.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	6	5.383125	0.897187	0.042551
2BG	6	2.755136	0.459189	0.026735
4BG	6	2.074912	0.345819	0.043728

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.017406	2	0.508703	13.50377	0.000442	3.68232
Within Groups	0.565068	15	0.037671			
Total	1.582473	17				

Appendix B

Table 1. Complete data of weevil number on EWM from in-situ exclosure cages used in analysis in the 2014 bluegill experiment.

bg = bluegill

asin = arc sine transformation

treatment	stems	adults	larvae	pupae	eggs	weevils	damage	w/s	%damaged	log damage	asin
control	90	0	4	5	0	9	33	0.1	36.666667	1.5759572	0.650432
2bg	91	0	0	0	0	0	10	0	10.989011	1.0787834	0.33789
4bg	91	0	1	0	0	1	9	0.010989	9.8901099	1.0370323	0.319915
control	57	0	1	4	0	5	21	0.087719	36.842105	1.5779753	0.652251
2bg	77	0	0	0	0	0	3	0	3.8961039	0.6898506	0.19869
4bg	94	0	0	0	0	0	0	0	0	0	0
control	89	0	37	21	0	58	75	0.651685	84.269663	1.9307945	1.16297
2bg	118	0	0	2	0	2	30	0.016949	25.423729	1.4219941	0.528478
4bg	152	0	1	0	0	1	27	0.006579	17.763158	1.2733059	0.435059
control	69	12	1	22	0	35	48	0.507246	69.565217	1.8485907	0.986422
2bg	94	3	3	5	0	11	23	0.117021	24.468085	1.4059963	0.517435
4bg	88	0	1	0	0	0	8	0	9.0909091	1.0039303	0.306277
control	82	3	4	2	17	26	52	0.317073	63.414634	1.8089845	0.921208
2bg	116	1	0	0	4	5	28	0.043103	24.137931	1.4003295	0.513586
4bg	99	0	0	1	0	1	13	0.010101	13.131313	1.1501825	0.370811
control	53	1	5	5	0	11	38	0.207547	71.698113	1.8615231	1.009841
2bg	80	0	0	0	0	0	30	0	37.5	1.5854607	0.659058
4bg	64	0	1	4	0	5	23	0.078125	35.9375	1.5674675	0.64285
totals	1604	20	59	71	21	170	471	0.119674	31.926903	1.3454533	

means										
control	73.33333	2.666667	8.666667	9.833333	2.833333	24	44.5	0.311879	60.4094	1.7673042
2bg	96	0.666667	0.5	1.166667	0.666667	3	20.66667	0.029512	21.069143	1.2637358
4bg	98	0	0.666667	0.833333	0	1.333333	13.33333	0.017632	14.302165	1.0053198

Appendix C

Table 1. Weevil counts on EWM from in-situ exclosure cages used in 2013 in the bluegill predation experiment.

	Bluegills	STEMS	ADULTS	LARVAE/PUPAE	EGGS	Total	Damage	Weevils/stem	Damage/stem
Location									
1	0	128	1	5	0	6	21	0.046875	0.1640625
2	2	111	1	2	0	3	16	0.02702703	0.144144144
3	4	132	0	0	0	0	7	0	0.053030303
4	8	94	2	4	2	8	8	0.08510638	0.085106383
5	0	115	29	14	27	70	101	0.60869565	0.87826087
6	2	97	1	2	7	10	21	0.10309278	0.216494845
7	4	126	1	3	0	4	19	0.03174603	0.150793651
8	8	105	1	3	0	4	13	0.03809524	0.123809524
9	0	114	5	1	2	8	17	0.07017544	0.149122807
10	2	114	1	0	7	8	11	0.07017544	0.096491228
11	4	91	0	0	0	0	4	0	0.043956044
12	8	92	0	0	0	0	3	0	0.032608696
13	0	97	3	0	6	9	16	0.09278351	0.164948454
14	2	92	0	0	0	0	1	0	0.010869565
15	4	84	0	0	0	0	3	0	0.035714286
16	8	78	0	0	0	0	1	0	0.012820513
A4	N/A	41	0	0	0	0	2	0	0.048780488
A8	N/A	46	0	1	0	1	1	0.02173913	0.02173913
A12	N/A	47	0	1	0	1	0	0.0212766	0
A16	N/A	45	0	0	0	0	0	0	0

Table 2. Totals by treatment of weevils on EWM from in-situ exclosure cages used in 2013 in the bluegill predation experiment.

	STEMS	ADULTS	LARVAE/PUPAE	EGGS	Total	Damage
Control	454	38	20	35	93	155
2 Bluegills	414	3	4	14	21	49
4 Bluegills	433	1	3	0	4	33
8 Bluegills	369	3	7	2	12	25
Adjacent	179	0	2	0	2	3

Table3. Mean values by treatment of weevils on EWM from in-situ exclosure cages used in 2013.

w/s = weevils per stem

d/s = weevil damage per stem

	STEMS	ADULTS	LARVAE/PUPAE	EGGS	Total	Damage	w/s	d/s
Control	113.5	9.5	5	8.75	23.25	38.75	0.20463	0.3391
2 Bluegills	103.5	0.75	1	3.5	5.25	12.25	0.05007	0.117
4 Bluegills	108.25	0.25	0.75	0	1	8.25	0.00794	0.07087
8 Bluegills	92.25	0.75	1.75	0.5	3	6.25	0.0308	0.06359
Adjacent	44.75	0	0.5	0	0.5	0.75	0.01075	0.01763

Appendix D

Table 1. Complete contents of the experimental tanks. P = Present, R = Rare, A =Abundant. DR Fly = Dragon Fly larva present.

Location	D-fly start	D-fly end	Adults	Pupae	Larvae	Eggs	Total	Mayflies	Mites	Sideswimmers	Notes
1	0	1	7	2	1	0	10	A	A	A	
2	3	2	3	3	2	0	8	A	A	A	
3	6	6	2	0	2	0	4	P	A	A	
4	12	9	7	1	1	0	9	R	A	A	
5	0	5	16	1	17	2	36	A	P	P	Dr Fly
6	3	2	6	7	2	17	32	P	P	P	
7	6	6	15	2	14	0	31	P	P	P	
8	12	13	8	2	8	6	24	R	0	0	
9	0	0	11	3	42	0	56	P	P	P	
10	3	3	13	6	3	0	22	A	A	A	
11	6	5	7	13	7	0	27	A	0	0	Dr Fly
12	12	8	6	1	8	10	25	P	0	0	
13	0	0	10	3	4	0	17	P	A	A	
14	3	2	10	2	4	0	16	P	0	0	
15	6	3	9	3	9	0	21	0	0	0	
16	12	8	7	4	10	8	29	A	P	P	Dr Fly
17	0	0	4	0	0	5	9	P	P	P	
18	3	2	5	1	3	2	11	P	A	A	
19	6	1	7	0	3	2	12	P	A	A	
20	12	11	3	2	2	0	7	R	0	0	

	Adults	Pupae	Larvae	Eggs	Total
totals	156	56	142	52	406
control	48	9	64	7	128
3	37	19	14	19	89
6	40	18	35	2	95
12	31	10	29	24	94
means					
control	9.6	1.8	12.8	1.4	25.6
3	7.4	3.8	2.8	3.8	17.8
6	8	3.6	7	0.4	19
12	6.2	2	5.8	4.8	18.8

Appendix E

Figure 1. Size in centimeters and weevils (Wvs) found in the stomach contents of bluegill from the in situ cages in Lake Joanis in 2013. Two large mouth bass (LMB) and three bullheads (BH) were found in the cages. Three weevil larvae (L) were found in bluegills.

cage	Size -Wvs	Size -Wvs	Size -Wvs	Size -Wvs	Size -Wvs	Size -Wvs	Size -Wvs	Size -Wvs	Size -Wvs	Size -Wvs	Size -Wvs	Size -Wvs	Size -Wvs
1	5.0- 0												
2	7.1-0	4.7-0	4.8-0	6.9- **1**	5.2-0	6.2-0	5.5-0	5.1-0	4.6- **2L**	4.4-0			
3	7.6-0	8.5-0	8.2-0	7.0-0	5.6-0								
4	7.6-0	7.3-0	8.1-**1**	7.8-0	6.0-0	6.8-0	6.5-0	5.2-0	5.1-0	4.6-0	5.0-0	3.1-0	**BH** 4.9-0
5	**LMB** 5.4-**1**												
6	6.5-**1+1L**	7.9-0	6.2-0	5.5-0	4.6-0								
7	5.6-0	7.5-0	6.8-0	9.7-0	6.5-0	5.6-0							
8	7.0-0	8.0-0	7.2-0	4.8-0	**LMB** 4.1-0	5.1-0	5.9-0	6.6-0	6.5-0				
9	1.8-0	2.1-0											
10	5.5-0	3.2-0	5.2-0	5.4-0	6.9-0	6.4-0	4.9-0	2.3-0					
11	8.1-0	3.0-0	3.1-0	10.2-0	7.0-0	5.1-0	6.4-0	7.2-0					
12	6.6-0	6.0-0	6.9-0	7.1-0	7.5-0	7.0-0	7.8-0						
13	6.6-0	**BH** 5.6-0	3.1-0										
14	2.0-0	2.3-0	3.0-0	**BH** 5.2-0	6.3-0	1.4-0	2.9-0	2.4-0					
15	9.1-0	7.5-0	7.4-0	8.9-0									
16	3.2-0	5.2-0	6.0-0	6.9-0	7.4-0	7.2-0	6.4-0	7.1-0					