thiamethoxam, all measurements took place under red light. If a larva was found that had exited the lagoon prematurely and was not pupating, the replicate was removed from the experiment. Pupal mass and date of pupation (±three days) were also recorded. Once pupation had commenced, remaining non-pupating replicates were checked for pupation twice daily. Pupae were weighed on a 0.001 g resolution balance, and individually placed in labelled 50 mL tubes with netting secured over the opening, with a small amount of tissue paper to absorb any excess moisture. These tubes were stored in the dark at 21 °C and five days after pupation were checked twice daily for emergence.

Adult measurements

Upon emergence, adults were colour-marked on their thorax denoting their treatment group with a spot of non-toxic enamel paint, released into a flight cage (60 cm × 45 cm × 60 cm), and provided with pollen, water, and a 15% sucrose solution for one week. To observe and compare the behaviour of individual flies, seven-day old adults were individually placed into a smaller cage of the same design (30 cm × 20 cm × 25 cm), provided with water and 15% sucrose solution in feeders and a small amount of pollen. They were given 1 min to acclimatise. Using an instantaneous sampling technique (following similar protocols in *Gilbert, 1985*), behaviour was then recorded for 10 min. These behavioural activity budgets were categorised as: stationary, grooming, walking, flying, probing through the cage netting with their proboscis, feeding on nectar, pollen or water (grouped together as feeding) and moving which involved remaining stationary whilst making small jerking motions of their body.

Statistical analysis

All statistical analyses were carried out using SPSS (v. 21; IBM SPSS Inc. Armonk, NY, USA). Data from the four experiment replicates were pooled for all analyses. The significance threshold was set at 0.05.

Larval development

Data were tested for normality using the Shapiro–Wilk statistic and visual inspection of Q–Q plots, and homogeneity of variance was tested using Levene's statistic. A one-way ANOVA was used to determine the effect of thiamethoxam on pupal weight. Due to deviations from normality a Kruskal–Wallis *H*-test was used to investigate the effect of treatment on larval development time (five-day old larvae to pupation). Log-transformed larval weight data was compared between treatment groups using a generalised linear mixed model (GLMM) with treatment (thiamethoxam presence or control) and time (day 3, 6, 9 or 12) as fixed factors, 'experiment round' (1, 2, 3 or 4) was included as the random effect, and 'scaled identity' for the repeated measures covariance structure. We first fitted a full model and systematically omitted interaction terms if they did not increase model fit. Model fit was compared using the Akaike Information Criterion (AIC). AIC was also used in selecting the repeated covariance type in models with repeated measures structure. Fisher's exact test (2 × 6) was used

Table 1 Larval survival, development time and average pupal weight from six different larval populations reared in substrate contaminated with thiamethoxam.

Treatment group	Number of larvae that reach pupation (total *n* of group)	Survival (%)	Average pupal weight (g) ± SD
Control (A)	30 (36)	83.3	0.249 ± 0.0049
5 ppb (A)	27 (36)	75	0.240 ± 0.0056
15 ppb (A)	20 (33)	63.6	0.255 ± 0.0086
50 ppb (A)	27 (35)	77.1	0.250 ± 0.0064
100 ppb (A)	27 (35)	77.1	0.247 ± 0.0057
500 ppb (B)	5 (38)	13.2	0.227 ± 0.0129

Note:
Treatments sharing the same letter did not differ significantly at $P < 0.05$ (post-hoc test: pairwise log-rank).

to analyse the distribution of count data between treatment type and the likelihood to exit a lagoon prematurely or remain in lagoon.

Survival analysis

Larvae that reached the pupal stage were counted as survivors, irrespective of whether they later successfully completed metamorphosis (*Haider, Dorn & Müller, 2013*). Survival of the larvae across the treatment groups was analysed using Kaplan–Meier survival analysis, and the log-rank test with a Bonferroni correction was applied to test for differences between survival distributions across treatment groups. Replicates where larvae were found in the leaves but were not pupating were completely removed from the experiment. Once individuals reached pupation they were treated as 'censored data' (i.e. the number of larvae reaching pupation). Censored data across treatment groups was dissimilar and are therefore reported (Table 1). Median lethal concentration (LC_{50}) was calculated by probit regression analysis.

Adult behaviour

The total amount of time spent carrying out each behaviour was compared between treatment groups. Assumptions of normality were not met for each group of the independent variables as defined by the Shapiro–Wilk statistic and visual inspection of histograms, and so individual non-parametric Kruskal–Wallis *H*-tests were used to investigate the effect of thiamethoxam treatment on adult behaviour.

RESULTS

Larval development

Across treatments, 27 larvae exited the lagoons prematurely and were found in the dried leaves. By the end of the experiment, for the control 5 and 50 ppb groups, four larvae (of 40 replicates in that treatment group) had exited prematurely (10%). Most larvae that were found in the leaves were in the 15 ppb group (7/40, 17.5%) with the least in 500 ppb (2/40, 5%); but overall there was no effect of treatment on exiting larvae (Fisher's exact test, $P = 0.656$). The lower figure for the positive control (500 ppb) is probably due to the elevated mortality levels of larvae in this treatment. These replicates were removed from all further statistical analyses.

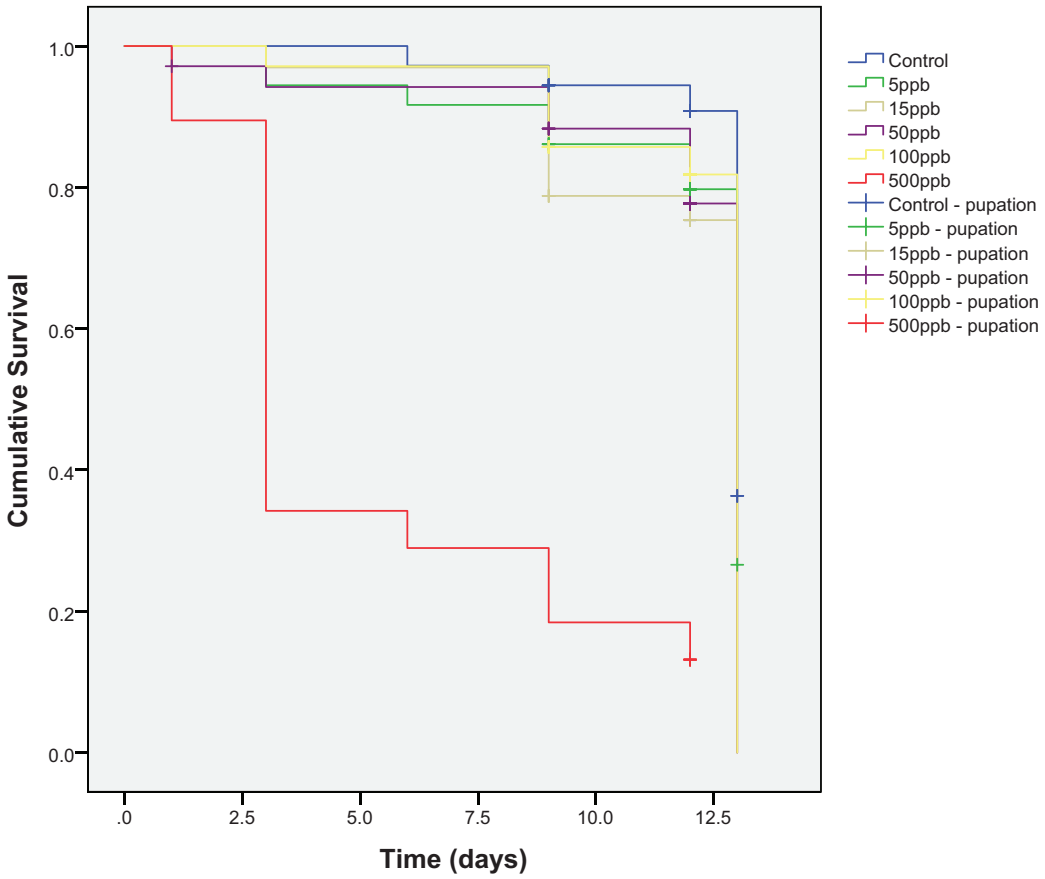

Figure 1 Cumulative survival of *Eristalis tenax* larvae (N = 33–38 per treatment) when reared in substrate contaminated with five different concentrations of thiamethoxam, plus control. Crosses indicate individuals that reach pupation (censored data). Many individuals pupated at the same time and so crosses are nested underneath one another. Post-hoc pairwise comparisons (Kaplan–Meier analysis, pairwise log-rank tests) showed significant differences between all groups with 500 ppb.

Full-size DOI: 10.7717/peerj.4258/fig-1

There was no significant effect of treatment on development time, which was 9–13 days (Kruskall–Wallis; $H(5) = 3.367$, $P = 0.644$; median for all groups—12 days), and no effect of treatment on pupal weight (one-way ANOVA, $F_{5, 129} = 1.029$, $P = 0.403$). Larval weight did not significantly differ between treatment groups (GLMM; $F_{5, 762} = 0.326$, $P = 0.897$).

Survival

Mortality across the six treatment groups was significantly different (Kaplan–Meier, log-rank; $\chi^2(5) = 122.27$, $P = <0.001$) and post-hoc pairwise comparisons showed significant differences between all treatment groups and the 500 ppb group (Kaplan–Meier analysis, pairwise log-rank test: control—500 ppb $\chi^2(1) = 50.172$, $P = <0.001$; 5–500 ppb, $\chi^2(1) = 39.272$, $P = <0.001$; 15–500 ppb, $\chi^2(1) = 35.431$, $P = <0.001$; 50–500 ppb, $\chi^2(1) = 36.280$, $P = <0.001$; 100–500 ppb, $\chi^2(1) = 41.112$, $P = <0.001$) (Fig. 1). Percentage survival was lowest in the 500 ppb group (13.2%), and highest in the control (83.3%) (Table 1). The LC_{50} for thiamethoxam and *E. tenax* was 215 ppb.

Adult behaviour

Distribution shapes were similar for all behaviour groups across treatments as assessed by visual inspection of a box plot. Median scores for all behaviours were not significantly different across treatments (Kruskal–Wallis; time spent: stationary $H(5) = 4.989$, $P = 0.417$; grooming $H(5) = 8.217$, $P = 0.145$; walking $H(5) = 6.960$, $P = 0.224$; flying $H(5) = 0.980$, $P = 0.964$; probing $H(5) = 3.188$, $P = 0.671$; feeding $H(5) = 7.497$, $P = 0.186$; moving $H(5) = 5.571$, $P = 0.350$).

DISCUSSION

While thiamethoxam has been detected in waterbodies on and near to farmland (*Samson-Robert et al., 2014*) with the potential for harming non-target species (*Pisa et al., 2015*; *Morrissey et al., 2015*) we report little or no effect of larval exposure to field-relevant doses of the neonicotinoid thiamethoxam via contaminated substrate. Our results indicate that *E. tenax* larvae are insensitive to field-realistic doses of thiamethoxam with no significant likelihood of direct mortality, or impacts on growth, development time or activity budgets in the resulting adults. These are the first known published data on the effects of a neonicotinoid on the insect family Syrphidae.

Within the field of aquatic toxicology, the chironomids (Diptera) are widely used in laboratory tests, with most work being undertaken at the organismal level by measuring larval survival and growth (*Saraiva et al., 2017*). A comprehensive review by *Morrissey et al. (2015)* looked at the lethal concentration in water (LC_{50}) and the EC_{50} values (where 50% of the pesticide's maximal effect is observed) for 214 acute (24–48 h) and chronic studies (7–28 days) for 48 species of aquatic invertebrate species. The geometric mean taken from the range of the $LC[E]_{50}$s for all Diptera and neonicotinoids tested was 32.9 ppb, and was 9.3 ppb for *Chironomous dilutes* (Diptera: Chironomidae) specifically. Aquatic invertebrate species also appear to vary in their sensitivity with *C. dilutes* being found to be the most sensitive of the three most common aquatic invertebrate species tested (compared to *Daphnia magna* (Cladocera; geometric mean: 23,690 ppb)) and *Gammarus pulex* (Amphipoda; geometric mean: 235.8 ppb)) (*Morrissey et al., 2015*), which emphasises the importance of testing a wide range of species in addition to a range of chemicals.

From this same review, only two studies examining effects of thiamethoxam on Diptera (Culicidae) were reported: *Aedes aegypti* (24 h) and *Chironomus riparius* (48 h) resulting in an $LC[E]_{50}$ of 183 and 35 ppb, respectively. Thiamethoxam is an order of magnitude less toxic than two other neonicotinoids, imidacloprid and clothianidin, to all life stages of *C. dilutes* over a 14-day exposure. The 14-day median lethal concentrations for imidacloprid, clothianidin and thiamethoxam were 1,520, 2,410 and 23,600 ppb. The 40-day median effect concentrations (emergence) for imidacloprid, clothianidin and thiamethoxam were, 390, 280 and 4,130 ppb, respectively (*Cavallaro et al., 2016*). Other studies demonstrate that toxicity can differ strongly between closely related species; the chronic LC_{50} of imidacloprid to *Chironomus tentans* is just 0.91 ppb (*Stoughton et al., 2008*). Unfortunately, a lack of studies on the effects of thiamethoxam on Diptera prevents much in the way of comparison. Our study estimated the LC_{50} for *E. tenax* to be

much higher at 215 ppb. It seems possible that thiamethoxam has a generally lower toxicity to aquatic invertebrates when compared to imidacloprid or clothianidin, but clearly more comparative studies are needed to draw firm conclusions.

Earlier larval instars have been consistently shown to be more sensitive to contaminants due to differences in biomass and bioaccumulation after exposure to a contaminant (*Heinis, Timmermans & Swain, 1990*). Our experiment commenced with five-day old larvae (which was essential to allow handling of larvae), it is possible that if eggs were laid directly in contaminated water, hatching or commencement of growth could be adversely affected.

Despite ensuring the lagoons were not exposed to UV light for the duration of the experiment (as UV is the major component contributing to thiamethoxam's photolytic decomposition; *Gupta, Gajbhiye & Gupta, 2008*), it is possible that during the experiment the thiamethoxam degraded over time due to the physicochemical properties of the matrix or bacterial action. Thiamethoxam in contaminated waste water rapidly degrades in darkness and this degradation has been attributed to the presence of microorganisms using the neonicotinoid as an energy source; a lagged effect was noticed as the microorganisms adapted to using the thiamethoxam (*Peña, Rodríguez-Liébana & Mingorance, 2011*). It is thus possible that the bacterial content of the lagoons resulted in biodegradation of the pesticide. However, if so, we would expect much the same to occur in the field.

Larvae of *E. tenax* mature in stagnant, anaerobic ponds and water-courses where they filter-feed on microbes associated with rotting organic material and faecal matter (*Hayes, Levine & Wilson, 2016*). It is possible that, due to being adapted to exploit these fetid environments, they are naturally robust and capable of coping with toxins. It is also feasible that their cuticle is impermeable therefore may prevent absorption of the chemical, reducing contact toxicity.

Interestingly, some larvae prematurely exited the lagoon before pupation; some exited just three days after transfer. We found no effect of treatment on the likelihood to exit a lagoon. We therefore hypothesise that larvae may be capable of detecting different conditions, which may be unfavourable compared to those in which they started development. Larvae are known to travel up to 10 m in search of favourable pupation habitats (*Fischer et al., 2006*), so searching for more favourable larval habitats, or the original habitat from which they were displaced may also be possible.

Evidence from studies on honeybees and bumblebees suggest that there is a latent effect of larval neonicotinoid exposure on the behaviour of the resulting adult. For example, larvae of *Apis cerana* (Apidae) exposed to low doses of imidacloprid (0.24 ng/bee) exhibited significantly impaired olfactory learning when tested as adults (*Tan et al., 2015*); the same effect was seen in *Apis mellifera* alongside higher brood mortality and reduced adult lifespan (*Peng & Yang, 2016*). Exposure to thiamethoxam specifically during larval development of the bumblebee can result in decreased memory function (*Stanley, Smith & Raine, 2015*), and reduced emerging queen body weights, reduced ovary weights, and lowered sperm counts in the honeybee (*Gajger, Sakač & Gregorc, 2017*). In this study, we found larval exposure to thiamethoxam and its metabolites to have no latent effect on in-situ adult hoverfly activity budgets, though we did not test for

effects of high level behaviours such as learning and memory. It is noted that the nervous system of adult insects is very different from that of the larvae, with the structures targeted by neonicotinoids, such as the mushroom-bodies in the brain, being undeveloped in the larvae (*Farris et al., 1999*). Further work is warranted on adult exposure to pollen and nectar containing field-relevant levels of neonicotinoids, as they pose the same potential risk of harm to hoverflies as they do to bees.

Research is most often focused on the effects of singular chemical exposures. However, fields can be treated with a large number of chemical compounds, with pesticides regularly applied as mixtures of similar or different active ingredients being common practice (*Cavallaro et al., 2016*; *Botías et al., 2017*). This potential exposure to a cocktail of chemicals in agricultural run-off is not addressed in this study and has not been commonly addressed in the wider field of investigations on the effect of pesticides on non-target organisms in general. Further research should examine exposure to field-realistic mixtures of chemicals (*Rodney, Teed & Moore, 2013*).

In summary, we found that thiamethoxam exposure results in elevated mortality of *E. tenax* larvae only at concentrations above those normally found in field-realistic situations. The larvae of this species appears to be less sensitive to thiamethoxam than some other aquatic insects that have previously been examined. Further research is required to investigate possible adverse effects via adult exposure, or from larval exposure to other neonicotinoids and currently used complex mixtures of pesticides. Farmland management may benefit from including hoverfly larval habitat to maintain an important pollinating species which, at least in the larval stage, appears to not be highly susceptible to at least one commonly used pesticide.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Kate Basley conceived and designed the experiments, performed the experiments, analysed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Balin Davenport performed the experiments, reviewed drafts of the paper, assisted with data collection.
- Kate Vogiatzis performed the experiments, reviewed drafts of the paper, assisted with data collection.
- Dave Goulson conceived and designed the experiments, analysed the data, wrote the paper, reviewed drafts of the paper.

Data Availability

The following information was supplied regarding data availability:

The raw data has been provided as Supplemental Dataset Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.4258#supplemental-information.

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