

Disinfection of Nauplii of *Artemia franciscana* by Ozonation

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Abstract.—The use of ozone to improve water quality in recirculation aquaculture systems is widespread. In these same systems, the use of brine shrimp *Artemia* spp. as the first food item for larval fish is also very common. The potential of brine shrimp to inoculate culture water with pathogenic bacteria is well understood, and the increasing availability of ozone makes it reasonable to consider ozone as a potential disinfectant of brine shrimp. In this study, brine shrimp nauplii were exposed to ozone (0.75 g/h) for various periods (10, 15, 20, 25, and 30 min). Survival of nauplii was greater than 90% in all but the 30-min exposure (84.4%), and bacterial reduction was nearly 100% for all exposures. Ozone, because of its demonstrated potential to disinfect brine shrimp without causing significant mortality, is a viable alternative to chemical disinfection of nauplii before feeding to larval fish.

Nauplii of brine shrimp *Artemia* spp. are widely used as the initial food for many larval fish cultured in intensive systems. Brine shrimp nauplii have also been reported as a potential source for the introduction of pathogenic bacteria into these systems (Soorgeloos et al. 1986; Dehasque et al. 1991; Rodriguez et al. 1991). Chemical methods for disinfecting nauplii have been reported (Gomez Gil-RS et al. 1994); however, there are growing regulatory concerns about the use of chemicals to kill bacteria and environmental concerns over the chemically treated water that is subsequently discharged. The ability of ozone to kill bacteria is considerable and well documented (McCarthy and Smith 1974; Roselund 1975; Oakes et al. 1979; Lohr and Gratzek 1984; Longley 1986; Tipping 1988; Liltveld et al. 1995). Ozone is already present at many recirculating-water aquaculture operations as a means of improving water quality (Oakes et al. 1979; Rosenthal and Otte 1980; Williams et al. 1982; Sutterlin et al. 1984; Paller and Lewis 1988; Reid and Arnold 1992; Rueter and Johnson 1995; Brazil et al. 1996; Summerfelt and Hocheimer 1997). Therefore, we postulate that

ozonation might offer a more acceptable alternative to disinfection of brine shrimp than chemical methods. This study was designed to evaluate the effectiveness of ozone for the disinfection of live brine shrimp nauplii before being offered as food to larval fish.

Cysts of Utah strain *Artemia franciscana* (2 g/L) were cultured at 27°C and 12‰ salinity for 24 h. Culture conditions included a 75-L conical tank, constant illumination, a submerged 20-W full-spectrum fluorescent light, and aeration. Hatched nauplii were placed in a 150- μ m sieve, washed, and diluted with tapwater (approximately 10 L) to a density of 1,000 nauplii/mL. Salinity was re-adjusted to 12‰. A silicone-based deionizer was added (4 mL) to prevent foaming during exposures. One liter (approximately one million nauplii) of brine shrimp was placed in each of six 1-L exposure columns (three treatments and three controls). Exposure columns were made of 575-mm-tall capped polyvinyl chloride pipe (54 mm inner diameter). In the first experiment, treatment nauplii were exposed to ozone for 10, 15, 20, 25, and 30 min at a rate of 0.75 g ozone/h and an oxygen flow rate of 28.3 L/h. Ozone was produced by a corona discharge ozone generator (with an oxygen flow rate of 28.3 L/h, producing ozone at 0.75 g/h, Aqua Flow model CD-06-P). Control nauplii received pure oxygen at the same flow rate and exposure times as the treatment nauplii, and each exposure time had its own controls. Brine

TABLE 1.—Survival (mean \pm SE) of brine shrimp nauplii exposed to ozone (0.75 g/h).

Exposure time (min)	Survival ^a (%)
10	99.1 \pm 0.49
15	96.9 \pm 0.24
20	99.2 \pm 1.08
25	96.2 \pm 2.58
30	84.4 \pm 3.52

^a Percent survival = mean of 100 \times percent survival of each replicate/mean of control percent survival.

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TABLE 2.—Mean (\pm SE) of bacterial counts (CFU^a) after various ozone exposures (0.75 g/L).

Exposure (min)	Control (CFU)	Treatment (CFU) ^a
10	62,667 \pm 13,615	34.00 \pm 55.5
15	14,707 \pm 11,484	1.34 \pm 2.3
20	3,267 \pm 3,384	2.67 \pm 2.3
25	1,133 \pm 702	1.33 \pm 2.3
30	12,667 \pm 13,429	0.33 \pm 0.6

^a CFU = colony forming units.

shrimp survival rates were estimated from manual counts of nauplii in three replicate 0.1-mL samples collected from each exposure column. Water samples from each column were sieved through sterile 25- μ m filters, serially diluted from 1 to 0.001 mL for treatments and from 0.1 to 0.001 mL for the controls, and then plated on plate count agar. The plates were incubated at room temperature for 24 h, and bacterial colonies were counted.

A second experiment was conducted to look at long-term survival of brine shrimp after ozone exposure. Nauplii were exposed to ozone for 15, 20, 25, and 30 min, as described for the first experiment, and survival rates were estimated for: 0, 30, 60, 120, and 240 min after ozone exposures. Data (percentages were arcsine-transformed) were analyzed by one-way analysis of variance (ANOVA) ($\alpha = 0.05$) and Duncan's multiple-range test.

Naupliar survival is expressed as a percent and is relative to the corresponding control (i.e., the mean percent survival for an exposure time is divided by the mean percent survival of the corresponding control and multiplied by 100). This was necessary to factor out the variability in survivorship between controls of different exposure times.

Brine shrimp nauplii survival was above 90% in all treatments and controls except for the 30-min exposure, in which survival averaged 84.4% across three replicates (Table 1). The ANOVA indicated a significant difference ($P = 0.001$) over time and between treatments. Duncan's multiple-range test indicated no differences between 10-

15-, 20- and 25- min ozone exposures but showed a significant difference between 30 min and all other exposures.

There was little bacterial growth on any of the incubated treatment plates. However, all control plates had heavy bacterial growth (Table 2). Analysis of variance indicated significant differences ($P = 0.001$) between the controls and the treatments. While the Duncan's multiple-range test indicated no difference between any of the treatments, it did, however, indicate that the bacterial counts for the 10-min control exposure differed from all other control results.

No statistical difference existed in the long-term survival of brine shrimp nauplii between treatments for any exposure level tested. A slight increase in mortality was observed at the 30-min exposure (Table 3), although no statistically significant difference was found.

Reid and Arnold (1994) reported that Pacific white shrimp *Penaeus vannamei* were more tolerant to ozone than red drum *Sciaenops ocellatus*. Although the shrimp were able to tolerate residuals of 1 mg/L for 24 h, the red drum in their study were only tolerant of 0.1 mg/L for 24 h. This information was the basis for the hypothesis that brine shrimp could tolerate a "disinfection treatment" via ozone exposure.

Our long-term survival experiment suggested no significant decrease in survival across all exposure levels tested, up to 4 h postexposure. Despite the slight decrease in survival of nauplii at time 0 and at the maximum exposure time of 30 min in our initial experiment, it is important to emphasize that naupliar survival was never below 84.4% and that the reduction in bacterial loading in all treatments was virtually 100%. In conclusion, we believe that ozone is a viable alternative to chemical disinfection of nauplii before feeding to larval fish because of its demonstrated potential to disinfect brine shrimp without causing significant mortality.

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TABLE 3.—Long-term survival^a (mean percent \pm SE) of brine shrimp nauplii exposed to ozone (0.75 g/L).

Exposure time (min)	Minutes after ozone exposure				
	0	30	60	120	240
15	100.4 \pm 0.75	98.5 \pm 1.10	98.0 \pm 0.49	96.7 \pm 1.2	96.4 \pm 1.10
20	98.9 \pm 0.22	98.6 \pm 0.33	99.0 \pm 0.57	96.2 \pm 0.82	95.7 \pm 1.16
25	100.3 \pm 0.97	97.7 \pm 0.62	96.9 \pm 1.06	96.0 \pm 0.23	95.2 \pm 0.54
30	98.7 \pm 1.16	95.9 \pm 1.15	94.9 \pm 1.03	94.7 \pm 1.23	93.0 \pm 1.03

^a Percent survival = mean of 100 \times percent survival of each replicate/mean of treatment control percent survival.

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