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APPLIED ISSUES

Emergent body size of mayfly survivors

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SUMMARY

1. Employing field-deployed mesocosms, we examined the effects of 12-h pulse and 20-day press (continuous) exposures of the common agricultural insecticide, imidacloprid, on nymph abundance, emergence patterns and adult body size of *Epeorus* spp. (Heptageniidae) and *Baetis* spp. (Baetidae).

2. In press exposures, reduced nymph density was driven by reduced survivorship; in pulse exposures, reduced nymph density may reflect increased emergence because of stress.

3. Once exposed to imidacloprid, *Epeorus* and *Baetis* mayflies developed less and emerged smaller than their control counterparts. Concentrations as low as 0.1 μg L^{-1} (12-h pulse) reduced head length in *Baetis* and thorax length in *Epeorus*.

4. In all of the *Baetis* and *Epeorus* examined, effects were only found in males. Male survivorship and body size can affect population dynamics. Sublethal doses of this widely applied agricultural insecticide have the potential to reduce reproductive success of mayfly populations.

Keywords: *Baetis*, *Epeorus*, imidacloprid, mayflies, sublethal

Introduction

Environmental stressors can cause mayflies to emerge earlier and at a smaller body size as a result of a trade-off between suboptimal reproduction (e.g. fewer eggs) and adult survivorship (Scrimgeour, Culp & Wrona, 1994; Peckarsky *et al.*, 2001). As optimal development and body size of insects is related to the attainment of sufficient food resources (Sibly & Calow, 1986), indicators of development, such as adult body size, are important sublethal indicators of stress (Buffagni & Comin, 2000). Various organic pesticides that interact with the nervous system, such as acetylcholinesterases that affect muscle control (Hart, 1993; Donkin *et al.*, 1997), are particularly prone to causing reduced feeding and development. Therefore, if mayfly nymphs have reduced feeding because of sublethal exposure to an insecticide, adult mayflies will probably have a corresponding reduction in adult body size and fecundity.

One such pesticide that induces a feeding inhibition response is imidacloprid, an insecticide that is commonly applied in agricultural catchments throughout North America (Dunn, 2004). Imidacloprid has a number of properties that pose potential risk to riverine communities. For example, it is a chemically stable mimic of nicotine which is highly soluble (510 mg L^{-1}), mobile in soil (Ambrust & Peeler, 2002) and persistent (>400 days; Felsot & Ruppert, 2002). Although originally designed to be specific for the nicotinic acetylcholine receptor (nAcChR) in targeted pest species, such as the Colorado potato beetle (Moffat, 1993), this receptor is highly conserved and common to most invertebrate taxa. In fact, imidacloprid can induce toxic effects in a variety of non-target species, such as aquatic arthropods (Song, Stark & Brown, 1997) and honey bees (Suchail, Guez & Belzunces, 2001). Moreover, imidacloprid has been frequently detected in the water of agricultural stream

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ecosystems at levels ranging from 0.25 to >15 μg L^{-1} (Dunn, 2004; Phillips & Bode, 2004).

As mayflies are sensitive to pesticides (see Hatakeyama, Shiraishi & Uno, 1997) an examination of the effects of imidacloprid on lotic grazers is particularly relevant. Specifically, this research examines the impact of the common insecticide imidacloprid on adult body size in two mayfly genera, namely *Baetis* spp. and *Epeorus* spp. The following study compares the effects of press (20-day continuous) versus pulse (12-h only) exposures on both benthic and emerged mayfly populations, as well as the effects of imidacloprid on mayfly density, emergence and adult body size.

Fig. 1 A modular mesocosm table containing eight replicate artificial streams each covered in emergence traps.

Methods

The experiment was conducted from 20 June to 10 July, 2004 and was designed to examine the effects of pulse (12-h) versus press (20-day) concentrations of imidacloprid (pulse: 0, 0.1, 0.5, 1, 5 and 10 μg L^{-1} and press: 0, 0.1, 0.5 and 1 μg L^{-1}) on the size of adult insects and the development of larval insects. In the following experiment, we inoculated 10 artificial streams (mesocosms) with a benthic invertebrate community collected in the Nashwaak River, New Brunswick, Canada (46°14294′N, 66°36722′W). Substratum (coarse and fine) and benthic invertebrates (<5 cm) were collected, subsampled (into four equal portions) and placed into treatment streams dosed with imidacloprid. Each modular mesocosm table (Culp *et al.*, 2003) contained eight replicate streams (Fig. 1). Three streams (from different mesocosm tables) were inoculated per subsample and 27 subsamples were taken to inoculate a total of 80 streams (10 treatment tables × 8 replicate streams; Fig. 2).

Study species

The mesocosms were inoculated with a portion (<5 cm) of a benthic invertebrate stream community. The mayfly grazers *Epeorus* spp. (Heptageniidae) and *Baetis* spp. (Baetidae) were chosen for this study because they are both highly abundant in North American streams and sensitive lotic species (Edmunds, Jensen & Berner, 1976). Additionally, the choice of study genera was appropriate as our previous work indicates that *Epeorus* nymphs have lower feeding rates when exposed to imidacloprid (Alexander *et al.*, 2007). Nymphs and adult specimens of *Epeorus* from our artificial streams have been

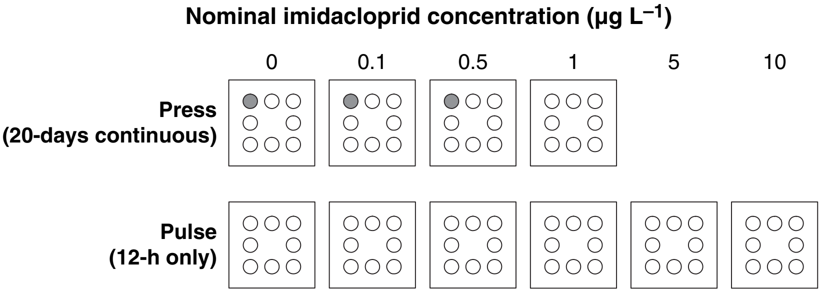

Fig. 2 Design: each square represents a mesocosm treatment table and each circle represents a replicate artificial stream (eight replicates per mesocosm treatment table). Above the shaded circles represent the systematic inoculation scheme where one-quarter of each invertebrate subsample would be allocated to the same replicate stream on a different mesocosm treatment table (square). Press treatments received 20-day of imidacloprid exposure. Pulse treatments received an initial 12-h dose of imidacloprid, then control groundwater for the remaining 19.5-day. In the actual experiment, treatments were allocated randomly. Also, the imidacloprid concentration does not differ between 0.1 and 0.5 pulse treatments according to chemical analysis.

identified as *Epeorus longimanus* (Eaton). Likewise, larval *Baetis* specimens in the mesocosm have been tentatively identified as *Baetis brunneicolor* (McDunnough). However, given the difficulties in correctly identifying adult Baetidae (Morihara & McCafferty, 1979) and adult females of most mayfly genera, we have conservatively referred to the mayflies by their genus names throughout the manuscript.

Epeorus mayfly nymphs are dorso-ventrally flattened, reside on cobble substratum in fast flowing riversand may reside in streams for long periods (7–8 months) as they develop (Edmunds *et al.*, 1976). *Epeorus* adult males tend to be larger than females and males may copulate with multiple females over the course of a 4-day swarming event (Edmunds & Allen, 1964; Flecker, Allan & McClintock, 1988). Similarly, *Baetis* nymphs, also found in cool, fast flowing rivers, are excellent swimmers, have uni- or bivoltine life histories and emerge in large swarms in early to mid-summer (Edmunds *et al.*, 1976). Males of this genus tend to be smaller than females (Beerstiller & Zwick, 1995; Peckarsky *et al.*, 2002) and possess turbinate compound eyes that are thought to enhance their ability to successfully navigate within mating swarms (Land, 1997).

Establishment of treatments

Mesocosms were deployed at the Agriculture and Agri-Foods Canada facility adjacent to the Saint John River, approximately 10 km southeast of Fredericton, New Brunswick, Canada. The partial flow-through mesocosms were designed to simulate lotic habitats by encompassing a portion of the benthic community in a controlled setting (Culp & Baird, 2006). Each mesocosm table represented one treatment group and contained eight replicate streams. There were 80 streams total, each having a planar area of 0.065 m^2 and a 10-L volume. The polyethylene tables housed a reservoir of the imidacloprid treatment solution and manifolds to distribute the treatment solutions at uniform flow rates to each replicate stream. Groundwater from the extensive Saint John River aquifer was used to provide water to the mesocosms. Wastewater from the mesocosms was passed through carbon filters (Culligan Inc.; activated carbon filter cylinder, Moncton, NB, Canada) to remove all contaminants before any water was discharged to the environment.

Imidacloprid solutions were mixed in two stock tanks (5 and 30 μg L^{-1}) by diluting a 1-mL aliquot of 240 g L^{-1} Admire® (Bayer CropScience; Calgary, AB, Canada). The insecticide mixture was delivered to a treatment reservoir in each mesocosm table by positive displacement pumps (Viking Pumps; Pulsefeeder 25-H duplex pump, Cedar Falls, IA, U.S.A.). Water was continuously pumped and was completely exchanged every 7 min. Each replicate stream was screened with 400 μm Nitex© mesh (Aquatic Ecosystems Inc.; Apopka, FL, U.S.A.) to prevent nymphs from escaping as well as to capture emerging insects. Water velocity in the stream was 11–12 cm s^{-1} which is similar to the typical substratum–water interface in rivers (Culp *et al.*, 2003).

Chemical analysis determined the actual concentrations of imidacloprid in each treatment (Table 1). When the two lowest pulse doses (0.1 and 0.5) did not receive different concentrations of imidacloprid only nine treatments (not 10) appear in the histograms throughout this manuscript. Chemical analyses were conducted at the National Water Research Institute (Environment Canada) in Saskatoon, SK, Canada on a Micromass Quattro Ultima liquid chromatography mass spectrometer (LC-MS) equipped with a stainless steel column (100 × 2.2 mm; MS Xterra C-8; 100 × 2.2 mm; Waters, Milford, MA, U.S.A.). Samples for imidacloprid analyses were taken from each mesocosm table at the onset, during and at the end of the imidacloprid pulse and every 5 days in the press exposures. These samples were collected in 1 L, amber vials (EPA vials; Fisher scientific; Fair Lawn,

Table 1 Comparison of the nominal to actual concentrations in the press and pulse mesocosm experiment

Nominal	Actual	±1 SE
Press experiment (20-day continual)		
0.1	0.1	<0.01
0.5	0.3	0.01
1	0.8	0.04
Pulse experiment (12-h only)		
0.1	0.1	<0.01
0.5	0.1	0.01
1	0.3	0.02
5	3.9	0.21
10	9.1	0.49

Overall, calibration yielded a fit of $y = 2.8595x + 2.709$ with an $R^2 = 0.9629$. All concentrations are in μg L^{-1}. Please note that the achieved concentration in the two lowest doses (0.1 and 0.5 μg L^{-1}) of the pulse experiment is the same (both 0.1 μg L^{-1}).

NJ, U.S.A.) and stored at 4 °C until shipment to the laboratory (within 10 days). The samples were directly injected into the LC-MS system. The mobile phase was 40% aqueous acetonitrile and 0.2% formic acid (v/v). The column flow rate was 200 μL min^{-1} and injections volumes were 10–20 μL.

Prior to initiating the experiment, benthic substrata were introduced into each replicate stream. A realistic benthic substratum was created in the mesocosms by inoculating each stream with a mixture of 25% fine gravel (2–4 mm) and 75% gravel (4–30 mm) that was obtained from gravel beds adjacent to the invertebrate sampling site on the Nashwaak River. Cobblestones (7–10 cm) were also collected from this site with five stones randomly assigned to each replicate stream. Cobble and gravel were gently washed to remove any attached invertebrates while maintaining the periphyton community. This procedure established a lotic substratum consisting of a 2–3 cm layer of gravel–cobble plus surface stones that were covered with periphyton. Once inoculated with cobble, the streams were permitted to equilibrate for 2 days prior to the addition of benthic invertebrates.

Benthic invertebrates were collected upstream of the gravel collection site on the Nashwaak River with *U*-nets (area = 0.06 m^2). Five *U*-nets were collected, pooled and subsampled into four equal portions for every three streams that were inoculated (135 *U*-nets total, in 27 subsamples to inoculate 80 replicate streams). Five *U*-nets for every three streams was used to slightly increase (*c.* 10%) the ambient density of aquatic invertebrates in the streams offsetting mortality because of transport from the river to the mesocosm test site. Three of the invertebrate assemblage subsamples were inoculated into three different treatment streams (one per treatment table). Once inoculated with invertebrates, the streams were permitted to equilibrate for 1 day before beginning the insecticide exposures. The fourth portion of the subsampled invertebrate assemblage was retained and preserved in 10% formalin (transferred to 70% ethanol after 1 week) for subsequent determination of the original benthic community composition and size (prior to inoculation into the mesocosm).

Endpoints

At the end of the 20-day experiment, the mesocosms were dismantled the contents of the replicate streams sieved. Water samples, periphyton samples and invertebrates were collected from each replicate stream. Nymphs were collected and preserved (10% formalin) for subsequent laboratory sorting and identification using LEICA© (Leica Microsystems Ltd., Cambridge, U.K.) dissecting microscopes. In the interest of time, nymphs of the orders Ephemeroptera, Plecoptera, Trichoptera and Coleoptera were identified to Family, Diptera were identified to subfamily while other orders such as, Oligochaeta, Nematoda and Collembola were identified only to Order. For our two families of interest (Heptageniidae and Baetidae) benthic density was determined by dividing the count of each family per replicate stream by the stream's planar area (0.065 m^2). Also, over the course of the 20-day experiment, adult insects were collected in 2-day intervals with an aspirator and preserved in 80% ethanol. Adult insects, which are easier to identify and were less numerous, were identified to genus and the head length, thorax length and abdomen length measured to the nearest 0.02 mm with the aid of an ocular micrometer. All three lengths were necessary to evaluate total length and confirm that body segments covary with total insect body size. Thorax and head length are suitable measurements of mayfly body size because both of these segments preserve well because of the abundance of chitin as well as being integral to the fitness and function of these insects (see Discussion). In the case of male *Baetis* with large turbinate eyes, measurements may better reflect eye size than head length.

Statistical analyses

Larval density of mayfly families (Heptageniidae and Baetidae) was compared using one-way ANOVA. Body size comparisons of the adult thorax and head length for the two genera were conducted using the two-way MANOVA sequence in the SAS program (Version 8.02; SAS Institute, Cary, NC, U.S.A.). The analysed variables included head length and thorax length versus imidacloprid treatment concentration and press versus pulse categories. MANOVA was deemed to be an appropriate statistical procedure as we were interested not only in the changes in adult body size because of imidacloprid treatment but also in the partitioning of changes in body size between male and female mayflies. Repeated measures ANOVA was used to evaluate changes in body size over time.

Post hoc comparisons, where applicable, were conducted using the sequential Bonferroni test (Winer, Brown & Michels, 1991; Underwood, 2002). All data sets were analysed for homogeneity of variance (Cochran's C test), normality (Shapiro–Wilks W test; Underwood, 2002) and residuals were checked, no transformations were required.

Results

The riverine subsamples (preserved and identified) generate a snapshot of the initial aquatic community. Nymphs from the original riverine subsamples (a one-quarter portion of the four equal portions inoculated into the mesocosm artificial streams) each had *c*. 747 ± 36 aquatic invertebrates. The total count of benthic invertebrates in the 27 original riverine subsamples was *c*. 20 thousand individuals. The density of mayflies did not differ between subsample groups ($P > 0.05$). Neither Heptageniidae nor Baetidae nymphs from the original riverine community differed in abundance between the 27 river subsamples (both Heptageniid and Baetid nymphs averaged *c*. 62 ± 3 individuals per replicate stream). *Epeorus* and *Baetis* nymphs were measured in two of the 27 riverine subsamples (>100 individuals of each genus) and did not differ in body size: head length; thorax length; thorax width; abdomen length or total length ($P > 0.05$). Therefore, changes in adult body size in the mesocosm experiment cannot be explained by the ambient variation in mayfly abundance and body size. Changes in larval density and body size cannot be explained by temperature, water chemistry or algal biomass. Over the course of the 20-day experiment, neither temperature (every 10 min) nor water chemistry (daily) differed between treatments. Algal biomass, measured as chlorophyll-*a* and ash free dry mass, did not differ at the end of the 20-day experiment in either the press (Wilks-$L = 0.72$, $F_{6,22} = 0.66$, $P = 0.68$) or pulse (Wilks-$L = 0.45$, $F_{10,34} = 1.66$, $P = 0.13$) treatments.

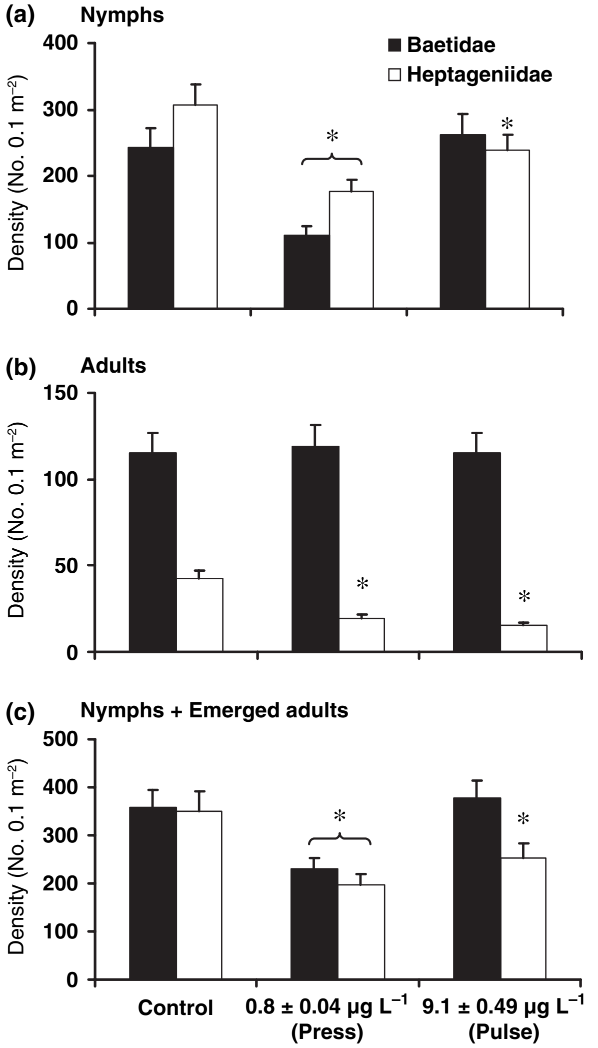

Fig. 3 Comparison of mean (±1 SE) nymph (a), adult (b) and nymph + emerged adult (c) density (counts per 0.1 m^2) from the highest press and pulse treatments. Treatments were 0.8 ± 0.04 µg L^{-1} press (20-day continual) and 9.1 ± 0.49 µg L^{-1} pulse (12-h only). Stars (*) indicate significant differences in treatment levels as compared with the control. Baetidae are not compared with the Heptageniidae, where both are significant it is in relation with their families' control insect density.

Therefore, changes in larval density (Fig. 3a) reflect reduced nymph survivorship in some treatment groups and/or adult emergence from the artificial streams. Heptageniid nymph density was severely reduced in the 0.8 ± 0.04 µg L^{-1} (press) and significantly, although less severely, reduced in the 9.1 ± 0.49 µg L^{-1} (pulse) treatment ($F_{2,11} = 2.93$, $P < 0.01$). In contrast, Baetid nymph density was only affected in the highest press exposure (0.8 ± 0.04 µg L^{-1}). Similarly, Baetid emergent density was unaffected by imidacloprid treatment (Fig. 3b) although there was a marked effect on Heptageniids ($F_{2,11} = 2.10$, $P < 0.05$). When these groups were combined (Fig. 3c) the response of nymph plus emergent adult was indistinguishable from the initial nymphal graph. This reflects the relatively small number of insects emerging (>400 mayflies; >1000 insects) compared with the abundance of nymphs in the treatment streams. In press exposures, reduced nymph density was driven by reduced survivorship; in pulse exposures, reduced nymph density of Heptageniids may reflect increased emergence because of stress.

Upon emergence, adult *Epeorus* males had significantly reduced thorax lengths in response to imidacloprid treatment (Fig. 4). Male head length was not affected ($F_{5,35} = 2.61$, $P = 0.08$). The negative effects of imidacloprid on *Epeorus* were most pronounced in the 0.25 ± 0.01 and 0.8 ± 0.04 μg L^{-1} press and the 9.1 ± 0.49 μg L^{-1} pulse treatments where no males emerged (NE). In contrast, female *Epeorus* emerged in all imidacloprid treatment groups ($N_{female} = 28$). Therefore, some *Epeorus* cohorts, exposed to the >0.25 ± 0.01 μg L^{-1} (press) and 9.1 ± 0.49 μg L^{-1} (pulse) of imidacloprid, were entirely female. However, unlike in males, female thorax length was unaffected ($F_{9,35} = 1.27$, $P = 0.28$) by press or pulse imidacloprid treatment and the average female thorax length was 1.37 ± 0.18 mm. Female head length was also not affected by imidacloprid treatment ($P > 0.05$).

Adult male *Baetis* head length was significantly shorter in response to imidacloprid press and pulse treatments (Fig. 5). All imidacloprid concentrations examined for both press (0.1, 0.3 and 0.8 μg L^{-1}) and pulse (0.1, 0.3, 3.9 and 9.1 μg L^{-1}) treatments resulted in adult males with smaller heads compared with control individuals ($F_{5,87} = 3.05$, $P = 0.014$). Female head length was unaffected by imidacloprid treatment ($F_{9,147} = 0.92$, $P = 0.51$) and was 0.44 ± 0.03 mm throughout the experiment. Unlike *Epeorus*, *Baetis* mayflies emerged throughout the experiment in all treatment concentrations and duration regimes. Although more female *Baetis* ($n = 163$) than males ($n = 115$) emerged. Thorax length was not significantly different in male or female Baetids. As in *Epeorus*, neither head length nor thorax length was significantly different in female Baetids.

As a result of the large number of *Baetis* emerging ($n > 250$) it was possible to compare the head length of adults in the control and imidacloprid treatments over time (Fig. 6). The 10 day divisions in Fig. 5 were arbitrarily chosen to divide not only the experimental

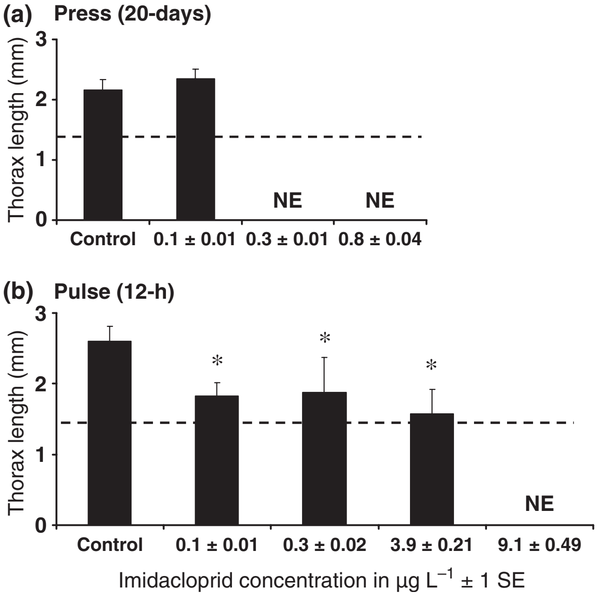

Fig. 4 Comparison of mean adult male *Epeorus* thorax length (in mm ±1 SE) across imidacloprid treatments: (a) press (20-day exposure) concentrations and (b) pulse (12-h exposure) concentrations. Significant differences are marked by a star (*) as determined by Bonferroni adjustment ($P < 0.02$). Female *Epeorus* thorax length (dashed line) was not significantly different between treatments (1.37 ± 0.18 mm). Lack of bar (NE) indicates no males emerged in treatment group. Females emerged throughout.

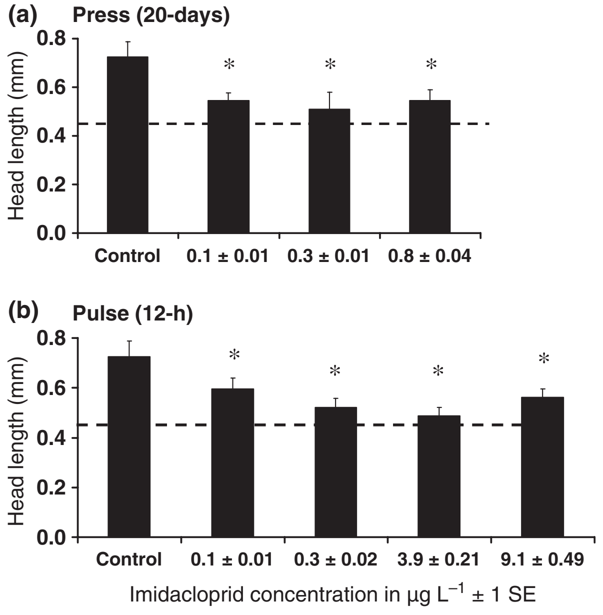

Fig. 5 Comparison of mean adult male *Baetis* head length (in mm ±1 SE) across imidacloprid treatments: (a) press (20-day exposure) concentrations and (b) pulse (12-h exposure) concentrations. Significant differences are marked by a star (*) as determined by Bonferroni adjustment ($P < 0.008$). Female *Baetis* head length (dashed line) was not significantly different between treatments (0.44 ± 0.03 mm). Males and females emerged throughout.

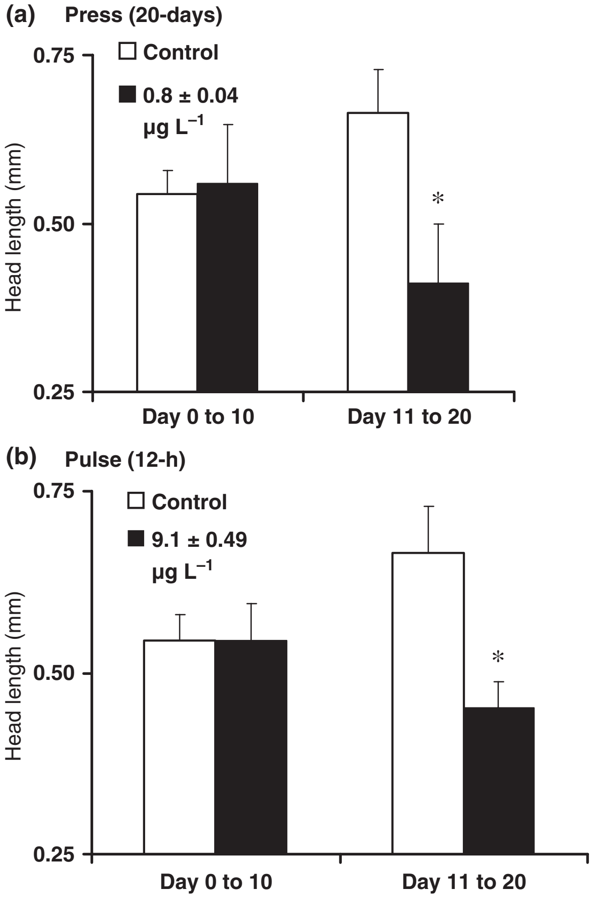

Fig. 6 Comparison of mean adult male *Baetis* head length (in mm ±1 SE) over time: (a) press (20-day imidacloprid exposure) and (b) pulse (12-h imidacloprid exposure). Significant differences are marked by a star (*) according to Bonferroni adjustment ($P < 0.02$). Female head length was not significantly different over time (not shown).

period in half but also to split the *Baetis* into two groups of approximately the same size (males and females *c.* 50). Male head length was reduced over time because of imidacloprid treatment ($F_{5,89} = 2.443$, $P = 0.04$). Both the highest concentration press and highest concentration pulse treatments, 0.8 ± 0.04 µg L^{-1} and 9.1 ± 0.49 µg L^{-1}, respectively, resulted in similar reductions in head length, irrespective of the duration of exposure. We found no difference in the effect of continuous or pulse exposure on male (or female) *Baetis* body size over time ($F_{1,89} = 3.697$, $P = 0.06$). However, male *Baetis* emerging prior to day 10 were larger than the mayflies emerging after day 10 ($F_{1,89} = 4.268$, $P = 0.04$). The positive increase in the control groups in both the press and pulse graphs corresponds to the growth of the *Baetis* over the course of the 20-day experiment – which may be due to the reduced predation pressure in the artificial streams. The other treatments examined, press (0.1 and 0.3 µg L^{-1}) and pulse (0.1–3.9 µg L^{-1}), were not different from the control male *Baetis* head length over time ($P > 0.05$). No effects on females were detected.

Once exposed to imidacloprid, male *Epeorus* and *Baetis* mayflies developed less and emerged smaller than their control counterparts. In particular, it was determined that concentrations as low as 0.1 µg L^{-1} (12-h pulse) reduced head length in *Baetis* mayflies and thorax length in *Epeorus*. At higher concentrations of longer duration, even more serious effects on male emergence and male body size occurred.

Discussion

Soluble agricultural insecticides such as imidacloprid can impair the development and growth of larval mayflies, potentially reducing their reproductive success. Previous studies have shown that mayflies emerge earlier and at a smaller body size in response to sublethal stresses such as temperature (Atkinson, 1995), predation (Scrimgeour *et al.*, 1994; Peckarsky *et al.*, 2001) or toxic substances (Lowell, Culp & Wrona, 1995). These are examples of trade-offs that mayflies experience between reaching a suitable reproductive stage and achieving the optimal body size. This study documents the reduction of *Baetis* head length and *Epeorus* thorax length because of exposure to the insecticide imidacloprid at environmentally-relevant concentrations.

In the highest press exposures (*c.* 1 µg L^{-1}), larval density of both Baetid and Heptageniid mayflies was reduced. However, as so many Baetid mayflies emerged over the course of the mesocosm experiment (virtually all *Baetis* spp. and some *Acentrella* spp.), we might conclude that decreased larval density was caused by increased emergence, i.e. Baetid adults were 'escaping' our larval estimates. In contrast, the Heptageniid nymphs were also less abundant in the press exposure but did not increase in adult density, suggesting larval mortality. For the Heptageniidae, the trend of reduced density recurs in the highest concentration pulse exposure, although the Baetid mayflies are consistently abundant throughout. As Baetidae and Heptageniidae were equally abundant in our original river community (and mesocosm inoculations) we might conclude that the Heptageniidae were more sensitive of the two families to imidacloprid. Heptageniidae sensitivity to imidacloprid may be particularly significant in natural streams; these mayflies spend 7–8 months as nymphs prior to emergence as adults (Edmunds *et al.*, 1976). Organisms that are slow to develop could be exposed to multiple pulses over the course of their development and be at higher risk than more rapidly developing groups.

By measuring adults, this study was able to detect patterns of effects that may impact the long-term fitness of mayfly populations. Although changes in larval mayfly density generally occurred in the press treatments, sublethal effects on body size occurred in both press and pulse exposures. These sublethal effects would have gone undetected using standard biological assessment methods (i.e. algal biomass or community larval abundance) in much the same way that we were unable to detect density-mediated effects in periphyton abundance or larval diversity. At the environmentally-relevant concentrations of imidacloprid examined, there was insufficient larval community mortality to release the periphyton from feeding inhibition rendering standard biological assessment methods unsuitable. In particular, standard approaches may not have detected effects between male and female mayflies that appear to be responding differently to imidacloprid treatment.

No male *Epeorus* emerged in some treatment groups: entire cohorts could be female. Although female parthenogenesis (virgin females laying eggs) has been widely described in some mayfly genera, it is generally thought that entirely female populations are unstable and contribute to an overall reduction in fitness (Funk, Jackson & Sweeney, 2006). Harker (1997) has previously reported that unfertilized eggs take longer and are less probably to hatch in two mayfly species. Although this study did not detect effects on female mayfly emergence patterns or body size for either *Baetis* or *Epeorus*, future studies should evaluate egg hatching success. If female parthenogenesis can destabilize *Epeorus* populations over time, mayfly populations exposed to concentrations of imidacloprid >0.3 ± 0.01 μg L^{-1} (press) or 9.1 ± 0.49 μg L^{-1} (pulse) may suffer local extinctions. As concentrations in this range have been reported (i.e. Dunn, 2004), either because of persistent groundwater contamination or episodic rain events, mayfly populations in general may be seriously impacted.

Body size has important implications to food webs (e.g. Woodward *et al.*, 2005) and although effects on males are an unusual endpoint to study, we are able to link body size with male success. Adult *Epeorus* males mate in flight, participate in multiple copulatory pairings and are generally larger than their female counterparts (Edmunds & Allen, 1964; Flecker *et al.*, 1988). As large body size is related to strength and longevity (Marden, 1989; Taylor, Anderson & Peckarsky, 1998), it is probable that *Epeorus* males would benefit from a larger thorax length (e.g. for robust wing attachment). Flecker *et al.* (1988) have previously described that male *E. longimanus* caught within a mating swarm are larger than those caught outside of the swarm. This implies that body size is an important indicator of male fitness.

Imidacloprid also caused reductions in adult head length in *Baetis* mayflies. All concentrations and treatment groups demonstrated this effect. Although the loss of adult emergence was not observed in the *Baetis* mayflies, the marked reduction in head length at very low concentrations suggests that the lowest effect level has yet to be determined. Additionally, *Baetis* responded to imidacloprid over time, indicating that the effect of imidacloprid is immediate and prolonged since the duration of the exposure (12-h or 20-day) demonstrated the same effect. This may be caused by imidacloprid-induced lethargy (e.g. Nauen *et al.*, 1998), reducing the accrual of sufficient nutrients and foodstuffs at critical developmental (larval) stages. While control groups increased in adult size throughout the experiment, *Baetis* nymphs exposed to imidacloprid had reduced head length at the end of the experimental period in treatments containing 0.8 ± 0.04 μg L^{-1} imidacloprid for 20-day and at 9.1 ± 0.49 μg L^{-1} for 12-h.

Unlike *Epeorus*, *Baetis* males are smaller than their female counterparts and do not mate in flight (Edmunds *et al.*, 1976; Peckarsky *et al.*, 2002). However, head size may be correlated to the function of male *Baetis* turbinate eyes. Turbinate eyes probably confer enhanced depth perception and may be important in the successful attraction and capture of females in mating swarms (Land, 1997). If this is the case, reduced head length may constitute reduced fitness (see Edmunds *et al.*, 1976; Merritt

& Cummins, 1996). Although no studies have examined the relationship between *Baetis* eye size and degree of development, reduced eye size has been shown to reduce territory size (Zollikofer, Wehner & Fukushi, 1995) and mate capture success (Rutowski, 2000) in other insect orders. If this is also the case in *Baetis* males, reduced eye size may affect male success (Land, 1997).

In summary, in the race to reproduce, mayflies are trapped in the aquatic environment until they achieve sufficient body size to successfully emerge and reproduce. We have found that by evaluating sublethal effects at low doses of imidacloprid that *Baetis* and *Epeorus* mayflies undergo changes in emergence patterns and body size that may not have been detected using routine risk assessment (larval) approaches. Once exposed to imidacloprid, *Epeorus* and *Baetis* mayflies developed less and emerged smaller than their control counterparts. In particular, it was determined that concentrations as low as 0.1 $\mu g\ L^{-1}$ (12-h pulse) reduced head length in *Baetis* mayflies and thorax length in *Epeorus*. At higher concentrations of longer duration, even more serious effects on body size and survivorship occurred. Although imidacloprid mostly affects insects who possess the nAcChR, streams dependant on insect processing to maintain function may be seriously impacted.

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References

Alexander A.C., Culp J.M., Liber K. & Cessna A.J. (2007) Effects of insecticide exposure on feeding inhibition in mayflies and oligochaetes. *Environmental Toxicology and Chemistry*, **26**, 129–135.

Ambrust K.L. & Peeler H.B. (2002) Effects of formulation on the run-off of imidacloprid from turf. *Pest Management Science*, **58**, 702–706.

Atkinson D. (1995) Effects of temperature on the size of aquatic ectotherms – exceptions to the general rule. *Journal of Thermal Biology*, **20**, 61–74.

Beerstiller A. & Zwick P. (1995) Biometric studies of some stoneflies and a mayfly (Plecoptera and Ephemeroptera). *Hydrobiologia*, **299**, 169–178.

Buffagni A. & Comin E. (2000) Secondary production of benthic communities at the habitat scale as a tool to assess ecological integrity in mountain streams. *Hydrobiologia*, **422**, 183–195.

Culp J.M. & Baird D.J. (2006) Establishing cause–effect relationships in multi-stressor environments. In: *Methods in Stream Ecology*, 2nd edn (Eds F.R. Hauer & G.A. Lamberti), pp. 835–854. Elsevier Academic Press, Boston, MA.

Culp J.M., Cash K.J., Glozier N.E. & Brua R.B. (2003) Effects of pulp mill effluent on benthic assemblages in mesocosms along the Saint John River, Canada. *Environmental Toxicology & Chemistry*, **22**, 2916–2925.

Donkin P., Widdows J., Evans S.V., Staff F.J. & Yan T. (1997) Effect of neurotoxic pesticides on the feeding rate of marine mussels (*Mytilus edulis*). *Pesticide Science*, **49**, 196–209.

Dunn A. (2004) *A Relative Risk Ranking of Pesticides Used in Prince Edward Island*. Surveillance Report EPS-5-AR-04-03. Environment Canada Environmental Protection Branch, Dartmouth, NS.

Edmunds G.F. & Allen R.K. (1964) The rocky mountain species of *Epeorus* (Iron) Eaton (Ephemeroptera: heptageniidae). *Journal of the Kansas Entomological Society*, **37**, 275–288.

Edmunds G.F., Jensen S.L. & Berner L. (1976) *The Mayflies of North and Central America*. Burns & MacEachern Ltd., Don Mills, ON.

Felsot A.S. & Ruppert J.R. (2002) Imidacloprid residues in Willapa Bay (Washington State) water and sediment following application for control of burrowing shrimp. *Journal of Agricultural and Food Chemistry*, **50**, 4417–4423.

Flecker A.S., Allan J.D. & McClintock N.L. (1988) Male body size and mating success in swarms of the mayfly *Epeorus longimanus*. *Holarctic Ecology*, **11**, 280–285.

Funk D.H., Jackson J.K. & Sweeney B.W. (2006) Taxonomy and genetics of the parthenogenetic mayfly *Centroptilum triangulifer* and its sexual sister *Centroptilum alamance* (Ephemeroptera: Baetidae). *Journal of the North American Benthological Society*, **25**, 417–429.

Harker J.E. (1997) The role of parthenogenesis in the biology of two species of mayfly (Ephemeroptera). *Freshwater Biology*, **37**, 287–297.

Hart A.D. (1993) Relationships between behavior and the inhibition of acetylcholinesterase in birds exposed to organophosphorus pesticides. *Environmental Toxicology and Chemistry*, **12**, 321–336.

Hatakeyama S., Shiraishi H. & Uno S. (1997) Overall pesticide effects on growth and emergence of two species of Ephemeroptera in a model stream carrying pesticide-polluted river water. *Ecotoxicology*, **6**, 167–180.

Land M.F. (1997) Visual acuity in insects. *Annual Review in Entomology*, **42**, 147–177.

Lowell R.B., Culp J.M. & Wrona F.J. (1995) Toxicity testing with artificial streams: effects of differences in current velocity. *Environmental Toxicology & Chemistry*, **14**, 1209–1217.

Marden J.H. (1989) Bodybuilding dragonflies – costs and benefits of maximizing flight-muscle. *Physiological Zoology*, **62**, 505–521.

Merritt R.W. & Cummins K.W. (1996) *An Introduction to the Aquatic Insects of North America*, 3rd edn. Kendall Hunt Pub, Dubuque, IA.

Moffat A.S. (1993) New chemicals seek to outwit insect pests. *Science*, **261**, 550–551.

Morihara D.K. & McCafferty W.P. (1979) The *Baetis* larvae of North America (Ephemeroptera: baetidae). *Transactions of the American Entomological Society*, **105**, 139–221.

Nauen R., Hungenberg H., Tollo B., Tietjen K. & Elbert A. (1998) Antifeedant effect, biological efficacy and high affinity binding of imidacloprid to acetylcholine receptors in *Myzus persicae* and *Myzus nicotianae*. *Pesticide Science*, **53**, 133–140.

Peckarsky B.L., McIntosh A.R., Caudill C.C. & Dahl J. (2002) Swarming and mating behavior of a mayfly *Baetis bicaudatus* suggest stabilizing selection for male body size. *Behavioral Ecology and Sociobiology*, **51**, 530–537.

Peckarsky B.L., Taylor B.W., McIntosh A.R., McPeek M.A. & Lytle D.A. (2001) Variation in mayfly size at metamorphosis as a developmental response to risk of predation. *Ecology*, **82**, 740–757.

Phillips P.J. & Bode R.W. (2004) Pesticides in surface water runoff in south-eastern New York State, U.S.A.: seasonal and stormflow effects on concentrations. *Pest Management Science*, **60**, 531–543.

Rutowski R.L. (2000) Variation in eye size in butterflies: inter- and intraspecific patterns. *Journal of Zoology (London)*, **252**, 187–195.

Scrimgeour G.J., Culp J.M. & Wrona F.J. (1994) Feeding while avoiding predators – evidence for a size-specific trade-off by a lotic mayfly. *Journal of the North American Benthological Society*, **13**, 368–378.

Sibly R.M. & Calow P. (1986) *Physiological Ecology of Animals: An Evolutionary Approach*. Blackwell Scientific Publications, Oxford.

Song M.Y., Stark J.D. & Brown J.J. (1997) Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods. *Environmental Toxicology & Chemistry*, **16**, 2494–2500.

Suchail S., Guez D. & Belzunces L.P. (2001) Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environmental Toxicology & Chemistry*, **20**, 2482–2486.

Taylor B.W., Anderson C.R. & Peckarsky B.L. (1998) Effects of size at metamorphosis on stonefly fecundity, longevity, and reproductive success. *Oecologia*, **114**, 494–502.

Underwood A.J. (2002) *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*, 6th edn. Cambridge University Press, New York.

Winer B.J., Brown D.R. & Michels K.M. (1991) *Statistical Principles in Experimental Design*, 3rd edn. McGraw Hill, New York.

Woodward G., Ebenham B., Emmerson M., Montoya J.M., Olesen J.M., Valido A. & Warren P.H. (2005) Body size in ecological networks. *Trends in Ecology and Evolution*, **20**, 402–409.

Zollikofer C.P., Wehner R. & Fukushi T. (1995) Optical scaling in conspecific *Cataglyphis* ants. *The Journal of Experimental Biology*, **198**, 1637–1646.

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