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An experimental comparison of sediment-based biological filtration designs for recirculating aquarium systems

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Abstract

Common sediment filtration designs for recirculating systems designed for marine ornamentals fall largely into two major categories: sandbed and plenum-based systems. To date, there has been no experimental comparison of the relative performance of these methods for handling nitrogenous wastes in marine aquaria. We compared nutrient levels in a factorial design of aquaria: (1) with or without a plenum; (2) with deep (9.0 cm) or shallow (2.5 cm) sediments; and (3) with coarse (2 mm) or fine (0.2 mm) mean particle sizes. None of these experimental treatments have a significant advantage in the processing of nitrogenous wastes in recirculating aquaria; final ammonia and nitrite concentrations were below detectable levels, and nitrate concentrations did not differ significantly among the experimental treatments. After an initial stabilization period, most experimental treatments responded equivalently to continuous ammonium input of up to 0.5 mg/l/day. Results were qualitatively similar whether experiments were carried out in the absence of animals in a lab with nutrient input via measured dosing of ammonium chloride, or in aquaria with live animals and natural sediments. Sediment depth and particle size had significant effects on a variety of water parameter measures throughout the experiment. Overall, coarse sediments had lower buffering capacity (pH, calcium and alkalinity) and much higher final phosphate concentrations than fine sediments. Death rates in the live animal experiments containing shallow sediments were roughly twice (2.91 ± 0.46) those of the deep sediment trials (1.47 ± 0.46). Regardless, the presence or absence of a plenum had little effect on water parameters throughout the experiment. These results suggest that there is little benefit to be gained from the addition of a plenum plate beneath the sediments in recirculating aquarium designs.

Keywords: Recirculating aquarium; Nitrification; Denitrification; Ammonia; Nitrite; Nitrate

1. Introduction

In addition to traditional public and private aquarium applications, there is growing interest in recircu-

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lating systems from aquaculture (reviewed by van-Rijn, 1996). Interest in zero emission recirculating systems has been fueled by a combination of limited suitable water supplies and stringent control of wastewater and nutrient discharges from pond and raceway facilities (e.g., Palacios and Timmons, 2001; Suzuki et al., 2003; Tango and Gagnon, 2003). Public aquaria and hobbyists at home have long used a variety of recirculating systems based on some form of sediment filtration to aid in the processing of nitrogenous wastes produced by tank inhabitants (e.g., Adey and Loveland, 1991; Atkinson et al., 1995; Carlson, 1999; Borneman and Lowrie, 2001). The design of the most common sediment filtration units for recirculating systems designed for marine ornamentals fall largely into two major types: sandbed and plenum-based systems. A third popular design, the "Berlin" system, involves only a shallow coating of coarse sediments on the bottom of the aquarium and reliance on "live rock" and protein skimming as the primary filtration for the aquarium rather than the sediments. However, there is considerable debate about the relative performance of these various systems, and no controlled and replicated experiments to test their relative performance.

Plenum-based systems gained widespread interest when Jean Jaubert worked with the Monaco Aquarium in transporting a complete live portion of a coral reef from the Red Sea for display at the Aquarium. Jaubert worked extensively with naturally collected coral substrates to enhance captive biological filtration in captive aquaria, and was granted a French patent for the plenum design in the late 1980s followed by a US patent in 1991. This system consists of essentially a mesh partition suspended just above the bottom of the aquarium onto which the sediments are piled. This design ensures that there is a sediment-free "void space" beneath the aquarium gravel, but the open mesh allows water and dissolved nutrients easy access to the void space by diffusion through the sediments. Based largely on the success of the Monaco Aquarium's "Microcean" display, this plenum-based aquarium design has become one of the primary design methods used by public aquaria around the world, and for nearly a decade was almost equally popular among hobbyists maintaining home aquaria.

However, in the past decade, more US hobbyists have begun to simply deposit an equivalent volume of sediments directly onto the bottom of the aquarium, and these "deep sandbed" systems have supplanted plenum-based systems in popularity. Thus, in a deep sandbed system there is no sediment-free void space beneath the sediments. The reliance on a thick bed of carbonate sediments is essentially the same as that of plenum-based system, but the necessity and utility of the void space beneath those sediments has been vigorously questioned.

There have been numerous articles and books written in the aquarium hobby about the advantages and disadvantages of such systems to recirculating systems (e.g., Adey and Loveland, 1991; Tullock, 1997; Goemans, 1999; Shimek, 2001; Hovanec, 2003; Delbeek and Sprung, in press), and there remains considerable debate about the most efficient design of a sediment bed for processing nutrients in a recirculating system. However, to date, nearly all public aquaria continue to follow the plenum-based tank design, but there has been no comparative experiment to determine whether or not the presence of a void space beneath the sediments confers any advantage relative to the presence of the deep sediments themselves. Likewise, the optimal sediment particle size and the depth of the sediment bed, are also hotly debated in the popular literature (reviewed by Toonen, 2000a,b). The plenum-based system remains the most common design used by large public aquarium facilities. Among home aquarists, the Berlin and plenum-based systems are most popular in Europe, whereas the deep sandbed system seems to be the more popular design in the United States.

Despite the widespread utility of these methods to academic institutions, Museums and Aquaria, and the general hobbyist, to date there has been no systematic test of the relative nutrient processing capacity of these basic designs for recirculating systems. Therefore, this study tests the relative nutrient processing capacity of recirculating systems based on in-tank sediment filtration designs. Here we examine the relative contribution of a void space (sediment bed with or without plenum), the sediment depth (2.5 cm versus 9.0 cm), and the mean particle size of sediments (2.0 mm versus 0.2 mm mean particle diameter) in aquaria to their nutrient processing capacity and performance as a lone filtration method for recirculating aquaria.

2. Materials and methods

2.1. Aquarium dosing experiments

We set up a factorial design experiment with three replicate tanks (27 cm long × 17 cm wide × 30 cm high) for each factor: with or without plenum, deep or shallow, and coarse or fine sediments for a total of 24 experimental aquaria (Fig. 1). Treatments were assigned to aquaria by use of a random number generator; if an aquarium was already assigned to a previous treatment, another random number was drawn until all treatments were assigned to a single aquarium. Aquaria were maintained in a temperature-controlled room (25 ± 0.5 °C) in constant darkness other than when water samples were being tested. Water tests were conducted within a consistent 3-h time frame every other day.

Deep sandbed treatments had sediments deposited directly onto the aquarium bottom. Plenum treatments had sediments suspended on a plate constructed from fine nylon shade cloth attached to a 1-cm plastic "egg-crate" light diffusion grating with hot-melt glue. This plate was suspended 1.5 cm from the bottom of the tank by five 1.27 cm (½ in.) ID diameter PVC rings attached with hot-melt glue to each corner of the plate and one in the center. Wet sediments were deposited carefully onto this plate prior to addition of water to the aquaria to minimize any sediments percolating through the nylon mesh into the plenum void space.

Deep sediments treatments contained 9.0 l of wet sediment to provide a constant depth of roughly 9.0 cm. Shallow sediment treatments contained 2.5 l of wet sediment to provide a constant depth of roughly 2.5 cm. Florida crushed coral (mean particle size ~2.0 mm × 4.0 mm) was used for the coarse sediments and Southdown Tropical Play Sand (mean particle diameter ~0.2 mm) was used for fine sediments. Twenty-five kilograms of each was purchased from a local pet shop and autoclaved prior to use. Autoclaved sediment of each type was placed into separate containers held in a single large holding tank. The holding tank

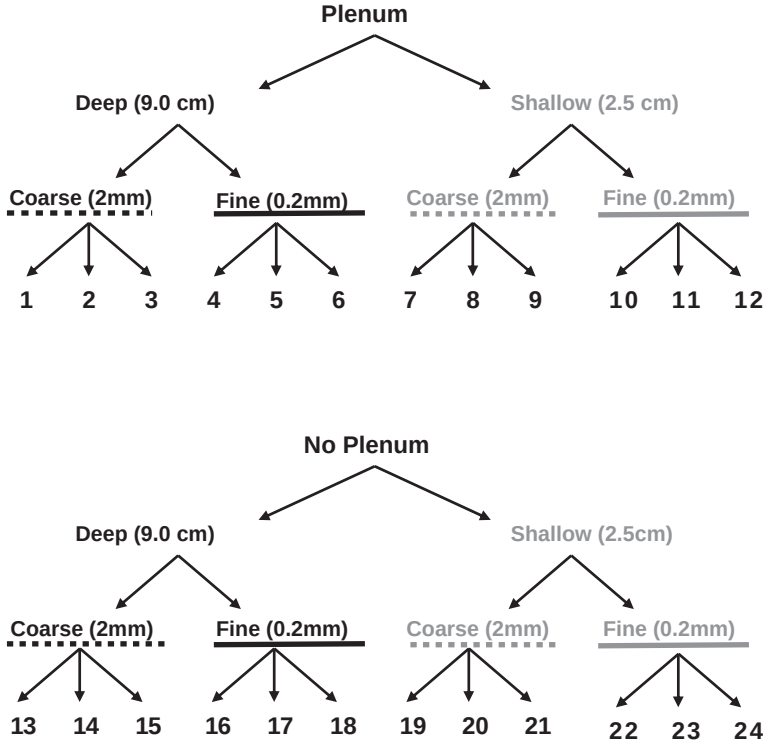

Fig. 1. Schematic of aquarium design experiment to compare directly the effects of the presence or absence of a plenum, the depth of the sediment bed, and the mean particle size of sediments in recirculating aquarium systems.

was filled with ~400 l of 2.0 μm-filtered natural seawater and 30 ml of homogenized frozen squid (*Loligo* sp.) was added as a nutrient source to facilitate growth of bacteria on the sterile sediments. Sediments were well mixed by hand every other day for 8 weeks until total ammonia readings in the holding tank dropped to undetectable levels with a standard aquarium test kit.

After sediment treatments had been placed in each tank, 8.0 l of 2.0 μm-filtered natural seawater was trickled into each aquarium to prevent any disturbance of the sediment beds. Water was circulated in the aquaria using a Catalina Aquariums CAP-180 powerhead set to 50% (~190 l/h) flow placed at the end of each aquarium such that the top of the powerhead was even with the surface of the aquarium water. 3M ammonium chloride (NH_4Cl) was added to each tank the following day to a final concentration of 8 mg NH_4^+ per liter. Twenty-eight days after that initial dose of ammonium, dosing resumed with 3M NH_4Cl at the rate of 0.5 mg NH_4^+/l/day. High-density aquaculture systems typically generate 0.5–2.0 mg NH_4^+/l/day (Tseng and Wu, 2004). Thus, our nutrient loading is at the low end of production aquaculture systems, but considerably higher than typical of marine ornamental systems. The experiment was continued for an additional 111 days without any water exchange. To account for evaporation within experimental aquaria, deionized distilled water was added to reduce the salinity to ~53 mS after each testing period.

2.2. Live animal aquarium experiments

Three replicate tanks for each factor were set up exactly as outlined above (Fig. 1). Sediments from the previous experiment were removed from each tank and combined together along with an equivalent volume of natural sediments of roughly equivalent size collected from the lagoon at Coconut Island (Hawaii Institute of Marine Biology, Kaneohe, HI). These sediments, along with the natural infauna, were mixed thoroughly by hand and then redistributed among the aquaria as detailed above. Aquaria were allowed to settle for 1 week without any nutrient additions prior to the introduction of live animals. Aquaria were maintained outside under shade cloth at the Hawaii Institute of Marine Biology. Lighting and temperature fluctuated with ambient throughout the experiment. Water parameters were tested (see below) for each aquarium at the end of this week to determine the starting conditions for each trial aquarium.

After the 1 week stabilization period, we added 1 kg of "live rock" (consisting of 1–3 pieces of natural coral rubble collected from the nearby reef), a single Hawaiian sharpnose puffer (*Canthigaster jactator*), a small rock urchin (*Echinometra oblongata*), 10 left-handed hermits (*Calcinus laevimanus*), and 10 snails (5 *Littorina* sp. and 5 *Nerita* sp.) to each aquarium. Fish were fed squid (*Loligo* sp.) pellets ad libitum each week day (unfed on weekends) until they did not ingest the final pellet offered to them (after Pawlik et al., 1995). The final uneaten pellet was left in the aquarium to provide food for scavengers in the tank. Any deaths of animals in the aquaria were recorded at each testing period, and replacement animals were added as necessary to maintain a constant bioload of each treatment throughout the experimental period.

The experiment was continued for 111 days after the addition of live animals without any water changes, for a total of 118 days experimental run. Again, the salinity of each aquarium was adjusted to ~53 mS every other day as outlined above.

2.3. Aquarium water testing

All tanks were initially filled from a large holding tank of well-mixed natural seawater, and a single 50 ml sample of this water was collected and frozen at −80 °C pending water analyses. Likewise, a single 50 ml sample from each treatment aquarium was collected on day 111 of the dosing experiments and day 118 of the live animal treatments, and also frozen at −80 °C. At the completion of the experiment, all water samples were transported frozen to the University of Hawaii at Manoa and inorganic nutrient concentrations were determined using colorimetric methods (APHA, 1998) on a Technicon AutoAnalyzer as outlined in Laws et al. (1999).

Each experimental aquarium was also tested at least twice per week for salinity, pH, ammonia, nitrite, nitrate, oxygen, phosphate, calcium, alkalinity, and organics using aquarium testing equipment. Salinity was determined using an electronic PinPoint salinity meter (calibrated to 53.0 mS using IAPSO seawater) and pH was measured with the electronic PinPoint pH

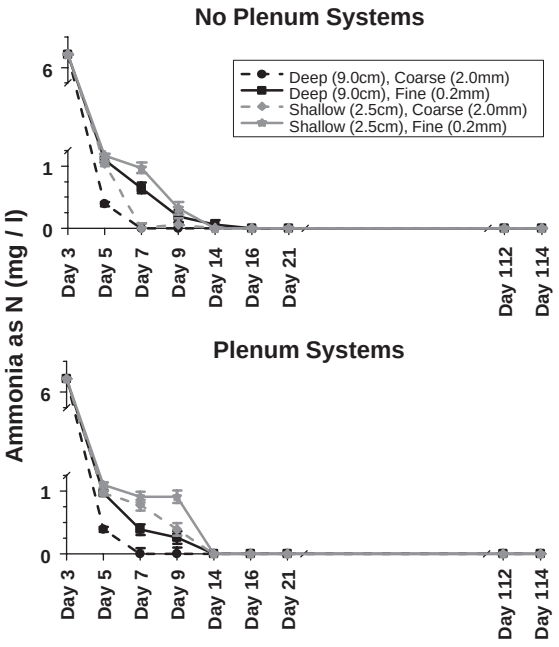

Fig. 2. Time series plot of the mean ammonium (NH_4^+ as N) concentration (mg/l) in experimental aquaria.

probe (after 2-point calibration to 7.0 and 10.0). All other water parameters were measured using hobbyist Salifert aquarium test kits compared to colorimetric standards. Comparisons of the nutrient concentration in initial and final water samples determined by Auto-Analyzer to the results obtained from the Salifert aquarium test kits were sufficiently correlated ($r^2=0.71$, $F=21.38$, $p<0.01$) to use the aquarium test kit values as a relative measure of aquarium nutrients throughout the experiment.

2.4. Statistical analyses

All statistical analyses were carried out using an analysis of variance as implemented in JMPin ver. 4.0.2 Academic Version (SAS Institute Inc.). We first confirmed conformity to assumptions of normality using Shapiro–Wilks, and homogeneity of variance using Bartletts test ($\alpha=0.01$) as implemented in JMP. The full ANOVA model used as the presence or absence of a plenum, the mean particle size of sediments, the depth of the bed and interactions among them as fixed effects; the salinity, pH, ammonia, nitrite, nitrate, oxygen, phosphate, alkalinity, and calcium were measured as response variables. Significant differences among treatment pairs (plenum versus none; fine versus coarse particles; deep versus shallow sediments) were determined for each response variable through effect tests as implemented in JMP. Data were plotted using PSI Plot version 7.01 (Poly Software International, Inc.).

3. Results

3.1. Aquarium dosing experiments

Time-series of total ammonia ($NH_3+NH_4^+$ as N), nitrite (NO_2^--N), and nitrate (NO_3^--N) concentrations in aquaria showed little difference among treatments (Figs. 2–4). After the initial 21 days, there were no significant differences among total ammonia, nitrite, nitrate, pH or salinity measured for any treatments through the end of the experiment (data not shown). Analyses of variance also revealed no significant differences among the final concentrations of organics, ammonia, nitrite, nitrate, oxygen or salinity, nor were there any significant interactions among experi-

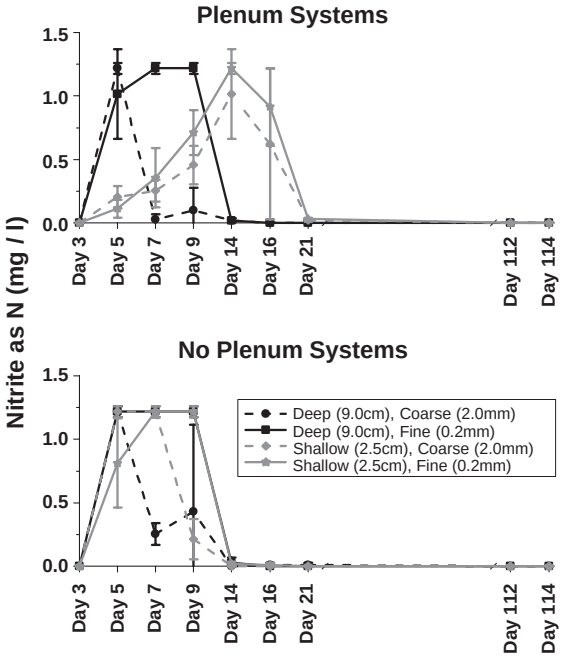

Fig. 3. Time series plot of the mean nitrite (NO_2^- as N) concentration (mg/l) in experimental aquaria.

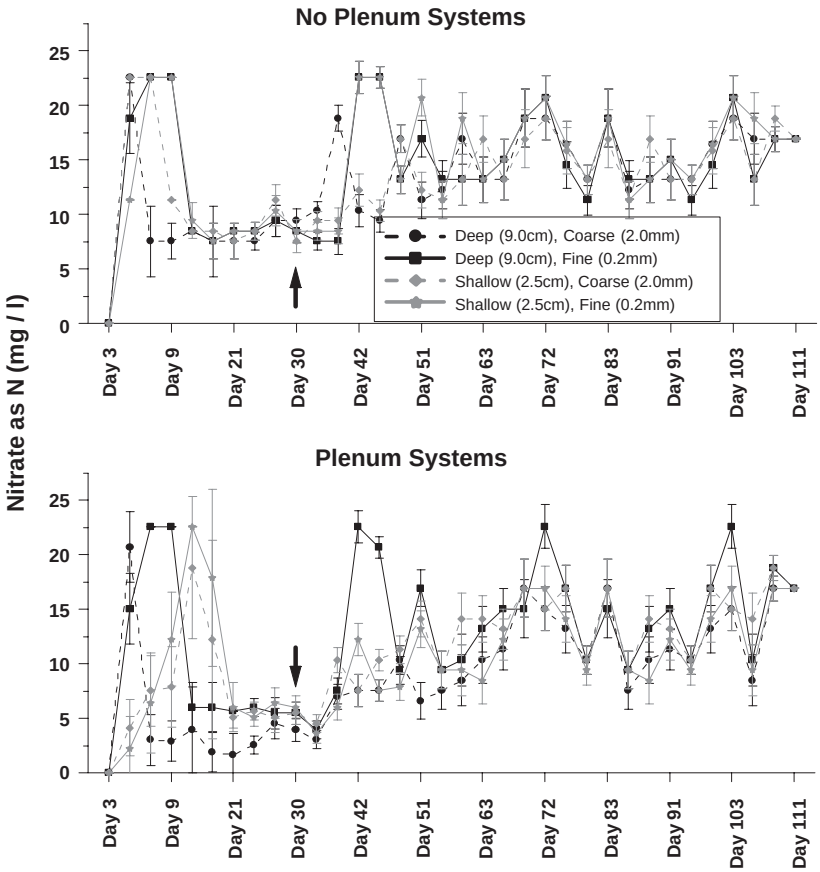

Fig. 4. Time series plot of the mean nitrate as N (NO_3^- as N) concentration (mg/l) in experimental aquaria. Arrows designate the start of continuous dosing of ammonium (see text).

mental treatments for any of these water parameters (data not shown). However, there were significant differences among treatments for each of the remaining water parameters: pH, phosphate, alkalinity and calcium. By the end of the experiment, pH was significantly higher in aquaria with fine (7.98 ± 0.01 S.E.) than coarse (7.91 ± 0.01 S.E.) sediments ($df=1$, $F=10.31$, $p<0.01$). Orthophosphate ended up significantly higher in aquaria with coarse (0.32 mg/l ± 0.01 S.E.) than fine (<0.01 mg/l ± 0.01 S.E.) sediments ($df=1$, $F=211.37$, $p<0.001$). Alkalinity was significantly higher in tanks with fine (2.36 meq $CaCO_3$/l ± 0.08 S.E.) than with coarse (1.80 meq $CaCO_3$/l ± 0.08 S.E.) sediments ($df=1$, $F=23.21$, $p<0.001$), and tanks with plenums (2.20 meq/l ± 0.08 S.E.) than without plenums (1.99 meq/l ± 0.08 S.E.) ($df=1$, $F=4.86$, $p<0.05$). No other source variables or interaction terms were significant for final pH, orthophosphate, or alkalinity values. Finally, calcium levels in the experimental aquaria were significantly different among depth and sediment particle size treatments, and there were significant interactions between the presence of a plenum and both particle size and sediment depth (Table 1).

Overall, the presence or absence of a plenum made little difference to the performance of sediments in processing aquarium nutrients (Fig. 5). There was no difference among the final concentrations of any nitrogenous waste in tanks with or without a plenum. Likewise, sediment depth had little overall effect on the ultimate values for water parameters in any of the experimental treatments (Fig. 6). The greatest effect observed in the dosing experiment was on parameters associated with the

Table 1
Analysis of variance for final calcium levels with respect to the presence or absence of a plenum beneath sediments (plenum), the mean particle size of sediments (size), and sediment depth (depth) in aquarium dosing experiments

Source	*df*	MS	*F*	*p*-value
Plenum	1	16.67	0.02	0.88
Size	1	4816.67	6.97	0.02
Depth	1	4266.67	6.17	0.02
Plenum × size	1	4816.67	6.97	0.02
Plenum × depth	1	4816.67	6.97	0.02
Size × depth	1	266.67	0.39	0.54
Plenum × size × depth	1	1666.67	2.41	0.14
Overall model	7	2952.38	4.27	0.007
Error	16	691.67		

buffering capacity of sediments in the mean particle size trials rather than nitrogenous waste processing capacity (Fig. 7).

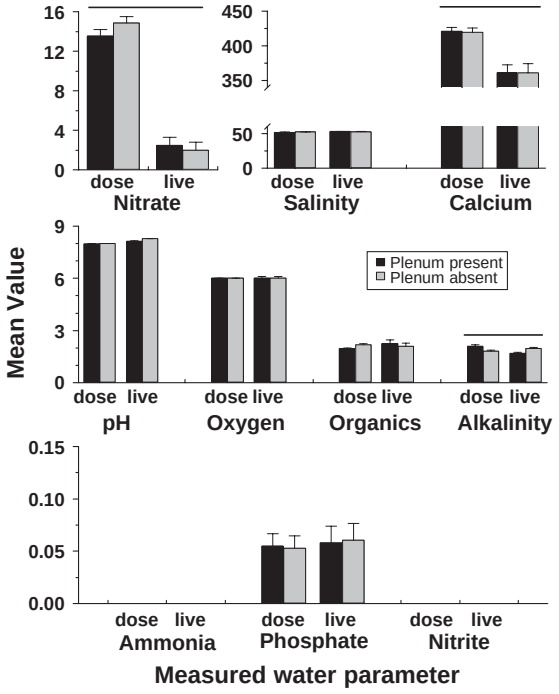

Fig. 5. Comparison of final nutrient concentrations in experimental aquaria with and without plenums. Results of aquarium dosing experiments (dose) and live animal (live) treatments are presented side-by-side; treatments that are significantly different are indicated by a line above the bars. Salinity is measured in mS, alkalinity in meq $CaCO_3$, and organics are presented as a relative colorimetric measure. Nitrate as N, calcium, oxygen, ammonium as N, orthophosphate as P, and nitrite as N are all presented in mg/l.

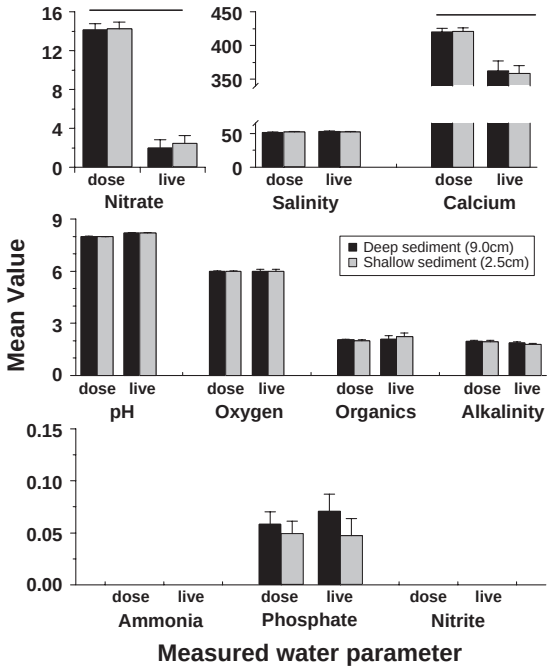

Fig. 6. Comparison of final nutrient concentrations in experimental aquaria with deep (9.0 cm) and shallow (2.5 cm) sediments. Results of aquarium dosing experiments (dose) and live animal (live) treatments are presented side-by-side; treatments that are significantly different are indicated by a line above the bars. Salinity is measured in mS, alkalinity in meq $CaCO_3$, and organics are presented as a relative colorimetric measure. Nitrate as N, calcium, oxygen, ammonium as N, orthophosphate as P, and nitrite as N are all presented in mg/l.

We also examined the rate of nutrient processing in the week after dosing each tank to 8 mg NH_4^+/l. Ammonia levels dropped faster in deep sandbed tanks than in aquaria with plenums ($df=1$, $F=8.21$, $p=0.01$), tanks with coarse sediments ($df=1$, $F=169.92$, $p<0.01$), and those with deep beds ($df=1$, $F=121.38$, $p<0.01$). There was also a significant interaction between the presence or absence of a plenum and the depth of sediments ($df=1$, $F=24.64$, $p<0.01$). Given the increased rate of ammonia processing, it is not surprising that nitrate accumulated faster over the first week in tanks with deep sandbeds than aquaria with plenums ($df=1$, $F=34.45$, $p<0.001$). As with ammonia, there was also an interaction between the presence or absence of a plenum and the depth of sediments ($df=1$, $F=19.74$, $p<0.01$). The rate at which nitrate levels subsequently decreased was roughly equivalent among treatments with the exception of shallow sedi-

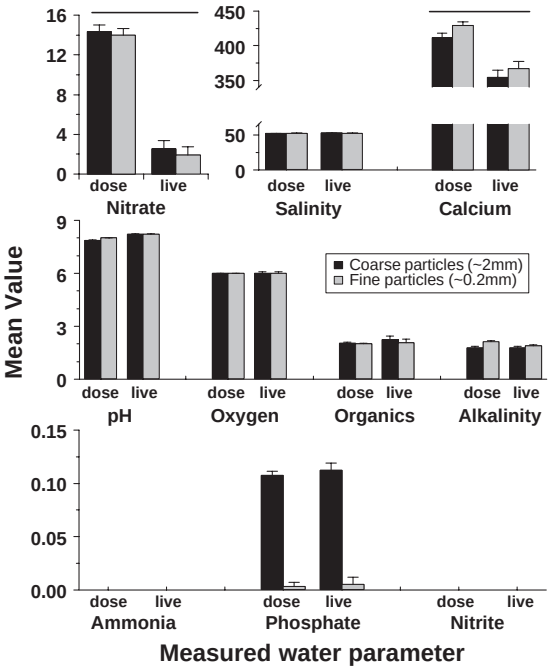

Fig. 7. Comparison of final nutrient concentrations in experimental aquaria with coarse (2.0 mm mean diameter) and fine (0.2 mm mean diameter) particles. Results of aquarium dosing experiments (dose) and live animal (live) treatments are presented side-by-side; treatments that are significantly different are indicated by a line above the bars. Salinity is measured in mS, alkalinity in meq $CaCO_3$, and organics are presented as a relative colorimetric measure. Nitrate as N, calcium, oxygen, ammonium as N, orthophosphate as P, and nitrite as N are all presented in mg/l.

ments above a plenum, which took significantly longer to drop to the same level as all other treatments (Fig. 4). There were no significant differences among the rate of nitrite change in the presence or absence of a plenum ($df=1$, $F=1.10$, $p>0.1$). The only significant effect on nitrite levels was particle size, in which tanks with fine particles took longer to process nitrite than tanks with coarse sediments ($df=1$, $F=51.19$, $p<0.01$).

3.2. Live animal aquarium experiments

Time-series plots of measured nutrients in the live animal experiments were very similar to those of the dosing experiments and are therefore not presented here. However, the variances are uniformly larger among treatments including live animals than in the dosing experiments. As with the dosing experiments above, time-series of pH, salinity, total ammonia, nitrite and nitrate concentrations in aquaria showed no significant differences among treatments (data not shown). Analyses of variance for each water parameter revealed no significant differences among the final salinity, ammonia, nitrite, oxygen, or organic concentrations, nor were there any significant interactions among experimental treatments for any of these water parameters (data not shown). There were significant differences among treatments for the remaining water parameters as outlined below.

By the end of the experiment, pH was significantly higher in aquaria with fine (8.22 ± 0.02 S.E.) than coarse (8.10 ± 0.02 S.E.) sediments ($df=1$, $F=7.68$, $p=0.01$). For final nitrate concentration, the overall analysis of variance was not significant ($df=7$, $F=1.25$, $p=0.34$). However, there was a significant particle size by depth interaction effect ($df=1$, $F=6.48$, $p=0.02$), in which deep, coarse (6.19 mg/l $\pm$ 2.07 S.E.) and shallow, fine (4.61 mg/l $\pm$ 1.22 S.E.) sediments have the highest average final nitrate concentration, while shallow, coarse (2.73 mg/l $\pm$ 1.46 S.E.) and deep, fine (0.15 mg/l $\pm$ 0.10 S.E.) sediments consistently had the lowest final nitrate as nitrogen concentrations. Orthophosphate ended up significantly higher in aquaria with coarse (0.35 ppm $\pm$ 0.02 S.E.) than fine (0.02 ppm $\pm$ 0.02 S.E.) sediments ($df=1$, $F=119.69$, $p<0.01$). Orthophosphate was also significantly higher among deep (0.22 mg/l $\pm$ 0.02 S.E.) than among shallow (0.15 mg/l $\pm$ 0.02 S.E.) sediment treatments ($df=1$, $F=5.70$, $p=0.03$), although this comparison becomes non-significant after Bonferroni correction. Alkalinity was significantly higher in tanks with fine (1.97 meq/l $\pm$ 0.06 S.E.) than with coarse (1.69 meq/l $\pm$ 0.06 S.E.) sediments ($df=1$, $F=12.03$, $p<0.01$). Finally, calcium concentrations were significantly higher in tanks with fine (340.42 mg/l $\pm$ 2.89 S.E.) than with coarse (327.92 mg/l $\pm$ 2.89 S.E.) sediments ($df=1$, $F=9.35$, $p<0.01$).

No other source variables or interaction terms were significant; however, trends in the data were very similar to those observed in the dosing experiments. Overall, all but one of the comparisons that were significant in the dosing experiments were also close to significant ($0.1<p>0.05$) despite the higher variability in live animal trials; these results suggest that an increased sample size may have shown identical trends among the two experiments. In turn, this similarity among treatments with and without live organisms suggests that the presence of organisms plays

little role in the nutrient processing. The only parameter that showed significant opposite effects between the dosing and live animal experiments was alkalinity in the presence or absence of a plenum (Fig. 5).

Finally, we also enumerated animal deaths in the live animal experiments. Each animal in the experiment was treated as equivalent, and the total number of individuals that required replacement (regardless of taxon) throughout the length of the experiment was compared among treatments. Although the overall analysis of variance was not significant ($df=7$, $F=0.88$, $p>0.5$), there was a significant effect of sediment depth on the death rate. On average 2.91 ± 0.46 animals had to be replaced in the shallow sediment treatments, whereas only 1.47 ± 0.46 animals had to be replaced in the deep sediment trials ($df=1$, $F=5.23$, $p<0.05$). No other treatment or interaction term significantly affected the death rate in these experimental aquaria. However, it is noteworthy that the variance in death rates among treatments was significantly greater (Bartlett's test, $F=3.75$, $p=0.05$) in tanks with plenums than those with a sandbed.

4. Discussion

Public aquaria and hobbyists at home have long used recirculating systems based on some form of sediment filtration to aid in the processing of nitrogenous wastes produced by tank inhabitants (reviewed by Carlson, 1999; Borneman and Lowrie, 2001). The design of popular sediment filtration units for recirculating systems to culture coral reef organisms falls largely into three major types: Berlin, sandbed and plenum-based systems. However the relative utility of each of these types, and the most effective means to design them are still a subject of considerable controversy (reviewed by Toonen, 2000a,b). Here, we present the first experimental data directly comparing these popular recirculating aquarium designs. Based on results from a factorial design experiment, we show that plenum and deep sandbed designs are approximately equally effective in maintaining suitable water parameters for recirculating aquarium systems; both depth and particle size have a greater effect on aquarium nutrient levels than does the presence or absence of a plenum. The shallow, coarse sediment treatment from the live animal trials is closest in design to that of the Berlin system, but without a protein skimmer no direct comparison can be made.

In terms of processing nitrogenous wastes from aquarium inhabitants (specifically ammonia, nitrite and nitrate), none of the experimental treatments appeared to have a significant advantage (Figs. 5–7). After the initial stabilization period, all experimental treatments responded equivalently to continuous input of up to 0.5 mg NH_4^+/l/day. None of the systems appeared to reach their maximum processing capacity, because within 2 weeks of the start of each experiment, no ammonia or nitrite was detectable using aquarium test kits through the end of the experiment (Figs. 2 and 3). Likewise, the rate of increase of nitrate in the experimental tanks was far less than the rate of addition to the aquaria (Fig. 4). Comparisons of the initial rate of nutrient processing after dosing aquaria to 8 mg NH_4^+/l show that deep beds of coarse sediments without a plenum processed nitrogenous wastes at a significantly higher rate than other treatments. However after the first week, a comparison of the rate of nitrate accumulation among treatments throughout the remainder the dosing experiments revealed that they were not significantly different (Fig. 4). Thus, each sediment filtration design tested herein appeared capable of handling the test bioload, and there did not appear to be any significant benefits for nitrogenous waste processing derived from any of these various experimental designs.

Denitrification must have occurred in all experimental treatments, because nitrate concentrations did not continue to climb throughout either the dosing (Fig. 4) or live animal (similar results, data not shown) experiments. Despite continued addition of 0.5 mg NH_4^+/l/day in the dosing experiment, it was metabolized quickly enough to keep total ammonia and nitrite at undetectable levels (Figs. 2 and 3). Nitrate concentration in each tank stabilized at a fairly constant level throughout the latter 60 days of the experiment (Fig. 4). However, similar to the results seen with ammonia and nitrite processing, there were no significant differences in the ability of any experimental design to reduce nitrate in these closed systems.

The significant differences among treatments in this experiment had little to do with nitrogenous waste processing capacity, and were instead related primarily to the buffering capacity of the sediments on the recirculating tank water (Figs. 5–7). The interac-

tion of sediment bed depth and particle size made the greatest difference to the overall performance of the system throughout these experiments, and the presence or absence of a plenum beneath those sediments showed no significant effect in any experimental parameter measured (Fig. 5).

With a single exception, the results of the live animal experiments were not qualitatively different from those of the animal-free dosing experiments (Figs. 5–7). The similarity of the dosing and live animal trials is noteworthy because of the presence of infauna as well as differences lighting and temperature between the trials. The dosing trials were conducted at a constant temperature (25 ± 0.5 °C) in complete darkness, whereas the live animal experiments were conducted under ambient temperatures and lighting. Despite the differences in environmental conditions between the trials, results were largely similar; only alkalinity showed the opposite pattern of significance in the presence or absence of a plenum among the dosing and live animal experiments (Fig. 5). The similarity in results between these trials suggests that temperature, lighting, and the presence of live rock and infauna make little overall difference to the outcome of the experiment.

Although final concentrations of nitrate and calcium did not vary among plenum, sediment depth or particle size treatments within either the dosing or the live animal experiments, both differed significantly between the two experiments. Nitrate concentrations (NO_3^--N) of experimental aquaria in the live animal experiments (3.42 ± 3.96) were significantly lower than those of the dosing experiments (14.18 ± 3.27) ($df = 1$, $F = 150.33$, $p < 0.01$). Likewise, final calcium concentrations of experimental aquaria with live animals (334.17 ± 11.81) were significantly lower than those in the aquarium dosing experiments (446.67 ± 37.15) ($df = 1$, $F = 199.95$, $p < 0.01$).

We cannot exclude the possibility that the presence of live animals in the aquarium may alter the buffering capacity or the rate of denitrification in the system. However, the most likely explanation for reduced final calcium concentrations is uptake by organisms in the trial aquaria. There are at least three additional potential explanations for the differences between the live animal and dosing trials in the final nitrate concentration. First, the presence of the coral rubble ('live rock') in the live animal trials may have increased the biological filtration capacity, and could provide increased denitrification and result in reduced final nitrate concentrations. Second, the waste introduced to the aquarium by the live animals was likely far lower than 0.5 mg NH_4^+/l/day. Based on a rough calculation of size-specific nitrogenous waste production from Qian et al. (2001), we estimate that the rate of ammonium production in the live animal trials was ~0.05–0.08 mg/l/day. Finally, the live animal trials were conducted in a protected outdoor shelter, rather than in a darkened temperature-controlled laboratory. Therefore, the presence of benthic algae in the live animal treatments could possibly account for substantial nitrate uptake relative to the aquarium dosing treatments. Further experimentation is required to address the specific cause of the reduced nitrate concentrations in the live animal trials, but ultimately the majority of the nutrient processing capacity appears to be explained by microbial processes and the presence of live animals had little overall effect on the patterns observed (Figs. 5–7).

Perhaps the most perplexing result from this experiment is the significant interaction of sediment particle size and depth in the aquaria. It is hard to explain why deep, coarse (6.19 mg/l ± 2.07 S.E.) and shallow, fine (4.61 mg/l ± 1.22 S.E.) sediments have the highest average final nitrate concentration, while shallow, coarse (2.73 mg/l ± 1.46 S.E.) and deep, fine (0.15 mg/l ± 0.10 S.E.) sediments consistently had the lowest final nitrate concentrations. Nitrate reduction in deep, fine sediments is easily explained by reduced oxygen penetration to the sediments. However, the higher final nitrate concentrations in aquaria with deep, coarse and shallow, fine sediments relative to the shallow, coarse treatment is harder to rationalize. Additional research will be required to explain the source of denitrification in shallow, coarse sediments and account for this unexpected result.

Overall, these results suggest that there is little difference among these common sediment filtration designs for maintaining suitable water parameters for recirculating aquarium systems. There were no significant differences among depth, particle size or plenum treatments for the processing of ammonia or nitrite in recirculating aquarium systems. Deep, fine sediments had the lowest average final concentration of nitrate in these trials, but these values were not significantly less than the average final concentra-

tion of nitrate in shallow, coarse sediment treatments. The presence or absence of a plenum had no significant effects on any measured water parameter in this study. Most significant differences among the experimental treatments were seen in the buffering capacity of the sediments rather than the processing of nitrogenous waste products. The largest differences among treatments were seen in the final concentration of phosphate: coarse sediment treatments had roughly 17 times the final concentration of aquaria in fine particle treatments. However, it is important to remember that the sediments for this experiment were purchased from a pet shop, and the chemical composition of fine and coarse sediments may also differ.

Ultimately, the exact concentrations of any of water parameter are likely of less concern to aquarists than whether or not animals survive in their aquaria. The only significant effect recorded in this experiment was that death rate in shallow sediments was significantly higher than that of tanks with deep sediments. In fact, the highest death rate of all designs was the shallow, coarse sediment treatments that most closely match the Berlin system (using only live rock and a very shallow gravel bed). In contrast to the differences in buffering capacity among experimental designs, there were no significant differences among any treatment in the ability of experimental aquaria to process nitrogenous wastes. Likewise, there was no significant difference in the mean number of animal deaths in plenum versus deep sandbed treatments, although the variance was significantly higher in plenum treatments. Thus, these results suggest that the espoused benefits of a plenum are unfounded, and there is no measurable advantage we can detect to the inclusion of a void space beneath the sandbed in recirculating aquarium designs.

5. Conclusion

1. Each aquarium design appeared capable of handling nutrient inputs up to 0.5 mg/l/day of ammonium. At this input level, final concentrations of total ammonia, nitrite and nitrate did not differ significantly among aquaria (1) with or without plenums, (2) containing deep (9.0 cm) or shallow (2.5 cm) sediments, or (3) containing coarse (2.0 mm) or fine (0.2 mm) mean particle sizes.
2. The greatest differences among experimental treatments were observed as decreased buffering capacity, and higher final phosphate concentration of aquaria with coarse sediments relative to those with fine sediments. However, overall death rates were roughly twice as high in aquaria with shallow sediments as in deep sediment treatments, and the highest death rates overall in the study were in coarse, shallow sediment treatments.
3. Experimental results were qualitatively similar among both the aquarium dosing and live animal experiments, suggesting the role of sediment infauna in nutrient processing within aquaria is minimal.
4. Throughout this experiment, there is no detectable advantage to the inclusion of a plenum beneath sediments in recirculating aquarium systems.

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