

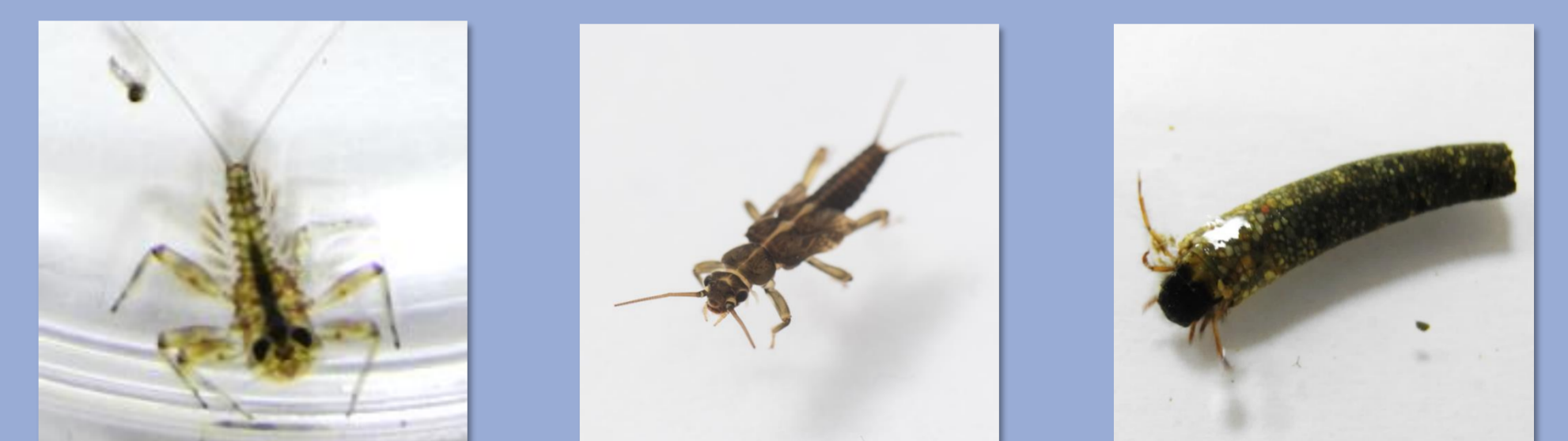
Working with EPT-Taxa - chronic testing of mayfly species in a test system simulating running waters

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1. Introduction

Lotic organisms belonging to the EPT-Taxa (Fig. 1) are exposed to releases of plant protection products, which are mainly applied to agricultural areas and washed off into adjacent running waters. Especially mayfly larvae are considered to be very sensitive against exposure to several pesticides. However, there are only few toxicity data available dealing with chronic effects of pesticides on these species. A new test system has been developed taking into account the specific characteristics of running waters regarding flow, oxygen level, temperature, light and food supply. Results of a 28-day study with mayfly larvae (Ephemeroptera, Heptageniidae) exposed to the ubiquitously used neonicotinoid Imidacloprid are presented and compared to previous data with stonefly larvae *Protonemura sp.*.



Ephemeroptera Plecoptera Trichoptera

Figure 1: Mayfly, stonefly and caddisfly larvae (Ephemeroptera, Plecoptera, Trichoptera).

2. Test Design

- Test System: 10 single test vessels each circulate inside test containers filled with test medium (Fig. 2), thereby creating a target flow of about 0.1 m/s
- Test vessels: Small cages made of stainless steel equipped with gravel mats and black glass nuggets simulating the natural habitat of test organisms
- Replicates: 10 replicates per concentration with one test organism per test vessel, resulting in 10 pseudo-replicates per test container → evaluation of individuals
- Test organisms: Field collected mayfly larvae of the family Heptageniidae which were in an early development stage
 - *Epeorus sylvicola*
 - *Ecdyonurus venosus*
- Temperature: 10 ± 1 °C
- Test substance: Imidacloprid
- Concentrations: Control + 0.03, 0.10, 0.30, 1.00 and 3.00 µg/L
- Medium: Moderately hard reconstituted medium according to EPA^[1]
- Medium renewal: Once per week (semi-static)
- Food: Algae *Navicula pelliculosa* grown on glass nuggets (Fig. 3)
- Test duration: 28 days
- Acclimation: 7 days before test start
- Endpoints: Growth and Mortality
- Test performance: 3 tests performed in (1) September, (2) October and (3) November 2018

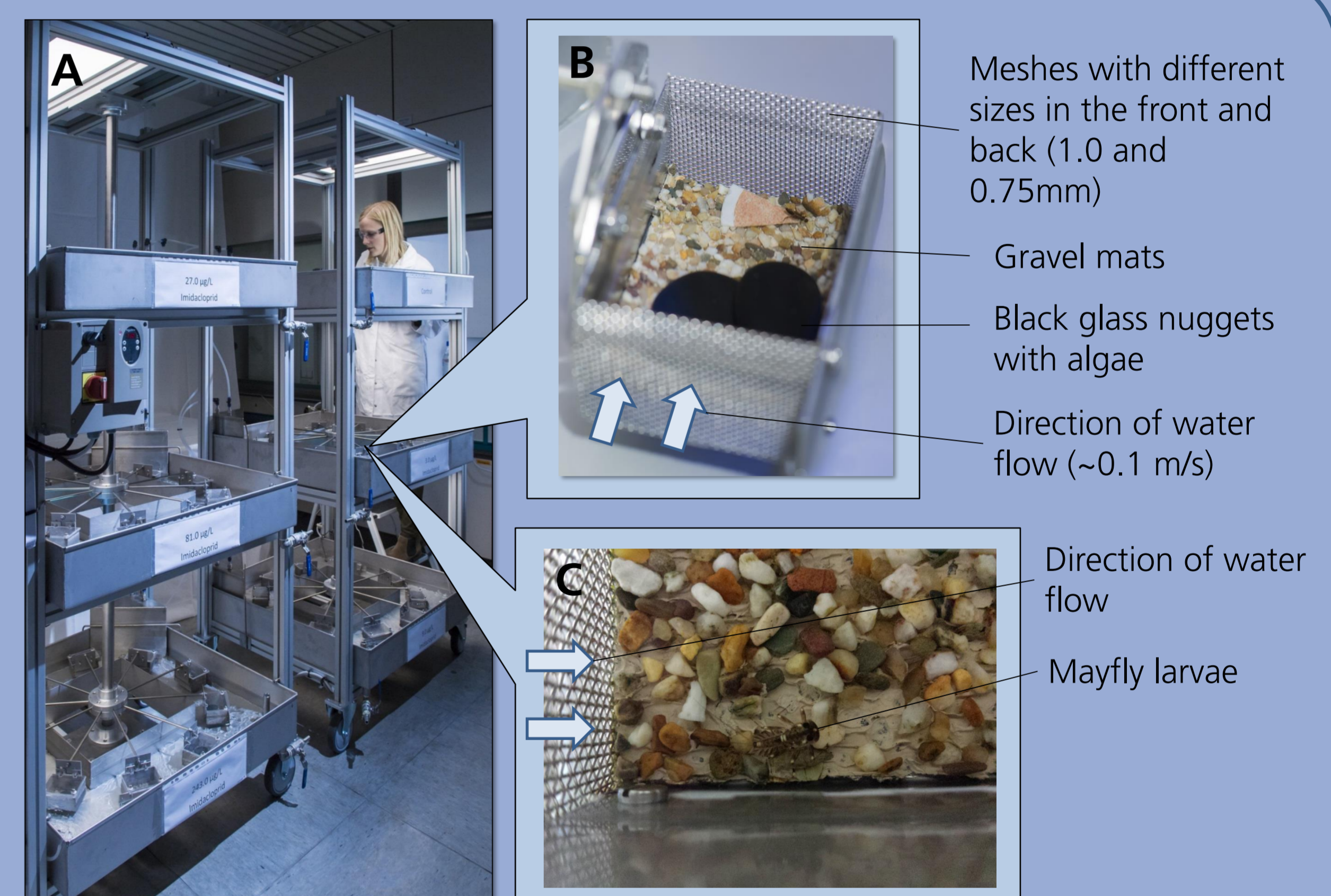


Figure 2: (A) Test system with six treatments. (B) & (C) Test vessel with test organism.

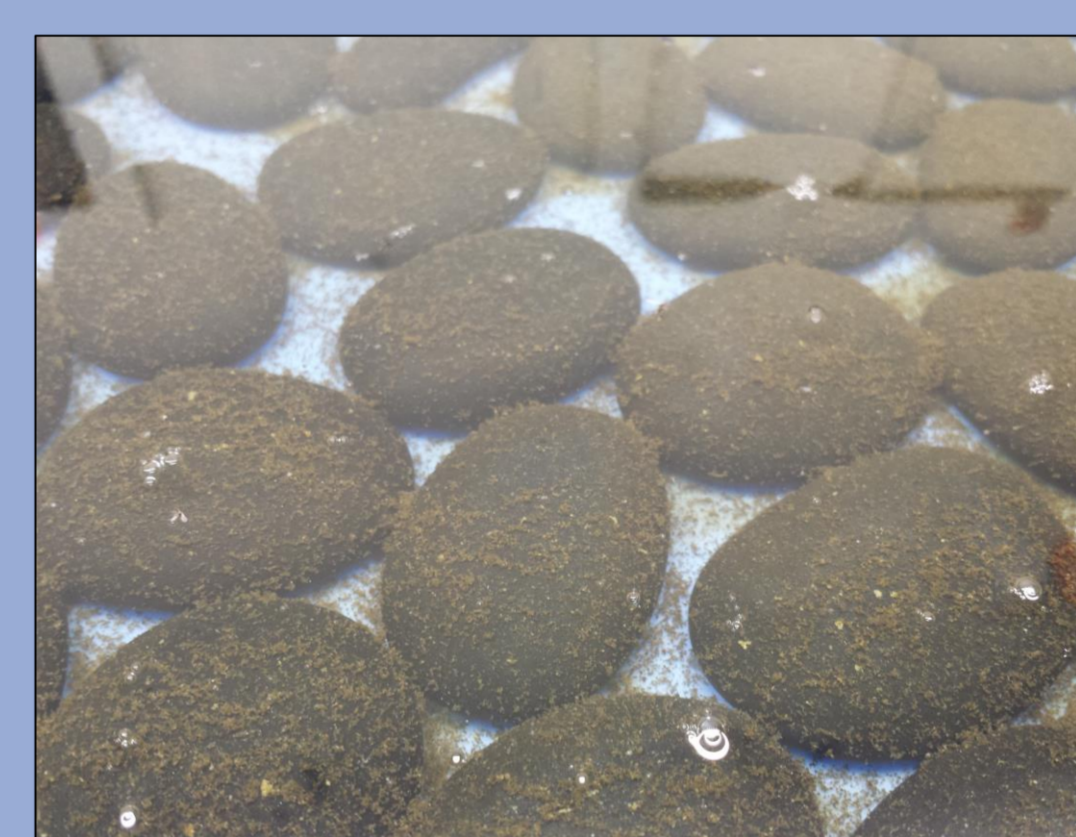


Figure 3: *Navicula pelliculosa* grown on glass nuggets used as food source.

3. Results

- Mayfly larvae were between 0.24 – 1.41 cm (1st study), 0.30 - 0.63 cm (2nd study) and 0.25 – 0.95 cm (3rd study) at test start
- Dose dependent effect of Imidacloprid on growth of mayfly larvae in all tests (Fig. 4)
- Results of repeated study are in the same order of magnitude and, thus, demonstrate the reproducibility of study results within the same test system

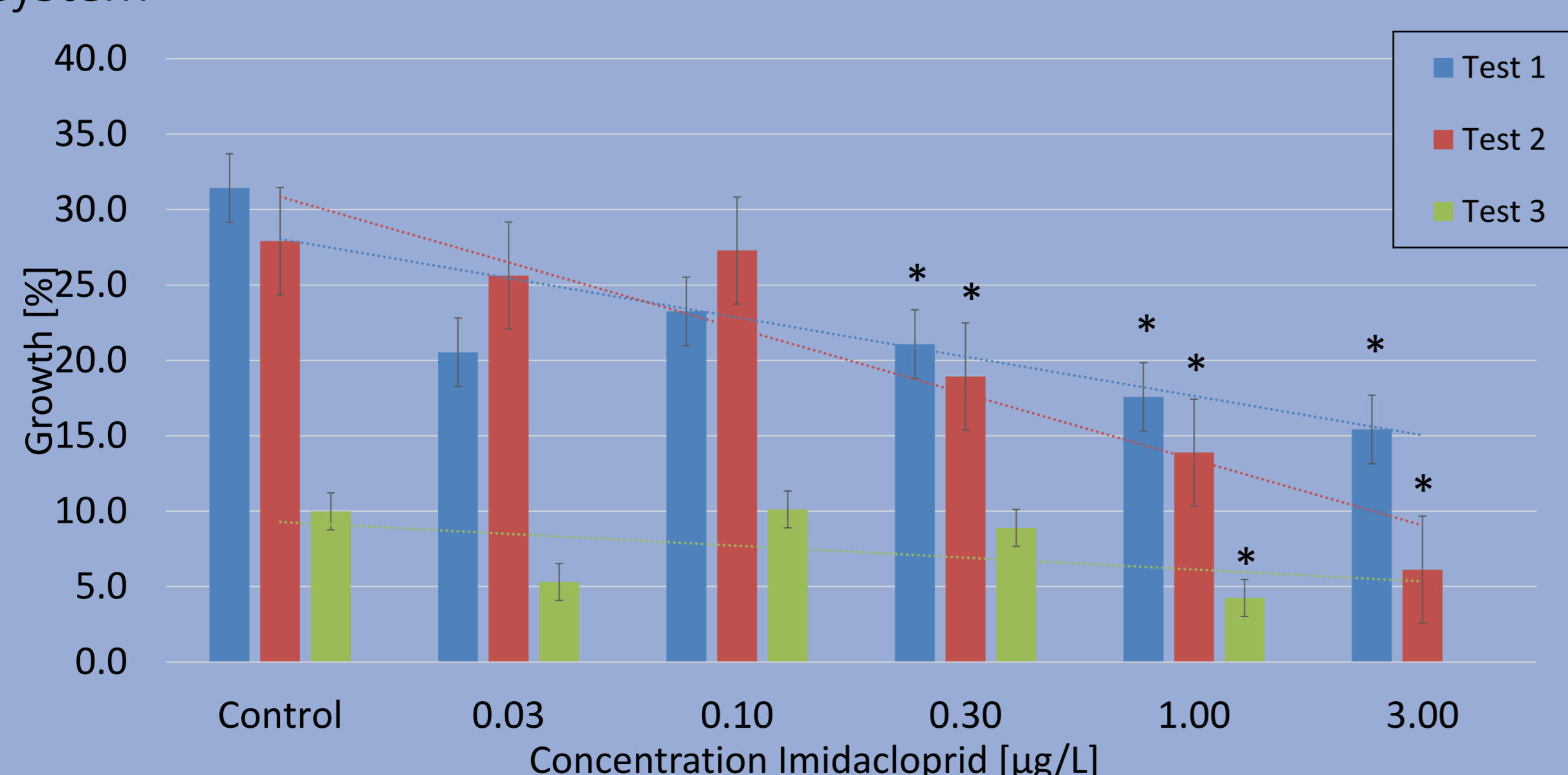


Figure 4: Growth of mayfly larvae at the end of the 28-day exposure to Imidacloprid. (*: significant different from control; alpha = 0.05, one-sided smaller;)

4. Comparison of sensitivities

- In 2017, the same test system was used for investigations of Imidacloprid on stonefly larvae over an exposure period of 21 days
- Stonefly larvae were about 30 – 90 times less sensitive than mayfly larvae

Table 1: Comparison of sensitivities for mayfly and stonefly larvae for endpoint growth after exposure to Imidacloprid.

Period of the study	September 2018	October 2018	November 2018	November 2017
Test organism	Mayfly larvae	Mayfly larvae	Mayfly larvae	Stonefly larvae
Test duration	28 days	28 days	28 days	21 days
EC ₁₀ [µg/L] (95% - CL)	n.d.	0.04 (0.01 – 0.13)	0.13 (n.d.)	11.2 (0.14 – 23.1)
EC ₅₀ [µg/L] (95% - CL)	2.92 (0.04 – 203.03)	0.68 (0.36 – 1.28)	0.68 (n.d.)	56.4 (31.3 – 193.2)
NOEC [µg/L]	0.10	0.10	0.30	9.0

n.d.: not determined

5. Conclusion & Outlook

Results of the performed studies underline the successful adaptation of the test system and the reproducibility of the test results for mayfly larvae. This increases the number of potential test species regarding the chronic testing for SSD (species sensitivity distribution). In further studies, the test system should be adapted for chronic testing of caddisfly.

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As many other non-target arthropods, mayfly larvae originating from small creeks and streams are exposed to releases of plant protection products, which are mainly applied to agricultural areas and washed off into adjacent running waters. Even though mayfly species are considered to be very sensitive against exposure to several pesticides, there are only few toxicity data available dealing with chronic effects of pesticides on these species. Therefore, we developed a test system for the chronic testing of lotic insect larvae.

The test system differs from usually known stream test systems particularly in its construction. In order to create a particular flow, small cages inside a test container filled with test medium circulate through the water phase thereby creating the target flow in the cage. The cages are equipped with gravel mats on the bottom and two black glass nuggets to simulate the natural habitat conditions of the larvae. Each cage serves as individual compartment for testing of single organisms to allow an organism specific evaluation of sublethal effects. The test system can provide data of chronic toxicity tests with different aquatic insect larvae, which can be used for a SSD (Species Sensitivity Distribution) approach for the risk assessment of pesticides.

In first studies performed with the new test system, the chronic effects of the neonicotinoid Imidacloprid on field collected mayfly larvae of the species *Epeorus* and *Ecdyonurus* belonging to the family of Heptageniidae were assessed in a 28 day exposure period. Larvae, which were in an early development stage, were adapted to laboratory conditions for up to 14 days before test start. The study was carried out in moderately hard reconstituted water (according to EPA) with five test concentrations and a control. The grazing larvae were fed with green algae *Desmodesmus subspicatus*, which were grown on the glass nuggets before test start. As sublethal endpoint, the growth of the test organisms was evaluated by measuring the length of each individual larvae at test start and test end. In addition, mortality of larvae was recorded weekly.

In previous studies, the test system was already used for chronic toxicity testing with stonefly larvae *Protonemura sp.* exposed to Imidacloprid for 21 days. In addition to the results of the performed mayfly study, a comparison of effects and sensitivities of mayfly and stonefly larvae to Imidacloprid will be presented.