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THE EFFICACY OF UV IRRADIATION IN THE MICROBIAL DISINFECTION OF MARINE MAMMAL WATER[1]

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Abstract: A study was made on the efficacy of a commercial ultraviolet (UV) sterilizer in reducing the number of bacteria and yeasts in a saline, closed-system marine mammal complex. UV irradiation was effective in lowering bacterial counts in the effluent of the unit (>75% reduction), but bacteria in more remote parts of the water system reached levels equal to or greater than pre-UV counts. Yeast reduction was considerably less, and a trend similar to that of the bacteria was observed in remote sections of the water system. It is concluded that UV irradiation is of limited value in the disinfection of marine mammal water. Factors contributing to the poor performance of the sterilizer were the long recycle time of the water and lack of a residual effect.

INTRODUCTION

Marine mammals used for research or public display commonly are kept in closed-system pools in which the water is filtered, disinfected, and recirculated. In such environments nutrients for microbial growth may accumulate, resulting in possible increases in the number of disease-producing organisms. Spotte and Adams,[14] for example, showed that the increase of total organic carbon in closed-system marine mammal pools was linear throughout the duration of their experiments (3 months). This is tentative evidence that equilibration of nutrients may be prolonged, placing considerable stress on the disinfection process.

The control of microorganisms can be approached indirectly by reducing the nutrient concentration in the water, or directly by refining the mode of disinfection. This paper describes a direct approach: ultraviolet (UV) irradiation.

MATERIALS AND METHODS

The water system of Mystic Marinelife Aquarium's whale/dolphin complex includes a main pool, two satellite pools (called the North and South pools), and a holding facility for California sea lions. The water is saline (S = 26 0/00), consisting of industrial grade sodium chloride dissolved in tap water. Twice-daily additions of liquid 15% sodium hypochlorite provide low-level chlorination for disinfection (free and combined available chlorine average 0.02 and 0.38 mg/l, respectively). Muriatic acid (30% HCl) is added intermittently for pH control (mean pH is 7.0), which makes chlorination more effective. A refrigeration unit keeps the temperature constant at 19 C. Alum (aluminum sulfate) is added by hand to the filter influent line twice daily at a rate of 2.27 kg/day to control turbidity. The water is processed continuously by eight rapid-sand pressure filters containing 0.49 mm silica sand with a support bed of silica pea

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gravel. Each filter tank is 244 cm in diameter. Total volume of the water system is 1.55×10^6 l, and turnover rate through the filters is 380 l/sec. No new water is added except to replace what is lost in backwashing the filters, or from draining the North and South pools periodically to examine animals.

A UV sterilizer [2] was installed 30 March 1978, but at no time was routine chlorination discontinued. The unit was positioned on the filter effluent side of the system and adjusted to a flow rate of 15 l/sec. Sampling ports were drilled in the influent and effluent lines of the sterilizer. Water samples were collected from the sampling ports and from remote areas of the main water system (North and South pools). Samples before and after exposure to UV were collected on 24 occasions between 11 April and 13 October 1978, and consisted of eight groups, each collected on three consecutive days. Two additional sets (three days each) of pool water samples were collected starting 20 March and 4 April 1978. Samples were collected in 1-l sterile brown polypropylene bottles containing sodium thiosulfate to neutralize chlorine.[1] All samples were returned immediately to the laboratory and processed within 1 hr.

Bacterial counts were made by spread plating[3] on Bacto-Plate Count agar,[3] [1] prepared with both distilled water (DW) and 3% NaCl solution. Incubation was at 25 and 35 C, with subsequent counting after 5 to 7 and 3 days, respectively. Yeast counts were made by filtering 200 to 500 ml of water through 0.8 μm black membranes. [4] Filters were placed on the surface of Bacto-Sabouraud Dextose agar [3] containing 150 mg/l of chloramphenicol. [5] Duplicate plates were incubated at 25 and 37 C; counts were recorded after 5 and 3 days, respectively.

On two sampling dates (29 September and 11 October), dominant bacteria based on colony morphology were isolated from Plate Count agar prepared with both DW and NaCl. Pure cultures were tentatively identified to genus based on the Gram stain and other reactions.[12]

RESULTS AND DISCUSSION

Table 1 shows average bacterial and yeast counts immediately before and

TABLE 1. Average microbial counts before and after IV treatment in the North and South pools.

Culture conditions		Pre-UV	Post-UV	N pool	S pool
Bacteria per ml (x 10^2)					
DW medium					
	25 C	8.34	1.73	9.54	13.18
	35 C	8.07	1.11	5.78	10.31
3% NaCl medium					
	25 C	15.49	0.98	3.24	8.83
	35 C	8.40	0.86	2.62	6.34
Yeasts per liter					
	25 C	7.58	4.83	60.58	30.42
	37 C	2.25	2.00	10.92	10.42

[2] Model No. AL-CSL-24, Aquafine Corp., Burbank, California 91504, USA.

[3] Difco Laboratories, Detroit, Michigan 48232, USA.

[4] AABGO47SO, Millipore Corp., Bedford, Massachusetts 01730, USA.

[5] Chloramphenicol, Sigma Chemical Co., St. Louis, Missouri 63178, USA.

after UV irradiation, and in the more remote North and South pools. The immediate effect of UV is obvious, particularly with respect to bacteria. However, bacterial counts increased with passage of water into the pools. Feces from the animals, in addition to bits of food (raw fish), no doubt contributed to these higher counts. Yeast populations showed the same general trend, although the impact of UV was considerably less and the pre-UV samples (those collected from the sampling port on the influent side of the sterilizer) showed a greater reduction in numbers compared with pool waters. This may have been a function of greater yeast size (more easily retained in the filters).

Table 2 shows the average percent reduction in bacterial and yeast counts achieved by UV irradiation. As noted previously, the unit was more efficient at eliminating bacteria than yeasts. Although yeast counts in the system overall were low (always < 100 cells/l), these organisms assume major significance as etiological agents of disease in captive aquatic environments used to maintain cetaceans. Several yeasts that are potentially pathogenic have been isolated from the water of this facility, including *Candida albicans*.[2] Candidiasis has been implicated in the deaths of several species of cetaceans at a number of oceanariums in North America.[9,10,14]

Average bacterial and yeast counts from each sampling period (mean of three consecutive days) are shown in Figures 1 and 2. There was no clear effect of UV — either immediate or long-range — in either pool on counts of any group of microorganisms studied. Both bacterial and yeast counts varied among samples, which probably reflected chlorine level, type and concentration of dissolved organic matter, and the availability of free-floating particulate material.

The dominant bacteria present at two sampling periods were species of *Achromobacter-Alcaligenes* (32% of isolates), *Aeromonas-Vibrio* (18%), "coryneforms" (18%), *Pseudomonas* (18%), *Micrococcus* (9%), and *Flavobacterium-Cytophaga* (5%). All these genera are characteristic of freshwater and marine habitats and are probably introduced constantly into marine mammal water with food. None of these pose significant threats to captive animals. We did not culture specifically for bacteria associated with diseases of cetaceans,[15] because this facility does not have a history of such problems. No members of the Enterobacteriaceae dominated on platings. Enteric bacteria, if originally present in small numbers, are reduced substantially by chlorination and UV

TABLE 2. Average percent reduction in microbial counts before and after UV treatment.

Culture conditions		No. of samples	% reduction
Bacteria			
DW medium			
	25 C	24	75.9
	35 C	21	77.0
3% NaCl medium			
	25 C	24	90.7
	35 C	22	85.9
Yeasts			
	25 C	14	20.7
	37 C	24	—[1]

[1]In 23 of 24 samples, counts were < 1 per liter

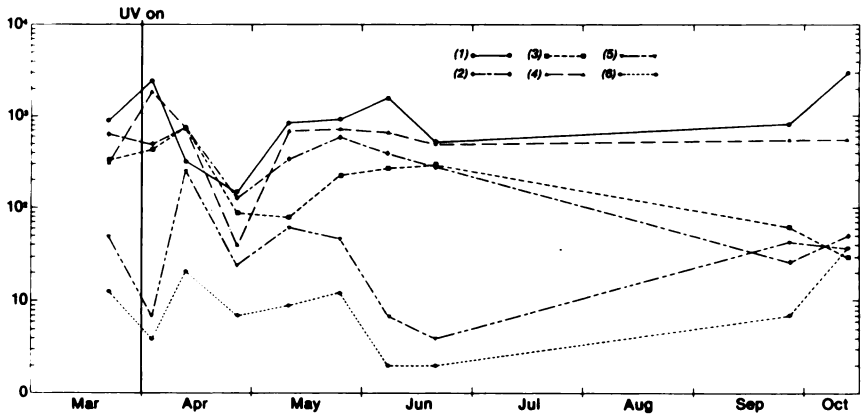

FIGURE 1. Average of each sampling period (3 consecutive days) in the North pool. Legend is: (1) bacteria (DW, 25 C); (2) bacteria (DW, 35 C); (3) bacteria (NaCl, 25 C); (4) bacteria (NaCl, 35 C); (5) yeasts (25 C); (6) yeasts (37 C).

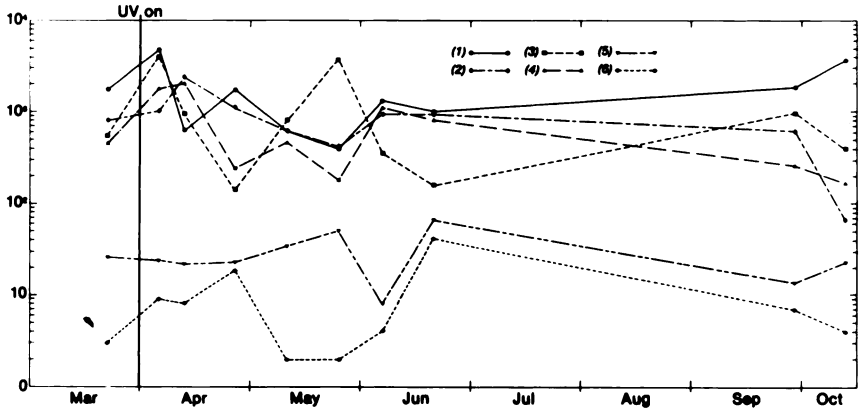

FIGURE 2. Average of each sampling period (3 consecutive days) in the South pool. Legend the same as in Fig. 1.

irradiation.[5,7] Considerably less information is available on the response of yeasts to physical and chemical agents, but strains of the pathogen *C. albicans* react variably to chlorine[8] and UV.[4,11]

Our data show that UV irradiation, as evaluated by these experiments, is of limited use in reducing the number of microbes in environments used to maintain captive marine mammals. Because the volume of the water system is large and the capacity of the UV sterilizer is comparatively small, microbial counts rose rapidly with increasing distance from the source of treatment. Treatment was effective inside the sterilizer, particularly against bacteria, but clearly inadequate to maintain low counts of microorganisms in areas of the water system that were recontaminated continuously. Since the unit was installed, two of the five Atlantic bottlenosed

dolphins *(Tursiops truncatus)* have shown signs of candidiasis, both cutaneous and systemic, and a beach-stranded Atlantic pilot whale *(Globicephala melaena)* died 30 November 1979 with apparent *C. albicans* involvement.

Clearly, additional or improved water treatment is necessary to eliminate yeasts from captive environments, but UV irradiation does not appear to be a viable technique. It is doubtful whether even a larger sterilizer or higher dosage levels of UV radiation would have given improved performance. The efficiency of UV irradiation in closed systems is further nullified by the lack of a residual effect, which would kill microbes beyond the contact site.[6]

Engineering parameters that work effectively in wastewater treatment have few direct applications in the disinfection of marine mammal water. Facilities for treating wastewater are essentially open (flow-through) systems in which the influent is treated in several stages. The sequential steps employed — settling, clarification, filtration, disinfection, and discharge — do not involve the continuous introduction of new contaminants. At no time is the water recontaminated, recirculated, and reused. Adequate disinfection of marine mammal water is possible only with a disinfectant such as chlorine, which forms a residual compound that persists long enough to kill microorganisms throughout the system, and not just at a central source.

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