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# Development of rearing and testing protocols for a new freshwater sediment test species: The gastropod *Valvata piscinalis*

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**Abstract**

This paper aimed at proposing rearing and testing protocols for *Valvata piscinalis*, a new potential species for sediment toxicity testing. Such tests were developed since this species reliably represents the bio/ecological characteristics of other gastropods. It may thus be representative of their sensitivity to chemicals.

*V. piscinalis* was successfully cultured in our laboratory for six generations. Cultures provided a high productivity for a low working time and low costs. The tests conditions we proposed seemed to be relevant for the development of reliable tests with this species. Indeed, hatching probability of egg-capsules, as well as embryo, newborn and juvenile survival rates, were close to 100%. Moreover, growth rates and fecundity were significantly higher than in field and in other laboratory studies.

Partial life-cycle tests on clean sediments were achieved for various feeding levels to determine survival, growth and reproduction patterns, ad libitum feeding level and life cycle parameters values. Ad libitum feeding levels for newborn, juveniles and adults were 0.1, 0.4 and 0.8 mg Tetramin®/individual/working day.

Growth tests with zinc-spiked sediments provided a no-effect concentration and a lowest effect concentration of respectively 200 and 624 mg zinc/kg dry sediment. Other growth tests on spiked sediments we ran at our laboratory with second, third and fourth instars larvae of *Chironomus riparius* pointed out that *V. piscinalis* was more sensible to zinc than the chironomid, which is a routine test species in ecotoxicology.

According to these results, *V. piscinalis* is a promising candidate species for sediment toxicity testing.


*Keywords:* Mollusks; Cultures; Life cycle tests; Zinc; Species sensitivity

## 1. Introduction

Despite their ecological relevance, mollusks have been seldom used as test species: only 47 species have been used in toxicity tests at the lab (EPA, 2005). Indeed, culturing might be difficult for some of them, thus

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preventing the use of juveniles, which are probably the most sensitive life stage (Giesy and Hoke, 1989). Furthermore, the closing of shells might make it difficult to assess lethality and actual exposure dose (Giesy and Hoke, 1989). These practical issues have been solved thanks to methodological innovations. Therefore, tests with mollusks are nowadays widely used for toxicity assessment of marine sediments (Traunspurger and Drews, 1996): results for 358 lab or field toxicity tests with marine mollusks can be found in the Aquire database (EPA, 2005). Anyway, only a few species are used for freshwater toxicity tests (7 of the 47 previously cited) (EPA, 2005). The gastropod *Lymnea* sp. and the bivalve *Dreissena* sp. are the most commonly tested ones (EPA, 2005). Among gastropods, studies mainly focused on *Lymnea stagnalis* (EPA, 2005). Anyway, this species is not of common occurrence in field (Mouthon, 1999). Its size (4–8 cm in adults) and its pulmonate breathing might represent practical difficulties for toxicity tests in the laboratory. We think that the development of rearing and testing protocols for a new gastropod species should permit to overcome the existing practical difficulties of rearing and testing at the lab and fill the lack of ecological relevance of the current gastropod test species. It would offer the opportunity to assess more easily toxic effects on gastropods, which zoological group is poorly represented in the lab, although it is of great ecological importance and of great sensitivity to several families of toxicants (e.g. endocrine disruptors: see for instance Huet et al., 1995; Oehlmann et al., 2000; Duft et al., 2003).

Little attention has been accorded to *Valvata piscinalis* [*Prosobranchia*, *Valvatidae*] although this gastropod could be a relevant novel species to be used for sediment toxicity tests. Indeed, it is easily identified in field (Binder, 1967). It is commonly found in the superficial layer of soft sediments and mud, in number of aquatic ecosystems i.e. canals, ditches, ponds, lakes and from rhithron to potamon of rivers (Boycott, 1934; Cleland, 1954; Mouthon and Dubois, 2001). It is often a dominant community member in lakes and rivers in Europe (Mouthon, 1999), Canada, USA (Fretter and Graham, 1978) and Western Asia (US FWS, 2004). This might facilitate the linking of laboratory data to field studies. Densities can reach 5000 individuals/$m^2$ in favorable habitats (Van den Berg et al., 1997). This abundance permits the gastropod to have a significant part in ecosystems functioning. Indeed, its selective feeding influences the characteristics of the superficial sediment layer (Cleland, 1954). It also constitutes a common food source for bottom fish (Young, 1975). As a number of mollusks, it tolerates large abiotic factors variations (Boycott, 1934; Fretter and Graham, 1978; Mouthon, 1996), thus favoring its acclimatization and rearing in the laboratory. Juveniles and adults live in the sediment whereas newborn live in the water column, fixed to macrophytes (Cleland, 1954). This variety of microhabitats probably allows the gastropod to be a relevant species for both water only and water/sediment toxicity tests. Large amounts of fine particles are ingested while searching for the nutritive matter belonging to the sediment (Cleland, 1954), so that this organism might be greatly exposed to sediment-bound toxicants. Furthermore, this species is quite similar to other gastropods concerning their bio/ecological characteristics (Ducrot et al., 2005). It may thus be representative of their sensitivity to chemicals.

A few laboratory studies already focused on *V. piscinalis* (Tsikhon-Lukanina, 1961a,b; Hoffmann and Neumann, 1990). However, its life cycle under laboratory conditions has not yet been investigated. Furthermore, this species has not yet been used for toxicity tests.

This paper aimed at developing rearing and testing protocols with *V. piscinalis*. When developing rearing protocols, we focused on determining the appropriate substrate, food source and water quality to ensure high production rates in our cultures. When developing testing protocols, our aim was to determine appropriate testing media, testing conditions, feeding level and endpoints measurement methods for survival, growth and reproduction tests.

Tests were conducted in uncontaminated sediments to estimate the optimum feeding diet and to assess the survival, growth and reproduction patterns. This permitted us to fully characterize the life cycle of the gastropod in the laboratory. Furthermore, its sensitivity to zinc-spiked sediment was assessed using a 28 days growth test.

This study thus provides insights for the following issues: is *V. piscinalis* a good candidate to laboratory acclimatization, rearing and handling? What are its life cycle characteristics in laboratory conditions? Is it possible to develop both acute and chronic endpoints with this species? May it be appropriately sensitive to sediment-bound chemicals?

## 2. Materials and methods

### 2.1. Biology and ecology

*Valvata piscinalis* is a branchiate gastropod, which is found on/in soft sediments, feeding on small-sized vegetal detritus, diatoms and bacteria that are either filtered or scraped. During the breeding season, adults mate in the sediment and then migrate to aquatic plants for laying. Each egg-capsule is heavily yolked and contains from 1 to 60 eggs (Heard, 1963), depending on the quality and quantity of food available for the snail from the sediment. Reproduction is hermaphroditic,