Aquaculture 311 (2011) 201–208

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

The relationship between enrichment, fatty acid profiles and bacterial load in cultured rotifers (*Brachionus plicatilis* L-strain) and *Artemia* (*Artemia salina* strain *Franciscana*)

Rémy Haché [a,*], Sébastien Plante [b]

[a] Institut de Recherche sur les Zones Côtières, 232B rue de l'Église, Shippagan, NB, Canada, E8S 1J2
[b] Université de Moncton, Campus de Shippagan, 218 boul. J.-D. Gauthier, Shippagan, NB, Canada, E8S 1P6

ARTICLE INFO

Article history:
Received 11 August 2010
Received in revised form 22 November 2010
Accepted 23 November 2010
Available online 27 November 2010

Keywords:
Rotifers
Artemia
Essential fatty acids
Live prey enrichment

ABSTRACT

Rotifers and *Artemia* are generally used as first foods in marine finfish aquaculture. Because of their poor nutritional value and the incapacity of marine fish to elongate or desaturate 18-carbon of longer polyunsaturated fatty acids (PUFA), it is a common practice to enrich live foods with commercial products. Since live foods represent a significant vector for transmitting bacterial contaminants, this study describes the impact of using different enrichment strategies for rotifers and *Artemia* on bacterial load, in addition to fatty acid profiles. Rotifers were reared in continuous culture while *Artemia* were obtained from cysts; both were enriched for 24 h. Total bacterial counts were obtained after a 7-d incubation on marine agar. Docosahexaenoic acid (DHA) levels varied from 9.8 to 34.4% and from 8.3 to 23.2% respectively, for rotifers and *Artemia*. Eicosapentaenoic acid (EPA) levels ranged from 3.2 to 7.3% for rotifers and from 5.1 to 9.0% for *Artemia,* while arachidonic acid (ARA) levels varied, respectively, from 0.7 to 2.9 and from 1.4 to 3.7. Total bacterial counts varied from 0.9×10^8 to 56.6×10^8 for rotifers and from 0.2×10^9 to 11.7×10^9 for *Artemia*. These results demonstrate the importance of the enrichment strategy on the fatty acid composition and the bacterial contamination of live food.

1. Introduction

Rotifers and *Artemia* sp. are widely used as first foods in the culture of almost all marine fish species. The rotifer species commonly reported to be used in commercial hatcheries are *Brachionus plicatilis* and *B. rotundiformis*. Their species identification is generally based solely on morphological criteria. Recent work (Gilbert and Walsh, 2005; Papakostas et al., 2006a; Papakostas et al., 2006b; Papakostas et al., 2009) using DNA, indicates that rotifer species used could be more varied than originally anticipated. In the wild, larvae generally feed on a mixture of zooplankton, mainly composed of copepods sp. that are rich in polyunsaturated fatty acids (PUFA). Problems with reliable supply sources of copepods as well as the increased risks of disease from high density culture methods have limited the use of copepods in marine fish culture (Holmefjord et al., 1993).

When evaluating the nutritional composition of fish larvae feed, lipids are a key factor (Koven et al., 1990; Czesny et al., 1999; Copeman et al., 2002) as they form the basis of cellular membranes; they are also responsible for transporting important compounds such as vitamins and hormones into the blood stream. Because marine fish species cannot elongate or desaturate 18-carbon or longer PUFA, the importance of PUFA in particular essential fatty acids (EFA), has been extensively investigated during the past 25 years (Watanabe, 1982; Watanabe et al., 1983; Watanabe and Kiron, 1994; Sargent et al., 1999b).

Although it has been observed that rotifers might have the ability to produce PUFA, the quantities synthesized are too low to assure high larval survival; *Artemia,* on the other hand, are naturally poor in EFA (Koven et al., 1990; Czesny et al., 1999; Copeman et al., 2002; Bell et al., 2003). Therefore, both preys are considered to be suboptimal for larval nutrition, especially when compared to wild copepods (Sargent et al., 1997; Nanton and Castell, 1999; Han et al., 2000; Hanaee et al., 2005). It is common practice to enrich rotifers and *Artemia* with phytoplankton (Gatesoupe, 1991) or with commercial products rich in EFA. Rodríguez et al. (1997) observed that rotifers had a better eicosapentaenoic acid (EPA) retention rate compared to docosahexaenoic acid (DHA) regardless of the ratio available in their enrichment. The DHA level in *Artemia* is particularly hard to increase through the use of any enrichment process. This can been explained by the following factors: 1) there is a naturally high lipid content of *Artemia;* 2) the structural fatty acids of *Artemia* are of poor nutritional value; 3) there is a relatively high EPA content in unenriched nauplii; and probably most important of all 4) there is a rapid retroconversion

* Corresponding author. Tel.: +1 506 336 6600; fax: +1 506 336 6601.
E-mail address: remy.hache@irzc.umcs.ca (R. Haché).

0044-8486/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2010.11.034

of DHA to EPA by *Artemia* (Tuncer and Harrell, 1992; Navarro et al., 1999; Bell et al., 2003).

The biochemical composition of eggs, more precisely of the yolk, generally reflects the basic early nutritional requirements of fish larvae (Rainuzzo et al., 1997; Sargent et al., 1999b). Therefore, enrichment of live food should be species specific. This represents a major challenge for selecting an enrichment strategy for larval fish.

Disease transmission has become a major issue with the development of intensive techniques of live food production in larval hatcheries. Rotifers carry a large bacterial load in their digestive tract (Nicolas et al., 1989; Munro et al., 1999; Skjermo and Vadstein, 1999). Therefore, it has been well-established that live foods represent a significant vector for transmitting bacterial contaminants and subsequent infection diseases in larval cultures (Nicolas et al., 1989; Lopez-Torres and Lizarraga-Partida, 2001). Furthermore, intensive fish production stimulates the proliferation of non-desirable bacteria populations such as *Vibrio* or other pathogens (Skjermo and Vadstein, 1999). Several studies have established a direct relationship between bacterial flora found in the larvae and in the live food (Nicolas et al., 1989; Munro et al., 1994; Munro et al., 1999) with the successful bacterial colonization of the larval gut (Munro et al., 1994). These studies have also concluded that the quantitative and qualitative properties of the bacterial flora of live food must be controlled to reduce the negative effects of bacteria overload.

The aim of this study was to determine the best enrichment strategies by comparing the complete fatty acids profiles of both cultured rotifers and *Artemia* after 24 h enrichment process with several commercial enrichment products; we compared the incorporation of fatty acids through the use of live food. This study also compared the bacterial load between the different enrichment strategies by using total colony counts on marine agar. These results could be used by hatchery managers to select the most efficient enrichment techniques based on the specific nutritional needs of the culture species.

2. Materials and methods

2.1. Rotifers cultures

Rotifers (*B. plicatilis* L-strain, Florida Aqua Farms, Florida, USA) were reared in three 1800-L conical tanks at room temperature under 24 h artificial illumination and pure oxygen was constantly added at a rate of 1 L min^{-1} by air stone. Rotifers were fed daily at 9 am with 15% v/v *Isochrysis galbana* (strain TISO) at a cell concentration of 8×10^6 mL^{-1} and again at 3 pm and 9 pm with 0.03 g L^{-1} of SAF-instant dry baker's yeast (*Saccharomyces cerevisiae*).

2.2. Rotifers enrichment

Fifty million rotifers were harvested from the continuous culture and transferred to a 100-L conical tank containing 80 L of seawater (27–30‰ salinity) and 20 L of TISO algae. Six enrichment strategies were tested. Rotifers were enriched twice daily (9 am and 9 pm) with one of the following: 1) 0.2 g L^{-1} of Algamac 3050™ (Aquafauna Bio-Marine Inc., Hawthorne, USA); 2) a mix of 0.05 g L^{-1} DHA Protein Selco™ and 0.075 g L^{-1} Algamac 3050™; 3) a mix of 0.1 g L^{-1} DHA Protein Selco™ and 0.15 g L^{-1} Algamac 3050™; 4) 0.2 g L^{-1} of DHA Protein Selco™ (INVE, Americas, Inc. Mountain Green, USA); 5) a mix of 0.05 g L^{-1} of DHA Protein Selco™ and 0.075 g L^{-1} of Algamac 2000™ (Aquafauna Bio-Marine Inc., Hawthorne, USA); and 6) 0.2 g L^{-1} Selco Plus™ (INVE, Americas, Inc. Mountain Green, USA). When converted to dry matter per million of rotifers, this produced the following results : 1) 0.0393 g of Algamac 3050™; 2) a mix of 0.0095 g DHA Protein Selco™ and 0.0015 g Algamac 3050™; 3) a mix of 0.0190 g DHA Protein Selco™ and 0.0295 g Algamac 3050™; 4) 0.0380 g of DHA Protein Selco™; 5) a mix of 0.0095 g of DHA Protein Selco™ and 0.0145 g of Algamac 2000™; and 6) 0.0285 g L^{-1} Selco Plus™. Rotifers were enriched twice a day, at 9 am and 9 pm, based on the manufacturer's recommendations and on the technical feasibility of a semi-commercial hatchery operation. Rotifers were harvested after 24 h on a 50-μm filter and gently rinsed with filtered seawater for 5 min before sampling for nutritional analysis and bacterial count. The first three strategies were tested together and the last three strategies were tested a week later. All treatments were made in triplicate.

2.3. Artemia culture

Dry *Artemia* cysts (*Artemia salina* strain *Franciscana*, Salt Creek Inc, Utah, USA) were decapsulated as described by the manufacturer. The cysts were hatched and reared in 140-L pyramidal incubators containing 28 °C seawater (27–30‰ salinity) and 2 g L^{-1} of baking soda under high-light intensity, oxygenation using pure oxygenation, and vigorous aeration.

2.4. Artemia enrichment

Hatched *Artemia* nauplii were rinsed with UV-sterilized seawater for at least 15 min. *Artemia* were enriched in five 140-L pyramidal tanks at a maximum concentration of 100,000 *Artemia* L^{-1} and held at room temperature under constant moderated aeration (1 L min^{-1}) and oxygenation (0.5 L min^{-1}). Five enrichment strategies were tested. *Artemia* were enriched twice daily (9 am and 9 pm) with one of the following procedure : 1) 0.1 g L^{-1} of Algamac 2000™; 2) a mix of 0.1 g L^{-1} of DHA Protein Selco™ and 0.2 g L^{-1} of Algamac 3050™; 3) 0.2 g L^{-1} of Algamac 3050™; 4) 0.1 g L^{-1} of DC Selco™; and 5) a mix of 0.05 g L^{-1} of DHA Protein Selco™ and 0.075 g L^{-1} of Algamac 2000™. When converted to dry matter per million rotifers, this produced the following results: 1) 0.0965 g of Algamac 2000™; 2) a mix of 0.0950 g of DHA Protein Selco™ and 0.1966 g of Algamac 3050™; 3) 0.1966 g of Algamac 3050™; 4) 0.0711 g of DC Selco™; and 5) a mix of 0.0475 g of DHA Protein Selco™ and 0.0723 g of Algamac 2000™. The enrichment strategies for the *Artemia* were the same as those used for the rotifers. *Artemia* were harvested after 24 h on a 150-μm filter and gently rinsed with filtered seawater for 5 min before sampling. All treatments were made in triplicate.

2.5. Lipid analysis

Samples for lipid analysis were stored in amber glass vials with Teflon liner caps under N_2 in 2 mL dichloromethane and then frozen at −80 °C. Lipids were extracted following a modified Folch et al. (1957) procedure as described by Parrish (1999). Samples were ground with 3 mL dichloromethane-methanol (2:1, by vol.) in a 7 mL Tenbroeck tissue grinder. The grinder was rinsed twice with 1.5 mL dichloromethane–methanol (2:1, by vol.) and solutions were pooled together. The solution was mixed thoroughly with 2 mL of distilled water and centrifuged at 800×*g* for 2 min at 4 °C. Lipids were recovered from the lower organic layer and the solvent was evaporated under nitrogen.

Fatty acids were determined as methyl esters by gas chromatography. Fatty acid methyl esters (FAME) were prepared from the extracted lipid phase using 12% boron-trifluoride (BF_3) in methanol. FAME were run on a SRI 8610C gas chromatograph equipped with an DB-Wax fused-silica capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness, Agilent Technology, Palo Alto, CA). Hydrogen was used as the carrier gas (flow velocity: 80 cm s^{-1} at 145 °C). FAME were directly introduced into a glass liner (uniliner, drilled, 4 mm, 6.3 × 78.5 mm, Restek, Bellfonte, PA) that was maintained at 300 °C, internal pressure 40 psi for 4 min to ensure a complete transfer of the vapour sample to the column (Grob, 1999). The temperature was initially held at 58 °C for 4 min at 40 psi, followed by a series of temperature ramps: 20 °C min^{-1} to 170 °C at 20 psi; 1 °C min^{-1} to