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Burrowing behavior of *Chironomus yoshimatsui* larvae as an indicator of freshwater quality

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ABSTRACT

A new behavioral endpoint in ecotoxicity testing, burrowing behavior of *Chironomus yoshimatsui* larvae that survived pre-exposure to 0, 1, 5, and 10 mg/L of cadmium for 96 h was evaluated in a system composed of clean reconstituted water and sand for 5 h, at 30 min intervals. The effective time required for 50% of the introduced larvae to burrow (ET_{50}), the proportion of the maximum burrowing rate reached during a 5 h period (BR_{5h}), and the area under the curve at 5 h (AUC_{5h}) were calculated based on the fitted relationship between burrowing rate and elapsed time. These effect parameters were chosen because they take into account either the burrowing speed of the larvae (ET_{50}), or the proportion of the maximum burrowing rate reached during a 5 h period (BR_{5h}), or both (AUC_{5h}). We found that higher cadmium concentrations resulted in longer ET_{50} and lower AUC_{5h} values. Additionally, field water samples were collected from streams near residential, natural, agricultural, and industrial areas in Ansan City, Korea in 2014 and 2015, and were used to validate the proposed burrowing test method. The results of the burrowing tests with field collected water samples also confirmed the sensitivity of burrowing activity as an ecotoxicity endpoint, indicated by the large differences among the ET_{50}, BR_{5h}, and AUC_{5h} across the sampling sites and dates with industrial sites strongly affecting burrowing behavior. This study clearly showed that the proposed burrowing test could provide information that would not be detected with the existing acute toxicity tests, within a relatively short time, and can therefore be used as a complement to existing chemical analyses and acute toxicity tests.

1. Introduction

Most aquatic ecotoxicity studies have conventionally focused on assessing acute effects of compounds on survival and chronic sub-lethal effects on development or reproduction (Stadler, 2011). However, assessing chronic effects requires a substantial amount of time and costs depending on the life history and developmental characteristics of the target species (Melvin and Wilson, 2013). As an alternative endpoint, behavior of an organism can represent cumulative interactions with a variety of biotic and abiotic factors, and responses to internal (physiological) and external (environmental) factors (Dell'Omo, 2002). Moreover, since foraging, burrowing, and irrigation behaviors of macroinvertebrates can redistribute particles and fluids, the organisms themselves can strongly influence the physical, chemical, and microbiological properties of their habitat (Aller and Aller, 1986; Matisoff, 1995). The usefulness of the behavioral endpoints in ecotoxicity studies has been documented previously (Gerhardt, 2007; Stadler, 2011; Melvin and Wilson, 2013). Yet, Bonnard et al. (2009) reported that copper concentrations affecting burrowing behavior of polychaetes and bivalves varied six times depending on abiotic factors. Moreover, Francoeur and Dorgan (2014) found that the burrowing behavior of polychaete species varied in sediments with different physical properties. It is therefore necessary to develop a standardized test that minimizes the effect of substrate textures on behavior.

Midges of the genus *Chironomus* have been widely used as a model species to assess the quality of freshwater and sediments, because they are abundant in freshwater and are widely distributed all around the world (Ingersoll and Nelson, 1990; Callaghan et al., 2002; Charles et al., 2004). They also play an important role in freshwater ecosystems, because they represent a prominent part of benthic communities and link the producers and higher consumers in freshwater food webs (Péry et al., 2002). The life cycle of *Chironomus* comprises of aquatic stages (egg, four larval instars, and a pupal stage) and an aerial adult stage (Armitage et al., 1995). In particular, the burrowing behavior into the sediment during the larval stage is ecologically important, because they grow and complete their aquatic life cycle in these sediments (Armitage

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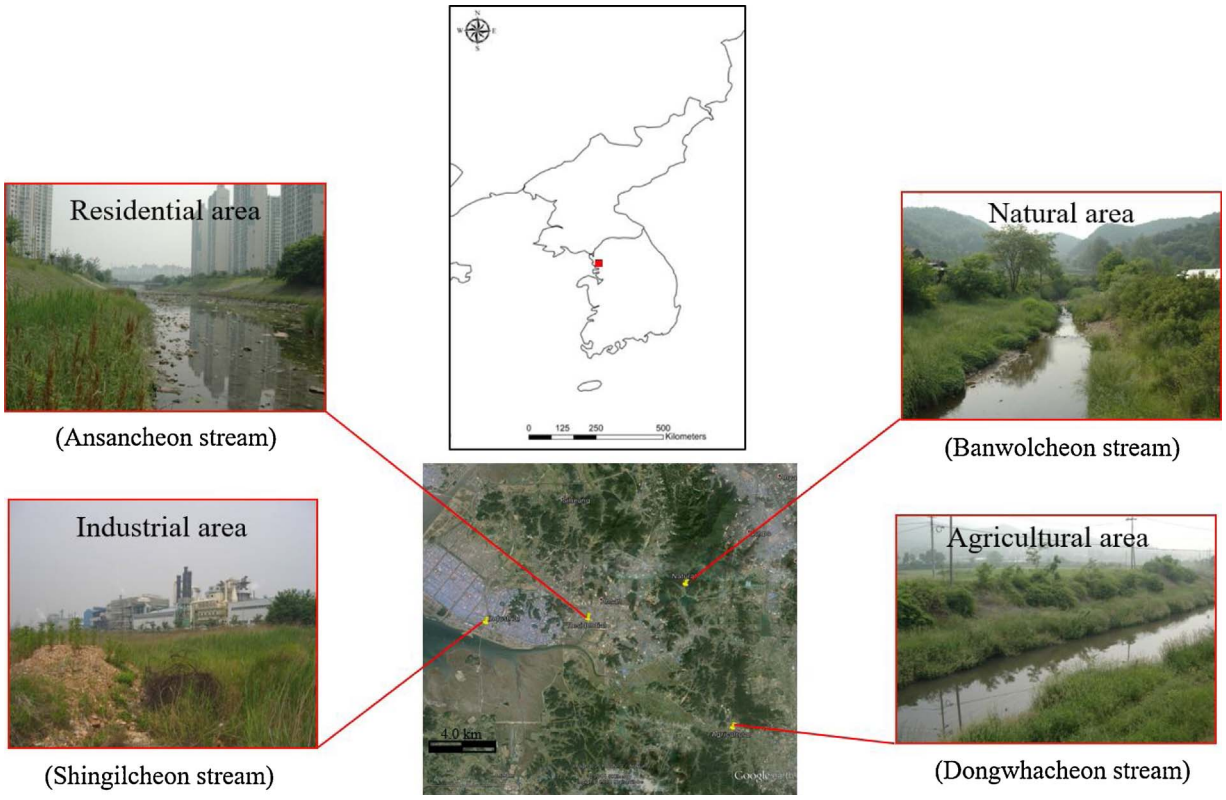

Fig. 1. Sampling sites and views of the streams.

et al., 1995). The burrowing behavior during the larval stages is essential for coping with hypoxia in the shallow water because these biogenic structures that pierce through the diffusive boundary layer can improve oxygen availability (Jørgensen and Revsbech, 1985). In addition, Schulz and Dabrowski (2001) reported that impaired burrowing behavior due to chemical exposure can increase the risk of mortality from fish predation. If burrowing behavior is impaired by chemical exposure or other environmental conditions, it can also affect population dynamics, as it is closely related to individual fitness (Brodin et al., 2014). Therefore, the use of burrowing behavior as an endpoint in ecotoxicity studies may be an effective tool for assessing the potential risk posed by water pollutants. In this study, *C. yoshimatsui* Martin *et* Sublette was used as a model species to develop a burrowing behavior test. *C. yoshimatsui* is widely distributed in Korea (Yoon and Chun, 1992) and is an internationally standardized species in ecotoxicological tests (OECD, 2010).

The objective of this study was first to investigate the feasibility of using the burrowing activity of *Chironomus yoshimatsui* larvae that survived pre-exposure to cadmium for 96 h as a new behavioral endpoint. Their burrowing behavior was evaluated for 5 h in a system composed of clean reconstituted water and sand. This procedure minimized the physical effects of sediment textures on the burrowing behavior. Secondly, we investigated the applicability of the proposed burrowing behavior test to evaluate the water quality of field-collected samples by observing their burrowing behavior after 96 h of exposure to the water samples experiencing different anthropogenic stresses.

2. Materials and methods

2.1. Model species for burrowing test

Egg masses of *C. yoshimatsui* were collected in the Jungnangcheon stream, eastern Seoul (N37°33′12″, E127°2′38″) and were maintained at 20 °C. Once larvae hatched from these egg masses, they were transferred into a 10 L glass aquarium tank filled with approximately 3 L of synthetic, moderately hard water (MHW; total hardness, 80–100 mg/L as $CaCO_3$) (US EPA, 1994) and an approximately 2 cm layer of acid-washed, burned commercial river sands (< 1 mm diameter) as sediment substrate. These aquarium tanks were kept in an incubator at 20 ± 1 °C with a photoduration of 16:8 h (L:D) and light intensity of 2,000 Lux, and air was gently supplied to the overlying water with an aquarium air pump. As a food source, 0.5 mg of ground Tetramin® fish flakes (Tetra Werke, Germany) per larva was provided every second day. The larvae were collected one day before the start of the experiments (9 d post hatching). These 10-d-old third instar larvae were used throughout the experiments.

2.2. Effect of cadmium on burrowing behavior in reconstituted water

A 1000 mg/L cadmium stock solution was prepared by dissolving cadmium chloride salts ($CdCl_2{\cdot}2.5H_2O$, purity ≥ 98%, Sigma-Aldrich, USA) in MHW. The stock solution was further diluted in MHW to prepare the final concentrations of 0 (control), 1, 5, and 10 mg/L. Cadmium concentrations in the spiked water samples were determined using an ICP-OES (730 Series ICP-OES, Agilent Technologies Inc., USA). The recovery of cadmium in the water samples was at least 90%. The cadmium-spiked reconstituted water treatments were prepared 24 h before the start of the experiment and kept under the same environmental conditions as described in Section 2.1.

At the beginning of the exposure, ten 10-d old 3rd instar larvae were transferred to a 250 mL glass beaker containing 100 mL of cadmium-spiked reconstituted water without feeding. All beakers were kept in an incubator at 20 ± 1 °C with a photoperiod of 16:8 h (L:D). All experiments were conducted in four replicates of 10 larvae each. Mortality was assessed 96 h after the establishment of the bioassays. The larvae were considered dead if they were unable to produce a coordinated response when lightly grasped with a pair of fine forceps. No larval mortality was observed in the control and cadmium treatments after 96 h of exposure. The surviving larvae were used to test their burrowing behavior. The surviving larvae from each beaker were