

1 **Defensive responses by a social caterpillar are tailored to different**  
2 **predators and change with larval instar and group size**

3

4 MELANIE McCLURE<sup>1</sup> AND EMMA DESPLAND<sup>1,\*</sup>

5 <sup>1</sup>*Biology Department, Concordia University, Montreal, Quebec, Canada*

6 *\*Corresponding author. E-mail: despland@alcor.concordia.ca; Tel.: 1 514 848-2424 ext*  
7 *3426*

8

9 **Key words:** antipredator, group behavior, predation, *Malacosoma disstria*, prey-predator  
10 interactions

11

12 **Abstract:**

13 Gregariousness in animals is widely accepted as a behavioral adaptation for  
14 protection from predation. However, predation risk and the effectiveness of a prey's  
15 defense can be a function of several other factors, including predator species, and prey  
16 size or age. The objective of this study was to determine if the gregarious habit of  
17 *Malacosoma disstria* caterpillars is advantageous against invertebrate natural enemies,  
18 and whether it is through dilution or cooperative defenses. We also examined the effects  
19 of larval growth and group size on the rate and success of attacks.

20 Caterpillars of *M. disstria* responded with predator-specific behaviors, which led  
21 to increased survival. Evasive behaviors were used against stinkbugs, while thrashing by  
22 fourth instar caterpillars and holding on to the silk mat by second instar caterpillars was  
23 most efficient against spider attacks. Collective head flicking and biting by groups of  
24 both second and fourth instar caterpillars were observed when attacked by parasitoids.

25 Increased larval size decreased the average number of attacks by spiders but  
26 increased the number of attacks by both stinkbugs and parasitoids. However, increased  
27 body size decreased the success rate of attacks by all three natural enemies, and increased  
28 handling time for both predators.

29 Larger group sizes did not influence the number of attacks from predators but  
30 increased the number of attacks and the number of successful attacks from parasitoids. In  
31 all cases, individual risk was lower in larger groups. Caterpillars showed collective  
32 defenses against parasitoids but not against the walking predators.

33 These results show that caterpillars use different tactics against different natural  
34 enemies. Overall, these tactics are both more diverse and more effective in fourth instar

35 than in second instar caterpillars, confirming that growth reduces predation risk. We also  
36 show that grouping benefits caterpillars through dilution of risk, and, in the case of  
37 parasitoids, through group defenses. The decreased tendency to aggregate in the last  
38 larval instar may therefore be linked to decreasing predation risk.

39

40 **Introduction:**

41 Many animals live in groups, and gregariousness has been shown to provide  
42 protection from predation in a variety of taxa such as anuran larvae (DeVito 2003; Smith  
43 and Awan 2009), fish (Krause and Godin 1995), invertebrates (Clark and Faeth 1997;  
44 Uetz *et al.* 2002; Lemos *et al.* 2005), small mammals (Hass and Valenzuela 2002;  
45 Rogovin *et al.* 2004), ungulates (Mooring and Hart 1992) and many others. Predation risk  
46 and the effectiveness of a prey's defense can be a function of several variables, including  
47 prey group size, and individual prey size as a function of age (Botham *et al.* 2006; Smith  
48 and Awan 2009). Although larger groups of prey may be more easily discovered and may  
49 suffer more frequent attacks due to increased conspicuousness, hunting success of  
50 predators and per capita predation risk of prey have also been shown to decrease in larger  
51 groups (Lawrence 1990; Clark and Faeth 1997; Hunter 2000; Botham *et al.* 2005). Group  
52 members may suffer a lower risk of capture because of cooperative defense, enhanced  
53 advertisement of unprofitability in aposematic species, shared and more effective  
54 vigilance and a reduced probability of predation by virtue of a dilution effect when a  
55 predator can take only a limited number of individuals from the group (e.g Seyfarth *et al.*  
56 1980; Peterson *et al.* 1987; Vulinec 1990; Mooring and Hart 1992; Uetz *et al.* 2002;  
57 DeVito 2003). In addition, animals in the centre of a group can decrease their risk of  
58 predation by surrounding themselves with others (Tostowaryk 1971; Mooring and Hart  
59 1992; Krause *et al.* 1998), which Hamilton (1971) termed the selfish herd effect.

60 As prey individuals grow, their vulnerability to predators can also change. Smaller  
61 predator species may not be physically capable of handling large prey, or the costs of  
62 subduing them may be too great (Peters 1983; Warren and Lawton 1987; Cohen *et al.*

63 1993), whereas larger predator species may avoid small prey because they are too costly  
64 to handle for the energy gains. Gaston *et al.* (1997) found that the body masses of the bird  
65 species feeding on successive instars of the mopane worm were strongly correlated with  
66 the larvae's mass. The ability of pentatomid predators to subdue caterpillars also depends  
67 on the larvae's size and behavior (Iwao and Wellington 1970).

68 Most prey are subject to predation from multiple predators, and different defenses  
69 are thought to have evolved in response to selective pressures from different types of  
70 predators. As such, different predators may elicit different responses, or a prey species  
71 may adopt a general response which provides protection from many different types of  
72 predator (Botham *et al.* 2006). Generalized rather than species-specific responses may  
73 benefit prey in species that co-occur with multiple similar predators (Webb *et al.* 2010),  
74 hence the importance of testing the effectiveness of a prey's defensive mechanisms  
75 against different predators. Yet many studies investigating behavioral responses in  
76 predator-prey interactions have focused on single predators, and experimental evidence  
77 that prey benefit in terms of survival by adopting different responses to different  
78 predators appears to be lacking (Botham *et al.* 2006; Castellanos and Barbosa 2006).

79 We examined the responses of *Malacosoma disstria* caterpillars against three  
80 natural enemies and tested the effects of larval growth and gregarious behavior on the  
81 rate and success of attacks. Caterpillars of *M. disstria* are gregarious until the final larval  
82 stadium, and decreased predation risk is often listed among the benefits of group-living  
83 for this (Parry *et al.* 1998) and other gregarious caterpillar species (Reader and Hochuli  
84 2003). *Malacosoma disstria* caterpillars are collective nomadic foragers and use  
85 pheromone trails to travel as a cohesive group between feeding sites. These caterpillars

86 hatch in early spring when food quality is high and they develop rapidly to escape  
87 predation (Parry *et al.* 1998), as predation risk is thought to decrease with increasing  
88 larval size (Costa 1993; Reavey 1993). The importance of predation in shaping the  
89 gregarious and fast-developing life history traits is not known, nor is the identity of the  
90 predators exerting the selection pressure. Caterpillars of *M. disstria* are unpalatable to  
91 most vertebrates (Heinrich 1983; Heinrich 1993a), but little is known of the defensive  
92 mechanisms against invertebrate predators (see Fitzgerald 1995). Synchronous flicking of  
93 the body has been described for many social caterpillars (see Fitzgerald and Costa 1999  
94 and references therein), and some, such as the closely-related *Malacosoma americanum*,  
95 also combine these displays with defensive regurgitation of enteric fluid containing host-  
96 derived benzaldehyde when attacked by predatory ants (Peterson *et al.* 1987).

97         The objective of this study was to determine if the gregarious habit of *M. disstria*  
98 is advantageous against invertebrate predation, and whether it is through dilution or  
99 cooperative defenses. We also hypothesized that the rate and success of attacks would  
100 decrease with increasing group size and caterpillar size (as a function of larval instar), but  
101 that these could vary between the three natural enemies tested, depending on the  
102 behavioral response exhibited in each case.

103

#### 104 **Methodology:**

105         Unhatched egg masses of *M. disstria* were collected from Southern Ontario,  
106 Canada (44°33.5N, 76°24.1W) in March 2009 and stored at 4°C with 80% R.H. until use.  
107 To minimize mortality from pathogens, egg bands were sterilized by soaking in 5%  
108 sodium hypochlorite as described by Grisdale (1985). Caterpillar colonies arising from a

109 single egg mass were kept in plastic rearing containers and kept in a rearing chamber  
110 under a controlled light and temperature regime of 21°C, 70% RH and 16L: 8D.  
111 Caterpillars were fed *ad libitum* on a nutritionally balanced, standard wheat germ-based  
112 meridic artificial diet (Addy 1969). Although *M. disstria* caterpillars have never been  
113 observed to regurgitate, gut content may affect predation and so caterpillars were given  
114 fresh leaves of their primary host, trembling aspen (*Populus tremuloides*), 24 hrs before  
115 being used in experiments with the walking predators. Leaves were collected from  
116 multiple trees in Montreal, Quebec and were sterilized using 1% hypochlorite solution  
117 and rinsed with tap water against the possible presence of pathogens. All experiments  
118 were conducted at temperatures ranging between 20-23°C and 50-60% RH.

119         Fifteen species of hemipteran stinkbugs are known to prey on tent caterpillars, but  
120 *Podisus maculiventris* Say is one of the most common and it is distributed over most of  
121 the United States and southern part of Canada. Stinkbugs overwinter as adults and are  
122 active in early spring, searching for prey and responding within a short distance or after  
123 physical contact (Evans 1982). When a prey is detected, stinkbugs stretch out their  
124 proboscis before eventually attacking by inserting their stylets. Stinkbugs appear limited  
125 to attacking caterpillars of 20 mm or less (Evans 1982). Beetles in the genus *Calosoma*  
126 are also well-known predators of tent caterpillars, which are grasped and cut in half with  
127 sharp mandibles (Fitzgerald and Costa 1999 and references therein). Spiders are also  
128 important generalist predators, especially of earlier instars (McClure and Despland 2010;  
129 Ronnas *et al.* 2010). Although many species of parasitoids attack the eggs or pupae of  
130 *Malacosoma*, a few families also attack the larval stage (see Fitzgerald 1995 and  
131 references therein; Williams *et al.* 1996). *Malacosoma* caterpillars are known for flicking

132 the anterior portion of their body when attacked by parasitoids, and this behavior quickly  
133 propagates through the group into a synchronized behavior. Prop (1960) found that such  
134 group displays in gregarious sawflies deterred oviposition by an ichneumonid parasitoid.

135 Three invertebrate predators, which co-occur with *M. disstria*, were therefore  
136 initially selected: stinkbugs (*Podisus maculiventris*) were obtained from The Bug Factory  
137 (Canada) and carabid beetles (*Calosoma sp.*) and spiders (*Thanatus vulgaris*) were  
138 collected in Montreal (Quebec, Canada). However, in preliminary trials (N=6) carabid  
139 beetles were found to be too mobile, with beetles escaping the set-up often without  
140 contacting the group of caterpillars (N=4), and were subsequently not used. A generalist  
141 parasitoid wasp (*Hyposoter fugitivus*) was also selected and was obtained from Dr.  
142 Stoltz's rearing colony (Dalhousie Univeristy in Halifax, Canada). All walking predators  
143 were starved 24h before use and a predator used in a test was not used again until it had  
144 fed and again been deprived of food. The predators were fed larvae of the greater wax  
145 moth, *Galleria mellonella*, and were supplied with moisture via a soaked paper towel.  
146 The parasitoids were fed with honey droplets. All walking predators were maintained in  
147 rearing chambers under a controlled light and temperature regime of 21°C, 70% RH and  
148 16L: 8D, and parasitoids were stored at 10°C until use.

149 Tested group sizes were of 2, 10 and 30 second or fourth instar caterpillars. Only  
150 second and fourth instar caterpillars were studied during our experiments, as they reflect  
151 distinct differences in both body size and group behaviour (older caterpillars exhibit more  
152 independent locomotion). The experimental set-up consisted of a plastic arena (43 cm  
153 long x 3 cm) covered in brown paper. The arena was balanced on rubber stoppers covered  
154 in acetate, placed in a tray containing 2 cm of water in order to prevent caterpillars from



155 leaving. Caterpillars were placed at one end of the arena 20 minutes before the  
156 introduction of a predator or 2 parasitoids to allow them to acclimatize and caterpillars  
157 were only used once. When using parasitoids, the experimental setup was placed in a  
158 mesh cage.

159 All group size, instar and natural enemy combinations were repeated 20 times.  
160 Experiments were terminated after 20 min for predators and 40 min for parasitoids. This  
161 was considered enough time to observe an attack, as on average predators attacked in less  
162 than 1 minute (mean±SE of 49.22±11.49 secs), and parasitoids did so in less than 8  
163 minutes (mean±SE of 7.96±1.10 min). A video camera was mounted above the arena and  
164 all experiments were recorded for further analysis. The likelihood of attack in each  
165 treatment was analysed using chi-square. A multivariate analysis of variance  
166 (MANOVA) was used for each natural enemy to determine if the number of caterpillars  
167 attacked and the number of those attacks that were successful was significantly affected  
168 by group size and/or larval instar. The MANOVA for both walking predators also  
169 included the latency to attack (i.e. the time from the moment the predator is introduced  
170 into the arena to the first attack observed) and the handling time (i.e. the time required for  
171 a predator to subdue its prey) as dependent variables. In addition, the MANOVA for the  
172 stinkbugs also included the time needed to perceive the caterpillars (determined as when  
173 the proboscis was raised). The MANOVA for the parasitoids included the time caterpillar  
174 groups spent head flicking after an attack as a dependent variable. Behavioral  
175 descriptions of predator or parasitoid attacks and escape responses of caterpillars were  
176 also noted for every predator-prey combination. Parasitizing success was determined by  
177 rearing some of the groups (a minimum of 7 replicates per group size-instar combination

178 was used for a total of N=45) until parasitoid emergence. Mortality risk per caterpillar  
179 from each natural enemy was also calculated by dividing the number of individuals  
180 within a group by the number of successful attacks and averaging them for all larval  
181 instar and group sizes.

182

### 183 **Results:**

184 Although these caterpillars are covered in setae, especially in the later instars,  
185 observations during this study gave no indication that they played any role in defense  
186 against the predators and parasitoids that were used. Because spiders and stinkbugs are  
187 only capable of preying on one individual at a time and require time to consume it, there  
188 could not be more than one successful attack per given trial. However, when  
189 unsuccessful, multiple attacks by these predators could be made within a single trial.  
190 *Malacosoma disstria* caterpillars were never observed to regurgitate. Group activity  
191 (defined as either active or resting) was never a significant predictor of either attacks or  
192 the success of these attacks for any of the natural enemies.

193

### 194 Carabid beetles

195 Preliminary trials with carabid beetles (N=6) were done with groups of 30 fourth  
196 instar caterpillars, but proved to be difficult as the carabid beetles were large and too  
197 mobile for the chosen experimental set-up. In 4 of the trials, the beetle repeatedly escaped  
198 the set-up without making contact with the caterpillars. In 2 trials, the beetle attacked 1  
199 caterpillar within the group and quickly devoured it. Predated individuals thrashed  
200 vigorously, but were never successful at escaping. The group's response consisted of all

201 caterpillars walking away and relocating at the opposite end of the bridge set-up while the  
202 predator was occupied with its prey. Although carabid beetles have been described as  
203 being aggressive predators which often attack multiple caterpillars within a group, only  
204 one individual was observed to be attacked (N=2). Both beetles subsequently escaped the  
205 set-up shortly after the predation events.

206

### 207 Spiders

208         Spiders attacked by pouncing on the caterpillars and rapidly piercing them with  
209 their chelicerae. Responses of caterpillars attacked by spiders were different for second  
210 and fourth instars (Table 1). Although 42% of second instar caterpillars thrashed when  
211 grasped and a small number bit the spiders (5%), this was never successful. Surprisingly,  
212 52% of the attacked caterpillars responded by gripping onto the silk mat: when spiders  
213 were unable to dislodge the caterpillar from its silk mat, they abandoned it. This tactic  
214 was successful in evading a predation event 80% of the time and bitten caterpillars that  
215 were abandoned always survived. Although this is not a group response per se, a group is  
216 needed to build a silk mat and this response was therefore not possible for individuals in  
217 groups of two. The larger fourth instar caterpillars were more aggressive in their  
218 responses. All individuals that were attacked thrashed vigorously. When not combined  
219 with any other behavior, this was successful in only 37% of attacks. Survival was similar  
220 when thrashing was combined with biting, but increased if caterpillars dropped off the  
221 bridge, which was always an effective evasive tactic. This would also be advantageous in  
222 the field as larger caterpillars can survive in the absence of conspecifics (Fitzgerald and  
223 Costa 1999).

224 For spiders, the time to attack (i.e. the latency for the spider to attack from the  
225 moment it is introduced) was not significantly influenced by group or larval size, but  
226 handling time was much longer for fourth instar caterpillars than for second instar  
227 caterpillars ( $162.00 \pm 33.87s$  vs.  $2.58 \pm 0.33s$ ; Table 2). The probability of at least one  
228 attack occurring during the trial decreased with larval instar ( $\chi^2=4.805$ ;  $df=1$ ;  $p=0.028$ )  
229 but was not affected by group size ( $\chi^2=1.669$ ;  $df=2$ ;  $p=0.434$ ). The number of attacks per  
230 trial increased with group size for second but decreased for fourth instar caterpillars (Fig.  
231 1). Attacks on fourth instar caterpillars were less likely to be successful than on second  
232 instar caterpillars. Attack success rate was not affected by group size (Table 2), and  
233 therefore the per capita mortality risk decreased in larger groups (Fig. 4). Position within  
234 the group was also found to be important, as the centre of the group sustained fewer  
235 attacks.

236

### 237 Stinkbugs

238 Caterpillars responded differently to stinkbugs, which after detecting the  
239 caterpillars raised their proboscis and approached them slowly, than they did to spiders.  
240 When second instar caterpillars detected the stinkbug before an attack (which occurred in  
241 10% of cases), they took evasive responses by jerking away (Table 1). This was always  
242 successful as stinkbugs retreated. Once the predator had inserted its stylets into the  
243 caterpillar, none succeeded in escaping despite 60% of caterpillars thrashing in response  
244 to the attack. Fourth instar caterpillars showed a larger range of behavioral responses to  
245 stinkbugs, which occurred either singly or in various combinations. Caterpillars took  
246 evasive measures in 37% of cases, either by walking quickly out of the predator's path or

247 by jerking away, and this was always successful in evading an attack. Predators were  
248 sometimes seen pursuing an escaping caterpillar with extended proboscis, but they never  
249 succeeded in catching them and quickly gave up the chase. Caterpillars responded to the  
250 stylets being inserted into their body by thrashing 56% of the time, but this was only  
251 effective in 17% of cases, even when combined with biting. Although only very few  
252 attacked caterpillars were able to both thrash and fall off the bridge (2%), this was always  
253 a successful tactic and these caterpillars always survived the piercing of their cuticle.

254 Whether stinkbugs attacked at least once was not significantly affected by either  
255 instar ( $\chi^2=1.634$ ;  $df=1$ ;  $p=0.201$ ) or group size ( $\chi^2=2.467$ ;  $df=2$ ;  $p=0.291$ ). Stinkbugs  
256 launched more attacks per trial against fourth instar caterpillars, but were more successful  
257 in capturing second instar caterpillars (Fig. 2). Again, because the number of successful  
258 attacks was not affected by group size, the mean mortality risk decreased with group size  
259 (Fig. 4). The time needed for stinkbugs to perceive the caterpillars (i.e. the time between  
260 introducing the stinkbug to the arena and the first moment they raised their proboscis)  
261 was not affected by either larval instar or group size (Table 2), but both the amount of  
262 time required to attack ( $35.68\pm 10.43s$  vs.  $200.57\pm 43.29s$ ; Table 2) and to subdue the prey  
263 (i.e. for the attacked caterpillar to stop moving) ( $25.58\pm 3.64s$  vs.  $168.95\pm 32.15s$ ; Table 1)  
264 was significantly longer for fourth instar caterpillars. Position within the group was again  
265 found to be important, as the centre of the group did not sustain any attack for either the  
266 second or fourth instar caterpillars.

267

268 Parasitoid wasps

269 Caterpillars reacted to parasitoid attacks, which stung the caterpillars with their  
270 ovipositor, both individually and as a group by flicking their heads, and in some cases,  
271 head flicking was accompanied by biting, which made it a far more effective tactic (Table  
272 1). Groups of two second instar caterpillars almost never reacted to being attacked.  
273 However, for both second and fourth instar caterpillars, groups of 30 were more likely to  
274 use biting along with flicking than groups of 10 caterpillars.

275 The probability of at least one attack by a parasitoid per trial significantly  
276 increased with group size ( $\chi^2=9.872$ ;  $df=2$ ;  $p=0.007$ ) but was not affected by larval instar  
277 ( $\chi^2=0.573$ ;  $df=1$ ;  $p=0.449$ ). The number of attacks increased with caterpillar instar (Fig.  
278 3), but the number of successful attacks (i.e. determined by the subsequent emergence of  
279 a parasitoid from the caterpillar) decreased with instar (Table 2). The number of attacks  
280 and of successful attacks increased with group size (Fig. 3), but the individual mortality  
281 risk still decreased with group size (Fig. 4). The time before an attack (i.e. time elapsed  
282 between the start of the experiment and the first attack observed) was not influenced by  
283 either caterpillar instar or group size, but the amount of time caterpillars spent head  
284 flicking after an attack significantly increased with group size (Table 2). Position within  
285 the group was again found to be important, as the centre of the group sustained fewer  
286 attacks.

287

## 288 **Discussion**

289 Caterpillars of *M. disstria* responded to attacks with predator-specific behaviors,  
290 which in many cases were successful in warding off attacks. When stinkbugs were used  
291 as predators, evasive behaviors were the most efficient in increasing survival, as has also

292 been observed for *Nezara viridula* (De Clercq *et al.* 2002) and *Bombyx mori* (Lemos *et*  
293 *al.* 2005) caterpillars. These behaviors were never observed against spiders. Many fourth  
294 instar caterpillars thrashed when attacked by either spiders or stinkbugs, but this behavior  
295 was most successful when used against spiders. Second instar caterpillars that were  
296 attacked by spiders sometimes responded by holding onto the silk mat. This behavior was  
297 never observed with stinkbugs, and it would not likely have been successful, as shriveled  
298 caterpillar carcasses are often found still attached to naturally occurring tents and silk  
299 mats of *Malacosoma* colonies attacked by *Podisus* stinkbugs. Head flicking and biting  
300 were observed in both second and fourth instar caterpillars when attacked by parasitoids,  
301 but not when attacked by walking predators. Predator specific responses of *M. disstria*  
302 groups were also observed during preliminary trials using *Calosoma* beetles: attacked  
303 individuals thrashed vigorously, but unsuccessfully as even fourth instar caterpillars are  
304 much smaller than the beetles. But while the beetle was busy with one prey, the rest of  
305 the caterpillar group moved away together and relocated to a new bivouac elsewhere,  
306 which is important because a single beetle can eradicate an entire colony (Fitzgerald and  
307 Costa 1999). Other studies (e.g. Clark and Faeth 1997) have shown that, if predators are  
308 not satiated by a single prey item, or if they show a strong and very rapid numerical  
309 response, they can annihilate entire groups and group relocation may be beneficial. Indeed,  
310 groups of *M. disstria* caterpillars have also been shown to relocate their bivouac in  
311 response to attacks by *Polistes* wasps (McClure and Despland 2010). However, relocation  
312 of the entire group before a food patch is depleted is likely costly, and it makes sense that  
313 this response would only be observed when caterpillar groups are attacked by predators  
314 capable of successfully preying on most, if not all, of the group.

315           Although different responses to different predators is believed to be adaptive and  
316 has previously been suggested, little experimental work has been done to empirically  
317 demonstrate the behavioral ecology of defenses (Botham *et al.* 2006).

318           The escape responses of *M. disstria* caterpillars to predator attacks also varied  
319 with larval instar. Smaller caterpillars had fewer defensive behaviors and never dropped  
320 off the bridge, probably because the cost of being separated from the group is much  
321 higher for younger caterpillars (Despland and Le Huu 2007). Although second instar  
322 caterpillars were at times aggressive against parasitoids, biting their legs and antennae,  
323 they never successfully bit either the spiders or the stinkbug predators, both of which are  
324 larger than the parasitoids. The larger fourth instar caterpillars, however, were more  
325 likely to defend themselves with aggressive retaliation such as biting against all  
326 predators, as the value of this defense increases with the size of the prey relative to its  
327 predator.

328           As such, the number of caterpillars successfully predated or parasitized decreased  
329 with increasing body size, and the time required to subdue the prey increased for both  
330 spiders and stinkbugs. However, stinkbugs and parasitoids did attack fourth instar  
331 caterpillars more often. Because stinkbugs are cautious predators that slowly approach  
332 their prey, failed attempts often occurred before any physical contact was made. As such,  
333 attempting to attack a larger caterpillar was possibly less costly for stinkbugs than for  
334 spiders, and in fact, stinkbugs were more likely to try again. However, this is likely to  
335 change with continued growth of the caterpillars and more aggressive defensive  
336 behaviours (Morris 1963), and in fact Evans (1983) observed that stinkbugs experienced  
337 increasing difficulty in capturing *Malacosoma* caterpillars as the season advanced. As the



338 caterpillars grew, they rapidly gained the ability to defend themselves from attacking  
339 adult stinkbugs by thrashing vigorously and forcing the timid stinkbugs to retreat and  
340 abandon the attack.

341         Although parasitoids can develop in second instar caterpillars, they face a higher  
342 risk of the host dying before the parasitoid larvae can complete its development (pers.  
343 obs.). Therefore fourth instar caterpillars are better hosts and this is most likely why  
344 parasitoids preferentially attacked more of the fourth instar caterpillars. Yet the  
345 proportion of caterpillars successfully parasitized decreased with increasing larval size,  
346 which suggests a trade-off for parasitoids. This may be due to both an increasing  
347 difficulty in successfully parasitizing the caterpillars due to defensive behaviours such as  
348 biting, and a stronger immune system in older caterpillars. As such, successful parasitism  
349 is likely to continue decreasing with increasing growth of the caterpillars. Thus overall  
350 increased body size lowers likelihood of successful attack for all three natural enemies  
351 but, at least for parasitoids, larval body size appears to increase attractiveness of prey.

352         Grouping appeared to lower individual risk from all three natural enemies via  
353 dilution and the selfish herd effect. In all three cases, individual risk decreased with  
354 increasing group size and individuals in the center of the group were at a lesser risk of  
355 sustaining attacks than individuals situated at the periphery.

356         For spiders and stinkbugs, group size had no effect on the number of attacks or  
357 the number of successfully captured caterpillars. Because the number of prey successfully  
358 attacked was never more than one per trial, mortality risk always decreased with group  
359 size. There were no group responses for either second or fourth instar caterpillars  
360 attacked by either of the walking predators and therefore, against these predators, dilution

361 of risk appears to be the only group benefit. Presumably, larger aggregations would be  
362 beneficial in the field if they do not attract more predators. For the gregarious caterpillar  
363 *Halisidota caryae* (Lawrence 1990), larger aggregations did not attract more invertebrate  
364 predators than did smaller ones, and so the likelihood of being taken was lower in a larger  
365 group. For *Malacosoma* species, Evans (1983) found that the density of caterpillars in a  
366 group was always high enough that the functional response of a pentatomid predator was  
367 independent of larval density.

368 By contrast, collective defense was observed against parasitoids. The parasitoids  
369 attacked more than one caterpillar once a group was located. However, despite multiple  
370 attacks and a higher attack success rate, mean mortality still decreased for individual  
371 caterpillars living in larger groups. The number of individuals successfully parasitized did  
372 not increase as rapidly as the number of individuals within a group. This may in part be  
373 because the optimal foraging time spent at a patch for parasitoids is limited by a  
374 diminishing return (Wajnberg 2006), but may also be due to the increasing difficulty in  
375 attacking defensive groups.

376 Indeed, although there was no evidence for group vigilance in trials done with  
377 spiders or stinkbugs, caterpillars appeared to benefit from the warning of a parasitoid's  
378 presence, possibly through the wing vibrations of parasitoids, vibrations in the silk mat  
379 generated by flicking caterpillars, and/or through the direct physical contact with flicking  
380 caterpillars, although they don't appear to respond to vibrations caused by approaching  
381 predators or by thrashing conspecifics. Caterpillars attacked by the parasitoids usually  
382 aggregated as tight flicking groups and displayed co-operative defenses such as  
383 simultaneous biting of the wasps' legs and antennae. Individuals who started flicking

384 before having sustained an attack themselves therefore appear to be benefiting from the  
385 signaling of other individuals, but those who have already been attacked also benefit as  
386 they may be attacked more than once (pers. obs.). Although groups of two caterpillars  
387 occasionally displayed these behaviors, they occurred less often, at a lower intensity and  
388 for a shorter time. The time spent flicking by groups after the first attack also increased  
389 with group size, which suggests that the effectiveness of this behaviour increases for  
390 larger groups.

391 In conclusion, we show that *M. disstria* exhibit different behaviours in response to  
392 different predators and at different larval stadia. Like guppies (Botham *et al.* 2006) and  
393 monkeys (Seyfarth *et al.* 1980), these caterpillars are able to discriminate between  
394 different predators, likely as a result of very different modes of attack, and respond  
395 appropriately. Indeed, this study shows experimentally that prey benefit in terms of  
396 survival by adopting different responses, although how these caterpillars are able to  
397 identify the predator and decide which response to make has yet to be determined.

398 In general, fourth instar caterpillars showed more varied defensive responses,  
399 including falling off the bridge and biting the aggressor, and were more successful  
400 against all three natural enemies. Our results confirm that larval vulnerability is greatest  
401 in the early larval instars, supporting the idea that rapid growth constitutes a defensive  
402 benefit. An extended development time in herbivorous insects increases larval exposure  
403 to natural enemies, termed the slow-growth-high-mortality hypothesis, and has been  
404 shown in many species (Schultz 1983; Benrey and Denno 1997). For example, Parry *et al.*  
405 (1998) found that survivorship of later hatching *Malacosoma* caterpillars was  
406 drastically reduced by invertebrate predation and Evans (1982) observed that during

407 unfavorable weather in the spring, the activity of predatory stinkbugs was temporarily  
408 suppressed and enabled the tent caterpillars to escape predation by growing to sizes too  
409 large to be subdued by the predators. We show that increased size is advantageous for  
410 caterpillars against three very different modes of attack, due not only to the predator's  
411 difficulty in handling larger prey, but also to the caterpillar's broader range of defensive  
412 behaviors. Our results also show a lower per capita predation risk in larger groups. In the  
413 case of spiders and stinkbugs, the benefits of grouping could only be attributed to dilution  
414 of risk, but against parasitoids, caterpillars also exhibited group defenses. Improved anti-  
415 predator defense has been suggested as a benefit to group living in a wide range of taxa  
416 (e.g. Hass and Valenzuela 2002; Uetz *et al.* 2002; e.g. DeVito 2003; Rogovin *et al.* 2004;  
417 Lemos *et al.* 2005; Smith and Awan 2009), including many caterpillars (see Vulinec  
418 1990). We confirm that grouping does indeed protect *M. disstria* caterpillars against  
419 predation and that they use group defenses in some contexts. Aggregations of early  
420 instars of *M. disstria* have also been shown to benefit from group thermoregulation  
421 (McClure *et al.* 2010) which enhances larval growth rates (Levesque *et al.* 2002), and  
422 thus the aggregated larval lifestyle may also indirectly reduce predation by decreasing  
423 exposure to predators. Grouping thus appears to protect *M. disstria* against predation via  
424 several simultaneously acting mechanisms: predator dilution, group defenses, faster  
425 development and possibly aposematism (Heinrich 1993b). Hunter (2000), who compared  
426 the shapes of published survivorship curves of gregarious and solitary Lepidoptera and  
427 Symphyta, concluded that there was something in addition of the possession of defenses  
428 that explains the higher larval survival of gregarious species. This study further supports  
429 their suggestion that dilution of risk, possibly in concert with increased group defense

430 behaviors, and reduced duration of exposure to enemies because of rapid development  
431 time may explain the survival advantage of gregariousness.

432         Finally, the decreased tendency to aggregate of later instars of *Malacosoma*  
433 species has been tied to an increase in food competition (Despland and Le Huu 2007) and  
434 a reduced need for thermoregulation (McClure *et al.* 2010); our results suggest that it may  
435 be further enabled by caterpillars' increased ability to defend themselves against  
436 invertebrate predators.

437

#### 438 **Acknowledgements**

439 Thanks go to Dr. Don Stoltz (Dalhousie Univeristy in Halifax, Canada) for providing the  
440 facilities and insects to complete the parasitoid experiments. Experiments done at  
441 Dalhousie were possible due to financial assistance by Dr. Stoltz and a travel scholarship  
442 to M.M. awarded by Centre d'Étude de la Forêt. Financial support was provided by the  
443 Canadian Natural Sciences and Engineering Research Council as a Discovery Grant to  
444 E.D., and by a Canadian Natural Sciences and Engineering Research Council Graduate  
445 Student Research Award to M.M. The experiments comply with the current laws of the  
446 country in which they were performed.

447

## References

- Addy ND (1969) Rearing the forest tent caterpillar on an artificial diet. *J Econ Entomol* 62:270-271
- Benrey B and Denno RF (1997) The slow-growth-high-mortality hypothesis: A test using the cabbage butterfly. *Ecology* 78:987-999
- Botham MS, Kerfoot CJ, Louca V and Krause J (2005) Predator choice in the field; grouping guppies, *Poecilia reticulata*, receive more attacks. *Behav Ecol Sociobiol* 59:181-184
- Botham MS, Kerfoot CJ, Louca V and Krause J (2006) The effects of different predator species on antipredator behavior in the Trinidadian guppy, *Poecilia reticulata*. *Naturwissenschaften* 93:431-439
- Castellanos I and Barbosa P (2006) Evaluation of predation risk by a caterpillar using substrate-borne vibrations. *Anim Behav* 72:461-469
- Clark BR and Faeth SH (1997) The consequences of larval aggregation in the butterfly *Chlosyne lacinia*. *Ecol Entomol* 22:408-415
- Cohen JE, Pimm SL, Yodzis P and Saldana J (1993) Body sizes of animal predators and animal prey in food webs. *J Anim Ecol* 62:67-78
- Costa JT (1993) Larval ontogeny and survivorship of eastern tent caterpillar colonies. *J Res Lepid* 32:89-98
- De Clercq P, Wyckhuys K, De Oliveira HN and Klapwijk J (2002) Predation by *Podisus maculiventris* on different life stages of *Nezara viridula*. *Fla Entomol* 85:197-202
- Despland E and Le Huu A (2007) Pros and cons of group-living in the forest tent caterpillar: separating the roles of silk and of grouping. *Entomol Exp Appl*

- DeVito J (2003) Metamorphic synchrony and aggregation as antipredator responses in American toads. *Oikos* 103:75-80
- Evans EW (1982) Influence of weather on predator/prey relations: stinkbugs and tent caterpillars. *N Y Entomol Soc* 4:241-246
- Evans EW (1983) Niche relations of predatory stinkbugs (*Podisus spp.*, Pentatomidae) attacking tent caterpillars (*Malacosoma americanum*, Lasiocampidae). *The American Midland Naturalist* 109:316-323
- Fitzgerald TD (1995) *The Tent Caterpillars*. Ithaca: Cornell University Press
- Fitzgerald TD and Costa JT (1999) Collective behavior in social caterpillars. In: Detrain C, Deneubourg JL, Pasteels JM (eds) *Information processing in social insects*. Birkhauser, Basel
- Gaston KJ, Chown SL and Styles CV (1997) Changing size and changing enemies: The case of the mopane worm. *Acta Oecol-Int. J Ecol* 18:21-26
- Grisdale D (1985) *Malacosoma disstria*. In: Singh P, Moore RF (eds) *Handbook of insect rearing*. Elsevier, Amsterdam, pp 369-379
- Hamilton WD (1971) Geometry for the selfish herd. *J Theor Biol* 31:295-311
- Hass CC and Valenzuela D (2002) Anti-predator benefits of group living in white-nosed coatis (*Nasua narica*). *Behav Ecol Sociobiol* 51:570-578
- Heinrich B (1983) Caterpillar leaf damage, and the game of hide-and-seek with birds. *Ecology* 64:592-602
- Heinrich B (1993a) *The hot blood insects: strategies and mechanisms of insect thermoregulation*. Harvard University Press, Cambridge, MA.

- Heinrich B (1993b) How avian predators constrain caterpillar foraging. In: Stamp NE, Casey TM (eds) Caterpillars: ecological and evolutionary constraints on foraging. Chapman & Hall, New York, pp 224-248
- Hunter AF (2000) Gregariousness and repellent defences in the survival of phytophagous insects. *Oikos* 91
- Iwao S and Wellington WG (1970) The western tent caterpillar: qualitative differences and the action of natural enemies. *Res. Popul. Ecol.* XII:81-99
- Krause J and Godin J-GJ (1995) Predator preferences for attacking particular group sizes: consequences for predator hunting success and prey predation risk. *Anim Behav* 50:465-473
- Krause J, Reeves P and Hoare D (1998) Positioning behaviour in roach shoals: the role of body length and nutritional state. *Behaviour* 135:1031-1039
- Lawrence WS (1990) The effects of group size and host species on development and survivorship of a gregarious caterpillar *Halisidota caryae* (Lepidoptera: Arctiidae). *Ecol Entomol* 15:53-62
- Lemos WP, Zanuncio JC and Serrao JE (2005) Attack behavior of *Podisus rostralis* (Heteroptera: Pentatomidae) adults on caterpillars of *Bombyx mori* (Lepidoptera: Bombycidae). *Braz Arch Biol Technol* 48:975-981
- Levesque KR, Fortin M and Mauffette Y (2002) Temperature and food quality effects on growth, consumption and post-ingestive utilization efficiencies of the forest tent caterpillar *Malacosoma disstria* (Lepidoptera: Lasiocampidae). *Bull Entomol Res* 92:127-136



- McClure M, Cannell E and Despland E (2010) Thermal ecology and behaviour of the nomadic social forager, *Malacosoma disstria*. Phys Entomol DOI:10.1111/j.1365-3032.2010.00770.x
- McClure M and Despland E (2010) Collective foraging patterns of field colonies of *Malacosoma disstria* caterpillars. Can Entomol 142:1-8
- Mooring MS and Hart BL (1992) Animal grouping for protection from parasites-selfish herd and encounter-dilution effects. Behavior 123:173-193
- Morris RF (1963) The effect of predator age and prey defense on the functional response of *Podisus maculiventris* Say to the density of *Hyphantria cunea* Drury. Can Entomol 95:1009-1023
- Parry D, Spence JR and Volney WJA (1998) Budbreak phenology and natural enemies mediate survival of first-instar forest tent caterpillar (Lepidoptera: Lasiocampidae). Environ Entomol 27:1368-1374
- Peters RH (1983) The ecological implication of body size. Cambridge University Press, Cambridge
- Peterson SC, Johnson ND and LeGuyader JL (1987) Defensive regurgitation of allelochemicals derived from host cyanogenesis by eastern tent caterpillars. Ecology 68:1268-1272
- Prop N (1960) Protection against birds and parasites in some species of tenthredinid larvae. Arch. Néerl. Zool. 13:380-447
- Reader T and Hochuli DF (2003) Understanding gregariousness in a larval Lepidoptera: the roles of host plant, predation, and microclimate. Ecol Entomol 28:729-737

- Reavey D (1993) Why body size matters to caterpillars. In: Stamp NE, Casey TM (eds) Caterpillars: ecological and evolutionary constraints on foraging. Chapman and Hall, New York, pp 248-279
- Rogovin K, Randall JA, Kolosova I and Moshkin M (2004) Predation on a social desert rodent, *Rhombomys opimus*: effect of group size, composition, and location. *J Mammal* 85:723-730
- Ronnas C, Larsson S, Pitacco A and Battisti A (2010) Effects of colony size on larval performance in a processionary moth. *Ecol Entomol* 35:436-445
- Schultz JC (1983) Habitat selection and foraging tactics of caterpillars in heterogeneous trees. In: Denno RF, McClure MS (eds) Variable plants and herbivores in natural and managed systems. Academic Press, New York, pp 61-90
- Seyfarth RM, Cheney DL and Marler P (1980) Monkey responses to three different alarm calls: evidence of predator classification and semantic communication. *Science* 210:801-803
- Smith GR and Awan AR (2009) The roles of predator identity and group size in the antipredator responses of American toad (*Bufo americanus*) and bullfrog (*Rana catesbeiana*) tadpoles. *Behaviour* 146:225-243
- Tostowaryk W (1971) Relationship between parasitism and predation in diprionid sawflies. *Ann Entomol Soc Am* 64:1424-1427
- Uetz GW, Boyle J, Hieber CS and Wilcox SR (2002) Antipredator benefits of group living in colonial web-building spiders: the 'early warning' effect. *Anim Behav* 63:445-452

- Vulinec K (1990) Collective security: aggregation by insects as a defense. In: Evans DL, Schmidt JO (eds) *Insect defenses. Adaptive Mechanisms of prey and predators.* State University of New York, Albany, New York, pp 251-288
- Wajnberg E (2006) Time allocation strategies in insect parasitoids: from ultimate predictions to proximate behavioral mechanisms. *Behav Ecol Sociobiol* 60:589-611
- Warren PH and Lawton JH (1987) Invertebrate predator-prey body size relationships: an explanation for upper triangular food webs and patterns in food web structure? *Oecologia* 74:231-235
- Webb JK, Du W, Pike D and Shine R (2010) Generalization of predator recognition: Velvet geckos display anti-predator behaviours in response to chemicals from non-dangerous elapid snakes. *Curr Zool* 56:337-342
- Williams DJM, Parry D and Langor DW (1996) Sampling and identification of forest tent caterpillar parasitoids in the Prairie Provinces. Canadian Forest Service NR, Northern Forestry Centre:Information Report NOR-X-345

## List of figures

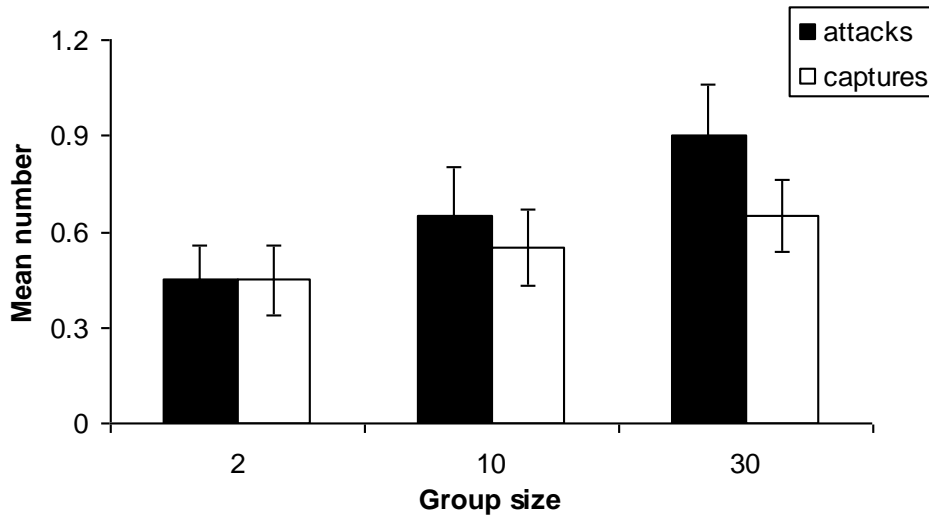
**Fig. 1:** Spiders: the average number of attacks and successful attacks ( $\pm$ SEM) for different group sizes of a) second and b) fourth instar caterpillars of *Malacosoma disstria* (N=20 caterpillar groups per treatment combination of instar and group size)

**Fig. 2:** Stinkbugs: the average number of attacks and successful attacks ( $\pm$ SEM) for different group sizes of a) second and b) fourth instar caterpillars of *Malacosoma disstria* (N=20 caterpillar groups per treatment combination of instar and group size)

**Fig. 3:** Parasitoids: the average number of attacks and successful attacks ( $\pm$ SEM) for different group sizes of a) second and b) fourth instar caterpillars of *Malacosoma disstria* (N=20 caterpillar groups per treatment combination of instar and group size)

**Fig. 4:** The mean mortality ( $\pm$ SEM) per capita of *Malacosoma disstria* caterpillars in different group sizes, for both a) second and b) fourth larval instar, for different invertebrate predators and parasitoids (N=20 caterpillar groups per treatment combination of instar and group size)

a)



b)

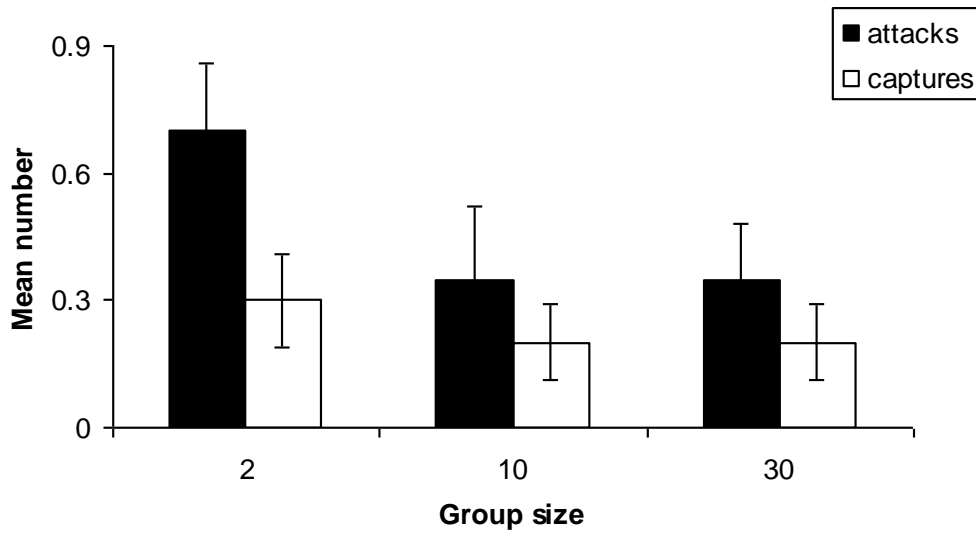
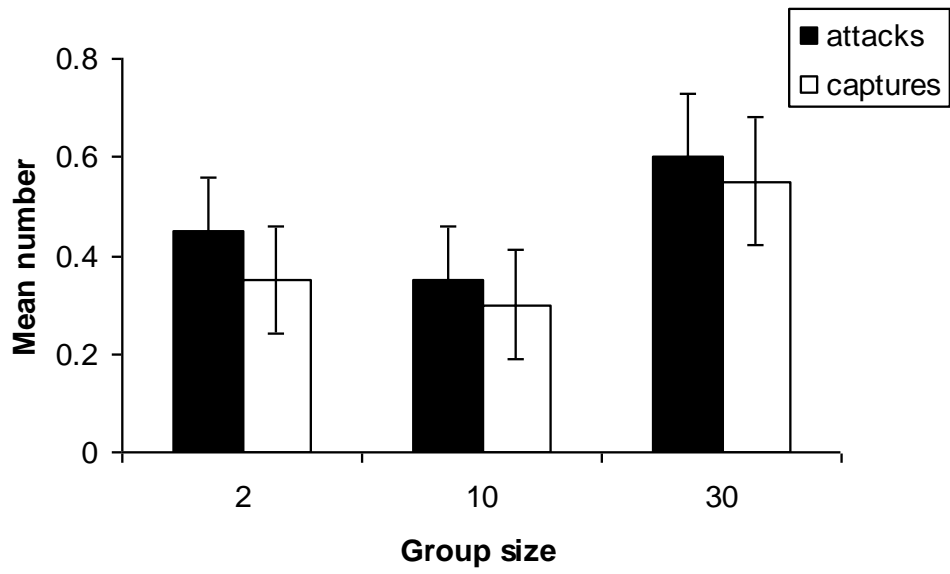
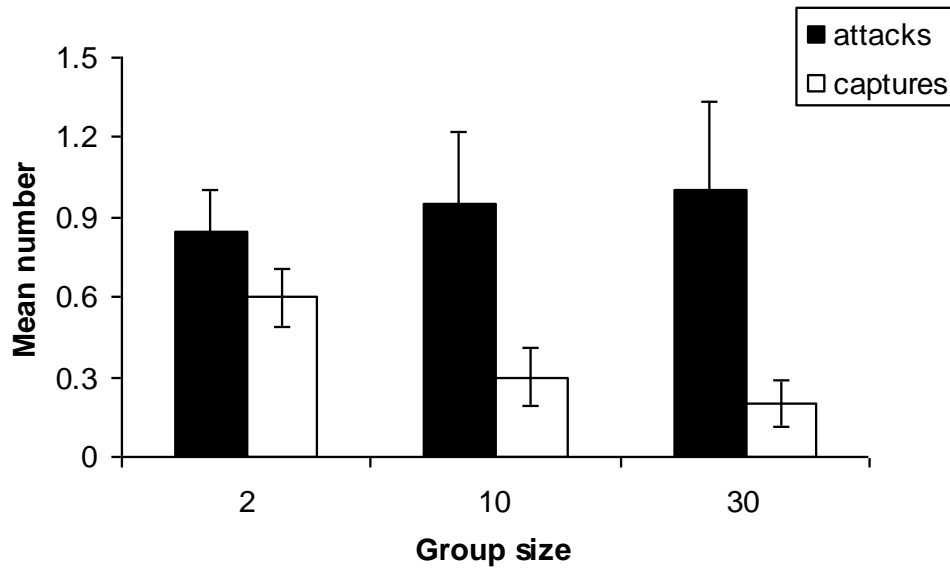


Fig. 1

a)

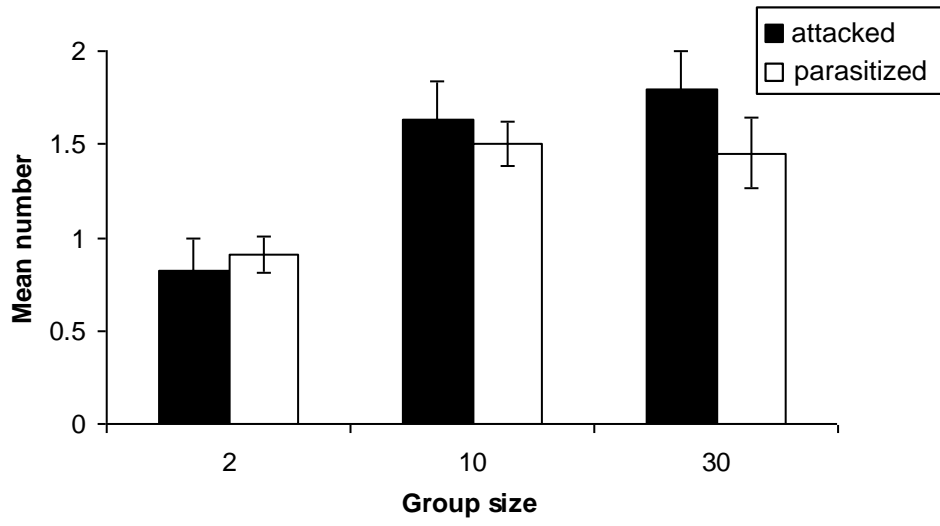


b)

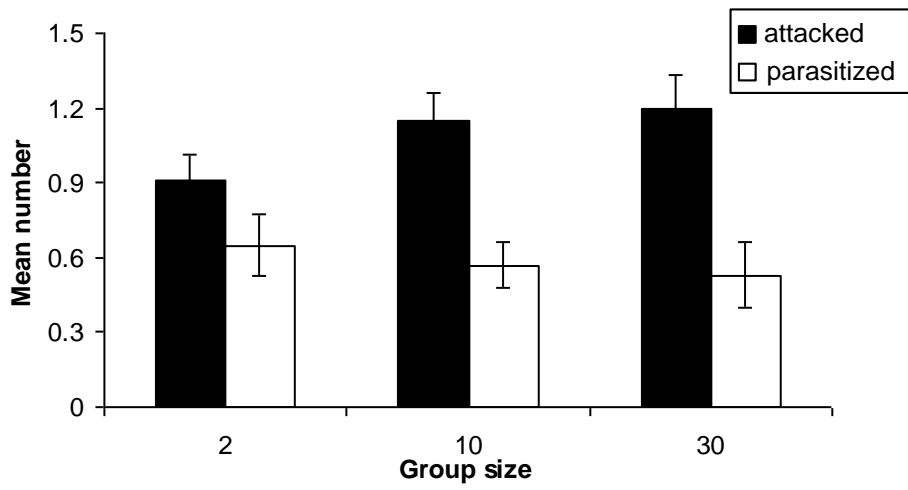


**Fig. 2**

a)

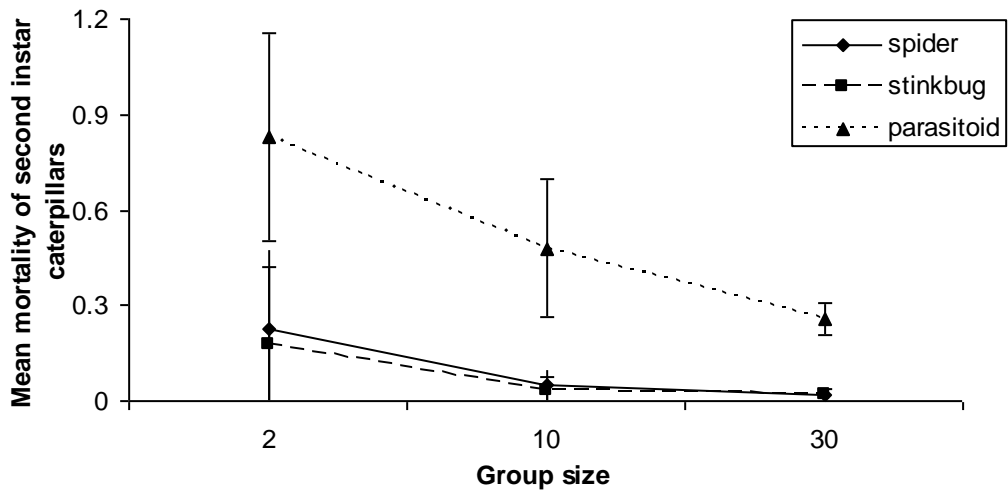


b)



**Fig. 3**

a)



b)

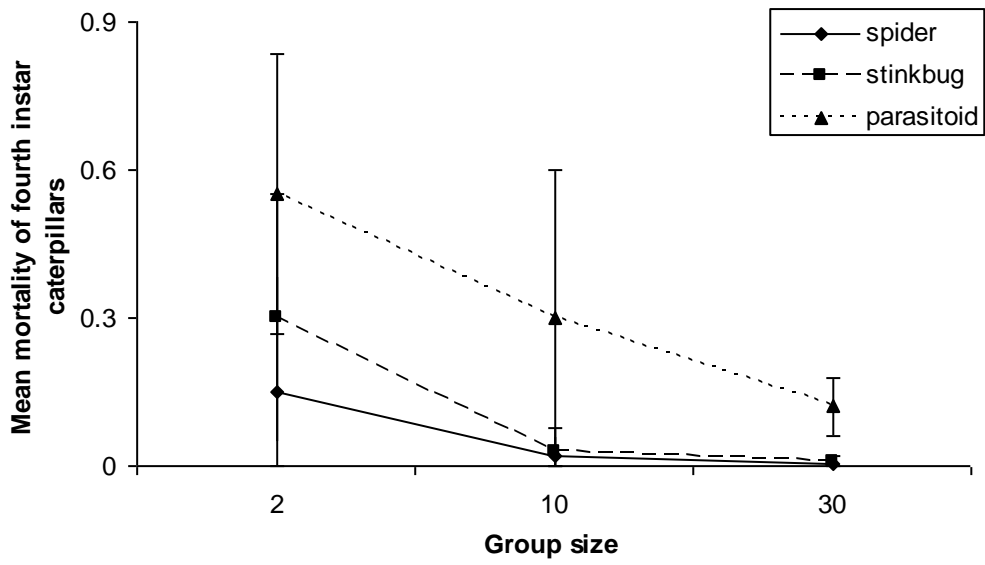


Fig. 4



**Table 1:** The behavioural response (when one was observed) elicited by an attack by an invertebrate predator or parasitoid, the proportion of *Malacosoma disstria* caterpillars responding and the proportion of those that were successful in escaping predation or parasitisation.

Type of predator	Instar	Behavioral response	% responding	% responding successfully
Spider	2	Thrashing	42	0
		Biting	5	0
		Holding the silk mat	53	80
	4	thrashing	37	38
		thrashing & biting	30	38
		thrashing & falling	33	100
Stinkbug	2	jerking back	11	100
		thrashing	61	0
	4	walking away	12	100
		jerking back	26	100
		thrashing	56	17
		thrashing & biting	5	0
		thrashing & falling	2	100
Parasitoid	2	head flicking	70	9
		head flicking & biting	30	30
	4	head flicking	66	32
		head flicking & biting	34	66

**Table 2:** Statistical results for 3 separate MANOVAs done for each natural enemy as a function of group size and larval instars of *Malacosoma disstria* caterpillars (N=120 groups per analysis).

Type of predator	Measured variable	Experimental factor	F value	df	p value	
Spider	Number of attacks	Caterpillar instar	2.7	1, 114	0.103	
		Group size	0.36	2, 114	0.701	
		Interaction	3.77	2, 114	<b>0.026*</b>	
	Number of captures	Caterpillar instar	12.26	1, 114	<b>0.001*</b>	
		Group size	0.27	2, 114	0.768	
		Interaction	1.02	2, 114	0.36	
	Time to attack (secs)	Caterpillar instar	0.15	1, 59	0.698	
		Group size	0.08	2, 59	0.923	
		Interaction	0.93	2, 59	0.402	
	Handling time (secs)	Caterpillar instar	86.38	1, 40	<b>&gt;0.001*</b>	
		Group size	0.81	2, 40	0.453	
		Interaction	0.85	2, 40	0.434	
Stinkbug	Number of attacks	Caterpillar instar	7.94	1, 114	<b>0.006*</b>	
		Group size	0.37	2, 114	0.695	
		Interaction	0.16	2, 114	0.851	
	Number of captures	Caterpillar instar	0.31	1, 114	0.58	
		Group size	1.27	2, 114	0.286	
		Interaction	4.42	2, 114	<b>0.014*</b>	
	Time to perceive (secs)	Caterpillar instar	1.6	1, 78	0.214	
		Group size	0.3	2, 78	0.741	
		Interaction	0.23	2, 78	0.798	
	Time to attack (secs)	Caterpillar instar	15.96	1, 78	<b>&gt;0.001*</b>	
		Group size	2.63	2, 78	0.087	
		Interaction	1.8	2, 78	0.181	
	Handling time (secs)	Caterpillar instar	14.28	1, 53	<b>&gt;0.001*</b>	
		Group size	0.01	2, 53	0.994	
		Interaction	0.02	2, 53	0.984	
	Parasitoid	Number of attacks	Caterpillar instar	4.16	1, 114	<b>0.042*</b>
			Group size	4.75	2, 114	<b>0.009*</b>
			Interaction	1.47	2, 114	0.232
Successfully parasitized		Caterpillar instar	16.69	2, 39	<b>&gt;0.001*</b>	
		Group size	20.02	1, 39	<b>&gt;0.001*</b>	
		Interaction	3.22	2, 39	0.051	
Time to attack (secs)		Caterpillar instar	6.29	1, 72	0.594	
		Group size	0.002	2, 72	0.998	
		Interaction	0.33	2, 72	0.719	
Time spent flicking (secs)		Caterpillar instar	0.6	1, 72	0.441	
		Group size	26.03	2, 72	<b>&gt;0.001*</b>	
		Interaction	1.32	2, 72	0.276	