

## The aquatic automated dosing and maintenance system (AADAMS)

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### Abstract

The maintenance and dosing of aquatic organisms, such as corals and mollusks, are essential for ecotoxicology studies, yet it is difficult to maintain many of these sensitive organisms for an extended period. Consequently, many previous aquatic ecotoxicology experiments have been limited in their number of replicates and maintained in one or a few experimental aquaria, with only a limited number of stressors tested in each experiment. Here we describe a modular system that overcomes many of the difficulties of maintaining large numbers of sensitive aquatic organisms in separate containers, and allows testing of a large suite of stressors in each experiment. The AADAMS (aquatic automated dosing and maintenance system) allows testing of 40 independent stressors with 10 independent replicates per stressor (400 individuals total). The AADAMS provides surge and regular water changes simultaneously with accurate dosing via Venturi valves. In a series of experiments over a 1-year period, the AADAMS was used to test the effects of various factors affecting water quality on Caribbean coral reefs. Roofing tar and road asphalt were two of the most damaging pollutants tested, with LD<sub>50</sub> values (lethal dose that killed 50% of the corals) of 0.013 g L<sup>-1</sup> and 0.079 g L<sup>-1</sup>, respectively, thus suggesting that runoff from roads and near-shore construction could be contributing to reef decline. The AADAMS is an accurate, reliable system for highly replicated ecotoxicological studies of sensitive aquatic organisms, which are important indicators of ecosystem health.

### Introduction

As human populations expand, marine ecosystems are becoming increasingly threatened, resulting in growing levels of mortality and disease (Harvell et al. 1999). Approximately

3 billion of the world's 6.3 billion people live in coastal areas, and this number is expected to double in the next 50 years (Brown 1997). These growing coastal populations cause many stresses to marine ecosystems, including eutrophication, sedimentation, and pollution (Brown 1997). However, the individual and synergistic effects of these stresses on marine organisms are largely unknown (Buddemeier and Smith 1999; Kennish 2001; Wilkinson 2002).

Tropical ecosystems, such as coral reefs, are particularly vulnerable to human impacts. Up to 75% of coral reefs, the most diverse of all marine ecosystems, are threatened globally (Wilkinson 2002, 2004) Upwards of 500 million people depend on coral reefs, and the greatest population increases are expected in tropical developing countries (Wilkinson 2002, 2004). Caribbean coral reefs have been especially affected by rapidly increasing coastal populations. In the past 10 years alone there has been a 20% increase in Caribbean

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**Table 1.** Summary of published coral ecotoxicology and environmental perturbation experiments.

Experiment	Stressors tested	Type of system	Water flow	Independent replicates/treatment
Alutoin 2001	Copper and salinity	Exposure tanks	Closed	1
Bhagooli and Hidaka 2003	Temperature and light	500-mL container	Closed	1
Bongiorno et al. 2003	Nutrients from fish farm	2 sites in field	Open	1
Cervino et al. 2003	Cyanide	Aquaria	Closed	1
Cox and Ward 2002	Ammonium	Microcosms	Open	3
Fabricius et al. 2002	Sediment and marine snow	Flow chambers	Closed	1
Ferrier-Pages et al. 2001	Iron and nitrate	Tanks	Open	2
Ferrier-Pages et al. 2000	Nitrogen and phosphorus	Peristaltic with tanks	Open	1
Grant et al. 2003	Copper	4-L containers	Closed	1
Grover et al. 2002	Ammonium	Beaker	Closed	1
Jones and Heyward 2003	Pore formation water (oil)	Plastic containers	Closed	3
Morgan and Snell 2002	Pesticides	Aquaria	Closed	1
Nordemar et al. 2003	Nitrate and temperature	85-L tanks	Closed	2
Nystrom et al. 2001	Copper and temperature	72-L tanks	Closed	1
Owen et al. 2002	Herbicide	Tanks	Closed	1
Porter et al. 1999	Salinity and temperature	Aquaria	Closed	1
Raberg et al. 2003	Herbicides	60-L containers	Closed	2
Reynaud et al. 2003	CO <sub>2</sub> and temperature	24-L tanks, reservoir with pump	Open	1

Independent replicates are defined as corals that are maintained so that they are not exposed to seawater that has been in contact with other replicates.

coastal populations, to over 110 million people (Wilkinson 2004). Associated with this rapid population increase has been a corresponding decline in Caribbean reefs, which have suffered an estimated 80% regional decline in coral coverage during the last 3 decades (Gardner et al. 2003). Furthermore, it is estimated that 64% of the remaining Caribbean coral reefs are threatened by increasing levels of human activities, with a high likelihood of further catastrophic loss (Wilkinson 2004).

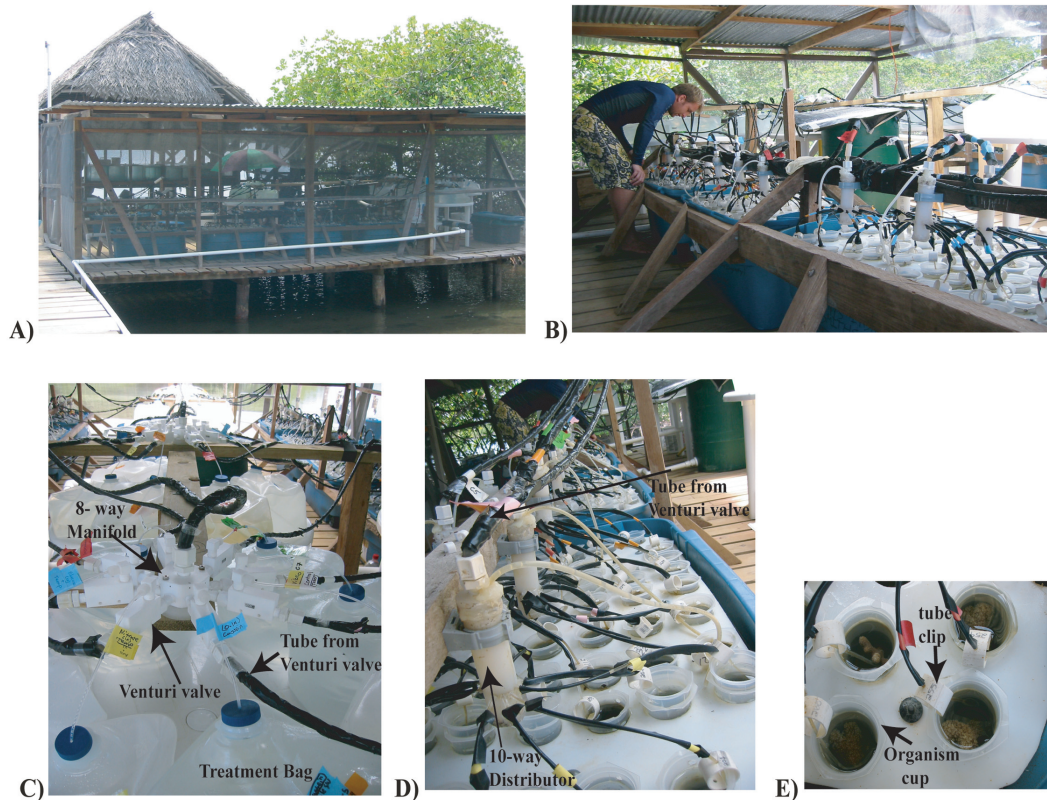
Despite the ecological and economic importance of coral reefs and their serious state of decline, the levels of marine pollutants that are damaging to reefs remain controversial. This is largely because of the difficulty in keeping corals healthy under controlled, experimental conditions. Correspondingly, most coral ecotoxicology and environmental perturbation experiments are performed with minimal replications and with replicate corals maintained in one or a few aquaria. In 18 recently published coral ecotoxicology and environmental perturbation studies, all had 3 or fewer independent replicates per experiment, and 12 of these studies had only 1 independent replicate per experiment. An independent replicate is defined here as corals that are maintained in separate containers, such that the seawater surrounding each coral, including its bacteria, mucus, etc., are not shared between replicates (Table 1). Having replicates maintained in separate containers minimizes the possibility that the mortality or illness of one replicate affects the others, reducing the potential for pseudoreplication (Hulbert 1984). Additionally, in 13 of these studies the corals were kept in closed systems with infrequent water changes. A closed experimental system affects the bacterial dynamics in the seawater and can lead to highly

accelerated bacterial growth rates due to the “bottle effect” [enhanced bacterial growth rates in the presence of a surface for attachment (Morita 1994)]. Moreover, in all 18 of these studies, only 1 or 2 stressors were tested in each experiment (Table 1). Here we describe a new system for rapidly testing toxicity on aquatic and marine organisms that would alleviate many of the previous experimental difficulties.

### Materials and procedures

**AADAMS statistical design**—The AADAMS used Venturi valves to automatically dose test organisms. In its current configuration, the AADAMS dosed 40 independent stressors, with 10 independent replicate test organisms for each stressor, for a total of 400 test organisms per experiment (Figures 1 and 2). A power analysis indicated that if stressors cause visible stress in 60% of the test organisms, then a statistical power of 90% would be achieved with 10 replicates per stressor (with proportions of  $p_1 = 0$ ,  $p_2 = 0.6$ , and  $\alpha = 0.05$ ). Furthermore, the system was designed so that the same stressor could be tested on multiple Venturi valves, resulting in greater statistical power if necessary (i.e., 3 Venturi valves could be used for each treatment to achieve 30 replicates per treatments with 13 treatments tested in an experiment). The system was also designed to be modular, so that additional Venturi valves could be added if a particular application required the testing of a greater number of treatments and/or increased statistical power.

**AADAMS construction and setup**—The AADAMS was constructed entirely of Teflon and polypropylene, to minimize contamination from plasticizers used in other plastics such as PVC. These plasticizers adversely affect bacteria and may harm



**Fig. 1.** Lay-out of the Aquatic Automated Dosing And Maintenance System (AADAMS) as set up at the Smithsonian Caribbean research station in Bocas del Toro, Panama. In A) the AADAMS is shown in the coral maintenance room built on the STRI dock. In B) the two rows of four water baths are shown. Each water bath has five distributors mounted above it, and 50 organism cups that are set in a frame to ensure that the organisms are maintained at reef water temperatures. In C) an 8-way manifold is shown with Venturi valves that aspirate concentrated chemicals from 10 l polypropylene treatment bags. In D) a close-up of a 10-way distributor is shown. The distributor delivers the dosed seawater to the 10 replicate organisms. In E) the 100 ml polypropylene cups are shown. The dosed seawater passes from the distributor through a Teflon tube, which is guided by a clip to the bottom of the cup. A hole at the 100 ml mark on the cup allows the seawater to drain.

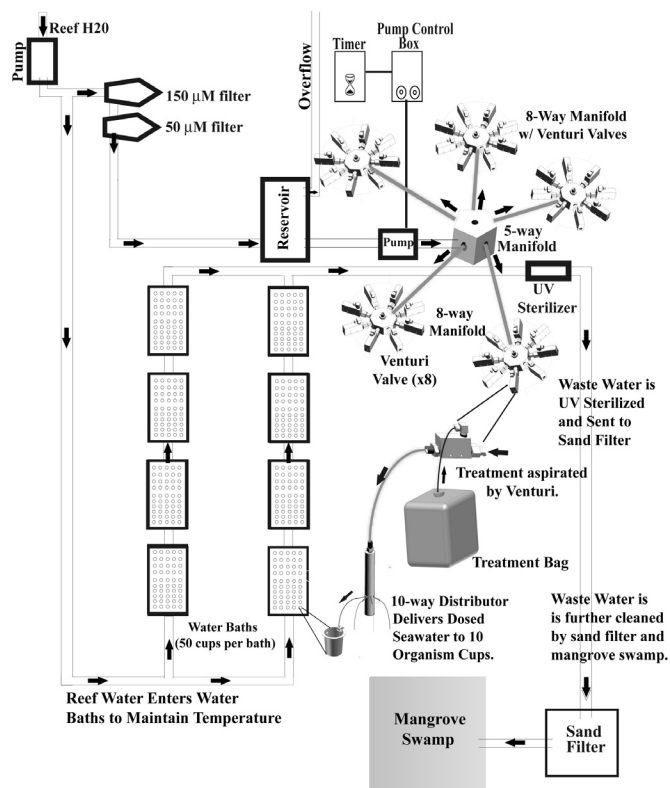
test organisms (Morita 1994; D.I.K. and F.R., unpublished results). A Jacuzzi pump (1.5 hp, 16 amp AC, Stareta #S48A53A03) transported reef water from approximately 3 m depth into a 150- $\mu$ m polypropylene bag filter, and then through a 50- $\mu$ m filter (series 400; Aquanetics; San Diego, CA, USA), removing large particles and sediments from the seawater that could clog the Venturi valves (Figure 2). The filtered seawater filled a 190-L polypropylene reservoir with an overflow tube at the 150-L level (Figure 2).

A second 0.5-hp, 90-V, DC Jacuzzi pump with a polypropylene pump head was connected at the base of the reservoir using polypropylene fittings and pipes. The pump was controlled by a timer/intervalometer (model 451; GraLab, Centerville, OH, USA) that was connected to an adjustable-speed DC pump controller (model KBMD-240D; Penta Power, Northampton, UK). The protocol that produced the most consistent Venturi rates, without backflow, was to have the intervalometer turn the pump on at full power for 6 s and then off for 54 s, for each minute throughout the experiment. This timing was determined by testing a range of settings to deduce which pro-

duced the most consistent Venturi aspiration rates (determined with a 20-mL graduated cylinder filled with seawater).

When the pump turned on each minute, it passed the filtered seawater through a Teflon "T" with 2 corrugated 1.9-cm (3/4-inch) diameter Teflon tubes that delivered the water to a 5-way Teflon manifold (Teqcom, Santa Ana, CA, USA) (Figures 2, 3A, and 4). The 5-way manifold was attached to the head of the pump and received seawater from both the top and bottom to achieve more equal distribution among the 5 ports (Figures 3 and 4). The seawater was then distributed through the 5-way manifold to five 8-way Teflon manifolds (Teqcom) (Figures 2, 3, and 4). Attached to each of the ports on the 8-way manifolds were Teflon Venturi valves (Teqcom) (Figures 2, 3, and 4). When the water passed through the Venturi valves, it caused concentrated chemical to be aspirated up the 40-cm (1/8-inch) long, 0.3-cm (1/8-inch) diameter Teflon tubing (Teqcom) that ran into the bottom of the 10-L polypropylene chemical bottles (#U-06100-30; Cole Palmer, Vernon Hills, IL, USA) (Figure 4). The aspirated concentrated chemical mixed with the seawater in the Venturi valve, and the dosed seawater





**Fig. 2.** Schematic of the AADAMS system. Black arrows represent the direction of water flow. The reef water is used both to maintain the water baths at reef temperature and to dose the organisms. The desired treatment is added to the seawater when it passes through the Venturi valve and is then delivered to the organism in the sample cup. The system uses a 5-way manifold to deliver the water to five, 8-way manifolds, which each has eight Venturi valves. The dosed seawater is delivered to the distributor, which divides the dosed seawater amongst the ten replicate organisms. The AADAMS provides surge, dosing of forty independent treatments, with ten replicates per treatment, for a total of 400 organisms per experiment.

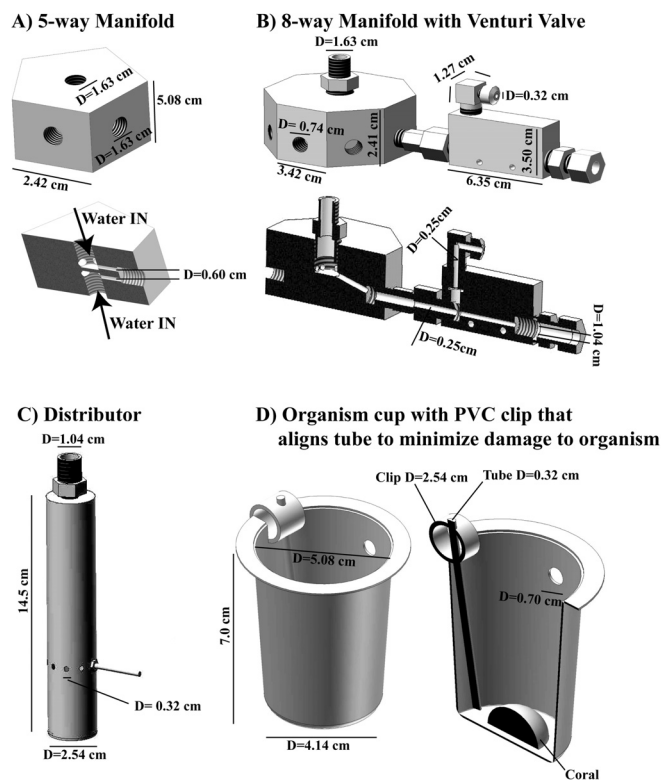
ter was then delivered from the Venturi valve through a 1.2-cm (1/2-inch) silicone tube (#U-96410-82; Cole Palmer), covered in black plastic to minimize algal growth, to a polypropylene 10-way distributor (Teqcom) (Figures 3 and 4). The 10-way distributor delivered the dosed seawater to 10 replicate samples held in 100-mL polypropylene cups (Figures 3 and 4). The average seawater flow rate from the Venturi valves to the distributors was  $132.4 \pm 6.0 \text{ mL min}^{-1}$  ( $\pm \text{SD}$ ).

The test organisms were maintained in 100-mL polypropylene cups with two 0.3-cm (1/8-inch) holes drilled at the 100-mL mark to allow for drainage of the water (Figures 3 and 4). Ten pieces of 0.3-cm (1/8-inch) Teflon tubing, covered in black plastic to minimize algal growth, delivered the dosed seawater from the 10-way distributor to the bottom of the sample cups. The tubes were held in place with a 2.5-cm (1-inch) PVC clip that was attached to the lip of the cup above the drainage holes so that the seawater never contacted the clip (Figure 3). With the intervalometer set to dose the corals for 6 s at full

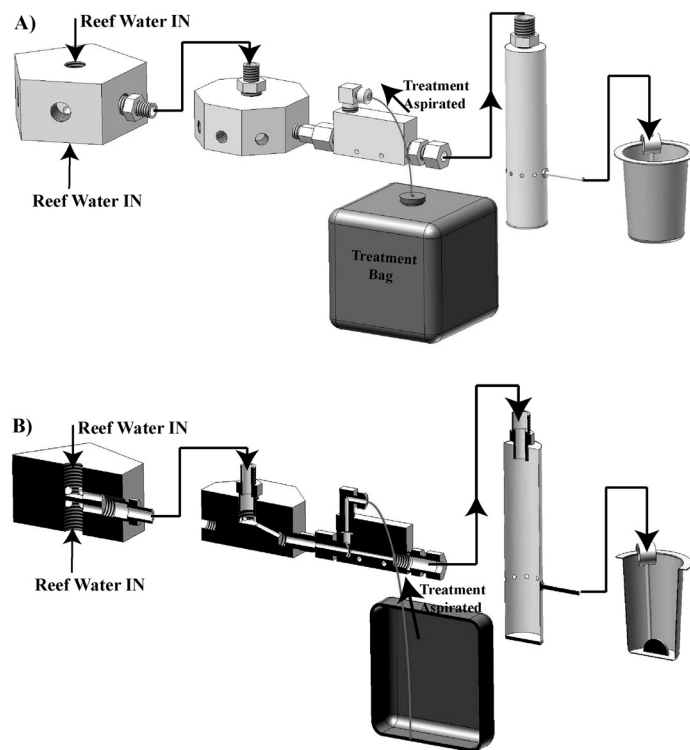
power every minute, the 100 mL of seawater in the cups was changed  $8.3 \pm 2.3$  times per hour ( $\pm \text{SD}$ ).

Five distributors were placed above each plastic water bath on a wooden support (Figure 1), and 50 sample cups were placed in a PVC frame that was held just above the level of flowing reef water to maintain the sample temperature close to that experienced on the reef (Figure 2). The water baths were arranged in 2 rows of 4 water baths, and the outflow from all of the water baths passed through a 25-W UV sterilizer (model 02025; Emperor Aquatics), and then down the length of the dock to a sand filter surrounded by mangrove trees (Figure 2). The outflow was passed through the UV sterilizer, sand filter, and mangrove forest to minimize pollution back into the marine environment. The outflow could be passed through additional sterilizers and filters depending on the experimental reagents being tested and the specific requirements of the country where the system was used.

The AADAMS was assembled at the Smithsonian Tropical Research Institute (STRI) Caribbean research station in Bocas del Toro, Panama. It was set up in a custom-made room to maintain the test organisms at seawater temperatures and light levels similar to those found on the reef (Figure 1). A sky-



**Fig. 3.** Detailed to-scale drawing of the main components of the AADAMS systems with measurements in centimeters and cut-away drawings that reveal the internal plumbing. Both the A) 5-way manifold and B) the 8-way manifold with a Venturi valve attached are made of virgin teflon, while, both the C) 10-way distributor and D) organism cup are made of polypropylene.



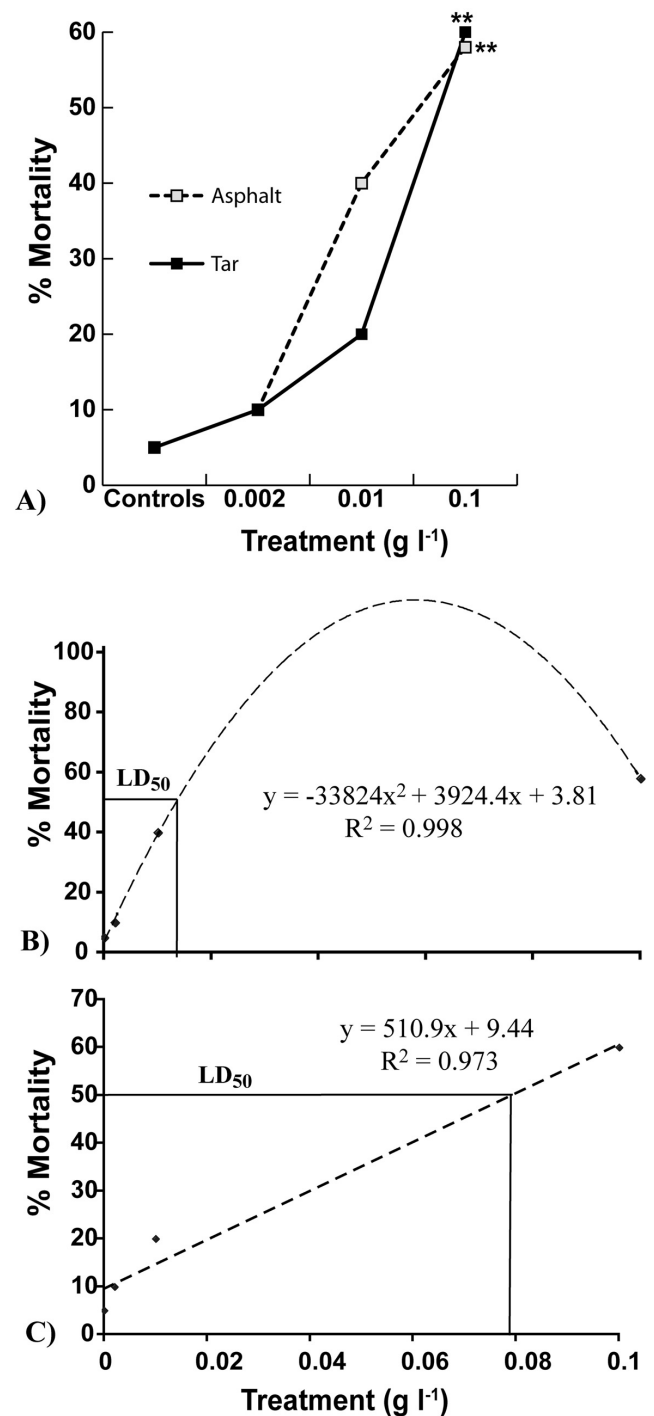
**Fig. 4.** In A) the flow path of the seawater in the system is diagrammed. The reef water is delivered to each of the Venturi valves, which aspirate the treatment and the dosed seawater is then delivered to the ten replicate corals. In B) a cutaway of each of the components reveals their internal plumbing.

light made of clear PVC sheets was used to increase the light level in the room, and walls made of layers of window screen were used to equalize light levels in the water baths. Light measurements (model #LI-193SA; Licor, Lincoln, NE, USA) (4 $\pi$  sensor, and 5-s average readings) were used to determine where to add extra screening to equalize light levels among the baths. Light levels above the baths around noon were  $153.6 \pm 39.6 \mu\text{Einsteins}$  ( $\pm$  SD) on an overcast day, and  $263.7 \pm 53.2 \mu\text{Einsteins}$  on a clear day. There was likely additional daily variability in light levels above the water baths depending on the angle of the sun and patchiness of cloud cover.

Window screening could be added or removed to modify light levels for the conditions required by the test organisms.

A 25-W UV sterilizer was also used to sterilize the seawater used to make up the concentrated chemicals to minimize bacterial contamination of the treatment chemicals. These chemicals were changed once every 2 to 4 days, depending on the Venturi rate.

*Dilution and fluorescein calculations*—Dilution rates were calculated for each Venturi valve at the beginning of each experiment by dividing the flow to the distributor by the volume aspirated from a graduated cylinder. The average dilution for the 40 Venturi valves was  $49.3 \pm 23.2 \text{ mL min}^{-1}$  ( $\pm$  SD). Each



**Fig. 5.** A) Killing curve for road asphalt and roof tar. Both treatments caused significant mortality (Kruskal-Wallis by ranks,  $df=4$ ,  $p<0.05$ ), with the 0.1 g l<sup>-1</sup> treatments causing significant mortality compared to the controls (\*\*, Tukey Honest Significant Difference post hoc test  $p<0.05$ ). B) Road asphalt exhibited a second order relationship between mortality and treatment concentration, causing a lowest dose that killed 50% (LD<sub>50</sub>) of the corals of 0.013 g l<sup>-1</sup>. C) Roofing tar exhibited a linear relationship with a higher LD<sub>50</sub> of 0.079 g l<sup>-1</sup>.

**Table 2.** Variation in the mean dilution, measured weekly, over a 1-month period.

Venturi valve #	Mean dilution								Average dilution	P value
	Week 1	SD	Week 2	SD	Week 3	SD	Week 4	SD		
1	35.9	0.1	35.9	0.1	35.9	0.1	35.8	0.1	35.9	0.5
2	40.9	1.1	41.0	1.1	40.9	1.2	40.9	1.2	40.9	0.4
3	40.3	0.1	40.3	0.1	40.3	0.1	40.3	0.1	40.3	0.7
4	78.5	0.2	78.4	0.0	78.5	0.1	78.5	0.1	78.5	0.6
5	70.5	0.2	70.2	0.4	70.3	0.4	70.6	0.4	70.4	0.5
6	55.5	0.1	55.3	0.3	55.4	0.1	55.4	0.1	55.4	0.6
7	33.7	0.0	33.7	0.4	33.7	0.1	33.7	0.1	33.7	0.7
8	46.3	0.3	46.3	0.3	46.3	0.3	46.3	0.3	46.3	1.0
9	57.9	0.5	57.9	0.6	57.9	0.5	57.9	0.6	57.9	1.0
10	28.3	0.1	28.3	0.1	28.3	0.1	28.3	0.6	28.3	0.9

P values are from a Kruskal-Wallis test. The Venturi valves did not have significantly different dilutions over a 1-month period ( $P \gg 0.05$ ).

treatment concentration was then multiplied by the respective dilution so that it was diluted to the proper concentration after passing through the Venturi valve. For example, for a Venturi valve with a measured dilution of 50, the treatment would be made up to 50 $\times$  so that the test organism would be dosed with the 1 $\times$  concentration. Dilutions were checked periodically throughout the experiments and adjusted if necessary.

Sodium fluorescein was used to determine the accuracy of the dilutions in the course of a 30-day experiment (Smart and Laidlaw 1977; Corbett et al. 2000). Fluorescein dilution rates were determined by aspirating a concentrated fluorescein solution and collecting the water flowing into 3 replicate cups from the distributor. The fluorescein absorbance of these water samples was then measured at a wavelength of 492 nm (Gaspar 1987; Corbett et al. 2000) and the dilution determined by comparison to a standard fluorescein dilution curve.

**Organism collection**—For corals, small colonies were collected using bone shears with care not to damage living tissue. Experiments were set up in a random block design, with 1 replicate for each treatment collected from a different coral colony. In an experiment with 400 corals, 40 replicate corals were collected from each of 10 different colonies. For mangrove oysters (*Isognomon alatus*), individuals were gently removed from mangrove roots and placed in sample cups. Ten oysters were used in each experiment.

**Test organism dosing experiments**—Dosing experiments were conducted using the water-soluble components of road asphalt and roofing tar to determine if there are potentially harmful compounds associated with development (roads and housing construction, respectively). Fresh road asphalt and roofing asphalt were purchased from a local hardware store, weighed, and placed into 50- $\mu$ m nitex plankton mesh bags. The plankton mesh bags were subsequently placed in the 10-L polypropylene chemical bottles that were filled with filtered seawater, and the bags with asphalt or tar were replaced weekly. Three quantities of roofing tar and road asphalt were

tested (0.002, 0.01, and 0.1 g L<sup>-1</sup>) and used in Venturi valves with similar dilution rates, 34.8  $\pm$  7.2 mL min<sup>-1</sup> ( $\pm$  SD), to ensure similar dilutions of the compounds across treatments. In all asphalt and tar treatments, only the dissolvable compounds that could pass through the 50- $\mu$ m nitex bags reached the corals, and at the end of the experiments the solids remained in the plankton bags. In all dosing experiments, mortality was determined visually, by the presence of bare white skeleton without clear bleached coral tissue, and was scored as 25%, 50%, 75%, or 100% for each coral fragment.

### Assessment

**Dosing accuracy**—To determine the consistency and accuracy of the Venturi valves, dilution measurements were made repeatedly on 10 Venturi valves, and dilution accuracy was determined on a subset of these valves using fluorescein measurements. The dilutions were measured 4 times in 10 of the Venturi valves each week during a 30-day experiment (Table 2). In all 10 of the Venturi valves tested, there was not a significant difference in the dilutions throughout the 1-month period (Kruskal-Wallis,  $P \gg 0.05$ ) (Table 2). Furthermore, dosing accuracy was determined at the beginning and at the end of a 30-day experiment, by using the formula:

$$\text{measured dilution/fluorescein dilution} \times 100$$

On day 1 of the experiment, the dilutions used had an accuracy of 91%  $\pm$  12.0%, and on day 27 the accuracy was slightly lower at 87.4%  $\pm$  13.6%. These results confirm that the dilutions determined using the Venturi rates were consistent and accurate throughout a 30-day experiment. The decrease in accuracy in the course of the experiment was likely due to partial clogging of the tubes in the distributors.

**Test organism survival rates**—The AADAMS system was run with 400 specimens in three 30-day experiments and one 60-day experiment. *Montastraea annularis* was used as a test coral, because it is the dominant reef-building coral on

**Table 3.** Mortality rates of 3 coral species and a mollusk after being maintained in the AADAMS for 30 to 60 days.

Species	No. of days	Experiment dates	Percent mortality
<i>Montastraea annularis</i>	29	15 Feb 03 – 16 Mar 03	2/78 corals 2.6%
<i>Montastraea annularis</i>	30	8 April 03 – 9 May 03	6/70 corals 8.5%
<i>Montastraea annularis</i>	30	25 Jun 03 – 24 Jul 03	3.25/50 corals 6.5%
<i>Montastraea annularis</i>	30	23 Sep 03 – 23 Oct 03	5.25/49 corals 10.7%
<i>Montastraea annularis</i>	60	25 Jun 03 – 23 Aug 03	7.25/50 corals 14.5%
<i>Agaricia tenuifolia</i>	30	23 Sep 03 – 23 Oct 03	1/10 corals 10%
<i>Porites furcata</i>	30	8 Apr 03 – 9 May 03	1/10 corals 10%
<i>Porites furcata</i>	30	23 Sep 03 – 23 Oct 03	2.4/9 corals 27%
Mangrove oysters	60	25 Jun 03 – 23 Aug 03	0/10 oysters = 0%

Fractions represent partial mortality.

Caribbean reefs (Goreau 1959; Knowlton et al. 1992). It had low mortality rates during 30-day experiments in the AADAMS system ( $7.1\% \pm 3.4\%$  mortality, range 2.6% to 10.7%,  $n = 247$  corals) (Table 3), and  $14.5\% \pm 8.9\%$  mortality after 60 days ( $n = 20$  corals). The AADAMS was also effective at maintaining other common Caribbean corals including *Agaricia tenuifolia* (10% mortality after 30 days) and *Porites furcata* (10% and 27% mortality after 30 days in May and October, respectively) (Table 3). In future experiments, the survival of *Porites furcata* could be improved by making stands so that the fragments sit upright in the maintenance cup and by increasing the surge frequency for this shallow-water coral. Mangrove oysters were successfully cultured with the AADAMS, with no mortality after 60 days, suggesting that the system would also be suitable for ecotoxicological studies with mollusks.

*Test cases with asphalt and roofing tar*—Exposure of corals to the water-soluble components of both road asphalt and roofing tar caused significant mortality in a 30-day experiment (Kruskal-Wallis by ranks,  $H = 8.92$ ,  $df = 4$ ,  $P = 0.030$  for asphalt and  $H = 11.20$ ,  $df = 4$ ,  $P = 0.025$  for tar). Furthermore, when post-hoc statistical analyses were performed, both road asphalt and roofing tar caused significant mortality compared to controls in the  $0.1 \text{ g L}^{-1}$  treatments (Tukey honest significant difference post-hoc test  $P = 0.027$  and  $P = 0.035$ , respectively) (Figure 5A). Road asphalt had a second-order polynomial relationship between concentration of asphalt and mortality ( $y = -33824x^2 + 3924.4x + 3.81$ ,  $R^2 = 0.99$ ), resulting in an  $LD_{50}$  of  $0.013 \text{ g L}^{-1}$  (Figure 5B). Roofing tar took longer to cause mortality with a linear relationship between grams of asphalt and coral mortality ( $y = 510.91x + 9.44$ ,  $R^2 = 0.97$ ), resulting in an  $LD_{50}$  of  $0.079 \text{ g L}^{-1}$  (Figure 5C). These results suggest that construction of roads and houses in coastal areas could result in coral mortality, and efforts should be taken to minimize development in near-reef areas. Future studies will determine the levels of hydrocarbons released by the asphalt and tar treatments in the experiment and compare it to levels in runoff, to provide further information useful to reef managers. The AADAMS could be used in efforts to develop and test new forms of asphalt and roofing tar that are less damaging to reef areas.

## Discussion

The AADAMS system made it possible to test a large suite of compounds with high levels of replication. Besides the asphalt and tar results that were presented here, other important coral ecotoxicological findings have been made using the AADAMS. One of our major findings was that routinely measured components of declining water quality (phosphate, nitrate, and ammonia) did not directly cause coral mortality, whereas rarely measured components (several forms of dissolved organic carbon) caused significant mortality (Kline et al. 2006). Furthermore, we were able to demonstrate that 3 different Caribbean coral species (*Montastraea annularis*, *Agaricia tenuifolia*, and *Porites furcata*) had different responses when exposed to several concentrations of organic carbon and nutrients, and that continuous exposure to several of the treatments resulted in an increasing probability of mortality over time (Kuntz et al. 2005). These experiments suggest that chronic exposure to a stressor increases the likelihood of mortality and that the response of coral reefs to common water contaminants will likely depend on the species of corals that are exposed and the duration of the exposure.

The AADAMS should be an important system for ecotoxicology experiments with sensitive marine organisms such as corals, and it will likely be useful for a variety of marine or freshwater ecotoxicology applications. For example, it could also be used to determine the genetic effects of pollutants using model organisms such as zebrafish. The AADAMS also makes it possible to test synergistic effects of human stressors to get a more realistic picture of human impacts on marine organisms. Coupled with sophisticated physiological measures of organism health, the AADAMS can improve our understanding of marine and aquatic ecotoxicology.

## Comments and Recommendations

There are several changes to the AADAMS that could improve the system and make it useable with a greater variety of aquatic organisms. The first improvement would be to increase the size of the accumulators to minimize the possibility of backflow, and to increase the size of the tubing delivering the water to the test organisms to increase the potential



flow rates. Another improvement would be to standardize the tube lengths between the 8-way manifolds and the accumulators. This would require redesigning the layout of the system so that it is centralized, but would make the rates more similar between the Venturi valves, allowing easier calibration. It would also be worthwhile to design tube clips that would standardize the orientation of the tubes going into the distributors, which would also help standardize rates across the Venturi valves. A final modification would be to increase the size of the sample cups to accommodate larger organisms in the system. The system could also be modified to provide more realistic hydrodynamic conditions, especially for shallow-water coral species. *Montastraea annularis*, a common mid-water coral species, had high survival rates in the system, suggesting that the surge created once a minute in the AADAMS was sufficient for these corals. However, *Porites furcata*, a shallow-water coral species, had lower survival rates, suggesting that more regular water changes might be necessary to mimic shallow-water hydrodynamic conditions and that stands are necessary to properly situate these corals. Future systems can be modified to allow for greater surge generation with more frequent dosing.

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