


# Testing the enemy release hypothesis in a native insect species with an expanding range

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The enemy release hypothesis (ERH) predicts that the spread of (invasive) species will be facilitated by release from their enemies as they occupy new areas. However, the ERH is rarely tested on native (non-invasive, long established) species with expanding or shifting ranges. I tested the ERH for a native damselfly (*Enallagma clausum*) whose range has recently expanded in western Canada, with respect to its water mite and gregarine parasites. Parasitism levels (prevalence and intensity) were also compared between *E. clausum* and a closely related species, *Enallagma boreale*, which has long been established in the study region and whose range is not shifting. A total of 1150 damselflies were collected at three 'old' sites for *E. clausum* in Saskatchewan, and three 'new' sites in Alberta. A little more than a quarter of the damselflies collected were parasitized with, on average, 18 water mite individuals, and 20% were parasitized by, on average, 10 gregarine individuals. I assessed whether the differences between levels of infection (prevalence and intensity) were due to site type or host species. The ERH was not supported: *Enallagma clausum* has higher or the same levels of parasitism in new sites than old sites. However, *E. boreale*  seems to be benefitting from the recent range expansion of a native, closely related species through ecological release from its parasites because the parasites may be choosing to infest the novel, potentially naïve, host instead of the well-established host.

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## 22 INTRODUCTION

23 The Enemy Release Hypothesis (ERH) predicts that a species will be successful in a new  
24 habitat when its former enemies (e.g. parasites) are not present (Keane & Crawley, 2002). This  
25 hypothesis has been widely applied to invasive species, plant or animal pests in new habitats  
26 (Colautti et al., 2004). There is mixed support for this hypothesis (Heger & Jeschke, 2014).  
27 review by Colautti et al. (2004) found that the ERH was supported in 11 of 13 such studies.  
28 However, in comparisons of co-occurring evolutionarily related species, where one was an  
29 established resident and the other a newcomer, the ERH was supported in only a third of the  
30 studies. The reason for this lack of support for the ERH could be that the established species has  
31 evolved defenses against the enemy, whereas the new species is still naïve (Colautti et al., 2004).  
32 In other words, the invading species is joining a community that already has a close relative to  
33 which enemies exist and so has enemies poised for potential attack on the invader, as seen in  
34 Darwin's Naturalization Hypothesis (Darwin, 1859; Daehler, 2001) or the Parasite Mediated  
35 Competition Hypothesis (Price, Westoby & Rice, 1988).

36 Since the review of Colautti et al. (2004), the ERH has continued to be debated: the  
37 hypothesis was not supported in some vertebrates such as bullfrogs (Dare & Forbes, 2013) and  
38 gobies (Gobiidae) (Kvach et al., 2014) or leafrollers (Tortricidae) (Buergi & Mills, 2014) but was  
39 supported in other invertebrate systems: ladybird beetles (Coccinellidae) (Comont et al., 2014)  
40 and brine shrimp (Artemiidae) (Rode et al., 2013). The ERH has been intensively studied with  
41 invasive species, particularly plants, where it has more support (Liu & Stiling, 2006). ERH has  
42 also been studied with host shifts (as enemy-free space), where the release from enemies comes  
43 not from removal in space but through movement to a species to a new host where enemies do  
44 not find that species (Heard et al., 2006). Other hypotheses, such as the Evolution of Increased  
45 Competitive Ability Hypothesis, have also been proposed as alternatives. This hypothesis

observes that invasive species evolve better competitive abilities than their close relatives over time (Blossey & Notzold, 1995). But in a meta-analysis, there was little support for it (Felker-Quinn, Schweitzer & Bailey, 2013). However, studies testing these hypotheses have almost exclusively focused on invasive species.

Invasive species are not the only species whose ranges are changing; a native species' range is not static (~~Chen et al., 2011~~) and can change with climate, or with changing landscapes (Chen et al., 2011, Burrows et al., 2014). Shifts in species ranges can have consequences for species interactions as a focal species encounters new partners or enemies and leaves old ones behind, but it is also worth noting that range shifts can also be driven by biotic interactions (Davis et al., 1998). However, there are few studies of species with shifting and expanding ranges (other than recently introduced species) that test and show support for the ERH. In a survey of common milkweed, *Asclepias syriaca* L. (Apocynaceae), natural populations at the geographical extremes of the ranges showed less herbivory (e.g. leaf damage and insect diversity) than those at the center of the range (Woods et al., 2012). Prior & Hellman (2013) studied the oak-gall forming wasp *Neuroterus saltatorius* Hartig (Cynipidae), whose range is expanding northward, and found support for the ERH in both natural and experimental settings because the wasp had greater demographic success and fewer parasitoids in the new range. The ERH was also supported in a marine whelk, *Kelletia kelletii* (Forbes) (Buccinidae), with an expanding range (Hopper et al., 2014). Hopper et al. (2014) found that despite poorer demographic performance in the expanded range populations, the whelk had only one fifth as many parasites as the historic-range populations.

I studied a native Nearctic damselfly, *Enallagma clausum* Morse (Odonata: Coenagrionidae), whose range is expanding westward in Canada. I tested the ERH by comparing prevalence and intensity of parasite infection in older, established populations of *E. clausum* (as a

baseline) to those in newly occupied sites. I also contrasted parasitism of *E. clausum* with that of close relative, *Enallagma boreale* Selys (Odonata: Coenagrionidae), which co-occurs at both the old and new sites. *Enallagma clausum* is a widespread species in Manitoba, Saskatchewan, and the northwestern United States (Walker, 1953) but has been moving westward into Alberta through the parklands ecoregion in the past few decades (Acorn, 2004). The reasons for expansion are unclear but it is most likely due to increased availability of new habitat with increased standing water in the prairies during the summertime due to recently built cattle dugouts, irrigation canals, waterfowl projects and power plants (Acorn, 2004). This westward expansion is being documented in many Lepidoptera as well as other odonates (Acorn, pers. comm.). *Enallagma boreale* is widespread throughout North America, its distribution reaching the Yukon, and has been established in Alberta for over 100 years (Walker, 1953).

The most common parasites of damselflies are water mites (Arthropoda: Acari) and gregarines (Protozoan: Apicomplexa) (Corbet, 1999). *Arrenurus* water mites (Arrenuridae) are external parasites of many insects associated with aquatic habitats (Smith, Cook & Smith, 2010). *Arrenurus* spp. attach phoretically to the larval damselfly host and then attach parasitically to the adult damselfly once it ecloses (Smith, Cook & Smith, 2010). Water mites feed on their hosts until fully engorged, and then drop back into the water to continue their life cycle. Gregarines, in contrast, are internal gut parasites of arthropods (Clopton, 2009). Gregarines develop and mate in the host mid-gut and are released into the environment as cysts. ~~It is believed that~~ gregarines are acquired from the environment; cysts have been observed on legs of prey items (Åbro, 1976).

The main objective of this study was to test the ERH by observing whether *E. clausum* is as frequently attacked by external and internal parasites in the newly occupied sites as in the 'old' sites where it has been long established. Secondly, by observing *E. boreale*, I also noted whether the arrival of *E. clausum* either dilutes the parasite pool and spares the well-established

close relative (Brown, McPeck & May, 2000), or increases the parasite pool and increases attack on both. I could therefore determine if at 'new' sites there are parasite populations that are locally adapted to their familiar host but not to the newly arriving one. If *E. clausum* is released from its enemies, I expect this species would have lower measures of parasitism in the new sites and there would not be a difference in parasitism in *E. boreale* between old and new sites. However, if there is another pattern of infection, such as differences in levels of parasitism in *E. boreale* or *E. clausum* not being freed from its parasites in the new sites, parasite-mediated competition, one host species benefits indirectly from the presence of a second related host species because the parasite has an alternate host (Price, Westoby & Rice, 1988) could be occurring.

## METHODS

### Host and parasite sampling

Hosts were collected at six sites in the parklands ecoregion of Canada (Fig. 1). No collecting permits were required for specimen collection. Lakes were chosen because of their environmental similarities and because they provide suitable habitat for *Enallagma* damselflies: all lakes are shallow, slightly saline, with sandy bottoms and grassy vegetation along the edges. Three 'old' sites in Saskatchewan: Lenore Lake (52°28'56.86"N, 104°56'55.88"W), Manitou Lake (52°46'25.61"N, 109°44'19.37"W), and Redberry Lake (52°41'30.30"N, 107°10'17.71"W) were chosen because both damselfly species were known from these sites for at least 60 years (Walker, 1953). The old sites are an average distance of 200 kilometers from each other. Three 'new' sites in Alberta: Gull Lake (52°29'28.87"N, 113°58'29.23"W), Sylvan Lake (52°20'42.34"N, 114°9'27.09"W) and Johnson Lake (50°35'44.60"N, 111°53'27.09"W) were selected based on *E. clausum* being first recorded in this area in the last decade (Acorn 2004). Sylvan lake is 20 kilometers from Gull lake and 240 kilometers from Johnson lake. As mentioned previously, these lakes are very ecologically similar other than size, which varied from Johnson lake covering one

118 km<sup>2</sup> to Gull lake and Redberry lake having an area of 80 km<sup>2</sup>, and presence of fish, which only  
119 Manitou lake is fishless.

120 Each site was visited twice ~~in two weeks~~ in the first two weeks of July 2012. At each site  
121 visit, 18 to 30 individuals of each damselfly species were collected by aerial sweep net by a  
122 single collector walking through the grass along the shoreline of the sites. *Arrenurus* spp.  
123 infection was tallied in the field. A total of 497 *E. clausum* and 653 *E. boreale* hosts were  
124 examined for *Arrenurus* parasitism. Female damselflies were omitted from analyses because of  
125 low sample size and the potential for sex bias in parasitism (Forbes & Robb, 2008). A subset of  
126 the damselfly specimens (342 *E. boreale* and 343 *E. clausum*) were collected and stored in  
127 separate vials in 95% ethanol for dissection in the lab to assess gregarine parasitism (see Table  
128 S1).

129 Water mites are usually located on either the ventral side of the thorax or the posterior  
130 ventral portion of the abdomen (Smith, Cook & Smith, 2010). All water mites were tallied and  
131 treated as a single taxonomic unit to compare prevalence and intensity of parasitism (Corbet,  
132 1999; Smith, Cook & Smith, 2010) because the effects on hosts are considered similar between  
133 water mite species (Åbro, 1982).

134 To determine gregarine infection, damselfly abdomens were dissected by tearing the  
135 membrane between the dorsal tergites and the ventral sclerites to expose the gut. Once the gut  
136 was exposed the gregarine individuals could be tallied under 10x magnification. As with water  
137 mites, all gregarines were combined as one taxonomic unit based on the assumption that different  
138 gregarine species affect hosts similarly.

## 139 Analyses

Prevalence (proportion of infected individuals per site) and mean intensity (average number of parasites per infected individual) were measured for each parasite group ~~separately~~. Confidence intervals for both measures were calculated using QP3.0 (Rózsa, Reiczigel & Majoros, 2000).

Each measure of parasitism of each parasite group was measured separately with species and site type as explanatory variables using two-way ANOVAs. Four two-way ANOVAs were performed, one for each measure of parasitism and for each parasite group. All analyses were performed in JMP v.11 (SAS 2013).

## RESULTS

### Water mite parasitism

Among sites, prevalence on *E. clausum* varied between 0.05 (0.01–0.1 95% Clopper-Pearson confidence interval) and 0.71 (0.58–0.82 95% CI; Table 1). Intensity varied between 12.8 (3.07–31.14 95% bootstrap CI) and 36.8 mites per infected host (19.8–50.7 95% bootstrap CI; Table 1). In *E. boreale*, prevalence varied between 0.11 (0.06–0.16 95% CI) and 0.65 (0.49–0.78 95% CI). Intensity varied between 8.05 (5.27–12.6 95% bootstrap CI) and 27.9 (20.8–37.1 95% bootstrap CI; Table 1).

There were significant differences in *Arrenurus* prevalence in the species by site interaction ( $F_{1,11} = 22.2$ ;  $P < 0.01$ ; Table 2a). *Enallagma clausum* had a significantly higher prevalence of *Arrenurus* parasites at new sites than at old sites (Fig. 2a). *Enallagma boreale* showed a higher prevalence of *Arrenurus* parasites at old sites (Fig. 2a). There was a significant difference in species and site type interaction for *Arrenurus* intensity as well ( $F_{1,11} = 6.16$ ;  $P = 0.02$ ; Table 2b), with the same pattern as prevalence to that in *Arrenurus* prevalence (Fig. 2b).

### Gregarine parasitism



Gregarine prevalence in *E. clausum* varied between 0.01 (0.00–0.08 95% CI) and 0.41 (0.29–0.54 95% CI; Table 1) over all the sites. Intensity varied between 3.50 (2.00–3.50 95% bootstrap CI) to 11.56 (7.80–16.48 95% bootstrap CI; Table 1). In *E. boreale*, gregarine prevalence varied between 0.09 (0.03–0.19 95% CI) and 0.48 (0.34–0.61 95% CI; Table 1). Intensity varied between 7.00 (2.00–13.50 95% bootstrap CI) and 22.43 (7.00–46.14 95% bootstrap CI; Table 1).

There were non significant difference in species by site type for gregarine prevalence ( $F_{1,11}=3.68$ ,  $P=0.07$ ; Table 3a), or in species by site type for gregarine intensity ( $F_{1,11}=2.47$ ,  $P=0.13$ ; Table 3b). However, the gregarine prevalence was close to significant, paralleling the pattern of *Arrenurus* mites where gregarine prevalence tended to be higher at new sites for *E. clausum* and at old sites for *E. boreale* (Fig. 2c,d).

## DISCUSSION

The ERH predicts that a species whose range is expanding should have lower levels of parasitism in ‘new’ sites versus ‘old’ sites. In this case, *E. clausum* had significantly higher prevalence and intensity of *Arrenurus* in ‘new’ sites than in ‘old’ sites. Although gregarine prevalence was not significantly different between the site types and species, more individuals of *E. clausum* at ‘new’ sites seemed infected by gregarine parasites. There was no significant difference in gregarine intensity. The expectation of the ERH is that a species with an expanding range should have lower levels of parasitism than a long-time resident in the same new geographic area. This effect was not observed in either measure of water mite or gregarine parasitism. Instead, I observed significantly higher *Arrenurus* parasitism in the newly occupied sites in *E. clausum*, the species whose range is expanding, compared to both *E. boreale* and to *E. clausum* populations at old sites. This could be consistent with the observations of less resistance to evolutionarily unfamiliar parasite populations, as seen in other odonate-water mite associations

(Forbes, Muma & Smith, 1999; Mlynarek, Knee & Forbes 2014b). ~~Based on the current observations of these particular host-parasite associations,~~ it is clear that the ERH is not documented in *E. clausum* infected by two of the most common parasites of damselflies: gregarines and water mites.

An alternative explanation for these results involves parasite-mediated competition, where a host species benefits indirectly because of the presence of a new closely related host (Price, Westoby & Rice, 1988) or Darwin's Naturalisation Hypothesis, where the new species will not be at an evolutionary advantage because a close relative is already present in those habitats and has enemies (Daehler, 2001). These two *Enallagma* host species are probably in competition because they are closely related (Brown, McPeck & May, 2000) and have similar ecological habits, other some behavioural difference due to *E. boreale* developing in fish and *E. clausum* in dragonfly habitats in Eastern North America (McPeck, 1990). In this study, both species were collected in sympatry, so it can be assumed that as larvae they developed in the same lake and were under the same pressure. *Enallagma boreale* may be better at evading these populations of parasites because it has had more time to coevolve with them at the sites ~~that are newly occupied by~~ *E. clausum*. *Enallagma clausum* and *E. boreale* obviously share a close evolutionary history (Brown, McPeck & May, 2000) and can share parasites across sites (Mlynarek, Knee & Forbes 2014a) but *E. clausum* may not share the evolutionary history with the particular parasite population at the new sites. The parasites have a naïve close relative to exploit (Maron & Vila, 2001) a pattern also observed in anolis lizards (Schall, 1992) and in oak herbivory, where there is increased herbivory on non-native oak species in the presence of native close relatives (Pearse & Hipp, 2014).

Another alternative explanation for the observed patterns in *E. clausum* may involve the range edge effect. There are many contributing factors that limit the extent of a species range

limit, one of which is increased parasite rates (Sexton et al., 2009). For example, populations of *Calopteryx maculata* at the northern edge of their Canadian range have higher levels of parasitism than those closer to the center of their range (J. Mlynarek unpubl.) ~~although the mechanisms could not be identified~~. Additionally, Kaunisto & Suhonen (2013) suggested that host damselflies at the edges of their ranges are under higher environmental stress, and therefore ~~cannot put as much energy into parasite evasion or immunity~~.

It is possible that *E. clausum* did undergo enemy release when it first arrived at the sites but the parasites subsequently quickly evolved to include them as a host. In this context, they are not supporting Evolution of Increased Competitive Ability Hypothesis either. However, a decade is a short time period of time in an evolutionary context, considering that water mites and damselflies tend to have univoltine life cycles in these areas (Smith, Cook & Smith, 2010). *Enallagma clausum* has recently expanded its range on a continent it has long occupied. It may, therefore, not be leaving its enemies behind, in the sense that the mites and gregarines from its former geographic range may be only slightly different from those in its newly occupied range. Over the course of the ever-changing post-Pleistocene environment, with changes in both climate and the distribution of appropriate habitats, damselflies and their parasites have likely existed in a continually shifting dynamic for millennia. Indeed, species that are expanding their range may actually be under higher parasitism pressure in new sites, whereas those already present may benefit from their arrival in the short term.

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Table 1: Prevalence and intensity of *Arrenurus* water mite and gregarines on *Enallagma boreale* and *Enallagma clausum* from six sites in Eastern Alberta and Western Saskatchewan (see supplemental Table S1 for raw data). Prevalence with Clopper Pearson 95% confidence limits and mean intensity with Bootstrap (BCa) 95% confidence limits with 2000 replications.

Species	Site Type	Site	N	<i>Arrenurus</i>		N	Gregarine	
				Prevalence	Intensity		Prevalence	Intensity
<i>E. boreale</i>	Old	Lenore	61	0.32 (0.24–0.49)	9.00 (5.27–12.64)	51	0.36 (0.26–0.54)	10.59 (6.05–21.20)
		Manitou	186	0.37 (0.31–0.46)	8.59 (6.24–12.27)	69	0.31 (0.22–0.46)	11.80 (8.26–18.48)
		Redberry	48	0.51 (0.49–0.78)	27.48 (20.81–37.10)	43	0.22 (0.15–0.44)	9.67 (4.83–16.50)
	New	Gull	131	0.18 (0.11–0.25)	7.92 (3.70–19.22)	65	0.09 (0.03–0.19)	7.00 (2.00–13.50)
		Johnson	59	0.24 (0.14–0.37)	17.47 (9.64–36.14)	59	0.45 (0.34–0.61)	9.79 (5.96–14.93)
		Sylvan	168	0.11 (0.06–0.16)	22.42 (14.72–41.78)	55	0.12 (0.05–0.24)	21.86 (7.00–46.14)
	Old	Lenore	67	0.08 (0.01–0.13)	10.67 (2.00–29.33)	48	0.09 (0.03–0.23)	9.00 (3.60–17.00)
		Manitou	38	0.06 (0.01–0.18)	16.67 (1.00–24.50)	37	0.08 (0.01–0.18)	2.25 (2.00–4.50)
		Redberry	174	0.05 (0.02–0.10)	38.45 (19.76–50.67)	70	0.03 (0.00–0.08)	17.50 (N/A)
<i>E. clausum</i>	New	Gull	83	0.17 (0.1–0.27)	20.00 (3.07–31.14)	65	0.07 (0.02–0.15)	3.80 (2.00–6.50)
		Johnson	73	0.29 (0.19–0.41)	13.77 (7.96–23.19)	61	0.42 (0.29–0.54)	11.41 (7.80–16.48)
		Sylvan	62	0.67 (0.58–0.82)	26.98 (20.39–36.41)	62	0.11 (0.06–0.24)	5.38 (2.88–9.50)

332 Table 2 – Results of two-way ANOVA testing for differences between host species and site for (a)  
 333 *Arrenurus* prevalence and (b) *Arrenurus* intensity infections in *Enallagma clausum*, a native  
 334 species whose range is expanding in western Canada, and *Enallagma boreale*, a closely related  
 335 long-established species.

Source	df	SS	F	P
(a) Prevalence				
Species	1	0.05	1.93	0.18
Site	1	0	0.18	0.66
Species*Site	1	0.53	22.18	<b>&lt;0.01</b>
(b) Intensity				
Species	1	3.51	0.1	0.75
Site	1	8.42	0.25	0.62
Species*Site	1	209.8	6.16	<b>0.02</b>

336 Table 3 – Results of two-way ANOVA testing for differences between host species and site for (a)  
 337 gregarine prevalence and (b) gregarine intensity infections in *Enallagma clausum*, a native  
 338 species whose range is expanding in western Canada, and *Enallagma boreale*, a closely related  
 339 long-established species.

Source	df	SS	F	P
(a) Prevalence				
Species	1	0.12	5.89	0.03
Site	1	0	0.05	0.83
Species*Site	1	0.08	3.68	0.07
(b) Intensity				
Species	1	23.1	7.41	0.01
Site	1	0.21	0.07	0.8
Species*Site	1	7.69	2.47	0.13



340 Figure 1: Map of study sites testing the enemy release hypothesis in the native species *Enallagma*  
 341 *clausum* in Saskatchewan and Alberta. Old sites: L = Lenore lake, M = Manitou lake, and R =  
 342 Redberry lake; New sites: G = Gull lake, J = Johnson lake, and S = Sylvan lake.

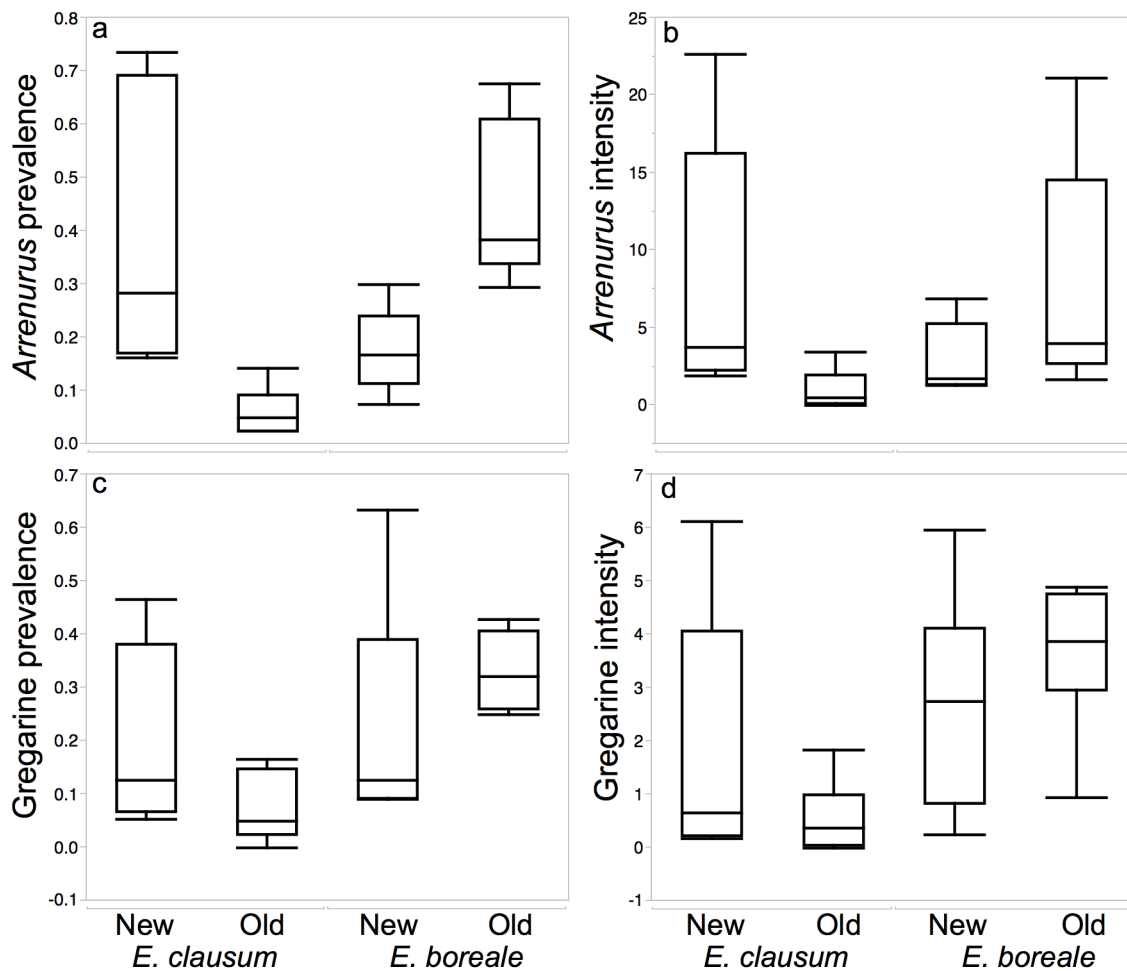


Figure 2: Boxplot of differences in (a) *Arrenurus* prevalence, (b) *Arrenurus* intensity, (c) gregarine prevalence and (d) gregarine intensity between old and new sites of *Enallagma clausum*, a native species whose range is expanding, and a closely related, well-established species *Enallagma boreale*.