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Bacteria contribute to *Artemia* nutrition in algae-limited conditions: A laboratory study

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ABSTRACT

We investigated the effect of the stimulation of bacterial growth on *Artemia* performance in combination with a standard and with a low algal feeding regime. In both regimes, organic carbon (supplied as sucrose or soluble potato starch) and ^{15}N labeled inorganic nitrogen (supplied as $NaNO_3$) were used to stimulate bacterial growth in the *Artemia* cultures at C/N ratio 10 and 50. After a culture period of 15 days, significantly improved biomass production was obtained in all treatments with the low algae feeding regime, supplemented by carbohydrate addition. In addition, results of ^{15}N accumulation and fatty acid analysis in *Artemia* indicated that *Artemia* utilized more bacteria in algae-limited conditions. Our study shows that bacteria can be used as a nutrient source for *Artemia* compensating for suboptimal algae supply. In *Artemia* pond cultures, carbohydrate addition may hence potentially be used to stimulate the conversion of nitrogen waste into heterotrophic bacterial biomass. This can be converted into protein-rich *Artemia* biomass, especially when algae are in sub-optimal supply. These findings open perspectives for alternative *Artemia* pond production protocols, in addition to the present management procedures that exclusively focus on phytoplankton blooms as nutrient source to sustain dense *Artemia* populations.

1. Introduction

Nauplii of the brine shrimp *Artemia* are the most commonly used live food in aquatic larviculture. Due to their nutritional value and size, *Artemia* nauplii, either freshly hatched or after nutritional enrichment, satisfy the nutritional requirements for early-stage fish and crustacean larvae (Sorgeloos et al., 2001). Being non-selective filter feeders, *Artemia* can feed on a wide range of diets such as micro-algae, bacteria, protozoa and small detritus particles. Fernández (2001) specified that the food size for *Artemia* metanauplii must range between 6.8 and 27.5 μm, with an optimum of about 16.0 μm. Its adults are able to ingest all particles less than 50 μm in size (D'Agostino, 1980; Dobbeleir et al., 1980). The use of bacteria, which are in the size range 0.6–3.0 μm (Palumbo et al., 1984), as food for *Artemia* has been reported by Intriago and Jones (1993). The ability of *Artemia* to graze on bacteria has further been demonstrated by studying the clearance rate when *Artemia* was fed radioactively labeled bacteria and measuring the amount of radioactivity accumulated in *Artemia* (Fernández, 2001).

Bacteria are easy to grow through administration of carbon and nitrogen (Gaudy and Gaudy, 1980), and the addition of carbohydrates into aquaculture systems has been reported to induce the conversion of nitrogen to bacterial protein (Avnimelech, 1999). Bacteria grown at high density tend to form bioflocs (Crab et al., 2007; De Schryver et al., 2008), which are conglomerates of bacteria, protozoa, algae, detritus etc. Bioflocs vary in size from 0.1 mm to a few mm (Avnimelech, 2011), and are thus of suitable size for uptake by aquaculture organisms such as Nile tilapia (*Oreochromis niloticus*) fingerlings (Avnimelech, 2007), white shrimp (*Penaeus vannamei*) from larvae to market size (Hari et al., 2004), and for fresh-water prawn (*Macrobrachium rosenbergii*) larvae (Crab et al., 2009a). The production of bioflocs induced by the addition of carbohydrates significantly increased the final survival and biomass production of these target animals. Additionally, promoting bacterial growth in aquaculture systems clearly reduced the demand of feed protein (Avnimelech, 1999; Burford et al., 2004; Crab et al., 2009b; Hari et al., 2004).

Artemia pond production of cysts and biomass is a profitable activity in solar saltworks in the Mekong Delta, Vietnam (Anh et al., 2009b; Baert et al., 1997). Thanks to its filtering feeding behavior, *Artemia* can be produced as a form of extractive aquaculture, lowering nutrient levels in aquaculture effluents and producing animal protein. The protein content of adult *Artemia* is around 50% of its dry weight (Anh et al., 2009a) and it can be used as an ingredient for shrimp feed, reducing

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the need for fish protein in shrimp culture. Traditionally, *Artemia* is cultured supplying animal wastes such as chicken manure, pig dung etc. and algae-rich green water from fertilizer ponds as food source. However, the carbon/nitrogen (C/N) ratio in *Artemia* food supplements is usually lower than the requirements needed to stimulate bacterial growth, e.g. within the range 4–8 for microalgae (Seixas et al., 2009) and 4–6 for soybean meal (Kuo et al., 2004). In biofloc production, this ratio is therefore increased by adding carbohydrates (Avnimelech, 1999; Crab et al., 2009b; Hari et al., 2006; Nootong et al., 2011). Avnimelech (1999) stated that C/N ratio 10 promotes bacterial growth. Later Asaduzzaman et al. (2008) and Hargreaves (2006) demonstrated that C/N ratio 10 or higher induces bacterial growth. According to other studies optimal biofloc production can be done at C/N ratio 15 (Schneider et al., 2005) or C/N ratio 20 (Asaduzzaman et al., 2008; Nootong et al., 2011). Furthermore, the growth of heterotrophic bacteria also depends on the source of carbohydrate supplied (Asaduzzaman et al., 2008; Kuhn et al., 2009).

In *Artemia* pond culture, quantification of the dietary contribution of bacteria is difficult to perform. Hence, in this study carbohydrate addition in an *Artemia* laboratory culture aimed to stimulate the conversion of nitrogen in the culture medium into heterotrophic bacterial biomass using different C/N ratios and carbohydrate sources. To clarify the possible positive and negative effects of bacterial growth in the culture medium and the effect of ingestion and assimilation of bacteria on *Artemia* performance, a broad range of C/N ratios was chosen in this study. We used C/N ratio 10 as lower value, because its effects on bacterial growth are relatively well documented in literature. As higher value C/N 50 was chosen as this is far above the range 10–20 described in literature. The contribution of the heterotrophic bacteria to the *Artemia* diet was assessed at different algal densities, and using *Artemia* survival, growth and total biomass production as criteria for culture success. The assimilation of bacteria was determined by the addition of ^{15}N-nitrogen into the cultures to label the bacteria (Avnimelech and Kochba, 2009; Burford et al., 2004) and subsequent measurement of the ^{15}N accumulation in *Artemia*. Moreover, as algae and bacteria are characterized by specific fatty acid profiles, and as dietary fatty acids are transferred conservatively into *Artemia* lipids (Intriago and Jones, 1993; Zhukova et al., 1998), the *Artemia* fatty acid profile was determined at the end of the culture period in order to assess the extent of assimilation of heterotrophic bacteria by *Artemia*.

2. Materials and methods

2.1. Experimental design

Artemia was cultured over a period of 15days under zero-water exchange. The different feeding regimes and different conditions stimulating bacterial growth were investigated for their effects on *Artemia* performance. *Artemia* were fed with microalgae concentrate as the main food source (see Section 2.2). From the first day after hatching (DAH1) to DAH4, *Artemia* were acclimated in identical culture conditions using a standard algal feeding (SF) regime without carbohydrate addition: preliminary tests had shown that due to the relatively low clearance rate of the youngest *Artemia* stages (Makridis and Vadstein, 1999), carbohydrate addition during this initial period resulted in quick biofloc formation due to poor uptake of bacteria by *Artemia*. From DAH5 onwards, carbohydrate was added to the cultures: the *Artemia* were split up into two groups under two different feeding regimes, standard and low (the latter being ¼ of the standard feeding regime). For each feeding regime, two different conditions of bacterial growth stimulation, C/N ratio 10 and 50, were applied. For each C/N ratio and feeding regime, two different carbon sources (sucrose and soluble potato starch) were used (Table 1). Soluble potato starch and sucrose were first dissolved in a limited amount of boiling water, left to cool down, and then provided to the *Artemia* cultures.

Table 1
Experimental set up; *Artemia* was reared over 15 days and fed on two different feeding regimes: standard feeding regime (SF) and low feeding regime (LF). C/N: carbon/nitrogen; S: sucrose; ST: soluble potato starch. No application is denoted by dash (–).

Treatment code	Algae ration		Carbon source	C/N ratio
	Days 1–4	Days 5–14		Days 5–14
1. SF (control 1)	SF	SF	–	5.7
2. SF + S10	SF	SF	Sucrose	10
3. SF + ST10	SF	SF	Soluble potato starch	10
4. SF + S50	SF	SF	Sucrose	50
5. SF + ST50	SF	SF	Soluble potato starch	50
6. LF (control 2)	SF	LF[a]	–	5.7
7. LF + S10	SF	LF	Sucrose	10
8. LF + ST10	SF	LF	Soluble potato starch	10
9. LF + S50	SF	LF	Sucrose	50
10. LF + ST50	SF	LF	Soluble potato starch	50

[a] LF = ¼ of SF.

C/N ratio calculation was based on a protein content of 54.66% for the *Tetraselmis* sp. concentrate used (information provided by Reed Mariculture Inc., USA) and a conversion factor to nitrogen of 1/6.25 for algae (Lourenço et al., 1998). Furthermore, as the carbon content of algae can be considered as around 50% (Behrens, 2005), the C/N ratio of the algae diet (which is approximately 5.7; information provided by Reed Mariculture Inc., USA), was lower than the optimum for subsequent complete N assimilation by bacteria. $NaNO_3$ was used as inorganic nitrogen source for all treatments (except for the controls) following the equation below:

$$\text{N needed (mg) per day} = \text{algae N content in SF (mg)} - \text{algae N content in LF (mg)}.$$

The carbon sources and inorganic nitrogen were daily adjusted according to the feeding regime (Table 2). ^{15}N–$NaNO_3$ was added into the *Artemia* cultures (except for the controls) once daily at a concentration of 0.1% of total nitrogen in the culture medium and in the diet to label bacteria (Burford et al., 2004). Each treatment was conducted in three replicates.

2.2. Food preparation

A marine *Tetraselmis* sp. concentrate (Instant Algae 3600; Reed Mariculture Inc., USA) was used. The microalgae concentrate contains intact cells that are non-viable. The latter was verified by the absence of pH change over a period of 6 h with continuous illumination ($\pm$41 μE/m^2 s) at an algae concentrate density of 1 g/L. As algae were metabolically non-active it is assumed that the nitrate assimilation in the experiments was done by the bacteria. The microalgae

Table 2
Feeding schedule for *Artemia* fed on microalgae (adapted from Naegel, 1999).

Day	*Tetraselmis* (10^6 cells/animal/day)
1	0.04
2	0.14
3	0.18
4	0.25
5	0.38
6	0.50
7	0.75
8	0.88
9	0.90
10–14	0.90