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Chronic testing of invertebrate species – Development of a testing method simulating running waters

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1. Introduction

Testing with insects from running waters provides important information for the risk assessment of insecticides, especially in the scope of regulation. There are three groups of lotic insects (mayfly (Ephemeroptera), stonefly (Plecoptera) & caddisfly (Trichoptera)) which were considered to be very sensitive to pollutants (EPT-Taxa)[1].
In order to broaden the spectrum of chronic tests for lotic invertebrates we developed a testing method for chronic testing of lotic insect larvae, which fulfills the characteristics of running waters regarding flow, oxygen level, temperature, light regime and food supply and is easy to handle under laboratory conditions at the same time.
For a pilot study field collected larvae (*Protonemura sp.*) were used adapted to laboratory conditions before test start.
An overview of the diversity, availability and development stages of lotic species during the course of the year is available.

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

Tab. 1: Availibility and development stages of different lotic species.

	Mayfly larvae			**Stonefly larvae**		
	Baetis sp.	*Epeorus sylvicola*	*Ecdyonurus venosus*	*Ephemerella ignita*	*Protonemura sp.*	*Isoperla sp.*
Spring	Y	Y	Y	XY	XY	X
Summer	XY	Y	Y	Y	Y	Y
Autum	X	X	X	Y	XY	Y
Winter	XY	X	XY	X	X	n.a.

X = early stages, Y = older stages; n.a. = no information available

2. Test System

The test system differs from usual stream systems. Not the water body itself but test vessels inside of test containers are moved and circulate through the test containers, thereby creating the target flow. Test vessels serve as individual compartments including one larva.

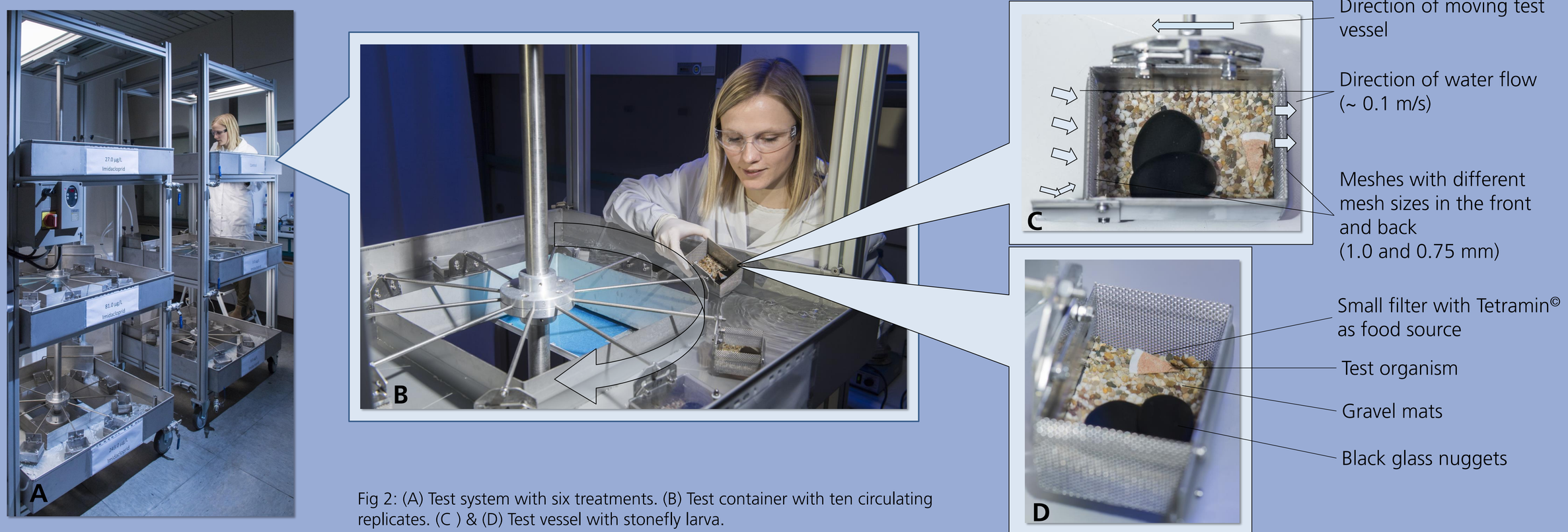

Fig 2: (A) Test system with six treatments. (B) Test container with ten circulating replicates. (C) & (D) Test vessel with stonefly larva.

3. Results of pilot studies

Two pilot studies were performed:
- Test organisms: Stonefly larvae (*Protonemura sp.*)
- Test item: Neonicotinoid Imidacloprid (Insecticide)
- Test performance: 1st study in winter; 2nd study in spring

Results:
- Dose dependent effect on growth and emergence
- Chronic toxicity endpoints were in the same order of magnitude
- Considered endpoints were season depending, related to the development stage of larvae

Tab 2: Assessment of chronic toxicity.

	1st Study	**2nd Study**
Endpoint	Growth	Emergence
EC_{10} [µg/L]	11.2	6.0
EC_{50} [µg/L]	56.4	37.1
NOEC [µg/L]	9.0	3.0

For details see presentation on Thursday May 11th, 9.35 am (ID: 468)

4. Advantages of the test system

- Individual testing of single organisms (one organism per replicate)
- Test conditions (flow, light, temperature) can be easily regulated
- One system can be used for testing of different species (EPT-Taxa)
- Semi-static exposure, flow through exposure and peak-exposure is possible
- Low potential for sorption of test substance (inert materials, no pumps needed to create flow)

5. Conclusion and Outlook

The applicability of the test system was proved for the stonefly *Protonemura sp.* In further studies the novel test system should be adapted for the testing of mayfly and caddisfly larvae.
Additionally, the novel test system provides a novel approach for the chronic testing of invertebrates particular with regard to provide toxicity data for a **SSD** (Species sensitivity distribution) approach.

[1] Weaver, P. C., et al. (2015). "Part 1: Laboratory culture of Centroptilum triangulifer (Ephemeroptera: Baetidae) using a defined diet of three diatoms." Chemosphere 139: 589-596.

Session: Late session
Poster
Exhibition Hall, Monday May 8th, 2017, ID: MO266

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Ecotoxicologial tests with invertebrate species are usually performed with organisms originate from standing water bodies, even though the part of potential sensitive species is higher in running waters. Thus, especially testing with insects from running waters provides important information for the risk assessment of insecticides, especially in the scope of regulation. There are three groups of stream-dwelling insects (mayfly, stonefly and caddisfly) which were considered to be very sensitive to pollutants in the stream environment.

We developed a testing method for chronic testing of mayfly and stonefly larvae which fulfills the characteristics of running waters regarding flow, high oxygen level, cold temperatures, light regime and food supply and at the same time is easy to handle under laboratory conditions.

We used field collected larvae (*Ecdyonurus venosus* and *Protonemura ssp.*) adapted to laboratory conditions before test start. Sampling of larvae over the whole year gave us an overview of the diversity and availability of organisms during the year.

The test system developed for chronic testing of mayfly and stonefly larvae differs from usual stream systems. Not the water body itself but the test vessel inside of test containers was moved. The test vessels circulate through the test container to create the aimed flow. As endpoints the length of the individual test organisms is determined at the beginning and the end of the test. Mortality is checked once per week. The test is conducted in Cu-free dilution water over 28 days, with a medium renewal once per week. Besides a semi-static exposure, a test under flow-through conditions or with peak exposures would be possible.

Contrary to usual model stream systems we are able to provide a test system which is inert to reduce sorption of test substances (no pumps needed to reach flow). Test vessels serve as individual compartments, for individual testing of larvae. The test system provides a constant flow which can be easily regulated, as well as temperature and light conditions. The test containers are smaller than outdoor stream system and thus, need less space and less test substance is necessary.

The developed test system provides a novel approach for the chronic testing of invertebrates particularly with regard to provide more organisms for a species sensitivity distribution in the scope of regulation. Its applicability was already proved with a pilot study with the neonicotinoid Imidachloprid.