



## Improvements and cost-effective measures to the automated intermittent water renewal system for toxicity testing with sediments



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### ABSTRACT

The push to make bioassays more sensitive has meant an increased duration of testing to look at more chronic endpoints. To conduct these longer bioassays through the use of traditional bioassay methods can be difficult, as many traditional bioassays have employed manual water changes, which take considerable time and effort. To that end, static-renewal systems were designed to provide researchers a technique to ease the manual water change burden. One of the most well-known static-renewal designs, the static intermittent renewal system (STIR) was produced by the United States Environmental Protection Agency in 1993. This system is still being used in laboratories across the globe today. However, these initial designs have become rather dated as new technologies and methods have been developed that make these systems easier to build and operate. The following information details changes to the initial design and a proof of concept experiment with the benthic invertebrate, *Chironomus tepperi*, to validate the modifications to the original system.

### 1. Introduction

Sediment bioassays are increasingly being used to assess ecological effects as part of aquatic risk assessments. Standardized techniques to address sediment toxicity have been adopted by the Organization for Economic Co-operation and Development, OECD (2004), the United States Environmental Protection Agency (2000), and American Society for Testing and Materials International (ASTM, 1997) among others. The need for water changes in bioassays (due to water quality issues) has become more critical as more sensitive and longer bioassay procedures, such as reproductive and other chronic evaluations, have been developed (ASTM, 1997; Ankley et al., 1993). Manual water changes, as one might suspect, are quite time-intensive and, if not carefully done, disruptive to the test organisms and test sediments in the bioassay. With the technical difficulties of performing manual water changes and the increasing size of bioassays (such as for toxicity identification evaluation bioassays), the need for automated water renewal procedures was obvious and automated procedures were developed. Even with these developments, many laboratories still manually perform water changes for bioassays. While the drawbacks to manual water changes revolve around the amount of time required to perform a change and the potential for re-suspension of sediments, the major drawback to the

automated water change hinges mainly on the initial cost and the technical expertise involved to build such a system.

Perhaps one of the most well known automated systems is the stationary and portable Sediment Testing Intermittent Renewal (STIR) system (Benoit et al., 1993). This system was designed to be economical and practical, while still being an effective and less time-consuming approach to conducting water changes in sediment bioassays. To date, the designs of the STIR as prepared in 1993 still meet those expectations. However, much of the information (e.g. cost, equipment choices, etc.), as detailed in that publication, has become dated, and modifications for easier construction with the use of new technologies have become possible, and they allow for construction that requires little to no building experience.

Similar to the initial publication describing the STIR system, the objective of this project is to provide researchers with enough detail to construct an automated system for sediment testing of their own. The information presented below is based on the construction of multiple automated sediment systems in laboratories in the United States, China, and Australia. With the construction of each system, modifications and improvements were made to make the system more user-friendly, while still reducing cost and space requirements. In some circumstances, proposed changes that we have utilized were suggested in other static

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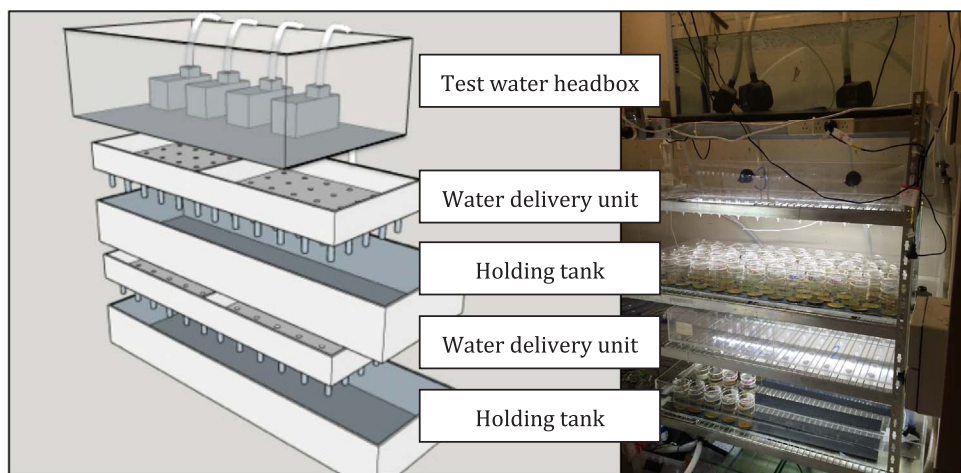


Fig. 1. Modified static-renewal system design.

renewal test designs (Rand et al., 2003; Zumwalt et al., 1994; Leppanen and Maier, 1998). The details provided here have further simplified the construction, making the system easier to use, increasing throughput while saving space, and/or making it more cost-effective to build in comparison to the STIR system.

## 2. Materials and methods

The original specifications for the STIR had five major components, but the modified system described has four: test water headbox, water delivery unit, holding tank, and exposure test chambers. The current setup does not require a water tank as specified in the original design as the holding tank itself doubles as a water bath. An optional filtration water discharge system will be also discussed. The proposed design does differ dramatically from the original schematics of the STIR design (as shown in Fig. 1). As space and cost are limitations for most laboratories, a description of how to build this system is provided with the understanding that modifications will be made based on an individual laboratory needs and resources.

### 2.1. Housing the system

The initial STIR system design was a table top design or was oriented more horizontally (Benoit et al., 1993); the proposed modified system described herein is a tiered system requiring ~ 2 m of vertical space, and ~ 1 m in width, and a depth of 0.5 m, which increases throughput and decreases space requirements (as it utilizes vertical space). Also, the new modified design can handle more replicates, as the initial design could conduct an automated change for 96 replicates for 12 different sediments, while the most recently created system (housed at the University of Melbourne) can house 120 replicates. This design neither limits the number of sediments that could be run simultaneously (thus, 120 different sediments could be evaluated if so desired) – the reason for this difference will be discussed in the delivery unit section. As this system is tiered and will be holding a considerable amount of water, suitable heavy-duty shelving that can handle large weights is required. This shelving houses the test water headbox, two water delivery units and two holding tanks as well as the exposure chambers. The weight of this system when used at full capacity (containing water and exposure beakers) can exceed 40 kg for an individual level of the shelf.

### 2.2. Test water headbox

The test water headbox as specified in the original STIR design was fabricated from welded stainless steel, with additional weldings being required for couplers that would connect the headbox to the rest of the STIR system (Benoit et al., 1982). Similarly, designs by Rand et al. (2003)

require the construction of a headbox that utilizes various glass compartments that are constructed using plate glass. In our automated system this headbox has been simplified dramatically. Large aquaria, positioned at the top of the unit, can be used as the test water headbox. If space is not a limitation, tubs or trashcans can be placed adjacent to the system as a substitute for holding the test water. The modified system proposed here uses submersible pumps and reinforced PVC plumbing hose to connect the test water headbox to the water delivery unit, eliminating the need for modifications to the test water headbox. This is different than most other designs which require the use of solenoids.

As for the choice of pumps, the main consideration should be the discharge rate. A fast discharge rate ensures that the water delivery unit will fill quickly and result in a uniform delivery of test waters. In the University of Melbourne system (four test water headbox units/30 replicates per unit), 230–240 V pumps with a Qmax of 2400 L/h were used. It should be noted that if the test water headbox is placed at the top of STIR system (as shown in Fig. 1), water would continually discharge even when the submersible pumps shut off (i.e. siphoning). Thus, the PVC hosing will need to be fitted with a PVC “T” fitting, which will discharge water back into the test headbox during water renewals. Once the pump shuts off, this fitting will allow air into the hose, which will stop the siphoning action.

The starting and stopping of the submersible pumps are controlled by electronic timers. The initial STIR design used solenoid valves (as have many past designs) that were to be wired into 24-h timers. This portion of the construction can now be avoided with the development of specialized timers. Since the publication of the initial STIR design, timers with the ability to complete cycles in the duration of seconds as well as having extensive programming capabilities have become readily available (for example MistKing Seconds Timer (Jungle Hobbies Ltd, Ontario, Canada). These specialized timers have an electrical socket so that pumps can be plugged directly into the timer. Calibration of the units (and hence the timers) will be discussed in greater detail below.

### 2.3. Water delivery unit

The water delivery unit in the initial STIR system pumps water into a holding tank containing up to eight exposure test beakers. This tank would fill slowly and replace water in the exposure test beakers through a water renewal hole in the exposure test beakers. The modified design uses a different technique than the initial STIR design that allows each exposure test beaker to be filled separately (similar to (Zumwalt et al., 1994; Leppanen and Maier, 1998)). In turn, this allows for beakers to be randomly distributed throughout the holding tank and for various levels of replication.

The PVC plumbing hoses that are attached to the pumps in the test water headbox are connected to the water delivery unit by a PVC fitting, which is positioned at the back of the water delivery unit. The water

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