Environmental levels of neonicotinoids reduce prey consumption, mobility and emergence of the damselfly *Ischnura elegans*

S. Henrik Barmentlo1 | Laura M. Vriend1 | Roy H. A. van Grunsven2 | Martina G. Vijver1

1Institute of Environmental Sciences, Leiden University, Leiden, The Netherlands
2Dutch Butterfly Conservation, Wageningen, The Netherlands

Correspondence
S. Henrik Barmentlo
Email: s.h.barmentlo@cml.leidenuniv.nl

Funding information
Nederlandse Organisatie voor Wetenschappelijk Onderzoek, Grant/Award Number: NWO-VIDI864.13.010; Horizon 2020 Framework Programme, Grant/Award Number: 824603

Handling Editor: Tadeu Siqueira

Abstract
1. Freshwaters are among the most endangered ecosystems in the world as a result of anthropogenic interference such as pollution. Pollution in the form of neonicotinoids has been intensively studied, but data thus far is often conflicted by contrasting responses between laboratory and field experiments. In addition, toxicity data are scarce and contradictory for insects such as Odonates (dragonflies and damselflies) and a potential risk to them may therefore be overlooked.

2. We investigate the potential risk of neonicotinoids to Odonates by exposing nymphs of the blue-tailed damselfly *Ischnura elegans* to environmentally relevant concentrations of the neonicotinoid thiacloprid. We consider *I. elegans* as an indicator species for other Odonates as it is an abundant, widespread and eurytopic species. We analyse the effects of thiacloprid on multiple endpoints (survival, consumption, growth, molting, mobility and emergence), using cage-experiments as well as controlled field observations in naturally colonized experimental ditches. In addition, we assess sensitivity by either feeding the damselfly nymphs with lab-cultured prey or by letting them feed freely on natural aquatic invertebrates.

3. All sublethal endpoints of *I. elegans* are affected to some degree, and strongly depend on the food offered; free-feeding nymphs are more sensitive than culture-fed nymphs. Environmental relevant concentrations of thiacloprid strongly reduce the emergence of *I. elegans* and this effect is more substantial in the natural populations compared to the caged damselflies. This is likely explained by exclusion of additional biotic pressures such as predation in the caged experiment.

4. **Policy implications.** Literature reports that one out of seven Odonates is threatened and 24% of the species have declining populations. Our observations show that current risks of neonicotinoids to Odonates are underestimated in laboratory experiments as the toxicity is governed by multiple biotic factors such as food quantity/quality and predation. Given the widespread abundance of blue-tailed damselfly *Ischnura elegans*, the observed sensitivity to neonicotinoids and current
1 | INTRODUCTION

Pesticides have been indicated as a potential pollution threat to freshwater ecosystems (Beketov, Kefford, Schäfer, & Liess, 2013; Tilman et al., 2001), one of the most endangered ecosystems in the world (Dudgeon et al., 2006). Currently, neonicotinoid insecticides receive much of the attention as their usage have increased sharply over the past two decades (Simon-Delso et al., 2015) and they are now the most commonly used insecticides globally (Jeschke, Nauen, Schindler, & Elbert, 2011).

Neonicotinoids combat insect pest species by binding to the nicotinic acetylcholine receptors (nAChR), which induces continuous excitation of the neuronal membranes leading to paralysis (Simon-Delso et al., 2015). Like most agricultural chemicals, neonicotinoids are often found in surface waters due to processes such as run-off, drift and leaching (Morrisey et al., 2015). The effects of neonicotinoids on non-target freshwater organisms have been studied intensively in the laboratory (e.g., Raby, Zhao, Hao, Poirier, & Sibley, 2018; Roessink, Merga, Zweers, & Brink, 2013), but the data are actually scarce for many groups, such as Odonates (dragonflies and damselflies). Consequently, scientists that focused on the risk of neonicotinoids to Odonates (e.g. Morrisey et al., 2015; Vijver et al., 2017) largely had to interpolate their assessment on only two bioassays (Beketov & Liess, 2008; Jinguji, Thuyet, Udá, & Watanabe, 2013). These studies showed that the survival and emergence of the dragonfly genus Sympetrum was negatively affected by concentrations that are higher than those typically found in natural environments. This contrasts with the study of van Dijk, Staalduinen, Sluijs, and Der, (2013) who indicated possible effects of current environmental levels of neonicotinoids on the abundance of the damselfly Ischnura elegans. Nevertheless, the low amount of data available and the contrasting findings indicate that the risk of neonicotinoids to Odonates is currently poorly understood.

On top of this, recent findings show that the toxicity of neonicotinoids to aquatic invertebrates is food quantity or quality dependent (Alexander, Luis, Culp, Baird, & Cessna, 2013; Barmentlo, Parmentier, Snoo, & Vijver, 2018; Ieromina et al., 2014). High food quality/quantity, as offered in standard laboratory experiments, may allow for higher energy expenditure to cope with the toxic stress (the Dynamic-Energy budget theory, see Nisbet, Muller, Li, & Kooijman, 2010). However, in the field, a plethora of (indirect) effects on the aquatic communities can limit food quality/quantity and consequently species sensitivity in a laboratory setup can differ from that in the field (Barmentlo et al., 2018).

Approximately one of seven Odonates is threatened with extinction in Europe and currently 24% of the species have declining populations (Kalkman et al., 2010). Since conservation science of Odonates is limited in general (Bried & Samways, 2015) and toxicity data are scarce and contradictory for this group, a potential hazard of neonicotinoids to Odonates could be overlooked. Therefore, we aimed to investigate if environmentally relevant concentrations of the neonicotinoid thiacloprid could affect a widespread, eurytopic, Odonate: the blue-tailed damselfly I. elegans. We investigated the effects of thiacloprid on several endpoints (survival, consumption, growth, molting, mobility and emergence) using an environmentally realistic set-up in naturally colonized ditches. Additionally, we investigated toxicity by either feeding the damselfly nymphs with laboratory-cultured prey to have culture-fed specimens versus nymphs that hunted natural aquatic invertebrates to account for the potential indirect effects affecting the food quantity/quality that in turn can alter toxicity. Finally, we compared our toxicity results by monitoring the emergence of natural populations of I. elegans that were exposed to the same spikes of thiacloprid.

2 | MATERIALS AND METHODS

2.1 | Test species

We selected I. elegans (Zygoptera: Coenagrionidae) for multiple reasons. First and most importantly, it is the most common damselfly in large parts of Europe (Boudot & Kalkman, 2015), including the Netherlands (Van Swaay et al., 2018). Because of its high abundance and widespread distribution, I. elegans has the potential to be used as an indicator species for damselflies in general. Second, I. elegans is eurytopic (i.e., it can adapt to a wide range of ecological conditions) and found in a range of lowland fresh and brackish waters; it is often the dominant damselfly in highly anthropogenic habitats (Brockhaus et al., 2015). As such, this species is likely to be found in the vicinity of anthropogenic disturbances such as neonicotinoid runoff from the agricultural landscape. Third, because of its preference for slow flowing or standing water and the fact that they can be identified at an early instar, it is a suitable test species for caged bioassays. Fourth and finally, I. elegans typically lives in the water up to nearly a year and has a very long emergence period in the Netherlands, from late April till late September (NVL, 2002). Therefore, I. elegans has high ecological relevance for both the aquatic and terrestrial food webs as it is an active opportunistic hunter and also because it is common prey for invertebrates, fish, birds and bats (Corbet, 1962).
2.2 | Experimental setup

2.2.1 | Test location

The experiment was conducted at the outdoor research facility of Leiden University: ‘the Living Lab’ (The Netherlands). The site consists of 36 experimental ditches (1,750 L per ditch) with a length of 10 m, a width of 0.8 m and a depth of 0.3 m that are connected to an adjoining lake that provides a natural input of flora and fauna (see Appendix Figure S1 for a photographic overview). After a 5-month colonization period and prior to the start of the experiment, these ditches were hydrologically isolated from the lake by placing a 1,000 × 500 × 2 mm acrylic plate firmly into the ditch banks and 15 cm deep into the sediment at the end of every ditch.

2.2.2 | In situ conditions

The damselfly nymphs used in the in situ experiment were caught from the adjoining lake of the experimental site by sweeping with a dipping net (mesh size 0.5 mm). The selected nymphs had an average length of 8 mm (SD 1.6 mm). Each nymph was placed in a cylindrical cage (ø: 5.5 cm, height: 6.8 cm), in an experimental ditch. Inoculation was performed 3 days prior to the start of the treatments (see Section 2.3.2.3) so that the nymphs could acclimatize to the cage. We also placed a thin twig in each cage for the nymphs to rest and hold onto. All cages contained one nymph and each experimental ditch received two cages. These two cages were nearly identical; high-density polyethylene cages with a 3.75 cm opening on one side (in the lid) covered with mesh. One of the cages was closed with a larger mesh size of 1,000 µm and the other with a finer mesh size of 150 µm. Smaller invertebrates, such as Daphniaidae, Cyclopidae and Diptera larvae, living in the experimental ditches could disperse freely in and out of the cages with the larger mesh, but not the finer. We did not actively feed the nymphs in the cages with the larger mesh. Instead, these nymphs fed on the natural invertebrates living in the experimental that freely dispersed into the cage. Nymphs in the cages with fine mesh were fed daily with <3 day old daphnids (Daphnia magna) retrieved from a longstanding culture. These two food conditions will hereafter be referred to as either the ‘Free-feeding’ or the ‘Fed’ treatment, respectively. During the acclimatization period, we fed the nymphs of the “Fed” treatment 5 daphnids per day. At the start of the experiment (the first thiacloprid spike, see Section 2.3.2.3), we started feeding the nymphs 10 daphnids daily and increased this number by 5 daphnids every week to fill the need of the growing nymphs, maintaining so-called ad libitum food conditions. However, if uneaten daphnids were observed, we subtracted the number of uneaten daphnids from the number of daphnids that we fed.

2.3 | Exposure conditions

After the initial acclimatization period of the nymphs, thiacloprid was spiked in the experimental ditches. Thiacloprid in Dutch surface waters is increasingly detected starting in April-May (see Barmentlo et al., 2018) with the highest numbers in June and July. This closely equals the season when I. elegans emerge. In May, we exposed the nymphs to four nominal concentrations of thiacloprid; 0 (control), 0.1, 1 and 10 µg/L with nine replicate ditches per concentration. These concentrations have all been measured in Dutch surface waters in the period 2013–2017, with the lower concentrations being observed more commonly (see Table 1, Leiden University & Rijkswaterstaat-WVL, 2018; Vijver, Van ‘T Zelfde, Tamis, Musters, & De Snoo, 2008). However, the octanol-water coefficient for thiacloprid is low (log Kow = 1.26 for thiacloprid, USEPA, 2003) and as such thiacloprid is removed rapidly of the aqueous phase and transported to the sediment. Consequently the chance of detecting the maximum concentrations (1.8% of all detects, as shown in Table 1) by grab sampling is low. The findings reported in Table 1 are thus likely an underestimate of the maximal concentrations in surface waters but do give guidance to determine environmentally relevant test concentrations. Our experimental concentrations are not exclusive to the Netherlands but are also commonly observed in other waterbodies (Morrissey et al., 2015; Sánchez-Bayo, Goka, & Hayasaka, 2016), thus we consider them environmentally relevant.

Stock solutions were prepared by dissolving thiacloprid (99.9% purity, purchased from Sigma-Aldrich) in demineralized water. These stocks were then diluted in a 10 L bottle with water originating from the ditch to which the thiacloprid was added and carefully poured into each ditch while spreading evenly. As >95% of the initial thiacloprid concentrations degrades after 2 weeks in our test system (DT90 = 11.1 days; shown by Barmentlo et al., 2018), a second spike was applied after two weeks in order to maintain experimental concentrations for one month. Water quality was monitored on a weekly basis by measuring water temperature, pH, oxygen concentration and conductivity using a portable HQ 40 days electronic multiparameter meter (Hach). We also monitored thiacloprid concentrations over time of exposure by collecting water samples 5 cm below the surface level of each experimental ditch daily during the first week after a spike and three times a week thereafter. These samples were then analysed using liquid chromatography–tandem mass spectrometry (Agilent Technologies; see Roessink et al., 2013 for the detailed procedure).

TABLE 1 Thiacloprid concentrations in Dutch surface waters over the period 2013–2017. In total 12,691 unique samples (time × location combinations over 746 locations) were analysed of which thiacloprid was detected in 801 samples. Data were retrieved from the Dutch Pesticide Atlas (Leiden University & Rijkswaterstaat-WVL, 2018).

| Concentration thiacloprid (ng/L) | No. of thiacloprid detects | % of total no. of detect samples |
|---------------------------------|-----------------------------|---------------------------------|
| <10                             | 299                         | 37.3                            |
| 10–100                          | 424                         | 52.9                            |
| 100–1,000                       | 64                          | 8.0                             |
| >1,000                          | 14                          | 1.8                             |

Note: Note that the detection limit was often approximately 10 ng/L.
2.4 | Damselfly performance

2.4.1 | Caged individuals

All nymphs were monitored daily on survival, consumption and molting. Nymphs of the ‘Free-feeding’ treatment (Figure 1b) were also monitored for emergence while the ‘Fed’ treatment (Figure 1a) was terminated after six weeks of inoculation. Food consumption was monitored in the ‘Fed’ treatment by counting the number of eaten daphnids that were offered the previous day. For the ‘Free-feeding’ treatment, we counted all invertebrates that were present in the cage and determined them as prey or non-prey (as according to Thompson, 1978). Non-edible species (such as snails) were removed from the cages. Moulting was monitored by collecting and counting the shed chitin moults in the cages. When animals were nearly ready to emerge — indicated by the presence of fully developed and non-transparent wing-sheaths, 50% of the water in the cage was removed. The twig was replaced with a broader shoot of dry Typha latifolia, being a naturally preferred substrate for emergence of I. elegans. Cages were returned to the experimental ditch at a slight downward angle so that the mesh remained completely covered with water, but an air pocket was created in the enclosure where the damselflies could emerge on the shoot of T. latifolia. Emergence was monitored daily for 5 weeks since the start of emergence. In addition to these daily observations, growth of the nymphs was determined on a weekly basis until the first emergence by temporarily and carefully removing the nymphs from their cages and photographing them on a Petri dish using an eScope DP-M17 USB-microscope camera. Body length was then determined from the top of the head to the start of the caudal gills using ImageJ (version 1.45S). Fleeing behaviour of the nymphs was tested on a weekly basis; swimming responsiveness was recorded from three weeks after the first spike of thiacloprid which was from the controls sixth instar onwards (Brochard & van der Ploeg, 2014). Swimming responsiveness was tested as activity occurring within 15 s of gentle stimulation using a plastic pipette, following the OECD guidance, for example, daphnids (OECD, 2012).

2.4.2 | Natural populations

To quantify the results from our controlled in situ setup to actual natural populations of I. elegans, natural emergence was monitored by placing one emergence trap above the middle of each ditch (Figure 1c). The traps consisted of a 60:60:74 (length:width:height) pyramid-form stainless steel frame fitted with No-See-Um netting (300 holes per cm$^2$) and the invertebrate collection system as according to Cadmus, Pomeranz, and Kraus (2016). The emergence traps were tightly secured to bamboo sticks that were placed horizontally in the ditch banks to hold them in place during the entire

---

**FIGURE 1** Graphical overview of the experimental setup. Shown is a lateral cross section of an experimental ditch with experiment (a) caged bioassay with Daphnia magna culture-fed nymphs, (b) caged bioassay with nymphs living of the natural food supply and (c) monitoring of emerged I. elegans of naturally colonized populations, exposed to four different concentrations of thiacloprid (0, 0.1, 1 and 10 µg/L). Note that invertebrates are not scaled to the equipment.
experimental period. The emergence traps were installed 3 cm below the water surface level so no emerging insects could escape the trap (or vice versa). Naturally emerging insects were subsequently continuously caught in 100 ml of 80% EtOH and collected from the trap two times per week (every Monday and Thursday) for the duration of three months. All emerged I. elegans were counted subsequently.

2.5 | Statistical analyses

We tested for the effect of thiacloprid on all different endpoints and the emergence of natural populations of I. elegans by using linear mixed-effect models (lme, package ‘nlme’) with the actual concentration of thiacloprid, time and their possible interaction as explanatory variables. As we remeasured the same individuals, we nested the nymphs within the respective ditch they resided in. We used a similar model for the emergence of the caged nymphs, but accounted for the binomial distribution of the data (glmer, package ‘lme4’). For the models investigating growth, molting and emergence we added the variables. As we remeasured the same individuals, we nested the nymphs within the respective ditch they resided in. We used a similar model for the emergence of the caged nymphs, but accounted for the binomial distribution of the data (glmer, package ‘lme4’). For the models investigating growth, molting and emergence we added the variables.

Prior to these analyses, we removed one individual from the data due to an accidental release. To further investigate the responsiveness of the nymphs in respect to mobility (swimming), we used Dose-response metrics (function drc, package ‘drc’). To find the Lowest Observed Effect Concentration (LOEC) of thiacloprid on this responsiveness, we used Dunnet’s post-hoc test when a significant effect of thiacloprid was observed. All statistical analyses were performed using R (version 3.5.0; R Core Team, 2019).

3 | RESULTS

3.1 | Treatments

Actual concentrations closely followed the nominally applied concentrations (see Table 2). Thiacloprid was rapidly removed from the aqueous phase as DT₅₀ and DT₉₀ values, calculated by first order kinetics, were 3.6 and 12.0 days, respectively. These values are very similar to those of the study of Barmentlo et al. (2018; DT₅₀ = 3.3 and DT₉₀ = 11.1 days), who also followed the degradation of thiacloprid in the same experimental ditches. Thiacloprid slightly decreased the time-weighted average (TWA) of conductivity by 21 µS/cm at the highest test concentration ($R^2 = 0.21, F_{1,34} = 8.9, p = 0.005$). Both the TWA of pH and oxygen concentrations increased slightly with increasing concentrations of thiacloprid as well ($R^2 = 0.52, F_{1,34} = 36.7, p < 0.001$ and $R^2 = 0.20, F_{1,34} = 8.3, p = 0.007$ respectively, see Appendix Table S1). These alterations in physicochemical characteristics were likely the result of altered primary production as also observed earlier in our experiments (see Barmentlo et al., 2018). The average water temperature was 22.4°C during the experimental period and was not influenced by thiacloprid ($p > 0.05$; see Appendix Table S1). In addition, oxygen concentrations were never below 9.65 mg/L, so no oxygen deficiency occurred.

3.2 | Effects on survival

Survival was 100% in all cases apart from one death individual in the ‘Fed’ as well as in the ‘Free-feeding’ treatment. Thus no significant effects of thiacloprid on survival were observed.

3.3 | Effects on consumption

The food consumption by the nymphs was clearly affected by thiacloprid. Nymphs that were fed consumed significantly less daphnids with increasing concentrations and this effect interacted with time of exposure (marginal $R^2 = 0.43, F_{1,34} = 7.0, p < 0.001$); effects were stronger during the first week of the experiment (Figure 2a). On the final day, the sum of consumed daphnids increased relative to the control on average by 210%, 225% and 469% for the actual spike concentrations 0.08, 0.85 and 9.86 µg/L, respectively ($R^2 = 0.27, F_{1,33} = 12.6, p = 0.001$). In contrast with the effect on food consumption, the total available number of prey (predominantly waterfleas and copepods, but also fly and midge larvae, mayflies, aquatic mites, caddisflies and ostracods) in the ‘Free-feeding’ cages did not follow a dose-response metric, but was significantly affected by thiacloprid (marginal $R^2 = 0.41, F_{1,34} = 474.7, p < 0.001$); we observed a decrease in potential prey by 24% at 0.08 µg/L and an increase by 67% at 9.86 µg/L thiacloprid compared to the control (Figure 2b).

**TABLE 2** Average thiacloprid concentrations (±SE, n = 9) of the two spikes and the time-weighted average (TWA) concentration per treatment

| Nominal conc. thiacloprid (µg/L) | Actual conc. thiacloprid (µg/L) | Actual conc. thiacloprid (µg/L) | TWA conc. thiacloprid first month (µg/L) |
|---------------------------------|---------------------------------|---------------------------------|------------------------------------------|
| First spike                     | Second spike                    |                                 |                                          |
| 0.0                             | <DL                             | 0.075 (± 0.003)                 | 0.030 (± 0.016)                           |
| 0.1                             | 0.093 (± 0.003)                 | 0.802 (± 0.015)                 | 0.284 (± 0.024)                           |
| 1.0                             | 0.894 (± 0.013)                 | 9.726 (± 0.248)                 | 3.300 (± 0.309)                           |
| 10.0                            | 9.662 (± 0.215)                 |                                 |                                          |

Note: DL, Detection Limit: 0.012 µg/L. "conc", concentration.
3.4 | Effects on growth and molting

We observed no effect of thiacloprid on the growth of nymphs in the ‘Fed’ treatment \((p > 0.05, \text{Figure 3a})\). However, growth of the nymphs in the ‘Free-feeding’ treatment was significantly delayed by increasing concentrations of thiacloprid interacting with time and the initial length per nymphs \((\text{marginal } R^2 = 0.71, F_{1,32} = 4.0, p = 0.047, \text{Figure 3b})\). The cumulative growth was slowest three weeks after the start of the treatments (one week after the second spike), by 28% at 9.86 µg/L thiacloprid. There was no significant difference in total growth at the end of the measurements \((p > 0.05)\). Similar to the results for growth, we observed no effect of thiacloprid on the cumulative number of molts of the culture-fed nymphs \((p > 0.05, \text{see Appendix Figure S2a})\), while molting of nymphs in the ‘Free-feeding’ treatment was significantly delayed \((\text{interacting with the initial length}; \text{marginal } R^2 = 0.61, F_{1,32} = 19.4, p < 0.001, \text{see Appendix Figure S2b})\). There was no significant difference in the total number of molts at the end of the measurements \((p > 0.05)\).

3.5 | Effects on mobility

Swimming ability of the nymphs declined in both feeding treatments with increasing concentrations \((\text{Figure 4a,b})\). In nearly all cases, the animals that started swimming did so within 1-2s after gentle stimulation \((\text{observation not shown})\). The LOEC of nymphs within the ‘Free-feeding’ treatment was 0.84 µg/L \((\text{Dunnett’s test}: t = -3.1, p = 0.010)\), which was close to our obtained 50% Effect Concentration \((\text{EC}_{50})\) value of 1.04 µg/L \((95\% \text{ CI: 0.38–1.71})\). After this initial decline, the swimming ability increased again over time \((\text{marginal } R^2 = 0.40, z = 2.2, p = 0.031, \text{Figure 4a,b})\). The decline was weaker in the culture-fed nymphs, for which maximally 44% reduction in swimming ability at the highest test concentration was observed versus 100% in the ‘Free-feeding’ treatment. In addition, the \(\text{EC}_{50}\) within the ‘Fed’ treatment was higher than the highest spike concentration \((>9.7 \mu g/L)\) and no statistically significant LOEC could be determined.

3.6 | Effects on emergence

At the end of the experiment, 89% of all caged damselflies within the control treatment emerged \((\text{Figure 5a})\). Only one individual within the control did not emerge as this individual lost two of its caudal appendages during the experiment. These appendages were regrown during the course of the experiment but resulted in a delayed emergence. Exposure to thiacloprid significantly reduced the cumulative emergence of damselflies within the ‘Free-feeding’ treatment \((\text{marginal } R^2 = 0.66, \chi^2 = 7.9, df = 3, p = 0.047)\). At the end of the experiment, 75% of individuals emerged at 0.85 µg/L exposure concentration and 56% at 9.86 µg/L and these concentrations no longer deviated statistically from the control \((p > 0.05)\). For the lowest spike concentration of 0.08 µg/L, only
11% emergence was observed ($z = -2.8, p = 0.015$; Figure 5a). We observed no effect of thiacloprid on the time until first emergence of individuals ($p > 0.05$). Thiacloprid significantly decreased the number of emerged damselflies of the natural populations residing in the ditches having the same thiacloprid concentrations as in the cages (marginal $R^2 = 0.34, F_{1,34} = 50.8, p < 0.001$; Figure 5b). In contrast with the caged damselflies, emergence decreased steadily with increasing thiacloprid concentrations (i.e., a dose-response manner). At the final day of measuring ($t = 55$), total emergence was reduced by 39%, 55%, and 65% for the actual spike concentrations 0.08, 0.85, and 9.86 µg/L, respectively.

**4 | DISCUSSION**

Many alarming reports are currently published on the decline of insects (e.g. Hallmann et al., 2017) with (agricultural) pollution often being recognized as one of the predominant drivers in intensively used environments (Dudgeon et al., 2006). This also holds true for neonicotinoids as it has been acknowledged that they are causing environmental risks higher than lab-based studies previously indicated (Barmentlo et al., 2018; EASAC, 2015; Goulson, 2013; Vijver et al., 2017). We have tested the effects of environmentally occurring thiacloprid concentrations on the Odonate *I. elegans* systematically using various endpoints, which allows to understand the mechanistic pathway of toxicity. If such eurytopic and widespread species is affected or even in decline, this is indicative that other Odonates are potentially threatened as well. We found clear effects of environmentally relevant neonicotinoid concentrations on both caged *I. elegans* and natural populations. All sublethal parameters selected were affected by thiacloprid to some degree, but the severity of effects was stronger in caged individuals feeding on natural food supplies compared to fed individuals. The severity of effects increased even more so when considering the emergence of natural populations, which decreased strongly due to neonicotinoid exposure in a dose-dependent manner.

In contrast with the observed strong effects for sublethal parameters, survival was not impacted in this study, which is consistent with current literature that indicate lethal effects of neonicotinoids on other Odonates at levels that are at least a threefold higher than our highest experimental concentration (Beketov & Liess, 2008; Jingui et al., 2013; Sugita, Agemori, & Goka, 2018). Sublethal effects of thiacloprid on food and prey consumption were found at concentrations as low as the monthly average thiacloprid concentration of 0.03 µg/L (spike concentration 0.08 µg/L); ‘Fed’ nymphs consumed significantly less daphnids with increasing concentrations. In addition, ‘Free-feeding’
nymphs had more natural prey leftover at the highest test concentration. These observations are underpinned by the mode of action of neonicotinoids to invertebrates, namely by blocking the nicotinic acetylcholine receptors within the nervous system (Simon-Delso et al., 2015). This can also explain the significant delays in both growth and moulting rate of the nymphs living off the natural food supplies. As well as that, these effects can also explain the reduced swimming performance of the nymphs. The observed strong decline in swimming ability can also be attributed to a physiological response; *I. elegans* nymphs are excellent swimmers, but are only able to do so starting from the sixth instar (Brochard & van der Ploeg, 2014). As all nymphs could eventually swim, it is likely that the delay in moulting subsequently led to a delay in reaching the sixth instar, making the nymphs temporally incapable of swimming. Irrespective of the causal mechanism, swimming behaviour of *I. elegans* and many other Odonate species is generally used to avoid predators such as fish or other invertebrates (Corbet, 1962). The temporarily reduced swimming capacity can thus have obvious negative effects for natural populations because the nymphs are more susceptible to predation. Fitness reduction throughout the life cycle of the damselflies can also explain the observed delay in emergence time in the ‘Free-feeding’ treatment. All our tested endpoints are connected through either energy transfer or a physiological response and hence the combined sublethal effects offer a mechanistic explanation for the delay in emergence of the damselflies.

Nymphs feeding on the natural food supplies consistently exhibited more toxic pressure than nymphs that were fed. This is shown by the number of endpoints that were affected; namely all sublethal endpoints for the ‘Free-feeding’ versus two for the ‘Fed’ nymphs. For example, growth rate was reduced only within the ‘Free-feeding’ treatment. The severity of effects for endpoints that were affected in both treatments also differed markedly; swimming ability was more affected in the ‘Free-feeding’ treatment. Such differences in neonicotinoid-induced toxicity due to differences in food quantity/quality has been observed earlier and explained as a compensatory feeding response to cope with toxic stress (see Alexander et al., 2013; Ieromina et al., 2014; Barmentlo et al., 2018). In our study, the food quantity/quality was indirectly determined by information on how many potential prey were not eaten on a daily basis. Strikingly, this number decreased by 24% at the lowest test concentration compared to the control within the ‘Free-feeding’ treatment, indicating that these nymphs were possibly feeding more and thus indicating compensatory feeding (Alexander et al., 2013; Barmentlo et al., 2018). Alternatively, a plethora of indirect effects can occur within the communities of the experimental ditches explaining the difference in toxicity between the treatments. The most obvious would be a secondary exposure to thiacloprid through contamination of the natural food supply. However, this is not that likely as neonicotinoids are highly soluble (USEPA, 2003) and thus generally considered to
have a low bioaccumulation potential. More likely are thiacloprid-induced alterations in the communities residing in the experimental ditches, which altered the amount of natural food supply that entered the cages. Thus it is possible that we observed less prey in the cages at the lowest test concentration (0.1 µg/L) due to less supply from the ditch community rather than due to compensatory feeding. This is supported by the observation that the delay in emergence of caged individuals was greatest at this lowest test concentration. In any case, the outperformance of the ‘Fed’ nymphs importantly showcases that optimal food conditions can alter neonicotinoid-induced toxicity. As the common laboratory-based ecotoxicological approach is to feed animals ad libitum (according all OECD regulations, for example OECD, 2012), the already scarce toxicity data for Odonates as collected in the laboratory setting may currently be underestimating the actual risks in the field.

The total emergence of the natural populations of *I. elegans* declined strongly (39%–65%) with increasing thiacloprid concentrations, whereas within our cage setup we mostly observed a delay in emergence time. The most likely explanation for this discrepancy is that in our cage setup many biotic interactions are excluded, for example, there was no predation pressure. These biotic interactions are obviously present within the natural damselfly populations. It is recognized that the effects of contaminants depend on ecological context (Clements, Hickey, & Kidd, 2012; Trekels, Meutter, & Stoks, 2011). Thus, it is probable that the temporarily neonicotinoid-induced weakening of the nymphs increased their vulnerability to biotic pressures such as predation or by rendering them competitively inferior to other less sensitive species (as shown several times before by, for example, Mills & Semlitsch, 2004; Trekels et al., 2011). The additional biotic pressure would thus reduce the actual population size and finally the total emergence of the damselflies. We stress these results as emergence is necessary for the reproduction of *I. elegans*, and all other Odonate species, and population sizes cannot be maintained without it. A delay in emergence as observed in the caged experiment could lead to a mismatch in emergence time between different sites (polluted-unpolluted) and thus a mismatch in reproduction for Odonates (although note that our test species, *I. elegans*, has a very long imago phase). The observed total decline in emergence of the natural populations suggests less reproduction and both findings can thus potentially lower the population sizes. Damselflies (and dragonflies) play an important role in controlling mosquitoes and agricultural pests (Painter, Tennessen, & Richardson, 1996; Sánchez-Bayo & Wyckhuys, 2019) because they are excellent predators on both nymphs and imagos of flying insects. Due to their function in the ecosystem as well as the fact that they can be inhabitants of highly anthropogenic areas, their role can be of great importance in natural pest control. Our results thus show that current environmental levels of neonicotinoids, insecticides that are meant to combat pest insects, might actually harm the natural pest control system.

Our experimental concentrations ranged from relatively low and commonly observed concentrations (0.1 µg/L) to less common higher end concentrations (10 µg/L) that are observed in surface waters all over the world. It should be noted that thiacloprid is not the only and also not the most common neonicotinoid polluting surface waters. Neonicotinoids are often found in mixtures and share a common mode of action (Morrissey et al., 2015) that can add up to toxicity (Maloney, Liber, Headley, Peru, & Morrissey, 2018). We compare our findings of strong decline in natural emergence at environmentally relevant concentrations to data on Odonate abundance and distribution trends collected by citizen scientist from the ‘Dutch Dragonfly Monitoring Scheme’ of the Dutch Butterfly Conservation (Van Swaay et al., 2018). This dataset consists of standardized transect counts since 1999 and of distribution data since 1991, based on opportunistic data analysed with occupancy models (Van Swaay et al., 2018). The surveillance data for our test species, *I. elegans*, shows a decline in both abundance and distribution since 2008 (Figure 6). This timeline matches that of sharp increases in the amount of neonicotinoids sold and used in 2008 as reported for other European countries (Sweden and the United Kingdom see fig. 2a and b in Simon-Delso et al., 2015). Building evidence, on the one hand our results show toxicity to *I. elegans* at environmental relevant concentrations, and on the other hand widespread decline of this species is observed. This indicates that neonicotinoids can be at least partially responsible (possibly together with other habitat altering parameters) for the current decline of the species *I. elegans*. This is in

**FIGURE 6** *Ischnura elegans* is declining in abundance and distribution in the Netherlands (index per year ± SE and a smoothed trend with 95% CI)
line with the study of van Dijk et al. (2013) who showed a decrease in natural I. elegans nymphs with increasing concentrations of the neonicotinoid imidacloprid. Such major declines in natural Odonate populations due to other systemic insecticides (imidacloprid and fipronil) have been shown before by Nakanishi, Yokomizo, and Hayashi (2018) for the species Symptenurum frequens. The transect surveillance data for all Odonates shows an initial increase in Odonates between 1990 and 2008. This is likely because of improved habitat and water quality in that period (Termaat, Grunsven, Plate, & Strien, 2015). However the data also shows that total Odonate distribution in the Netherlands is steadily declining, like for I. elegans, since the late 2000s (see Appendix Figure S3). Given that our test species is one of the most widespread and eurytopic Odonates in Europe (Boudot & Kalkman, 2015), the observed neonicotinoid-induced toxicity may thus be indicative for this overall decline of Odonates.

5 | CONCLUSIONS

Clear effects of environmentally relevant concentrations of the neonicotinoid thiacloprid on the life cycle of the Odonate I. elegans were shown. While no direct effects on mortality were observed at environmental relevant concentrations, all sublethal endpoints tested were affected. Our results strongly depended on the food offered, which indicates that current laboratory assessments performed at ad libitum food underestimate neonicotinoid toxicity in the actual environment. In addition, it appears that even our realistic exposure scenario using caged individuals in experimental ditches also underestimates toxicity as the emergence of natural populations was more strongly affected. This is likely because biotic pressures such as predation add to toxicity and these pressures are not included within the caged experiment nor the common laboratory approaches. Finally, our observed reduced fitness during the nymph stage and the strong decline in natural emergence can be indicative for neonicotinoids adding to the ongoing I. elegans decline.

ACKNOWLEDGEMENTS

We thank Justin Knetsch, Jo-Anne Bartels and Janneke van der Horst for their assistance with the experimental work. Furthermore, we thank all volunteers of the Dutch Butterfly Conservation for their valuable monitoring efforts. SB and MV were funded through a NWO-VIDI864.13.010 grant, awarded to MV. RG was supported by the ACTION project which has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement number 824603.

AUTHORS’ CONTRIBUTIONS

S.H.B. and L.M.V. conceptualized and executed the experiment; S.H.B. analysed the data and led the writing of the manuscript; all authors contributed to the interpretation of the data, writing of the MS and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data is available via Dryad Digital Repository https://doi.org/10.5061/dryad.0vh187] [Barmentlo, Vriend, Grunsven, & Vijver, 2019].

ORCID

S. Henrik Barmentlo https://orcid.org/0000-0001-5279-3047

REFERENCES

Alexander, A. C., Luis, A. T., Culp, J. M., Baird, D. J., & Cessna, A. J. (2013). Can nutrients mask community responses to insecticide mixtures? Ecotoxicology, 22, 1085–1100. https://doi.org/10.1007/s10646-013-1096-3

Barmentlo, S. H., Parmentier, E. M., de Snoo, G. R., & Vijver, M. G. (2018). Thiacloprid-induced toxicity influenced by nutrients: Evidence from in situ bioassays in experimental ditches. Environmental Toxicology and Chemistry, 37, 1907–1915. https://doi.org/10.1002/etc.4142

Barmentlo, S. H., Vriend, L., van Grunsven, R. H. A., & Vijver, M. G. (2019). Data from: Environmental levels of neonicotinoids reduce prey consumption, mobility and emergence of the damselfly Ischnura elegans. Dryad Digital Repository, https://doi.org/10.5061/dryad.0vh187

Beketov, M. A., Kefford, B. J., Schäfer, R. B., & Liess, M. (2013). Pesticides reduce regional biodiversity of stream invertebrates. Proceedings of the National Academy of Sciences, 110, 11039–11043. https://doi.org/10.1073/pnas.1305618110

Beketov, M. A., & Liess, M. (2008). Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthropods. Environmental Toxicology and Chemistry, 27, 461–470. https://doi.org/10.1897/07-322R.1

Boudot, J. P., & Kalkman, V. J. (2015). Atlas of the European dragonflies and damselflies. Zeist, Netherlands: KNNV Publishing.

Bried, J. T., & Samways, M. J. (2015). A review of odonatology in freshwater applied ecology and conservation science. Freshwater Science, 34, 1023–1031. https://doi.org/10.1086/682174

Brochard, C., & van der Ploeg, E. (2014). Fotogids Larven van Libellen. Utrecht, Netherlands: KNNV.

Brockhaus, T., Roland, H.-J., Benken, T., Conze, K., Günther, A., Leipelt, K. G., ... Willigalla, C. (2015). Atlas der Libellen Deutschlands (Odonata). Cadmus, P., Pomeranz, J. P., & Kraus, J. M. (2016). Low-cost floating emergence net and bottle trap: Comparison of two designs. Journal of Freshwater Ecology, 31, 653–658. https://doi.org/10.1080/02705060.2016.1217944

Clements, W. H., Hickey, C. W., & Kidd, K. A. (2012). How do aquatic communities respond to contaminants? It depends on the ecological context. Environmental Toxicology and Chemistry, 31, 1932–1940. https://doi.org/10.1002/etc.1937

Corbet, P. (1962). A biology of dragonflies. London, UK: H.F. & G. Witherby.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., ... Sullivan, C. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. Biological Reviews, 81, 163–182. https://doi.org/10.1111/j.1364-410X.2006.0006950

European Academies Science Advisory Council (EASAC). (2015). Ecosystem services, agriculture and neonicotinoids. Retrieved from https://easac.eu/publications/details/ecosystem-services-agriculture-and-neonicotinoids/F.

Goulson, D. (2013). An overview of the environmental risks posed by neonicotinoid insecticides. Journal of Applied Ecology, 50, 977–987. https://doi.org/10.1111/1365-2664.12111

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., ... Kroon, H. D. (2017). More than 75 percent decline over 27
years in total flying insect biomass in protected areas. PLoS ONE, 12, e0185809. https://doi.org/10.1371/journal.pone.0185809.

Ieromina, O., Peijnenburg, W. J. G. M., De Snoo, G., Müller, J., Knepper, T. P., & Vijver, M. G. (2014). Impact of imidaclopid on Daphnia magna under different food quality regimes. Environmental Toxicology and Chemistry, 33, 621–631. https://doi.org/10.1002/etc.2472.

Jeschke, P., Nauen, R., Schindler, M., & Elbert, A. (2011). Overview of the status and global strategy for neonicotinoids. Journal of Agriculture and Food Chemistry, 59, 2897–2908. https://doi.org/10.1021/jf10303g.

Jingui, H., Thuyet, D. Q., Ueda, T., & Watanabe, H. (2013). Effect of imidaclopid and fipronil pesticide application on Symptem infuscatum (Libellulidae: Odonata) larvae and adults. Paddy and Water Environment, 11, 277–284. https://doi.org/10.1007/s10333-012-0317-3.

Kalkman, V. J., Boudot, J., Bernard, R., Conze, K., Knijf, G. D., Dytatlova, E., ... Sahlén, G. (2010). European red list of dragonflies. Luxembourg: Publications Office of the European Union. https://doi.org/10.2779/84650.

Leiden University, and Rijkswaterstaat-WVL. (2018). Pesticide Atlas, version 2.0. Retrieved from www.bestrijdingsmiddelenatlases.nl. Accessed 11.22.18.

Maloney, E. M., Liber, K., Headley, J. V., Peru, K. M., & Morrissey, C. A. (2018). Neonicotinoid insecticide mixtures: Evaluation of laboratory-based toxicity predictions under semi-controlled field conditions. Environmental Pollution, 243, 1727–1739. https://doi.org/10.1016/j.envpol.2018.09.008.

Mills, N. E., & Semlitsch, R. D. (2004). Competition and predation mediate the indirect effects of an insecticide on southern leopard frogs. Ecological Applications, 14, 1041–1054. https://doi.org/10.1890/02-5134.

Morrissey, C. A., Mineau, P., Devries, J. H., Sanchez-Bayo, F., Liess, M., Cavallaro, M. C., & Liber, K. (2015). Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. Environment International, 74, 291–303. https://doi.org/10.1016/j.envint.2014.10.024.

Nakanishi, K., Yokomizo, H., & Hayashi, T. I. (2018). Were the sharp declines of dragonfly populations in the 1990s in Japan caused by fipronil and imidaclopid? An analysis of Hill’s causality for the case of Symptem frequens. Environmental Science and Pollution Research, 25, 35352–35364. https://doi.org/10.1007/s11356-018-3440-x.

Nederlandse Vereniging voor Libellenstudie (NVL). (2002). The Dutch dragonflies (Odonata: Zygoptera). Nationaal Natuurhistorisch Museum Naturalis, KNNV Uitgeverij & European Invertebrate Survey, Leiden.

Nisbet, R. M., Muller, E. B., Liia, K., & Kooijman, S. A. L. M. (2010). From hatching to final instar. Environmental Toxicology and Chemistry, 39, 8–27. https://doi.org/10.1002/etc.2201.

Nisbet, R. M., Liber, K., De Snoo, G., Mertens, V., Van den Brink, P. J. (2013). The neonicotinoid imidaclopid shows high chronic toxicity to mayfly nymphs. Environmental Toxicology and Chemistry, 32, 1096–1100. https://doi.org/10.1002/etc.2201.

Sánchez-Bayo, F., Goka, K., & Hayasaka, D. (2016). Contamination of the aquatic environment with neonicotinoids and its implication for ecosystems. Frontiers in Environmental Science, 4, https://doi.org/10.3389/fenvs.2016.00071.

Sánchez-Bayo, F., & Wyckhuys, K. A. (2019). Worldwide decline of the entomofauna: A review of its drivers. Biological Conservation, 232, 8–27. https://doi.org/10.1016/j.biocon.2019.01.020.

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Chagnon, M., Downs, C., ... Wiemers, M. (2015). Systemic insecticides (neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. Environmental Science and Pollution Research, 22, 5–34. https://doi.org/10.1007/s11356-014-3470-y.

Sugita, N., Agemori, H., & Goka, K. (2018). Acute toxicity of neonicotinoid and some insecticides to first instar nymphs of a non-target damselfly, Ischnura senegalensis (Odonata : Coenagrionidae), in Japanese paddy fields. Applied Entomology and Zoology, 53, 519–524. https://doi.org/10.1007/s13355-018-0583-7.

Termaat, T., Grunsven, R. H. A. V., Plate, C. L., & Strien, A. J. V. (2015). Strong recovery of dragonflies in recent decades in The Netherlands. Freshwater Science, 34, 1094–1104. https://doi.org/10.1086/682669.

Thompson, D. J. (1978). The natural prey of larvae of the damselfly, Ischnura elegans (Odonata: Zygoptera). Freshwater Biology, 8, 377–384. https://doi.org/10.1111/j.1365-2427.1978.tb01458.x.

Tilman, D., Fargione, J., Wolff, B., Antonio, C. D., Dobson, A., Howarth, R., ... Swackhammer, D. (2001). Forecasting agriculturally driven global environmental change. Science, 292, 281–284. https://doi.org/10.1126/science.1057544.

Trekels, H., Van de Meuteter, F., & Stoks, R. (2011). Effects of species-specific interactions with predation risk on the relative species sensitivities to a pesticide in water boatemans (Corixidae). Oikos, 120, 897–905. https://doi.org/10.1111/j.1600-0706.2010.18852.x.

United States Environmental Protection Agency (USEPA). (2003). Pesticide fact sheet, Thiacylord. Office of Prevention, Pesticides and Toxic Substances, Washington, DC.

Van Dijk, T. C. T., Van Staalden, M. A., & Van der Sijp, J. P. (2013). Macroinvertebrate decline in surface water polluted with imidaclopid. PLoS ONE, 8, e62374. https://doi.org/10.1371/journal.pone.0062374.

Van Swaay, C. A. M., Bos, G., van Grunsven, R. H. A., Kok, J., Huskens, K., van Deijk, J. R., & Poot, M. (2018). Vlinders en libellen geteld. Jaarverslag 2017. Rapport VS2018.006. De Vilsterdichting, Wageningen.

Vijver, M. G., Hunting, E. R., Nederstigt, T. A. P., Tamis, W. L. M., van den Brink, P. J., & van Bodegom, P. M. (2017). Postregistration monitoring of pesticides is urgently required to protect ecosystems. Environmental Toxicology and Chemistry, 36, 860–865. https://doi.org/10.1002/etc.3721.

Vijver, M. G., Van ‘T Zelfde, M., Tamis, W. L. M., Musters, K. J. M., & De Snoo, G. R. (2008). Spatial and temporal analysis of pesticide concentrations in surface water: Pesticides atlas. Journal of Environmental Science and Health, Part B, 43, 665–674. https://doi.org/10.1080/03601230802388728.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.