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Contribution of gut content to the nutritional value of *Brachionus plicatilis* used as prey in larviculture

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ABSTRACT

With the aim of assessing the potential value of gut content in the rotifer *Brachionus plicatilis* during the standard procedures of enrichment and posterior residence in the larval tanks, we have examined by image analysis the changes in gut volume during the filling and subsequent evacuation process when feed is no more available. The gut filling pattern has been examined at different microalgal concentrations of *Nannochloropsis gaditana* ranging between 0.4 and 20.8×10^6 cell ml^{-1}. The rotifer gut was completely filled in 120 min and the gut volume became significantly higher in rotifers fed at the highest microalgae concentrations tested. The gut volume accounted for up to 15% of the body volume. When harvested with a non-submerged filter and resuspended in clean seawater, the rotifers evacuated the gut quickly, losing 60% of its content in the first 5 min. Contrarily, when the rotifers were rinsed carefully using a submerged filter while maintaining the water volume, the gut was evacuated progressively and needed 1 h to lose 60% of the content. Moreover, we have examined the changes in dry mass and energy. After 2 h of gut evacuation the rotifers lost 20% of the initial dry weight, and 38% after 24 h of starvation. In terms of energy the rotifers lost 43% of the caloric content after 24 h of starvation. The findings of this study confirm the importance of performing appropriate feeding protocols to supply living prey of high nutritional quality in larviculture.

1. Introduction

Different species and lineages of the *Brachionus plicatilis* cryptic species complex are widely used as live prey to feed marine fish larvae during the first weeks. The advantages and constraints of their use as food in aquaculture are well known (Conceição et al., 2010; Lubzens and Zmora, 2003; Lubzens et al., 1989). One of the main concerns is the nutritional quality when the rotifers are produced in mass culture. Rotifers are produced under different methods such us batch, semi-continuous and continuous cultures, as well as under high-density superintensive, water recirculation and automatic-controlled systems (Alver et al., 2010; Bentley et al., 2008; Dhert et al., 2001; Kostopoulou et al., 2012; Lubzens et al., 1989; Yoshimura et al., 1997; Yúfera, 2001). The culture system may affect the body biochemical composition. In some cases, the food is almost exhausted at harvesting and the animals may present pre-starvation symptoms affecting the body dry mass and biochemical composition with the consequent poor nutritional quality (Kotani et al., 2009; Makridis and Olsen, 1999; Szyper, 1989). Likewise, the use of non-expensive food sources such as baker yeast, some commercial products, and some microalgae species usually yield rotifers with nutritional deficiencies of the essential fatty acids (Ben-Amotz et al., 1987; Rainuzzo et al., 1989, 1994; Watanabe et al., 1983), vitamins and minerals (Giménez et al., 2007; Hamre et al., 2008). The potentially inappropriate nutritional quality of rotifers as food for fish larvae has been solved by re-feeding them with some microalgae species, oil emulsions, commercial microparticulated products and tailored boosters (Demir and Diken, 2011; Fernández-Reiriz et al., 1993; Olsen et al, 1989; Palmtag et al., 2006; Rodríguez et al., 1996) before being supplied to the rearing tanks.

This post-harvesting enrichment process has a double purpose. One is the incorporation of desired specific nutrients to the rotifer tissues, process that requires between 12 and 24 h of enrichment (Kotani et al., 2010; Rainuzzo et al., 1994). The other is to use the rotifer body, and most specifically the gut, as a living capsule for transferring a given feed containing a specific compound to the fish larval gut (bioencapsulation). In this second aspect, the final quality of the ingested food depends widely on the larval feeding protocol. Gut transit time in rotifers is relatively fast and consequently the time the rotifers spend in the rearing tank before being eaten will affect the final quality as a prey (Olsen et al., 1989; Yamamoto et al., 2009). Furthermore, the survival and normal development of larvae depends not only on the adequate nutrients but also on the ingestion of the sufficient calories. Therefore, the nutritional value of rotifers depends on their biochemical composition as well as on their dry mass and caloric content (Lubzens et al., 1989).

The objective of this study is to assess the nutritional quality in terms of mass and caloric content of rotifers during the common practices of feeding larvae in the hatcheries. With this aim, we have

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firstly determined the gut filling pattern of rotifers fed with different microalgal cell concentrations as well as the evacuation pattern in clean and green seawater, and secondly we have compared the dry weight and the carbon, nitrogen and energy content of overfed and starved rotifers.

2. Material and methods

B. plicatilis sensu stricto, strain S-1 (Dooms et al., 2007; Yúfera, 1982) was cultured in 1 L flasks at a temperature of 20 °C and a salinity of 33 ppt with gentle aeration. The microalgae *Nannochloropsis gaditana* was provided as food in all experiments.

2.1. Gut filling pattern

To determine the gut filling pattern the rotifers were first maintained under starvation conditions during 24 h to empty the guts. The rotifers were fed with algae at six different concentrations, 0.4×10^6, 1.2×10^6, 2.6×10^6, 5.2×10^6, 10.4×10^6 and 20.8×10^6 cell ml^{-1}. The density of the rotifers (30–40 individual ml^{-1}) was low enough to avoid that the ingestion affected the microalgal cell concentration during the experimental time. Rotifers were sampled in the six treatments at different times for a period of 240 min and fixed in formalin (4%v/v). Then, the body and gut volume were measured as described in Morphometric analyses section, and to avoid the variability associated with body size the gut volume was presented as percentage of the total female body volume. The following asymptotic function was fitted to the set of data points showing the increase of gut volume:

$$V = a\left(1-e^{-bT}\right).$$

Excepting for the treatment with 0.4×10^6 cell ml^{-1}, in which the best fit was obtained with a sigmoid equation:

$$V = a/\left(1+e^{(c-T)/b}\right)$$

where V is the gut volume (% body volume), T is time (min), *a* is the asymptote, and *b* and c are regression parameters.

2.2. Gut evacuation pattern

To determine the evacuation pattern, rotifers were first overfed with microalgae at high concentration (20.8×10^6 cell ml^{-1}) for 180 min. Then, microalgal cells were removed by filtration through a 71 μm mesh sieve using three different procedures. In the first procedure (non-submerged filtration), the rotifers (rotifers + culture medium) were poured over the filter and the collected rotifers were rinsed and transferred to a new flask with clean seawater. The second procedure (non-submerged filtration + microalgae) was the same as the first one but after rinsing, the rotifers were transferred to a flask containing seawater with 0.4×10^6 cell ml^{-1} of *N. gaditana* in order to simulate the conditions of green water rearing techniques. In the third procedure (submerged filtration), the rotifers were poured over a sieve submerged in a recipient with water to maintain the water volume during the washing process. Then, clean seawater was added to recipient to remove the microalgal cells.

Rotifers were sampled at different times for a period of 180 min and fixed in formalin. The first sample was taken just before the filtration (considered as time 0).The declining of gut volume was presented as percentage of the total body volume. The following exponential equation was fitted to the experimental points:

$$V = ae^{-bT} + ce^{-dT}$$

where V is the gut volume (% body volume), T is time (min) and *a*, *b*, *c* and *d* are the regression parameters.

2.3. Morphometric analyses

Determination of body and gut volume was carried out by image analysis. Between 6 and 10 rotifers of each sample were photographed under a microscope at 250× magnification. The pictures were analysed using the free software UTHSCSA ImageTool (University of Texas Health Science Center, San Antonio, TX, http://ddsdx.uthscsa.edu/). Body width and length and the gut area were measured in each individual (Fig. 1). For the gut measurement, the criterion was to consider just the green-coloured area of the image. This area could be composed of either 1 or 2 spots, corresponding to the so-called stomach and intestine cavities (Kleinow et al., 1991). Rotifers after starvation may exhibit a nominal gut lumen volume of 0 μm^3, this is due to the absence of microalgae, i.e. there is no coloured part in the image, as shown in Fig. 1A.

Rotifers body volume was calculated according to an ellipsoid of revolution. For each individual, the width and length (μm) were obtained measuring the lorica without spines (Yúfera, 1982). Assuming the cross section in this *Brachionus* strain is circular; depth and width are equal, and the volume was calculated as follows:

$$\text{Rotifer volume } \left(\mu m^3\right) = 4/3\pi ab^2$$

where *a* and *b* are one-half of the major and minor axes (length and width), respectively.

The gut cavities were considered to have a spherical shape and the volume was calculated from the area. For the calculation of the volume, the following formula was used:

$$\text{Gut volume } \left(\mu m^3\right) = 4/3\left(A_1\sqrt{(A_1/\pi)}\right) + 4/3\left(A_2\sqrt{(A_2/\pi)}\right)$$

where A_1 and A_2 correspond to the area (μm^2) of the two gut cavities measured in the pictures.

2.4. Dry mass and energy content

Dry weight was determined in rotifers with guts full of microalgae (fed 120 min with 20.8×10^6 cell ml^{-1}), after 120 min of evacuation in clean water without microalgae, and after 24 h of starvation. Dry weight was determined by drying triplicate samples of 900–1000 individuals at 60 °C. The samples were obtained by concentrating the rotifers with a submerged filter. After rinsing with distilled water to remove saltwater and microalgae the filter was taken out of the recipient with water and a sample was placed in pre-weighed glass covers. The egg/female ratio was quantified due to its positive correlation to dry weight (Yúfera and Pascual, 1989). Carbon and nitrogen content were analysed in rotifers with their guts completely full and after 24 h of starvation. Three 1 mg subsamples per treatment were analysed using an elemental CNHS analyser (Thermoquest, mod. Flash 1112), using sulphanilamide as standard. Energy content was estimated using the factor of 43 J per mg of carbon (Yúfera et al., 1997).

2.5. Statistical analysis

In order to identify significant differences of rotifer gut fullness among the six treatments, the maximum experimental mean reached in a specific time of each treatment were compared. The maximum percentages of gut volume were contrasted using one-way ANOVA followed by a post-hoc Tukey's test to identify which groups were significantly different.

3. Results

The body volume of the rotifers ranged between 0.73 and 4.21×10^6 μm^3 (average: $2.05\pm0.68\times10^6$ μm^3) (Fig. 2). The maximum gut volume average obtained from rotifers once the asymptote