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Salt as a decontamination agent to control bacterial load in *Artemia salina* cultures

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ABSTRACT

Live food represents one of the major sources of bacterial contamination in larval culture. Since *Artemia* are widely used as live food, this approach has been thoroughly studied. Several techniques using antibiotics, ozone, chemical compounds, algae, and probiotics, have been tested to reduce bacterial load in *Artemia*. This research looked at the potential of salt to reduce bacterial contamination. Two salt treatments, consisting of 1 — a dip in a 200 g L^{-1} salt solution, and 2 — a 24 h enrichment in a 60 g L^{-1} salt solution, were compared to a commercial antimicrobial enrichment product (A1 DHA Selco), a mix of commercial antibacterial products and a non-antimicrobial enrichment product (AlgaMac) as well as a control (enriched with AlgaMac). All treatments induced a reduction of the bacterial count of over 99.999983%. It was expected that different enrichment products would induce variations in the nutrient profiles, but the levels of DHA were higher in the *Artemia* treated with either of the salt treatments, when compared to the control. The salt treatment did not affect EPA and ARA levels, neither did it affect the levels of vitamins C and E. Therefore, salt allows for a comparable or higher reduction of the bacterial load in *Artemia* nauplii, than that obtained with other techniques, with a limited effect on nutrient profiles. Even more, the use of salt does not limit the choice of the enrichment product used, since it can be used in combination with a non-antibacterial enrichment product.

Statement of relevance: Utilization of salt allows for a comparable or higher reduction of the bacterial load in *Artemia* nauplii than the ones obtained with more complicated or less durable techniques such as antibiotics, formaldehyde and ozone. As demonstrated in the present study, commercial enrichment products exist that allow for an achievement of similar results, but the use of salt does not limit the choice to enrichment products containing antimicrobial compounds, and produces minimal variations in the nutritional profiles of the *Artemia*. Therefore this new technique could be useful for all fish culture using *Artemia*.

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1. Introduction

Artemia are widely used as live food in aquaculture and the bacterial load associated with it has always been a major concern. Several studies have established a clear link between the bacterial present in larvae and the ones present in live food (Nicolas et al., 1989; Munro et al., 1994, 1999). Furthermore, studies have established that live food represents one of the major sources of bacterial contaminants that could lead to larval diseases (Nicolas et al., 1989; Blancheton and Canaguier, 1995; Munro et al., 1995; Planas and Cunha, 1999; Dhert et al., 2001; Lopez-Torres and Lizarraga-Partida, 2001; Sorgeloos et al., 2001).

Over many years, several techniques have been tested to reduce the bacterial load in live food: these include antibiotics (Benavente and Gatesoupe, 1988; Hameed and Balasubramanian, 2000; Hoj et al., 2009); probiotics (Makridis et al., 2000; Verschuere et al., 2000; Makridis et al., 2001; Interaminense et al., 2014); algae (Kogure et al., 1979; Austin et al., 1992; Olsen et al., 2000; Tolomei et al., 2004); ozone (Sugita et al., 1992; Liltved et al., 1995; Summerfelt and Hochheimer, 1997; Suantika et al., 2001; Tolomei et al., 2004); commercial enrichment products (Tolomei et al., 2004); ultraviolet light (Munro et al., 1999); chemicals (Gomez-Gil et al., 1994; Douillet, 2000; Hoj et al., 2009); hydrogen peroxide (Gimenez et al., 2006); and some have met with great success. The presence of antibiotic resistant bacteria has led to the reduction of the use of antibiotics as an option to reduce bacterial load (Skjermo and Vadstein, 1999; Verschuere et al., 2000).

Artemia are commonly used to feed marine fish species, and particular attention has been paid to essential fatty acids (EFA) since marine fish cannot synthesize desaturated 18-carbon or longer polyunsaturated fatty acids. Therefore, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) and their different ratios have been extensively studied in the past 25 years: (Watanabe, 1982; Watanabe et al., 1983; Watanabe and Kiron, 1994; Sargent et al., 1997; Sargent et al., 1999a,b; Han et al., 2001; Hanaee et al., 2005).

Artemia live in saltwater lakes where water salinity can reach over 200%; in fact, *Artemia* can survive in water up to 250% (Daintith, 1996).

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This tolerance to extreme salinity is quite interesting since salt has been used for decades to preserve food and disinfect wounds due to its ability to inhibit bacterial growth. The aim of this study was to determine the potential to reduce bacterial load in *Artemia* cultures of a salt solution without affecting their nutritional value. This objective was achieved by comparing two salt treatments to a commercial product renowned to have antimicrobial proprieties.

2. Material and methods

2.1. Artemia

Artemia salina (Salt Creek Inc., Utah, USA) nauplii were obtained from cysts, after decapsulation, and a 48 h hatching period as described in Haché and Plante (2011). *Artemia* were transferred into an enrichment pyramidal tank of 150-L at a density of 100,000 L^{-1}. The pH was maintained by adding 0.4 g L^{-1} of sodium bicarbonate and the oxygen was added through ceramic plates and maintained above 4 mg L^{-1}. An open-air line was installed at the bottom of the tank to keep the *Artemia* from settling. *Artemia* were harvested after a period of 24 h of enrichment.

2.2. Experimental design

The *Artemia* were divided into five groups, and one of the following treatments was randomly assigned to each group: 1 — control, 0.1 g L^{-1} of AlgaMac 2000 (INVE Aquaculture, Dendermonde, Belgium) was added at 9:00 and 21:00; 2 — *Artemia* were enriched with 0.1 g L^{-1} of AlgaMac 2000 at 9:00 and 21:00; after the enrichment period, the *Artemia* were rinsed and dipped into a salt solution of 200 g L^{-1} for 30 min; 3 — *Artemia* was enriched twice a day (9:00 and 21:00) with 0.1 g L^{-1} of AlgaMac 2000, but the salinity of the water in the enrichment tanks was adjusted to 60 g L^{-1}; 4 Z— *Artemia* were enriched twice a day (9:00 and 21:00) with 0.3 g L^{-1} of A1 DHA Selco (INVE Aquaculture, Dendermonde, Belgium); and 5 — *Artemia* were enriched twice a day (9:00 and 21:00) with 0.15 g L^{-1} of A1 DHA Selco and 0.05 g L^{-1} of AlgaMac 2000. Once the treatment completed, the *Artemia* were rinsed for 5 min on a 60 μm filter with filtrated and UV treated salt water. The *Artemia* formed a layer of about 1 cm thick on the filter. After the rinsing step was completed, absorbent paper towels were used to remove excess of water by sponging the bottom of the filter. The last step was repeated until no sign of humidity was present on the paper towel. Samples for bacterial count and nutritional analysis of *Artemia* were taken once the excess of water was removed. Treatments were replicated three times for bacterial count, but only the first two replicates were sent for nutritional analyses.

2.3. Bacterial count and nutritional analysis

Bacterial counts (CFU) were performed as described in Haché and Plante (2011). The nutritional analyses were performed by a private laboratory, the Centre de recherche et de développement des produits marins (CRDPM) of Shippagan, New Brunswick, Canada.

2.4. Statistical analysis

ANOVA analysis was performed on bacterial counts to establish differences induced by the treatment. Five MANOVAs were performed as follows: proximate analysis; EPA, DHA and ARA levels; vitamins; *n*-3 and *n*-6 totals; and fatty acid ratios. When significant differences occurred ($P < 0.05$), a Tukey–Kramer test was performed for pairwise comparison (Day and Quinn, 1989). The normal distribution and homoscedasticity were tested using the Shapiro–Wilk test and Levene's test, respectively (Sokal and Rohlf, 2011). When the data failed to meet these assumptions, an arcsin transformation was applied. The statistical analyses were made by using IBM SPSS statistics 19.0 (Chicago, IL).

3. Results

None of the tested treatments induce mortalities in *Artemia*. The length of the dip salt treatment was established on prior observations. Longer dip period induced ±15% and ±33% *Artemia* mortalities for treatment of 45 min and 1 h respectively. Table 1 shows the bacterial count (CFU) of *Artemia*. All treatments induced a significant reduction of over 99.999983% of the bacterial load, when compared to the control group, without any significant differences between the treatments.

Table 2 shows nutritional composition of *Artemia*. The use of salt seemed to have a limited effect on nutritional values of *Artemia* (Table 2), except for the quantity of dry matter. The major variation occurred in the level of the essential fatty acid DHA for *Artemia*, exposed to a salt solution that increased to a level in between the ones obtained when the A1 DHA Selco was used. As expected, variation in enrichment products (i.e. AlgaMac 2000 vs A1 DHA Selco) induced significant changes in the quantity of essential fatty acids ARA, EPA and DHA, as well as in the totals of *n*-3 and *n*-6. The level of vitamin E was only affected by the type of enrichment used when the level of vitamin C increased when the mix of enrichment or that the *Artemia* is exposed for a long period of time to salt.

Table 3 shows the levels of the essential amino acids in *Artemia* after the treatment. Neither, the use of salt nor the use of a different enrichment product, induced significant differences. Only the amino acids; isoleucine, leucine and lysine, showed levels of variance.

4. Discussion

The bacterial load of untreated *Artemia* reported in this present study (Table 1) was in accordance with ones previously reported (Verdonck et al., 1994; Oie et al., 1997; Hameed and Balasubramanian, 2000; Makridis et al., 2000; Hoj et al., 2009; Haché and Plante, 2011). The final bacterial load achieved with the different salt treatments was 2.096×10^3 and 7.651×10^2 per *Artemia* which represent a total reduction of 99.999983 and 99.99991%. Antibiotics have been exhaustively studied in order to reduce bacterial load in *Artemia* culture (Benavente and Gatesoupe, 1988; Gomez-Gil et al., 1994; Hameed and Balasubramanian, 2000; Hoj et al., 2009). The reduction of the bacterial load achieved with antibiotics were highly variable from the reported 46.3% (Hameed and Balasubramanian, 2000) to 99.98334% the latest representing a final load of 3.8×10^3 bacterial per *Artemia* (Interaminense et al., 2014). The antimicrobial proprieties of microalgae have also been greatly studied with reports of bacterial load reduction ranging from 66% to 99.66667% (Kogure et al., 1979; Austin et al., 1992; Olsen et al., 2000; Tolomei et al., 2004; Hoj et al., 2009; Interaminense et al., 2014). Chemical compounds such as formaldehyde, iodine and sodium hypochlorite (Gomez-Gil et al., 1994; Douillet, 2000) had also been studied to control the bacterial load in *Artemia* culture. Gomez-Gil et al. (1994) reported a diminution of 99.96029%, 12.61262% and 97.58065% for formaldehyde, iodine and sodium hypochlorite respectively but *Artemia* mortalities were observed in the formaldehyde and the sodium

Table 1
Bacterial colony of *Artemia salina* after seven-day incubation at 20 °C on marine agar (per *Artemia*).

Treatment	Colony counts (S.D.)	Percentage of the bacterial load reduction
Control	1.233×10^{10} (1.126×10^9)^b	
30 min in 200 g L^{-1} salt solution	7.651×10^2 (8.628)^a	99.999991
24 h in 60 g L^{-1} salt solution	20.960×10^2 (9.126)^a	99.999983
A1 DHA Selco	6.517×10^2 (10.051)^a	99.999994
A1 DHA Selco + AlgaMac 2000	11.413×10^2 (13.754)^a	99.999989

All values represent the mean (S.D.) (n = 3).
Different letters indicate significant differences ($P < 0.05$).

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