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

W. Tyler Mehler[a], Jing You[b,*], Michael J. Keough[a], Michael J. Lydy[c], Vincent Pettigrove[a]

[a] Department of Biosciences, Centre for Aquatic Pollution Identification and Management (CAPIM), The University of Melbourne, Royal Parade, Parkville 3010, Australia
[b] School of Environment, Guangzhou Key Laboratory of Environmental Exposure and Health, and Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, China
[c] Center for Fisheries, Aquaculture and Aquatic Sciences and Department of Zoology, Southern Illinois University, Carbondale, IL 62901, USA

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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

* Corresponding author.
E-mail address: youjing@jnu.edu.cn (J. You).

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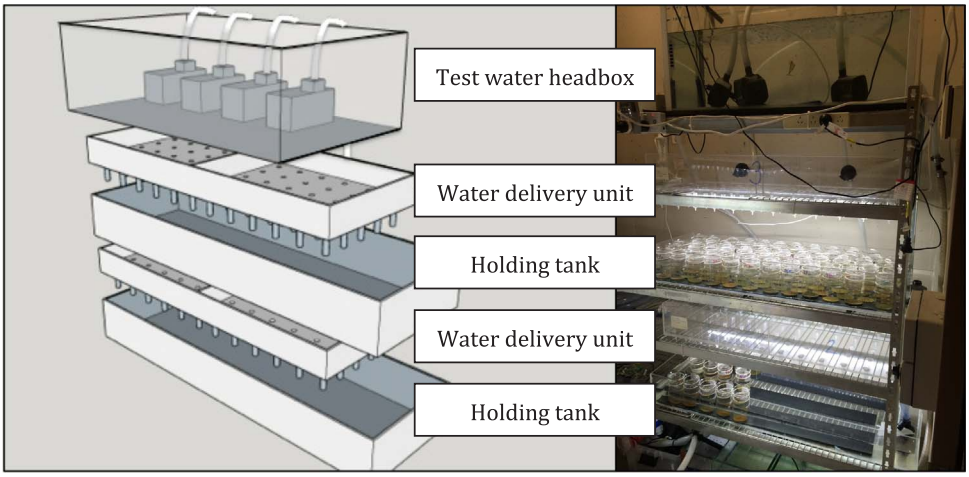

Fig. 1. Modified static-renewal system design.

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

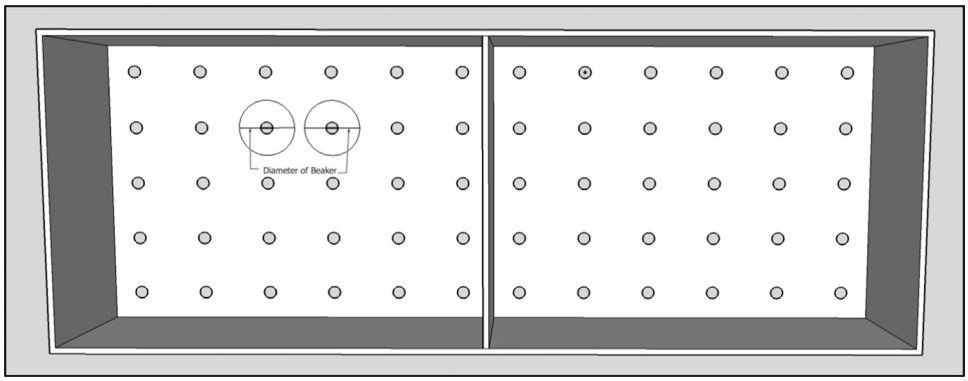

Fig. 2. Water-delivery unit design as built for the modified static-renewal system.

delivery unit itself is made entirely of poly (methyl methacrylate) – or more commonly known as acrylic glass (or by its trade name as Perspex™ or Plexigas™). The water delivery unit is essentially a modified open box (as shown in Fig. 1). Although the specifications for length and width of the water delivery unit are shelving dependent, the unit must be deep enough to contain the necessary water volume that is supplied to the test chambers. For the system as shown in Fig. 2 the depth is 14 cm. Similarly, the thickness of the glass for both the water delivery unit and the holding tank is important as thin acrylic glass has the potential to break or warp with high volumes of water (thicknesses of greater than 0.5 cm have been used successfully). Each water delivery unit is separated into two units (with each unit being filled by a separate pump). Each unit is able to yield 25–30 experimental replicates depending on design. The objective of separating the units into smaller blocks is so that the system can still be employed in smaller experiments, while using the necessary amount of water required for testing and also filling the chamber quickly to get more precise water volumes amongst replicates. The use of a poly (methyl methacrylate) solvent cement should be used when constructing the water delivery unit (as well as the holding tank) over silicon. These types of cement melts the glass together for a stronger and neater seal and should be used for the construction of both the water delivery unit as well as the holding tank.

The base of the water delivery unit has holes cut at a little more than a test chamber width apart (as shown in Fig. 2), which will house syringes (the use of syringes as part of a static renewal system has also been discussed in other designs (Rand et al., 2003; Zumwalt et al., 1994)). As the water delivery unit fills, these syringes will discharge the test water into the exposure test beakers, which sit below the unit in the holding tank. Various sizes of syringes (without the needle) have been used successfully in the past (10–50 mL) although the use of smaller volume syringes is encouraged as it allows for the delivery unit to fill more quickly (providing a more uniform water distribution), less disturbance to the sediments in the beaker, as well as saving vertical space. Syringe holes should be cut to the correct diameter to house the syringes to avoid leaks; in many cases a 'lip' on the syringes can be beneficial as it can provide additional area for a better seal.

Each separate system is calibrated before use and the volume and water change frequency is dependent on the research requirements. In general, we have typically employed a frequency of two water changes per day aiming for 100–150 mL per change. The system should be calibrated before use and in our experience each 30 replicate unit is able to produce volumes consistently that have less then a 10% relative standard deviation among all replicates. Water changes generally take between 14 and 17 s using the pumps that were mentioned earlier. Syringes can be adjusted vertically if the designed hole is cut snugly as suggested, to further aid in calibration. Intial setup of the design should not only determine the pump frequency and duration, but also the syringe to chamber distance that allows for the fresh water to circulate with the old, while still not resuspend the sediment in the chamber.

2.4. Holding tank

Similar to the water delivery unit, the holding tank has been made from acrylic glass and the specifications for length and width are again shelving dependent. The depth of the tank should be greater than that of the exposure test beakers (as the beakers will be discharging water into the holding tank). The only additional assembly required with the holding tank is the waste overflow PVC fitting. The fitting needs to be positioned at such a height that the water in the bath is lower than that of the release hole in the test beakers (as shown in Fig. 3). The location of the fitting can be either on the side of the box (as shown in the depiction) or on the bottom the tank. As mentioned earlier the holding

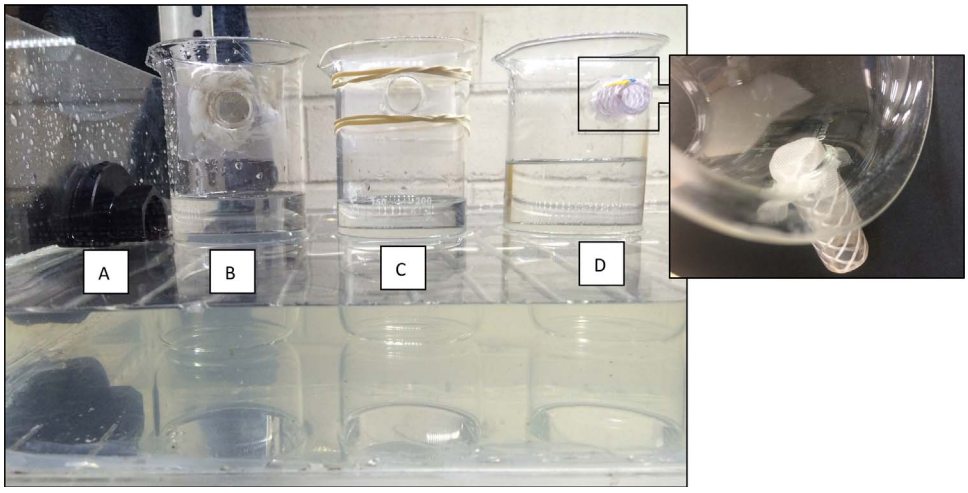

Fig. 3. Excess valve (A) and various test beaker designs (mesh screen (B) silicone, (C) held with rubber bands, and (D) hosing fitted with screen.

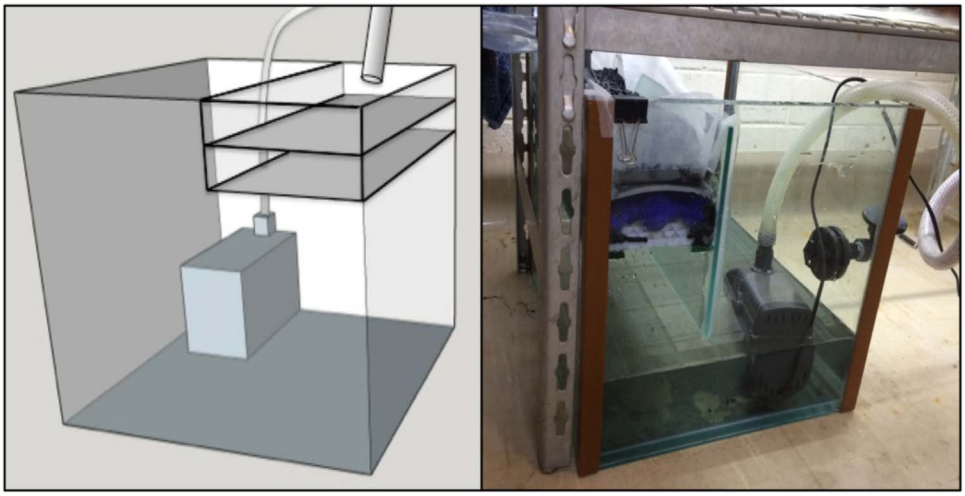

Fig. 4. Water discharge filtration system as built for the modified static renewal system.

tank also serves as a water bath. In many circumstances temperature controls are not required if the system is placed into a temperature-controlled room. If this is not possible, chiller units and heating units have also been successfully used to ensure that water temperature is maintained to the desired level.

2.5. Water discharge filtration system

The excess water from the test beakers will be discharged into the holding tank, which will dispose of this water through the excess flow valve. The flow valve is affixed with hosing which takes this water into the filtration water discharge system (see depiction in Fig. 4). This system has been put into place to ensure that any chemicals in the water are captured before the water is discharged as waste. The discharge system is fixed with a top compartment that can be filled with bioballs, activated carbon, or other agents to clean the test waters (depending on possible contaminants). After passing through the top compartment, the settled waters can be pumped out and disposed of as required.

2.6. Exposure test beakers

The size of beakers to be used for bioassays is laboratory specific as cost, availability, and types of bioassays being performed must all be taken into consideration. It is important to note that the size of the beakers will also play a large role into the size of the flow-through system – so the size of the beakers should be considered early on in the construction process. Previous designs have used standard beakers fixed with mesh over the top lip or have had a small notch cut at the top (Rand et al., 2003; Zumwalt et al., 1994; Leppanen and Maier, 1998). This technique works well with many organisms and provides an inexpensive means for exposure vessels; however, in the past we have had issues with overflowing and loss of test species especially when working with pelagic and/or small test organisms. For this reason, we use a similar construction method of exposure vessels as Benoit et al. (1993). The initial STIR design uses 300 mL beakers that have a hole drilled near the top of the vessel to release excess water. The hole should be positioned approximately 3–5 cm below the lip of the beaker (as shown in Fig. 3). In the construction proposed here, various types (customized beakers, mason jars) and sizes (200–600 mL) have been successfully used. The holes can be cut with a drill press using a diamond cutting bit with care being taken while cutting. Similar to the initial STIR design, the exposure test beaker hole should be covered with fine mesh screen (typically around 200–250 μm) to avoid the release of test organisms with the test water. This screen can be attached to beaker through the use of silicon or other non-toxic adhesives as mentioned in the initial design specifications (Fig. 3). However, as mentioned in the initial design, much care needs to be taken to avoid creating areas where organisms can become lodged. An additional concern with the attachment of the screen to the beakers via silicon was the cleaning of the test beakers – as in many laboratories (including the University of Melbourne) cleaning of test beakers is conducted using an acid-cleaning step, which over time destroyed the silicon and would cause breaks in the seal. To remedy the above issues, two different solutions have been devised and should be used dependent on the test organism being used. As shown in Fig. 3, besides the use of silicon, the mesh can be held on the beakers through the use of rubber bands, this has been shown to be effective for test organisms that reside in the sediment and are unlikely to be in the water column. The other, and more preferred option, is to use the mesh in combination with flexible tubing that fits within the diameter of the hole snugly. This is simple in practice – the mesh is placed over the hole from the outside and the tubing can be pushed through the hole to make a tight seal (as shown in Fig. 3). This option ensures that test organisms will not escape or be lodged in the crevices of the mesh, while still allowing for the test vessels to be easily cleaned. It is important to note that the mesh size should not to be too small as water tension and/or build up may block the vessel from releasing its water.

3. Results

The following discussion details the benefits and drawbacks of both water change approaches (manual water changes and via the static renewal system) in conjuction with a “proof of concept” experiment.

3.1. Proof of concept

As a means to evaluate the suitability of the static renewal system that has been developed, a control experiment (using 5-d growth and survival as well as emergence as endpoints) with a commonly used freshwater species in Australia (*Chironomus tepperi*) was employed to understand the difference among the various methods currently used (manual water changes with aeration, aeration only, manual water changes only, no manual water changes with no aeration) with this static renewal system. The methods for the experiment can be found in the Supplemental material. Water quality was acceptable for all of the methods tested with the exception of the method that used no manual water changes with no aeration (due to low dissolved oxygen < 50%). Interestingly, each method showed various water quality trends (pH (Fig. S1), conductivity (Fig. S2), and dissolved oxygen (Fig. S3)), which can be explained by the method employed; further details on those trends can be found in the Supplemental material. Control survival (Fig. S4) and growth (Fig. S5) using all five methods for the five day acute

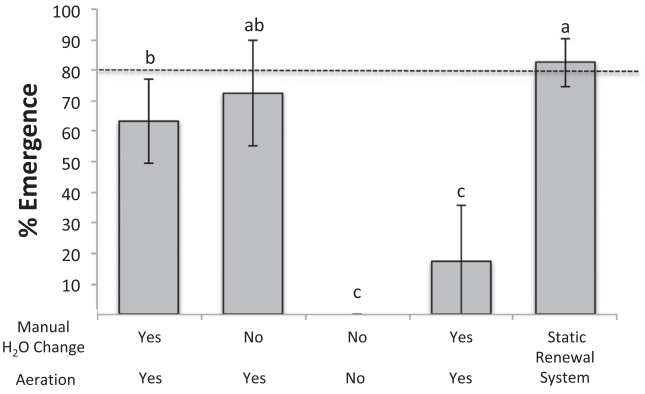

Fig. 5. Emergence (%) of *C. tepperi* for each control method tested after a 5-d bioassay period. Error bars are the standard deviation for each mean. The emergence threshold (± 80%) as required per the *C. tepperi* guidelines (Simpson and Batley, 2016) is depicted by the dashed line. Different letters indicate significant differences among the control methods tested ($p < 0.05$; single-way ANOVA).

portion of the experiment were comparable and not significant from one another (> 80% survival and growth ranged from 0.70 to 0.78 mg/individual) and survival met recently developed guidelines for *C. tepperi* (Simpson and Batley, 2016). However, it should be mentioned that significant differences were found between the treatments as the static renewal system had the highest survival (98% survival) and was significantly different then the manual water change with aeration method which just met the guideline threshold for control mortality (80%). These differences were further distinguished in the emergence results (Fig. 5), as only the static renewal system met the newly developed *C. tepperi* toxicity guidelines of > 80% (Simpson and Batley, 2016). This result was surprising, as the manual water changes with aeration method has been used in our laboratory successfully in the past using emergence as an endpoint (Boyle et al., 2016). The reason for the differences between the past studies that used aeration and manual water changes and those used here could be due to a smaller beaker (350 mL vs. 600 mL, respectively) with a smaller diameter being used (6 cm vs. 8.5 cm, respectively), possibly resulting in unwarranted disturbance to the organism. Issues with the sediment and/or fitness of the organism might have be an issue in the present study as emergence using the static renewal system (82.5%) was also just over the guideline threshold. Regardless, the results suggests that the newly-developed static renewal system can be employed with a great deal of confidence and may be more favorable then traditional methods. It also further shows that regardless of the method employed, control experiments should be conducted to ensure that the chosen method will work for the test organism of interest.

3.2. Time and cost savings

Manual water changes are quite time-intensive; in our experience testing using *Chironomus tepperi* (a benthic species) a water change takes 1–2 min per replicate per change. Most of the time required in the manual water change procedure is an attempt to avoid disturbing the sediment when adding fresh water. Additional time is required when conducting manual water changes for epi-benthic and pelagic species as these species reside in the water column itself and thus more care must be taken to avoid losing and/or stressing these organisms. The automated system described here can change water for approximately 120 replicates in less than 30 s and requires little to no researcher assistance during the change. Standard manual techniques conduct water changes every other day (for *C. tepperi*) or even less often for other test species (*Austrochiltonia subtenuis* (amphipod): one water change per week). To conduct a manual water change for 120 replicates, a single person would need to allocate at a minimum of 3–4 h to do the work. This automated technique not only is quicker, but also allows for additional water changes to be conducted per day. In contrast, the only responsibility of the researcher with an automated system is initial calibration, ensuring that the test water headbox is filled throughout the bioassay, and that the beakers are lined up under the syringes. In our experience, the system can easily be built within a few days and if constructed by the researcher under $1000 AUD (Perspex/plexiglass: ~ $600, pumps: ~ $100, and timers, hosing, syringes and pvc material: ~ $300). Even if one does not have the necessary resources to build a system, an outside entity can still produce a relatively inexpensive system. For instance, two entire systems (240 replicates) were built by a consultant for the University of Melbourne for less than $3000 AUD. If a laboratory is frequently running bioassays the amount of time and money saved will be obvious within the first few bioassays run.

3.3. Comparison with other static renewal systems

The merits of using a static renewal system over manual water changes are abundantly apparent. Comparing the strengths and limitations of specific designs to one another, however, becomes much more difficult. First and foremost, the system discussed herein can only be used for sediment exposures, while other systems (such as the mini-diluter system (Benoit et al., 1982)) can be used for both sediment and water exposures. The systems that can do both exposure types generally have many more moving parts to ensure that accurate dilutions can be made, but also so that the system can easily be taken apart for cleaning purposes (as the entirety of the system would need cleaned between bioassays). As such, these systems are more costly and generally harder to build. Additionally, as the use of sediments to assess risk is now being recognized by many in the field as a more environmentally relevant media for assessment purposes over that of overlying water, pore water, or effluent (Mehler et al., 2010; United States Environmental Protection Agency, 2007; Chapman et al., 2002), many laboratories may not need a diluter system and instead would focus solely on sediment toxicology (especially those facilities doing considerable biomonitoring work). In these circumstances, the described system, offers considerable advantages over older designs, not because these designs are flawed, but rather that this design utilizes the strengths of each of these past designs (Benoit et al., 1993; Rand et al., 2003; Zumwalt et al., 1994) while taking advantage of more reason technologies. It should be noted, however, that a variety of renewal systems exists (mainly for water only exposures) many of which were designed specifically for a type or class of organism (i.e. fish embryos (Lammer et al., 2009), pelagic microscopic organisms (Smith and Hargreaves, 1983; Lauth et al., 1996; Novak et al., 1982), and even larval or juvenile fish (Brenniman et al., 1976; Diamond et al., 1995) systems). As many systems exists, researchers should first determine the needs of the laboratory as well as budget constrains and then consult the literature for designs before embarking on building a system of their own.

4. Conclusions

The use of static-renewal systems provides researchers added flexibility, saves times and money, and produces consistent results for bioassays. The initial design produced in 1993 (Benoit et al., 1993) produced a system that met these specifications. With the following modifications, we feel that even less resources and/or money and less constructional “know-how” is required to build a static-renewal system. It is our opinion that if a laboratory is conducting sediment bioassays at regular intervals (i.e. at least once a month) that the construction of a static-renewal system, such as the one discussed here, is well worth the investment.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.12.051

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