THE IDENTIFICATION OF A SUITABLE CULTURE ORGANISM TO ESTABLISH A BIO-ASSAY FOR EVALUATING SEDIMENT TOXICITY

Organism culture investigation and stability of selected organisms for sediment toxicity testing

Report to the
Water Research Commission

by

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The publication of this report emanates from a project titled *The identification of a suitable culture organism to establish a bio-assay for evaluating sediment toxicity* (WRC Project No K8/946).

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EXECUTIVE SUMMARY

Sediments act as a source and sink for a variety of organic and inorganic contaminants. These contaminants accumulate, resulting in extremely high concentrations even once the overlying water concentrations are at or below acceptable water quality guidelines. Any changes in the physical parameters of the overlying water can cause these pollutants to be released back into solution. These accumulated contaminants can be released at even higher concentrations than previously detected. In recent years sediment contamination has highlighted the need to monitor these previously overlooked pollutant sources that have accumulated in aquatic ecosystems. When the contaminants bound to sediments become toxic they pose a risk both to the aquatic organisms as well as Human health.

South Africa does not currently have standardised methods to assess sediment toxicity. Although international methods exist, they are largely untested in South Africa and the organisms needed to conduct these tests are not readily available.

This Report on the Organism culture investigation and stability of selected organisms for sediment toxicity testing is the second deliverable of the WRC Project No K8/946 to identify a suitable culture organism to establish a bio-assay for evaluating sediment toxicity.

This report includes an update on the culturing of the four organisms identified which have been selected as preliminary candidates for sediment testing, the progress so far as well as the current methods which have been used.

From the Nineteen organisms identified in the Preliminary literature review, the preliminary organisms selected included:

- A Hydra sp.
- An Ostracod sp.
- A Chironomid sp.; and
- A Mollusc, *Melanoides tuberculata.*

Currently the cultures are stable, however it was decided that the newly hatched Ostracods are too small to manage as a laboratory culture and that this would hamper its use in toxicity testing. These organisms form part of a Toxkit, which will be assessed later in the study. The Culturing methods are being refined and adapted in order to optimize the culture conditions for the organisms. Once the culturing conditions have been stabilized, these methods will be compiled into training material and methods manual.

LIST OF ACRONYMS

DO	Dissolved Oxygen
DWA	Department of Water Affairs
RQS	Resource Quality Services
SSHW	Standard Synthetic Hard Water
US EPA	United States Environmental Protection Agency
WRC	Water Research Commission

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1. INTRODUCTION

The aim of this project is to successfully culture, as far as possible, a South African species for sediment toxicity testing. If this is not possible, the use of international accepted test kits for sediment toxicity will be considered and tested under South African conditions.

In the preliminary literature review for this project (WRC Project No K8/946, GARL 2010) the following sediment toxicity indicator organisms were identified (from studies conducted globally) as candidates for laboratory cultures and toxicity testing: *Vibrio fisheri* (Bacteria), *Selenastrum capricornutum* (Algae), *Spirostomum ambiguum* (Ciliated protozoa), *Daphnia magma*, *D. pulex* (Crustacea), *Thamnocephalus platyurus* (Crustacea), *Hydra vulgaris*, *H. varidissima* (Hydrozoa), *Heterocypris incongruens* (Ostracoda), *Hydropsyche angustipennis*, *Cyrnus trimaculatus* (Thrichoptera), *Chironomus riparius*, *C. tentans* (Diptera), *Lumbriculus variegates*, *L. terrestris*, *Eisenia andrei*, *E. fetida* (Oligochaeta), *Caenorhabditid elegans* (Nematoda) and *Hyallela azteca* (Amphipoda).

After careful consideration of both the positive and negative points associated with culturing the above named organisms under laboratory conditions, as well as using them for routine sediment toxicity tests, the following three organisms were chosen as preliminary culture candidates (Hydra sp., Ostracod sp., & Cironomid sp.) with the addition of a fourth organism (*Melanoides tuberculata*), which was not part of the original literature review.

This Report on the Organism culture investigation and stability of selected organisms for sediment toxicity testing is the **second deliverable of the WRC Project No K8/946 to identify a suitable culture organism to establish a bio-assay for evaluating sediment toxicity.**

2. SELECTED CULTURE ORGANISMS

The selected culture organisms were selected for various reasons including ease of culturing, availability and usability.

HYDROZOA

Hydras are easy to culture as they only require a sufficient medium containing the most basic ions and an adequate supply of food. Little bench space is required for their culture containers which makes the culturing of Hydra very cost effective under laboratory conditions (Holdway, 2005). A stable culture proliferates rapidly if they are fed on a regular

basis. Hydra sp. have been used in the internationally to test water, elutriate (Holdway, 2005) as well as sediment toxicity (Rosenkrantz et al., 2008).

OSTRACODA

Ostracods are easy to culture under laboratory conditions. They require a small amount of medium and feed on small food particles. An Ostracod culture does not take up a lot of bench space thus reducing the costs associated with regular culturing methods (Belgis et al., 2003).

Ostracods lay numerous eggs at a time and thus the population in a culture can increase rapidly. In nature Ostracods are constantly in contact with the sediment (Day et al., 2001) due to their feeding behavior, and thus they have been used in the past for sediment toxicity testing. An Ostracod test kit is also available internationally (Ostracod test kit F).

DIPTERA (CHIRONOMID SP.)

Chironomids have been cultured under laboratory conditions internationally for sediment toxicity tests (Pery et al., 2005) with relative ease. They can be cultured in any type of container and require a substrate to live in. Due to their size, Chironomids are easy to handle. They have a short life cycle and can produce egg sacs that can contain hundreds of eggs. Little bench space is needed for Chironomid culturing, thus reducing the costs (US EPA 2004).

Chironomids are in direct contact with the substrate as they build tubes from small particles and will borrow into the substrate (Pery et al., 2005). Their feeding habits also expose them to possible contaminants that may be present in the substrate, thus making them a suitable culture organism for sediment toxicity testing.

Another attribute of using Chironomids for sediment toxicity testing is that they can also be used for bioaccumulation (Roulier et al., 2008) and biomarker studies (Domingues et al., 2007) after they have been exposed to potentials contaminants. This can contribute to the knowledge of the specific sample and its potential effects on the environment.

MOLLUSCA

Snails are easy to culture as they only require a substrate and a sufficient amount of food. Snails are easy to handle and can be kept in a medium sized container. Minimal costs are required to sustain a culture. Snails can produce a large population of individuals quit rapidly. They are in direct contact with the sediment as they feed from its surface and some snails will even borrow into the substrate. Besides their use for sediment

toxicity tests they have been used in the past for biomarker (Moolman et al., 2007) and bioaccumulation (White et al., 2006) studies.

3. PRELIMINARY CULTURE METHODS

The following are preliminary culture methods for the selected culture organisms:

CNIDARIA

The Hydra species (Figure 1A) used for culturing was originally obtained from Happy Acres in Magaliesburg and were cultured at the University of Johannesburg. A subculture was obtained and placed into 3 ℓ Schott Duran Glass beakers (Figure 2A) with 500 ml of Standard Synthetic Hard Water (SSHW) prepared according to the US EPA 2002 method. No aeration or substrate was added.

The culture medium was agitated slightly every second day in order to add some oxygen. Originally a 200 ml water change was done every second day. The amount of medium in the beaker was then increase to 1 ℓ and the water changes were reduce to 500 ml once a week. The room temperature has been kept constant at a temperature of 22°C since the culture was started in August 2010. The culture is fed on live brine shrimp (*Artemia* sp.) or live *Daphnia pulex* every second day. The culture is stable and the Hydra population is increasing steadily. The culture methods are summarized in Table 1.

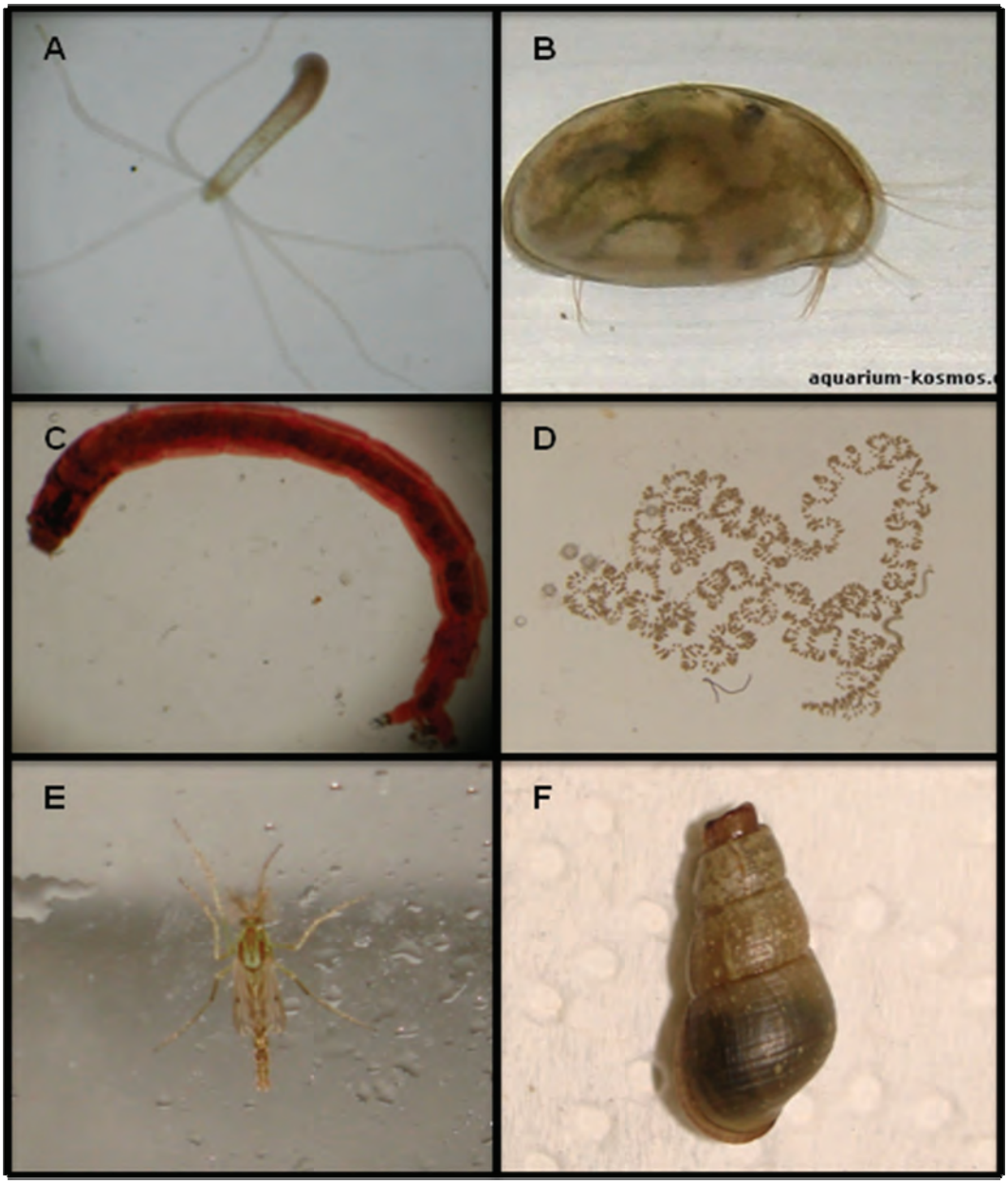

Figure 1: A Photo plate of the four culture organisms. A: Hydra sp. B. Heterocypris incongruens (Ostracod) (www.aquarium-kosmos.de). C: Chironomid sp. D: Chironomid egg mass. E: adult Chironomid. F: Melanoides tuberculata (Snail).

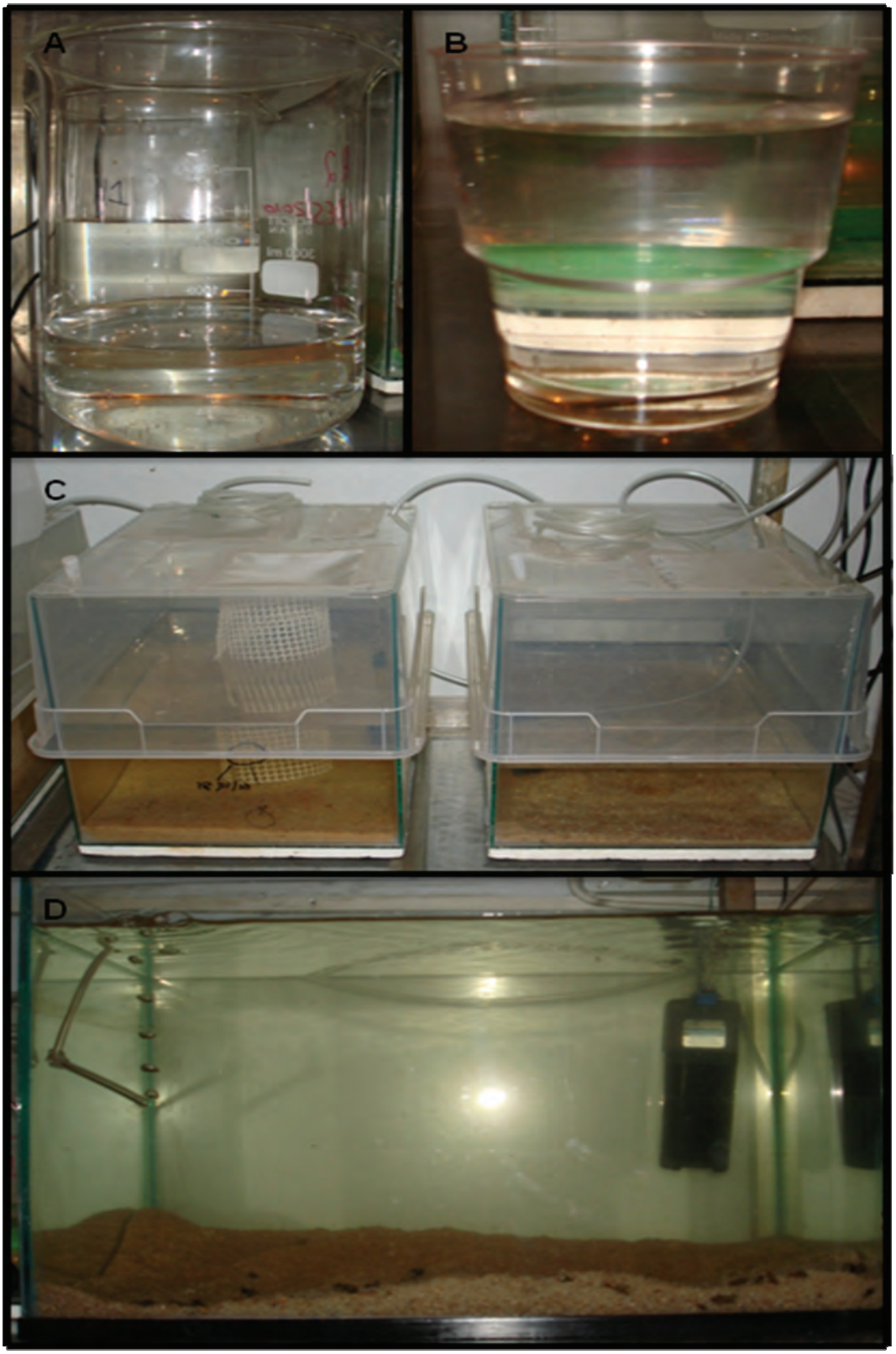

Figure 2: Culture Containers. A: 3 ℓ beaker for the Hydra culture. B: 250 ml cup for the Ostracod culture. C. 12 ℓ Tanks for the Chironomid culture. D: 30 ℓ tank for the snail culture.

Table 1: A Summary of the Organism Culture Conditions

Culture Organisms	Hydra sp.	Ostracod sp. *(Heterocypris incongruens)*	Chironomid sp.	*Melanoides tuberculata*
Temperature	22°C ± 1	22°C ± 1	22 ± 1	22 ± 1
Light quality	Ambient laboratory illumination	Ambient laboratory illumination	Ambient laboratory illumination	Ambient laboratory illumination
Photoperiod	12 Light: 12 Dark	12 Light: 12 Dark	12 Light: 12 Dark	12 Light: 12 Dark
Culture container size	3 ℓ Scott Duran Beaker	250 ml cup	12 ℓ glass tank	30 ℓ glass tank
Medium volume	1 ℓ	40 ml	3 ℓ	25 ℓ
Renewal of culture medium	500 ml once a week	20 ml once a week	1 ℓ once a week	3 ℓ once a week
Number of organisms per container	±100	5	±150	±30
Cleaning of container	Once every 6 weeks	Once every 6 weeks	Sides of tank once every second week	Sides of tank once every second week
Feeding regime	*Daphnia pulex* or Brines shrimp	200 µl Daphnia food every second day	15 ml Daphnia food and 1 ml fish food slurry	2 ml fish food slurry twice a week
Aeration	None	None	Light aeration	Medium aeration

OSTRACODA

The Ostracod *Heterocypris incongruens* (Figure 1B) used for culturing was originally obtained from the Ostracod test kit F. The Ostracods were placed into a 250 ml cup (Figure 2B) with 200 ml of SSHW. No aeration or substrate was added. Half of the water in the cup was changed every second day, but this was reduce to only one water change per week.

The room temperature was kept constant at 22°C and the Ostracods were fed 200 µl Daphnia food prepared according to the US EPA 2002 standard method. The adults laid eggs at the bottom of the cup, and these hatched. It was however decided that the newly

hatched Ostracods are too small to manage in a laboratory culture and that this would hamper its use in toxicity testing.

The Ostracod test kit F however is easy to use and would be the better method to use for the sediment toxicity test. The culture methods are summarized in Table 1.

DIPTERA

The Chironomid sp. (Figure 1C) was originally obtained from Aquaculture CC (Meyerton). A total of 150 individuals of different sizes were placed in 12 ℓ Glass tanks (Figure 2C) with 3 ℓ SSHW. A 1cm mixture of acid washed fine and coarse silica sand was added to each tank. Constant aeration was supplied with an air stone. The tanks were covered with shade cloth to prevent adults from escaping the tanks; this was later changed to plastic lids which were modified to allow for aeration.

A 1 ℓ water change was done every second day. This was later reduced a 1 ℓ water change only once a week. The room temperature was kept constant at 22°C. The culture is fed 15 ml of Daphnia food with a 1 ml Sera vipan flake slurry every second day. Once a week the water quality (pH, O_2% & O_2 mg/ℓ) is measured and recorded using a HACH sension 156 Multi-meter. The cultures are stable and have laid eggs (Figure 1D). These eggs have hatched, have grown into adults (Figure 1E), and then have reproduced again.

The eggs that are produced are carefully removed from the tanks and placed in a new 3 ℓ Schott Duran Glass beakers with aeration and SSHW in order to produce a monoculture. Specimens from the monocultures will be sent to Rhodes University for the identification of the species. There are two possible ways by which the larvae age can be determined, firstly is to note the specific date that they hatch from the egg sacs, and the second method is to determine the instar stage with the use of a microscope. The culture methods are summarized in Table 1.

MOLLUSCA

The snail species *Melanoides tuberculata* (Figure 1D) used for culturing was originally obtained from the University of Johannesburg. A subculture was placed into a 12 ℓ glass tank that has since been replaced with a 30 ℓ glass tank (Figure 2F) with 25 ℓ SSHW. A substrate of 1.5cm acid washed coarse silica sand was added the tank. Constant aeration was supplied with an airstone and the water is filtered with an internal carbon filter.

Half of the water in the tank was changed every second day but this has since been reduced to a 3 ℓ water change once a week. The room temperature was kept constant at 22° C and the snails are fed 2 ml Sera vipan slurry once a week. The culture is stable. The culture methods are summarized in Table 1.

4. THE WAY FORWARD

Currently, all the cultures are stable. The current culturing methods still need to be refined and adapted continuously in order to optimize conditions for the organisms. This will allow for more control and better quality assurance of the test organisms.

The following is the way forward:

- Once the culturing conditions have been stabilized, these methods will be compiled into training material and methods manual which will be provided to the WRC for review and approval. Arrangements will be made to move a subculture to the Department of Water Affairs (DWA) Resource Quality Services (RQS) Laboratory, and the laboratory personnel at RQS will receive training on the culture methods through a work shop and hands on experience.
- The Hydra sp. and Chironomid sp. will be sent to be identified at Rhodes University in order to determine which species have been successfully cultured and stabilized.
- Preliminary sediment toxicity test will be conducted in the future to determine the application of each organism to the sediment toxicity tests and to develop sediment toxicity test methods.

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