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13 How does a pesticide pulse increase vulnerability to predation? Combined effects on
14 behavioral antipredator traits and escape swimming

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23 Abstract

24 An increasing number of studies documented that sublethal pesticide exposure can
25 change predator-prey interactions. Most of these studies focused on effects of long-term
26 pesticide exposure on only one type of antipredator traits and did not directly link
27 changes in these traits to mortality by predation. To get a better mechanistic
28 understanding how short-term pesticide pulses make prey organisms more vulnerable to
29 predation, we studied effects of 24 h exposure to a sublethal concentration of the
30 insecticide endosulfan and the herbicide Roundup on the major antipredator traits and the
31 resulting mortality by predation in larvae of the damselfly *Enallagma cyathigerum*. A
32 pulse of both pesticides affected antipredator traits involved in avoiding detection by
33 predators as well as traits involved in escape after detection. After a pesticide pulse,
34 larvae increased activity levels and even further increased the number of walks when
35 predation risk was present. Further, an endosulfan pulse tended to reduce escape
36 swimming speed. In contrast, previous exposure to Roundup caused the larvae to swim
37 faster, yet less often when attacked. Importantly, although both studied pesticides induced
38 maladaptive changes in overall activity, only for endosulfan this resulted in an increased
39 mortality by predation. Our study highlights that considering changed predator-prey
40 interactions may improve ecological risk evaluations of short pesticide pulses, yet also
41 underscores the need (1) to consider effects on all important antipredator traits of the prey
42 as trait compensation may occur and (2) to effectively score the outcome of predator-prey
43 interactions in staged encounters.

44 *Keywords:* damselfly larvae survival; post-exposure effects; short-term exposure; anti-
45 predator response; endosulfan; Roundup

1. INTRODUCTION

The use of pesticides in agriculture is increasingly worldwide (Schwarzenbach et al., 2006). As a result, non-target aquatic animals in nearby ponds are increasingly exposed to pesticides through processes like runoff and through small streams (Liess et al. 2008). Traditionally, most focus has been on lethal effects, yet sublethal pesticide concentrations may negatively influence the population dynamics of non-target species (Clements and Newman, 2002). One important sublethal pathway is by changing interactions with natural predators (e.g. Broomhall, 2004), thereby causing a larger impact on nontarget organisms as could be foreseen by the currently used ecological risk evaluations (Rohr et al., 2006).

Many studies documented that pesticide exposure can change antipredator traits (reviews in Dell'Omo, 2002; Clements and Newman, 2002). Negative effects on two types of antipredator traits have been identified: traits involved in avoiding detection by predators (e.g. Teplitsky et al., 2005) and traits involved in escape after predator detection (e.g. Van Gossum et al., 2009). While studies tend to focus only on one type of antipredator trait, their combined study is important. This is because trait compensation may occur, a negative covariation pattern between two antipredator traits whereby (groups of) animals with poor values for one antipredator trait compensate for this by relying more on another antipredator trait to avoid a higher vulnerability to predation (Dewitt et al. 1999; Steiner and Pfeiffer 2007). For example, prey organisms with a reduced ability to escape by swimming may rely more on reducing their activity to avoid predator detection (Engstrom-Ost and Lehtiniemi, 2004). Furthermore, many studies considered maladaptive changes in antipredator traits as a proxy for increased mortality

by predation (e.g. Van Gossum et al., 2009). Only some of these studies directly linked effects on antipredator traits to changes in mortality by predation (see e.g. Reynaldi et al., 2011). Such studies that combine changes in antipredator traits and mortality by predation are important to mechanistically understand how pesticides make prey organisms more vulnerable to predation. Moreover, as changes in antipredator traits not necessarily translate into changes in mortality by predation (e.g. Van Buskirk and McCollum, 2000), potentially because of compensatory mechanisms, a more direct testing of the resulting mortality by predation is warranted.

Most studies on the effects of pesticides on antipredator traits considered effects during a long exposure period (e.g., Teplitsky et al., 2005; Van Gossum et al., 2009). This may overestimate effects on prey organisms as many pesticides are applied in pulses and degrade relatively quickly (Ashauer et al. 2006). Fewer studies examined whether effects on antipredator traits also occur after pesticide pulses and whether these effects are strong enough to generate increases in mortality by predation (but see e.g. Floyd et al., 2008; Reynaldi et al., 2011).

The objectives of this study were to determine the effects of short-term exposure to a sublethal pesticide concentration on two key antipredator traits (activity levels and escape swimming performance) and the resulting mortality by predation in the larvae of the damselfly *Enallagma cyathigerum*. This is a widespread species that in Flanders mostly occurs in fishless waters, with the largest populations typically situated in nature reserves. Damselfly larvae are very relevant study organisms to test for effects of pesticide pulses on antipredator traits and vulnerability to predation in ponds. Their ecology, including antipredator traits, is exceptionally well-known (Corbet, 1999; Stoks

et al., 2003; Stoks and McPeck, 2006). They are important intermediate predators in ponds preying on zooplankton and other aquatic insects including pest and vector mosquitoes and are themselves prey of fish and large dragonfly larvae (Corbet, 1999). Given their complex life cycle with an aquatic larval stage and a terrestrial adult stage Odonata have the potential to couple aquatic and terrestrial ecosystems (Stoks and Cordoba-Aguilar, 2012). Pesticide effects on their vulnerability to predators in the larval stage therefore may affect both the aquatic and terrestrial food webs. All Odonates are protected by law in Flanders making them also from a nature protection perspective important non-target species to consider when evaluating the impact of pesticide pulses. So far, only one study explored effects of pollutant exposure to antipredator traits in damselfly larvae (Van Gossum et al., 2009). This study looked at effects of long-term exposure to perfluorooctanesulfonic acid (PFOS) on general activity levels and swimming speed but did not consider behavioral responses to predators and the resulting effects on mortality by predation.

To start evaluating the generality of the effects of sublethal pesticide pulses we studied two pesticides: endosulfan and Roundup. Endosulfan, an organochlorine insecticide, is an antagonist of the GABA-receptor in the inhibitory synapse of signal transduction. By blocking the chloride channels it will overstimulate the central nervous system (Stenersen, 2004). Endosulfan has been shown to interfere with antipredator traits in aquatic organisms, both by increasing the probability of being detected by predators (Brunelli et al., 2009) and by reducing escape swimming speed (Ballesteros et al., 2009). Roundup is a commercial herbicide, consisting of the active ingredient glyphosate and the surfactant POEA (polyethoxylated tallowamine). Glyphosate functions as an enzyme

inhibitor by blocking the production of amino acids, thereby disturbing plant growth. It is supposed to have minor effects on animals. However, there is increasing evidence that Roundup has negative effects on the survival of aquatic organisms like larval amphibians (Relyea, 2005). Since the increasing use of glyphosate-based herbicides (Donaldson et al., 2002) it is important to obtain better insights in the way that this pesticide can influence aquatic organisms. For both pesticides, we expect previous pesticide exposure to (1) disturb the behavior involved in avoiding detection (e.g. Wolf and Moore, 2002), (2) reduce swimming escape performance (e.g. Van Gossum et al., 2009) and (3) increase vulnerability to predators (e.g. Reynaldi et al. 2011).

2. MATERIALS AND METHODS

2.1 Collecting and housing

Fifteen mated females of the damselfly *Enallagma cyathigerum* were collected in “De Ruiterskuilen” situated in a protected nature area without pesticide application history in Opglabbeek (Belgium). This is a fishless pond with larvae of the large dragonfly *Anax imperator* as the top predator. Female *E. cyathigerum* were kept in the laboratory for egg laying in plastic cups with wet filter paper as oviposition substrate. Initially, freshly-hatched larvae were kept in groups of 20 in 150 ml cups. Ten days after egg hatching, larvae were placed individually in 200 ml cups. Throughout their life, larvae were reared in a room with a constant temperature of 21°C and a photoperiod of L:D 14:10 hours. Damselfly larvae were daily fed ad libitum with *Artemia* nauplii six days a week (average daily dose = 204, SE = 36, n = 10).

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139 *2.2 Pesticide application*

140 We chose to apply both pesticides using a single 24h pesticide pulse in order to mimic
141 realistic field situations. Both endosulfan and glyphosate are applied in pulses and
142 degrade quickly. Endosulfan is used worldwide as broad spectrum organochlorine
143 insecticide and acaricide (US EPA, 1999) with several application pulses during one
144 growing season (Weber et al., 2010). After two days, endosulfan can drop to 12% of the
145 initial concentration (Trekels et al., 2011). Roundup is applied in short pulses, especially
146 in the beginning of the growing season (Giesy et al., 2000). After 4.2 days, Roundup
147 concentrations can drop to 50% of the initial concentration (Vera et al., 2010).

148 To select the pesticide concentrations for the pulse treatment, we first ran a range
149 finding experiment where we exposed larvae individually in glass vials (100 ml) for 24h
150 to several concentrations of each pesticide. Both pesticides were dissolved in mineral
151 water Spa Reine[®] which ensures constant rearing conditions. Pre-trials showed growth
152 rates and activity levels in this medium to be similar to those observed in natural pond
153 water (Janssens, unpublished data). For endosulfan we tested 50 µg/l, 75 µg/l and 100
154 µg/l (based on Trekels, 2006) and a solvent control, all containing 10 µl ethanol. We used
155 a solvent control because we dissolved 10 mg endosulfan powder in 1 ml ethanol for the
156 preparation of the stock solution. For Roundup we tested 1 mg/l, 2 mg/l, 3 mg/l and 4
157 mg/l of the active ingredient glyphosate (based on Relyea, 2005) and the mineral water
158 Spa Reine[®] as control. After 24 h exposure, we studied for each larva the activity in the
159 absence and presence of chemical predator stimuli (see 2.4 behavioral tests). We tested

five final instar larvae per pesticide concentration and selected per pesticide the lowest observed effect concentration (LOEC) where the activity of the larvae detectably differed from the (solvent) control. The selected nominal concentrations for the experiment were 50 µg/l endosulfan and 1.5 mg/l Roundup. The latter concentration was chosen because the number of feeding bites was affected at 1 mg/l and the number of walks at 2 mg/l (Janssens, unpublished data).

The selected LOEC concentrations of both pesticides were sublethal. From the range finding experiment, we know that endosulfan concentrations up to 100 µg/l are not lethal. From a previous experiment, we also know that 7-day exposure to Roundup concentrations up to 6 mg/l did not cause any mortality in the study species (L. Janssens, pers. observation). The concentrations used in our study were for both pesticides concentrations that have been documented in natural ponds. For endosulfan, the selected concentration (50 µg/l) is on the higher end of the concentrations found in Flanders (going up to 54.5 µg/l, VMM Flemish Environment Agency, 2006, unpublished data). Worldwide, peak concentrations up to 700 µg/l have been detected (Ernst et al. 1991). For Roundup, we used a concentration of 1.5 mg/l. The highest concentration reported in nature for the active component of Roundup, glyphosate, is 2.6 mg/l (Thompson et al., 2004). Scenarios for the future predict glyphosate concentrations up to 5.2 mg/l in fresh water systems (Giesy et al., 2000).

2.3 General experimental strategy

The overall strategy to test for delayed effects of the pesticides on predator-prey interactions was to expose larvae for 24h to the pesticide, transfer them to pesticide-free conditions and (1) test activity levels in the absence and presence of predator cues, (2) quantify escape swimming under a simulated attack and (3) finally monitor survival in the presence of a predator. For logistic reasons, we ran the experiments with both pesticides separately. One day after larvae molted into the final instar they were weighed to the nearest 0.01 mg (range 25.6-49.2 mg). Thereafter, larvae were randomly assigned to one of two pesticide treatments per pesticide [(solvent) control or pesticide] for 24h in a glass vial filled with 50 ml of the medium. By placing opaque dividers between the different vials we made sure that the larvae could not see each other. This was done because damselfly larvae are cannibalistic and thus can cause predator stress to each other (De Block and Stoks, 2004). During this 24h-exposure period the larvae were not fed to avoid any energetic carry-over effects due to changed food intake during the exposure period to the post-exposure period. In the field, damselfly larvae are often food-limited (Baker, 1989) and can endure more than seven days of starvation without any body mass loss (Stoks et al., 2006).

2.4 Behavioral tests

After the 24h-exposure period all larvae were transferred to mineral water to score their behavior in a series of three behavioral tests. First, we successively scored the activity of each larva in the absence and then in the presence of chemical predator cues. Damselfly larvae, including the study species, respond to larval dragonfly predators by reducing

activity levels (Stoks et al., 2005). Second, we determined the swimming propensity and swimming speed. *Enallagma* larvae use swimming to escape predation by dragonfly larvae (Stoks and McPeck, 2006; Strobbe et al., 2009). Third, we exposed the larvae individually to a predator, namely a large dragonfly larva of the species *Anax imperator* and we monitored the survival time. We ran 40 replicates for each pesticide treatment (80 larvae per pesticide, total of 160 larvae).

We scored larval activity in the absence and presence of predation risk based on the protocol by Chivers et al. (1996). For this, we placed a single larva in a 2 L container that contained 150 ml of mineral water. The larvae could acclimate in the container for 10 minutes before the experiment began. We started the behavioral experiment by injecting 4 ml of a standard solution of brine shrimp nauplii (ca. 1600 *Artemia* nauplii) into each container. Thereafter, we recorded the frequency of three behaviors (feeding bites, head bends toward the prey and walks) for seven minutes. A walk is defined as one bout of activity where the larva moved its legs to change position (Chivers et al., 1996). Next, the larva was transferred to an identical container that contained 150 ml mineral water to which 1 ml of predator medium was added to impose predation risk. After another acclimation period of 10 minutes we again added *Artemia* nauplii and recorded the frequency of the three behaviors for seven minutes. We transferred the larva to a new container to make the conditions during both observation periods as similar as possible except for the presence of predator medium. Using this procedure, also the second period started with a transfer of the larva to another container and after an acclimation period. Moreover, this way we ensured each observation period started with the same initial density of brine shrimp nauplii. Pre-trials showed that when larvae were transferred to

226 another container and tested for the second 7-min period in the absence of predator cues,
227 their behavior did not differ between both observation periods (Janssens, unpublished
228 data), indicating that any difference between both observation periods in current
229 experiment could be attributed to predation risk and was not confounded with other
230 factors like saturation. For each larva we studied first the behavior without predator cues
231 and then with predator cues to avoid any carry-over effects after the predator cues were
232 removed. Predator medium was prepared by homogenizing two conspecific damselfly
233 larvae in 10 ml of water where a large *Anax* dragonfly predator had eaten a conspecific
234 larva. This way, the larvae received chemical predator cues to which *Enallagma* larvae
235 are known to react with antipredator behavior (Mortensen and Richardson, 2008).

236 To measure the swimming performance, we transferred a single larva to a large
237 container (32 cm x 21 cm, 4 cm high), filled to height of 1.5 cm with mineral water. After
238 10 minutes of acclimation, we scored swimming performance using the methodology of
239 McPeck et al. (1996) and Stoks and McPeck (2006). The larva was stimulated five times
240 to swim by tapping it on the dorsal surface of the thorax with a plastic pipette. The
241 swimming propensity was defined as the number of times the larva swam (ranging from
242 zero to five). Three swimming bouts per larva were filmed with a digital camera (Basler
243 30Hz) connected to a computer using Streampix software. Larvae that swam less than
244 three times when testing their swimming propensity were stimulated further until three
245 swimming bouts were obtained. From these recordings we quantified per swimming bout
246 the swimming speed using Image Pro Plus v5. Swimming speed (cm/s) was calculated as
247 the distance the larvae covered during the first 15 frames of one swimming bout divided
248 by the duration (0.5 seconds). We chose to digitize the first 0.5 s to have enough frames

to accurately calculate swimming speed while this initial 0.5 s period is likely to be the most critical period for damselfly larvae to escape attacks from sit-and-wait predators such as dragonfly larvae that do not chase their prey after the initial attack (Dayton et al., 2005). Per larva, we averaged the speed of the three swimming bouts for later analysis.

Finally, we performed a survival test where we scored the survival time of each damselfly larva in the presence of a dragonfly predator. For this, the larva was transferred to a container (9 cm × 9 cm) with 150 ml of mineral water. After an acclimation period of 10 minutes we placed a final instar *Anax imperator* at the other side of the container. We scored the time that the predator needed to catch the damselfly larva. Survival times longer than 180 minutes were considered right-censored. Each predator was used several times with at least two days between successive trials. We measured the size of each predator to the nearest 0.01 mm using a caliper to include as a correcting factor in the analyses.

2.5 Statistical analyses

The experimental unit in the experiments was a single larva. Per behavioral response variable (number of walks, orientations and feeding strikes) we had two repeated observations for the same experimental unit (each larva was scored without and with predation risk). We therefore tested for effects of pesticide treatment and predation risk on the three log-transformed behavioral variables scored in the activity test by means of a repeated-measures MANCOVA. The preceding pesticide exposure treatment was the independent variable and the activity of each larva measured with and without exposure

271 to chemical predator stimuli were the repeats. Because larger larvae likely are more
272 active we added body mass as a covariate. Thereafter, to interpret the repeated-measures
273 MANCOVA we ran separate follow-up repeated-measures ANCOVAs per behavioral
274 variable. This hierarchical procedure avoids inflation of the type I error rate; the follow
275 up analyses can be considered “protected ANCOVAs” (Scheiner 2001).

276 Effects of the pesticide treatment on swimming speed were analyzed using
277 ANCOVA. In all these analyses the interactions between pesticide exposure and body
278 mass were not significant, so we removed these interactions from the model. To test for
279 effects of pesticide treatment on the swimming propensity, we used the Mann-Whitney
280 U-test because the dependent variable was not normally distributed.

281 Effects of the pesticide treatment on survival times in the presence of the predator
282 were tested with time-to-event analyses which allow taking into account right-censored
283 data, i.e. animals that survived up to 180 minutes. In a first step, we compared survival
284 curves between both pesticide treatments using a Gehan’s Wilcoxon test. This is the
285 preferred, most powerful test when comparing two groups with many right-censored data
286 (Fox, 2001). In a second step, we tested which of the measured behavioral variables
287 influenced the survival time and whether this differed among pesticide treatments using a
288 Cox regression. We started with a model with the behavioral variables, pesticide
289 treatment and their two-way interactions. We also entered the size of the predator and the
290 mass of the larvae in the model. We simplified this model by removing non-significant
291 terms, starting with the two-way interactions. Given the a priori expectations that higher
292 activity levels would increase and higher swimming speeds would reduce mortality rates
293 (Stoks et al., 2005; Strobbe et al., 2009, 2010) we present one-sided p-values for their

slopes. Statistical analyses were performed in STATISTICA 9, except for the Cox regression for which we used SAS 9.2.

3. RESULTS

3.1 Endosulfan

The repeated-measures MANCOVA showed that pesticide exposure ($F_{3,75} = 29.73$, $p < 0.001$), predation risk ($F_{3,75} = 4.60$, $p = 0.0052$) and the interaction between pesticide and predation ($F_{3,75} = 10.37$, $p < 0.001$) had an effect on the combined behaviors of the larvae.

After exposure to endosulfan, larvae had higher activity levels as reflected in the higher number of walks, orientations and feeding strikes (Table 1, Figure 1A-C). The significant predation risk $\times$ pesticide interactions for number of walks and feeding strikes indicated that there was a different response to predation risk in the two pesticide conditions (Table 1). While larvae that had not been exposed to endosulfan reduced feeding strikes and walks in the presence of predation risk, larvae previously exposed to endosulfan did not reduce feeding bites and even increased walks (Figure 1A-C). Although the interaction between pesticide and predation risk was not significant for the number of orientations (Table 1), the post-hoc Duncan tests also suggested that only the control larvae reduced the number of orientations in the presence of predation risk (Figure 1B).

Previous exposure to endosulfan had no effect on the swimming propensity (Mann-Whitney U: $U = 744$, $N_1 = 40$, $N_2 = 39$, $p = 0.72$), and caused a trend towards slower swimming ($F_{1,76} = 2.14$, $p = 0.097$) (Figure 2A-B).

Previously to endosulfan exposed larvae had significant shorter survival times than the control larvae in the survival test with the dragonfly predator (Gehan Wilcoxon test statistic = -2.34, $p = 0.019$) (Figure 3A). Larvae with a higher walking activity in the presence of predator stimuli showed a higher mortality rate in the survival test (Cox regression, $\chi^2 = 4.91$, $df=1$, $p_{\text{one-sided}} = 0.013$, slope = 0.64 (SE = 0.28), hazard ratio = 1.9). There was also a trend for larvae that swam faster to have a lower mortality rate ($\chi^2 = 1.80$, $p_{\text{one-sided}} = 0.09$, slope = -0.072 (SE = 0.054), hazard ratio = 0.93).

3.2 Roundup

The repeated-measures MANCOVA showed that pesticide exposure ($F_{3,76} = 34.17$, $p < 0.001$), predation risk ($F_{3,76} = 20.14$, $p < 0.001$) and the interaction between pesticide and predation ($F_{3,76} = 5.23$, $p = 0.0025$) had an influence on the combined behaviors of the larvae. After exposure to Roundup larvae had higher activity levels as indicated by the higher number of walks, orientations and feeding strikes (Table 1, Figure 1D-F). The effect of predation risk strongly depended upon previous exposure to Roundup (predation risk $\times$ pesticide interactions, Table 1, Figure 1D-F). Larvae from the pesticide control treatment reduced all three behaviors in the presence of predation risk. The larvae exposed to Roundup did not reduce the number of orientations and feeding strikes, and even increased the number of walks.

Previous exposure to Roundup had a negative effect on the swimming propensity (Mann-Whitney U: $U = 531$, $N_1 = 40$, $N_2 = 40$, $p = 0.0090$) and an unexpected positive effect on the swimming speed ($F_{1,78} = 4.39$, $p = 0.0039$) (Figure 2C-D).

Previous exposure to Roundup had no effect on the survival times in the survival test (Gehan Wilcoxon test statistic = -0.12, $p = 0.90$) (Figure 3B). Larvae with a higher walking activity in the presence of predator stimuli showed a higher mortality rate (Cox regression, $\chi^2 = 5.99$, $df=1$, $p_{\text{one-sided}} = 0.0072$, slope = 0.74 (SE = 0.30), hazard ratio = 2.10).

4. DISCUSSION

To avoid being detected prey organisms typically reduce their activity levels when they perceive predation risk (Werner and Anholt, 1993). Higher activity levels have indeed been associated with a higher vulnerability to predation in damselfly larvae (Stoks and Johansson, 2000; Brodin and Johansson, 2004) including *Enallagma* larvae (Stoks et al., 2005; Strobbe et al., 2011; this study). In line with this, larvae that were not exposed to a pesticide reduced walking, orientations toward prey and feeding strikes in the presence of predation risk in both experiments. This confirms previous studies on *Enallagma* damselfly larvae (McPeck, 1990; Stoks et al., 2003) including the study species (Stoks et al., 2005). Previous exposure for 24h to endosulfan and to Roundup drastically changed these behavioral antipredator traits in two ways: larvae increased the number of walks, orientations and feeding strikes in the absence of predation risk and did not reduce the number of orientations and feeding strikes and even increased the number of walks when predation risk was present.

Several mechanisms may explain these maladaptive activity changes after a pesticide pulse. A likely explanation for the overall increased activity levels after

pesticide exposure is that larvae were more actively foraging in response to higher energetic needs. Organisms are capable to cope with moderate concentrations of pollutants by upregulating processes linked to detoxification (Newman and Unger, 2003). Furthermore, exposed animals may protect themselves against cellular damage by energetically costly increases of antioxidant enzymes and stress proteins.(De Block and Stoks, 2008; Slos and Stoks, 2008; Slos et al., 2009; Stoks and De Block, 2011). In contrast to our results, other studies showed no effect on or a decreased food uptake in animals exposed to endosulfan (Ribeiro et al., 2001; Broomhall, 2004; Campero et al., 2007). These studies, however, exposed animals for longer times, while an upregulation of food intake may be more likely after a short pesticide pulse.

The above-mentioned increased energetic demands due to pesticide exposure may also explain why previously-exposed larvae did not reduce the number of orientations and feeding strikes and even increased the number of walks in the presence of predation risk. Alternatively, the pesticide may have changed the way the larvae perceived predation risk by interfering with neural processes. For endosulfan this seems a likely explanation, since it is known to interfere with the central nervous system by blocking the chloride channels. Similar maladaptive responses to predation cues after a pesticide pulse have been observed for other pesticides (e.g.: diazinon: Scholz et al. 2000; metachlor: Wolf and Moore 2002; fenvalerate: Reynaldi et al. 2011).

Once prey animals are detected, they will try to escape and avoid being captured during an attack. In damselfly larvae up to 60% can escape an attack by dragonfly larvae by swimming away (Stoks and McPeck, 2003). Experiments in field enclosures showed that *Enallagma* larvae that survived in the presence of dragonfly larvae had a higher

propensity to swim away and a higher swimming speed (Strobbe et al., 2009, 2010). In our study endosulfan tended to cause a decrease in escape swimming speed. There are several studies that showed a negative effect of endosulfan and other pesticides on the swimming capacities of prey, both during (e.g. Van Gossum et al., 2009; Trekels et al. in review) and after the exposure period (e.g. Hopkins et al., 2005; Beggel et al., 2010). One underlying reason for this is inhibition of muscle cholinesterases (Trekels et al., in review). In contrast, previous exposure to Roundup caused the larvae to swim faster, yet less often when attacked. The increase in swimming speed is unexpected given that Roundup inhibits the synthesis of amino acids as tyrosine, tryptophan and phenylalanine (Schneider et al., 2009). Similarly, after 4 h exposure to esfenvalerate *Pimephales promelas* fish larvae also swim less and tend to swim faster (Floyd et al., 2008).

While several studies documented how pesticide exposure affected antipredator traits, few of these also directly evaluated how this translated into a different mortality by predation (but see e.g. Floyd et al., 2008; Reynaldi et al., 2011). Given that both pesticides increased overall activity levels and caused a further increase in walking in the presence of predation cues, they likely increased the probability of detection by the dragonfly predators. Previous exposure to endosulfan indeed reduced the survival times, mainly caused by a higher number of walks. In another study on endosulfan, previously-exposed *Limnodynastes peronii* tadpoles also suffered a higher mortality by dragonfly predation (Broomhall, 2004). In contrast, previously to Roundup exposed larvae did not suffer more predation, although they also increased overall activity and even reduced swimming propensity. Possibly, the increase in swimming speed in the group of previously exposed larvae partly compensated for these maladaptive behavioral changes.

Note, that our study was not set up to attribute differences between both pesticides in how they shape the vulnerability to predation linked to their mode of action. Furthermore, the differential effects of both pesticides may be specific for the chosen LOEC concentration. It is indeed to be expected that at higher Roundup concentrations swimming speed will also decrease and vulnerability to predation will increase.

Our study adds to the insight that a sublethal pesticide pulse may increase mortality by making prey organisms more vulnerable to predation (e.g. Floyd et al., 2008). Ecological risk assessment evaluations ignoring these carry-over effects of pesticide exposure to the post-exposure period likely underestimate the response of non-target organisms to sublethal pesticide pulses. Importantly, although at the used LOEC concentration both studied pesticides, endosulfan and Roundup, induced maladaptive changes in overall activity, only for the former this resulted in an increased mortality by predation. This underscores the need to consider effects on all important antipredator traits of the prey as trait compensation may occur (Dewitt et al., 1999; Steiner and Pfeiffer, 2007), and to effectively score the outcome of predator-prey interactions in staged encounters. Given that damselfly larvae are important predators of pest and vector mosquitoes (Ohba et al., 2010) they are often exposed to pesticide pulses targeting the mosquitoes. Evaluating effects of these pesticide pulses on their survival is therefore highly relevant when assessing their potential as biocontrol agents (e.g. Mandal et al., 2008; Shaalan and Canyon, 2009).

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611 FIGURE LEGENDS

612 Figure 1. Effects of previous exposure to endosulfan (A-C) or Roundup (D-F) and actual
613 predation risk on activity-related variables of *E. cyathigerum* larvae: number of walks (A, D),
614 orientations (B, E), and feeding strikes (C, F). Given are least-squares means corrected for
615 body mass with 95% confidence intervals. Different letter codes indicate means that differ
616 based on Duncan posthoc tests.

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618 Figure 2. Effects of previous exposure to endosulfan (A-B) and Roundup (C-D) on variables
619 related to escape swimming of *E. cyathigerum* larvae: (A, C) swimming propensity and (B, D)
620 swimming speed corrected for body mass. Given are means with 95% confidence intervals..

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622 Figure 3. Survival curves of *E. cyathigerum* larvae when exposed to an *Anax* dragonfly
623 predator in function of their previous exposure to endosulfan (A) and Roundup (B). Larvae
624 alive after 180 min were considered right-censored.

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