

Chronic testing of mayfly and stonefly species – Development of a new approach

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1. Introduction

Aquatic organisms, especially lotic invertebrate species originating from running waters, are exposed to releases of plant protection products which are mainly used in agriculture. Since lotic invertebrate species (i.e. mayfly, stonefly & caddisfly (Fig. 1)) are regarded to be very sensitive but are hardly considered in chronic ecotoxicity testing, we developed a test system in order to investigate chronic effects on mayfly and stonefly species.

After successful development of a test method for stonefly larvae *Protonemura ssp.*, (presented at SETAC Europe in Bruxelles in 2017) the next step was the establishment of a method for testing of mayfly species.



Ephemeroptera Plecoptera Trichoptera

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

2. Test System

- Test containers filled with test medium include 10 single test vessels each
- Test vessels are circulated inside test containers, thereby creating a target flow (\neq usual known indoor stream systems)
- One test organism per test vessel, resulting in 10 pseudo-replicates per test container \rightarrow evaluation of individuals
- Gravel mats and black glass nuggets in each test vessel simulate the natural habitat
- Field collected organisms
- Endpoints: growth/emergence and mortality
- Test duration: 21 days

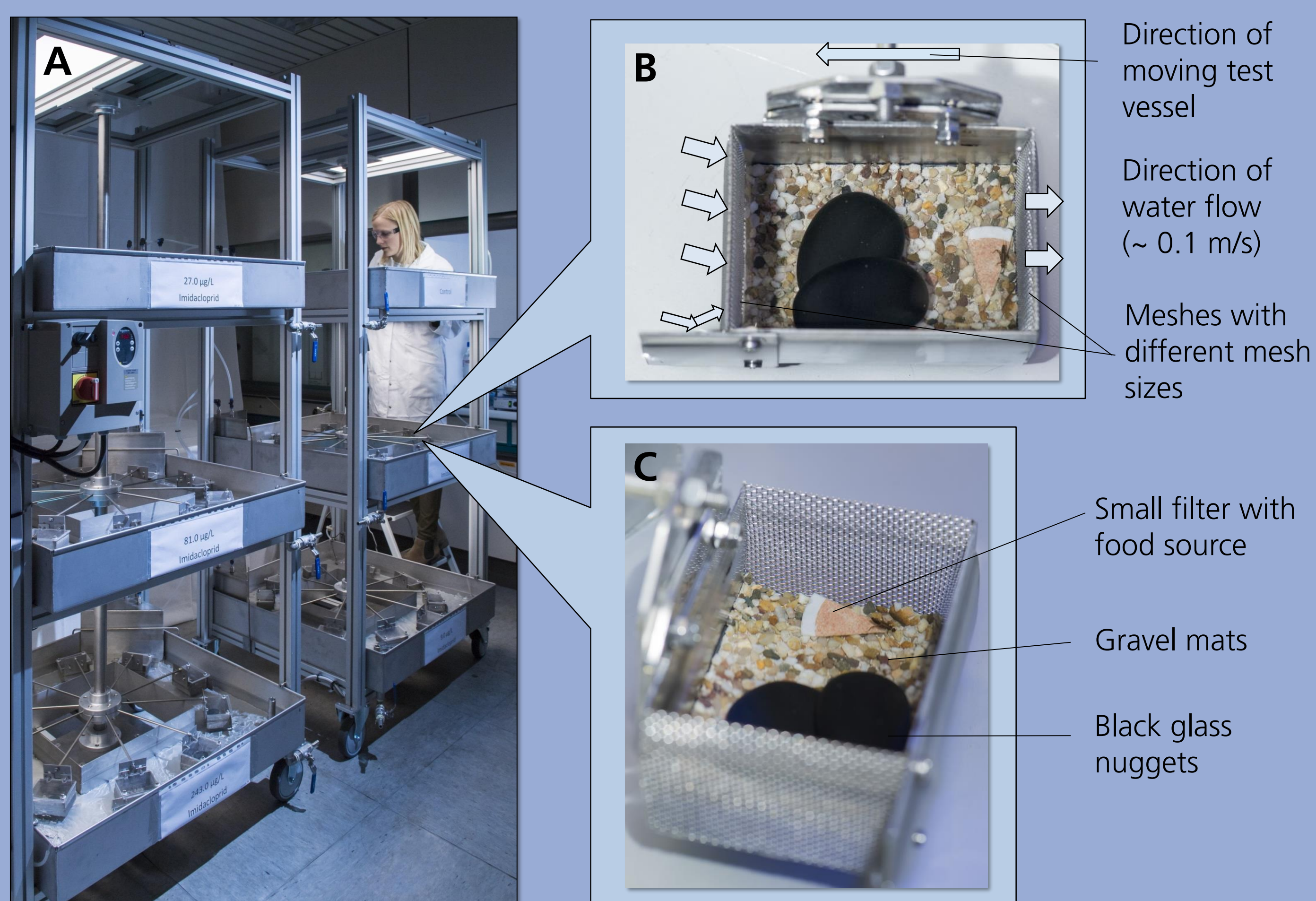


Figure 2: (A) Test system with six treatments. (B) & (C) Test vessel with test organism.

4. Conclusion

- Successful adaptation of easy to handle test system simulating running water for mayfly larvae in addition to stonefly larvae
- Highly variable natural stream water can be replaced by moderately hard reconstituted water according to EPA
- Increase of the number of test species regarding the chronic testing for SSD (species sensitivity distribution)

5. Outlook

- 21-day exposure test with Imidacloprid and mayfly larvae \rightarrow Comparison of sensitivity of mayfly and stonefly larvae
- Adaptation for testing of caddisfly

3. Adaptation of test system

The test conditions established for the testing of stonefly larvae were adapted to the requirements of mayfly larvae *Epeorus sp.* collected from a small german stream (Wester).

Test medium

- Testing of different media fulfilling the requirements of standardization (Cu-reduced dilution water, ISO-Medium according to OECD 243^[1], moderately hard reconstituted water according to EPA^[2])
- Fresh water from the original stream habitat (Wester) as reference \rightarrow Optimal development of mayfly larvae with moderately hard reconstituted water according to EPA

Food

- Investigation of different food quality (Tetramin®, algae *Desmodesmus subspicatus* presented on filter pieces, diatoms *Phaeodactylum tricorutum* and *Navicula pelliculosa* grown on black glass nuggets) \rightarrow Optimal development of mayfly larvae feeding on *Navicula pelliculosa* (Fig. 3)



Figure 3: Diatom *Navicula pelliculosa* growing on black glass nuggets.

Acclimatisation period

- Optimal development of mayfly larvae after 7 days before test start (stonefly larvae were acclimatized for 48 hours)

Proof of principle

- 21-day study with investigation of survival and growth of mayfly larvae *Epeorus sp.* \rightarrow Survival rates of *Epeorus sp.* in EPA medium and fed with diatom *N. pelliculosa* were similar to original stream water (Fig. 4) \rightarrow Growth of *Epeorus sp.* was reduced compared to original stream water (Fig. 5). However, the test system is expected to be still suitable for the indication of effects caused by a test substance

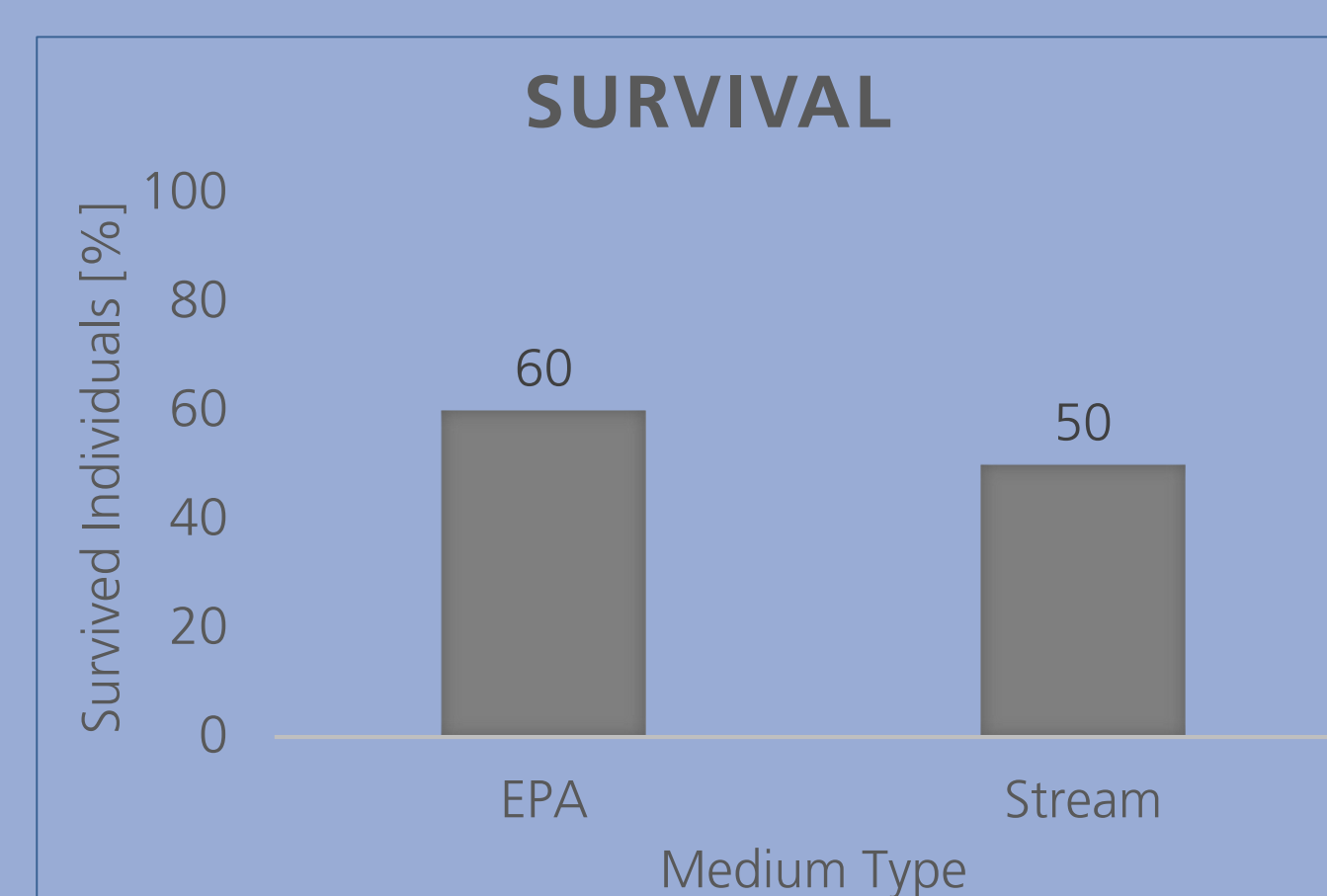


Figure 4: Survival of mayfly larvae *Epeorus sp.* in EPA medium and original stream water.

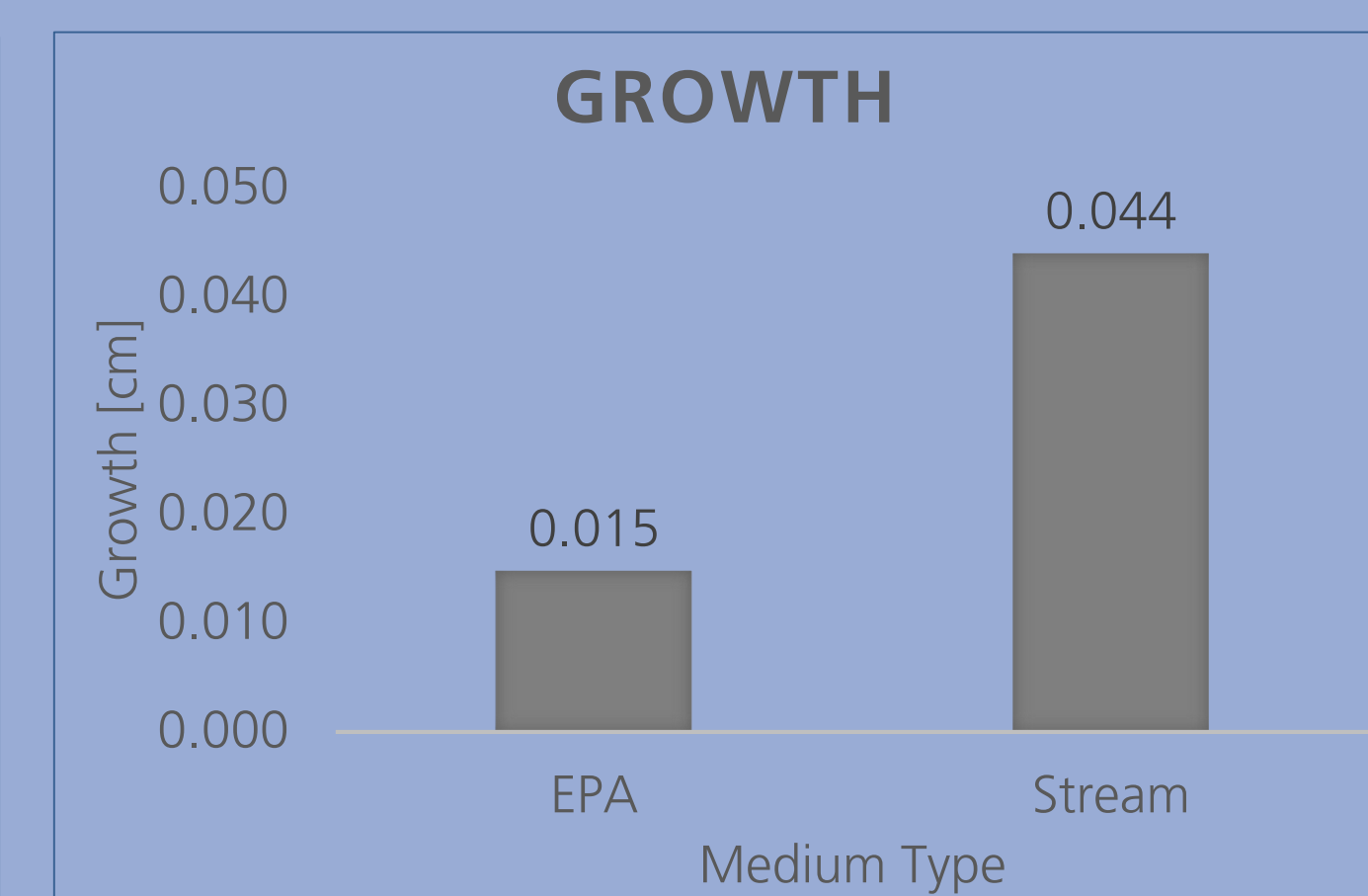


Figure 5: Growth of mayfly larvae *Epeorus sp.* in EPA medium and original stream water.

References

- [1] OECD Guideline for testing of chemicals, *Lymnaea stagnalis* Reproduction Test, adopted July 29, 2016
[2] Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th edition, EPA United States Environmental Protection Agency, October 2002

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Aquatic organisms, especially lotic invertebrate species originating from running waters, are exposed to releases of plant protection products which are mainly used in agriculture. Since lotic invertebrate species are regarded to be very sensitive but are hardly considered in chronic ecotoxicity testing, we developed a test system in order to investigate chronic effects on mayfly and stonefly species. After successfully development of a test method for stonefly larvae *Protonemura ssp.*, the next step was the establishment of a method for testing of mayfly species *Epeorus ssp.*

In the developed test system, contrary to usual indoor stream systems, not the water body itself, but test vessels inside test containers are circulated, thereby creating a target flow. The test containers are filled with medium and contains ten replicates each. Each replicate is a small cage, which serves as individual compartment for individual testing of one test organism. The surroundings are adapted to the natural habitat of the test organisms. As endpoints growth, emergence and mortality are observed during a 21 day exposure period. For the studies we use field collected larvae which are adapted to laboratory conditions before test start.

In a first step the test conditions were adapted to the requirements of mayfly larvae. Therefore testing of different media was performed. It turned out that, contrary to testing of stonefly larvae, which is performed in Cu-reduced dilution water, moderately hard reconstituted water (according to EPA) works best for testing of mayfly larvae. Instead of Tetramin®, which was used in stonefly testing, the green algae *Desmodesmus subspicatus* was used for feeding of mayfly larvae. In addition, while stonefly larvae were acclimated for 48 hours, the acclimation period was extended to seven days before test start. Under these conditions mayfly larvae showed an acceptable mortality of test organisms. The next step is to perform a test with the test substance Imidacloprid which will be exposed to mayfly larvae for 21 days. Afterwards sensitivity of mayfly and stonefly larvae to Imidacloprid will be compared.

The new testing method can provide toxicity data of chronic testing with different aquatic insect larvae, which can be used for a SSD (Species Sensitivity Distribution) approach. The developed test system, the results of the performed tests as well as a comparison between testing of mayfly and stonefly species will be presented.