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Short communication

Gut fluorescence analysis of barnacle larvae: An approach to quantify the ingested food

Chetan A. Gaonkar, Arga Chandrashekar Anil*

CSIR-National Institute of Oceanography, Dona Paula, Goa 4003 004, India

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ABSTRACT

Gut fluorescence analysis can provide a snapshot of ingested food and has been employed in feeding studies of various organisms. In this study we standardised the gut fluorescence method using laboratory-reared barnacle larvae (*Balanus amphitrite*) fed with mono-algal diet *Chaetoceros calcitrans*, a unicellular diatom at a cell concentration of 2×10^5 cells ml^{-1}. The gut fluorescence of IV–VI instar nauplii was found to be 370(±12) ng chlorophyll *a* larva^{-1} and in faecal pellets it was 224(±63) ng chlorophyll *a* larva^{-1}. A phaeopigment concentration in larval gut was found to be 311(±13) ng larva^{-1} and in faecal pellets it was 172(±61) ng larva^{-1}. The study also analysed larval samples collected from the field during different seasons from a tropical environment influenced by monsoons (Dona Paula bay, Goa, west coast of India), with characteristic temporal variations in phytoplankton abundance and diversity. Gut fluorescence of larvae obtained during the post-monsoon season was consistently higher when compared to the pre-monsoon season, suggesting the predominance of autotrophic forms in the larval gut during the post-monsoon season. Whereas, the low gut fluorescence obtained during the pre-monsoon season indicated the ingestion of food sources other than autotrophs. Such differences observed in the feeding behaviour of larvae could be due to differential availability of food for the larvae during different seasons and indicate the capability of larvae to feed on wide range of food sources. This study shows the value of the fluorescence method in feeding studies of planktotrophic organisms and in the evaluation of ecosystem dynamics.

1. Introduction

Barnacles are dominant in the intertidal region and development in these organisms includes planktonic naupliar stages followed by a pre-settling, non-feeding cyprid stage. Food available to the nauplii has a deterministic influence on the barnacle population in an area (Anil et al., 1995, 2001; Anil and Kurian, 1996; Pechenik et al., 1998; Desai and Anil, 2004). Laboratory studies have shown that diatoms are one of the food items most preferred by the larvae (Qiu and Qian, 1997; Anil et al., 2001; Desai and Anil, 2004; Desai et al., 2006). Recent studies have also shown that they can feed on wide range and size of food organisms (Turner et al., 2001; Vargas et al., 2006; Gaonkar and Anil, 2010) although the food preference and requirements of larvae in the wild is still not well understood.

A measure of gut fluorescence is one approach to quantify the food ingested by the larvae, even though there are certain limitations in the method, for example it can detect only the ingested phytoplankton and there is also a possibility of pigment destruction in the guts to non-fluorescent compounds (Conover et al., 1986; Head, 1992; Pasternak, 1994; Head and Harris, 1996; McLeroy-Etheridge and McManus, 1999 and the references therein). Analysis of pigments in the guts of grazers has become a popular tool for the last few decades for measuring the feeding activity of planktonic herbivores (Nemoto, 1968; Mackas and Bohrer, 1976; Baars and Oosterhuis, 1984; Kleppel et al., 1988; Durbin and Campbell, 2007; Lopez et al., 2007 and the references therein). However, this method has not been employed so far in the feeding studies of barnacle larvae.

The larval samples were collected from the Dona Paula Bay, Goa (located along the west coast of India), an area influenced by monsoons and with characteristic temporal variations in phytoplankton abundance and diversity. In this study, we initially standardised the gut fluorescence technique to assess the larval feeding using laboratory-reared larvae. This technique was then employed to evaluate the gut fluorescence of barnacle larvae collected from the field thus helping to understand the seasonal variability in availability of food for the larvae.

* Corresponding author.
E-mail address: acanil@nio.org (A.C. Anil).

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2. Materials and methods

The gut fluorescence method, described by Mackas and Bohrer (1976) to measure the *in situ* feeding rate of planktonic herbivores and then by Lopez et al. (2007) to estimate the copepod nauplii ingestion rates on phytoplankton, was adapted in this study to analyse the feeding of barnacle nauplii. Prior to analysing the gut fluorescence of field-collected larvae, experiments were conducted with laboratory-reared larvae to examine the influence of various factors on experimental procedure and to standardise the gut fluorescence protocol for the analysis of barnacle larval feeding.

2.1. Larval rearing in the laboratory

Larval development in barnacles consists of six naupliar instars and a pre-settling cyprid instar. The I instar nauplius is non-feeding and moults into II instar within a short time and the II to VI instars are phytoplanktivorus. Nauplii obtained from the adult broods of the barnacle *Balanus amphitrite* were mass reared in 2 L glass beakers on a daily diet of *Chaetoceros calcitrans*, a unicellular diatom at a cell concentration of 2×10^5 cells ml^{-1}. The cultures were maintained at 20 °C (±1 °C) temperature in the incubators at 12:12 h light:dark cycle. Larval density was maintained by monitoring the mortality of larvae every day, prior to changing the food.

2.2. Gut fluorescence analysis of laboratory-reared larvae

The larvae belonging to IV–VI instars were used for the analysis of gut fluorescence. Before analysing the fluorescence, larvae were washed 5–6 times with 0.22 μm filtered seawater to remove the adhering phytoplankton cells in which the larvae were reared. In preliminary experiments, to establish the minimum number of larvae needed to obtain valid gut fluorescence measurements, samples with different number of individuals (10, 20, 30, 40 and 50) were analysed and 30 individuals were found to be appropriate for the analysis of gut fluorescence. Background fluorescence of the larvae was also determined by starving the larvae for 24 h and it was found to be negligible. For each treatment, measurements were taken from 10 different replicates. To avoid gut evacuation and photodegradation of the gut contents, washing of the larvae, sorting and picking processes were carried out rapidly by working under a dissecting microscope with minimum exposure to light.

Pigments were extracted in 2 ml of 90% acetone, kept overnight at 4 °C in dark condition without homogenization (extraction was also tried with homogenised larvae and the differences in readings were found to be negligible). After extraction overnight, the solution was centrifuged and the upper clear solution was measured using a Turner Design (Model-Triology) fluorometer in the laboratory illuminated with dim light. Fluorescence was measured before and after acidification with HCl (Parsons et al., 1984) and the pigment concentration is expressed as nanograms per individual larva.

2.3. Fluorescence analysis of faecal pellets defaecated by laboratory-reared larvae

For the analysis of faecal pellets, larvae were reared until IV–VI instars and sorted (30 larvae) from the mass rearing beaker (2 L glass beaker) into a 50 ml beaker containing 0.22 μm filtered seawater and were incubated for 24 h for the evacuation of gut and to obtain pellets. After incubation, the larvae were separated out and the incubation water containing faecal pellets was filtered on to 0.22 μm filter paper by carefully rinsing the beaker. The pellets were then picked from the filter paper using a dissecting microscope with minimum exposure to light and were used for pigment extraction following the procedure mentioned above for larvae. Five replicate samples were analysed for this purpose.

Following the laboratory experiments, larval samples were collected from the field to measure the gut fluorescence of field-collected larvae and to study the temporal variations in gut fluorescence of field-collected larvae.

2.4. Field collection of larvae

Larval samples were collected from the Dona Paula Bay, Goa (west coast of India) with the help of a Haron–Trantor net of mesh size 100 μm (boat-towed horizontal plankton tows). Immediately after collection they were brought back to the laboratory and kept in 5 L containers with additional seawater (also collected from the same area from where the larvae were collected). The containers were supplied with moderate aeration and maintained at normal room temperature and natural light. Barnacle larvae of stages IV–VI instars were immediately sorted under the dissecting microscope into 0.22 μm filtered seawater and were washed 4–5 times with 0.22 μm filtered seawater to clean and remove the adhering phytoplankton cells and dirt from the surface of larvae; these were then used for the analysis of gut fluorescence following the above mentioned protocol for laboratory-reared larvae.

The study area is broadly categorized in to 3 seasons based on the influence of monsoons, i.e. the pre-monsoon (February–May), monsoon (June–September) and post-monsoon (October–January). Larval samples were collected during the post-monsoon and pre-monsoon seasons (samples were collected every alternate month in each season). Samples representing the post-monsoon season were collected during October (12th, 14th and 16th) and December (14th, 16th and 18th) 2009. Samples representing the pre-monsoon season were collected during March (15th, 17th and 19th) and May (17th, 19th and 21st) 2010. Five replicate samples were analysed from each sampling events.

2.5. Data analysis

Gut fluorescence estimations of larvae obtained from different sampling months were subjected to one-way analysis of variance (ANOVA) (Sokal and Rohlf, 1981) to evaluate the variance between different sampling months (temporal variations). Data were log transformed before being subjected to ANOVA to ensure normality and homogeneity of variance.

3. Results and discussion

Fluorescence analysis of the laboratory-reared larvae indicated that the mean chlorophyll *a* content of the larval gut is 370 (±12)

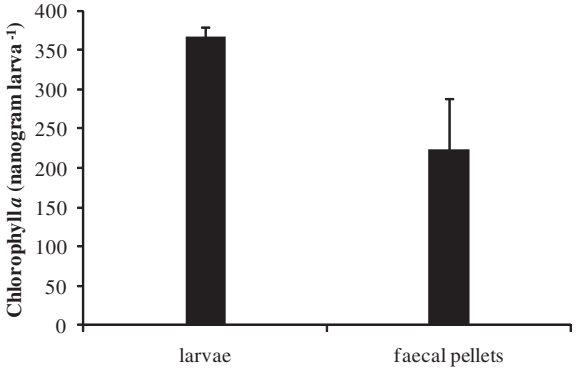

Fig. 1. Chlorophyll *a* content of the laboratory-reared larvae and their faecal pellets.