Influence of Diet on the Ingestion Rate of the Harpacticoid Copepod *Euterpina acutifrons* (Dana, 1847)

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ABSTRACT

The influence of the type of diet, concentration, and sex on the ingestion rate of the harpacticoid copepod *Euterpina acutifrons* was examined in controlled laboratory experiments. Four dietary treatments were investigated in 1:1 proportion: *Isochrysis galbana*, *I. galbana* and *Tetraselmis suecica*, *I. galbana* and *Dunaliella* sp., and *I. galbana* and baker's yeast (*Saccharomyces cerevisiae*). Furthermore, six different cell concentrations were tested for each combination: 500, 700, 1,000, 1,500, 10,000 and 20,000 cell ml^{-1}. All experiments were carried out under the photoperiod of 12h:12h (light:dark cycle), with salinity at 32±1 psu and at 28±2 °C for 24 hours. The results showed no difference in the ingestion rates among different diets or between male and female *E. acutifrons* ($P>0.05$). This indicates that mature *E. acutifrons* can feed on a variety of foods. However, different cell concentrations significantly influenced the ingestion rate of *E. acutifrons* ($P<0.05$). The highest ingestion rate (15,563 cells *E. acutifrons*$^{-1}$ day^{-1}) for *E. acutifrons* was observed when a combination of *I. galbana* and *Dunaliella* sp. at 20,000 cells ml^{-1} was provided. Further studies are needed to obtain the maximum ingestion rate for *E. acutifrons*.

Keywords: *Euterpina acutifrons*, ingestion rate, diet

INTRODUCTION

Rotifer and *Artemia* sp. are the most popular feeding options in hatchery-based larviculture (Kraul, 2006). Although these are easy to culture in high densities, they have low nutritional value for some larvae (Zaleha *et al*., 2012). Studies have shown that marine fish larvae fed with copepod nauplii had faster growth, enhanced nutritional content, and a higher survival rate compared to those fed with diets consisting solely of rotifers and *Artemia* sp. (Kraul *et al*., 1992; Nanton and Castell 1999; Zaleha *et al*., 2012). Harpacticoid copepods are of a suitable size for marine fish larvae and have high nutritional value (e.g. Johnson and Olson, 1984; Ajiboye *et al*., 2011). Moreover, harpacticoid copepods are easy to maintain in culture as they feed on a variety of food options: microalgae, bacteria, detritus, or artificial food, and are more tolerant to salinity and temperature alterations than calanoid and cyclopoid copepods (Delbare *et al*., 1996; Díaz *et al*., 2003), but few species can be grown under mass culture conditions. *Euterpina acutiforns* is one of the most common harpacticoid copepods in Thai waters (Jitchum and Wongrat, 2009; Maiphae and Sa-ardrit, 2011; Promkaew *et al*., 2012). A previous study attempted to use microalgae as feed for *E. acutiforns* (Camus and Zeng, 2012). However, Matias-Peralta *et al*. (2012) suggested that many harpacticoids are highly specific in their choice of food organisms either for survival or as a required stimulus for growth and reproduction. This study aimed to investigate the

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influence of diet on the ingestion rate of *E. acutiforns* to evaluate its potential as a live feed for the marine fish larvae industry.

MATERIALS AND METHODS

Euterpina acutifrons culture

E. acutifrons was collected from the Sikao canal and mangrove creeks in Sikao district, Trang, Thailand, using a 100 µm mesh size plankton net towed vertically. The concentrated zooplankton was rinsed and poured into bottles, kept in a cooler to maintain a stable temperature and prevent exposure to direct sunlight, and transported immediately to the laboratory at Rajamangala University of Technology Srivijaya (RMUTSV), Thailand. *E. acutifrons* was isolated by using the single cell technique under the stereomicroscope (Olympus SZ-40). Then *E. acutifrons* were fed with *Isochrysis galbana* Guisande *et al*., 1999) and maintained in autoclaved 1 µm filtered seawater 32±1 psu, at 25±1ºC, with a 12:12h light: dark cycle.

Diet preparation

The following phytoplankton species, *I. galbana*, *Tetraselmis suecica* and *Dunaliella* sp. were maintained in Conwy media (Walne, 1974) under similar conditions as described for *E. acutifrons* culture. Baker's yeast (*Saccharomyces cerevisiae*) was considered as a substitute for microalgae in this study because of their suitable particle size, high protein content, high stability in the water column and availability in local markets at a relatively low price. Baker's yeast (*S. cerevisiae*) was added to autoclaved 1 µm filtered seawater (0.4 g L^{-1}) at least 30 mins before the experiment started, under the same temperature and salinity conditions as before.

Ingestion rate experiment

To estimate the ingestion rate, we incubated 3 males and 3 females of *E. acutifrons* into 125 ml test tubes containing 25 ml of autoclaved 1 µm filtered seawater (6 *E. acutifrons* per diet), under the same conditions as before, except the temperature was 28 ±2ºC. Four dietary treatments were investigated as follows: 1) *I. galbana*; 2) *I. galbana* and *T. suecica* (1:1 density/density); 3) *I. galbana* and *Dunaliella* sp. (1:1); 4) *I. galbana* and baker's yeast (*S. cerevisiae*) (1:1). For each of these treatments, six different cell concentrations were used: 500, 700, 1,000, 1,500, 10,000 and 20,000 cell ml^{-1}. The experiments were set up by using a completely randomized design (CRD) with triplicates. The control incubations were grown without *E. acutifrons*. Before starting the experiment, the selected *E. acutifrons* were acclimatized in the same conditions as the assigned treatment for two days. The experiments lasted for 24h and three trials were run. During the experiments, the incubations were mixed gently every 4h to avoid settling of the microalgae and yeast. Microalgae and yeast counts were obtained at T_0 and T_{24} using a compound microscope (Olympus CH30).

Ingestion rates of *E. acutifrons* were determined from the decrease in diet concentrations over 24h compared to the growth of the control as described by Jakobsen and Hansen (1997). The ingestion rate *Uy* was estimated using the following equations:

$$\frac{dx}{dt} = \mu_x - U_y$$

$$\frac{dy}{dt} = \mu_y y$$

where (*x*) is the number cells ingested by the grazer (*y*). It is assumed that the diet (*x*) grows with a constant rate of μ_x. The mortality/decrease of the diet due to grazing is *Uy*, where *U* (cells *E. acutifrons*$^{-1}$ d^{-1}) is the per capita ingestion rate, which is independent on *x*. The ingestion rate (*U*) was iteratively calculated using "Prey" (by B. Vismann) software (Jakobsen and Hansen, 1997).

Data analysis

One-way ANOVA was used to test if the differences between the ingestion rates of *E. acutifrons* were influenced by different diets and diet densities. If significant differences ($P<0.05$) were found, Tukey's multiple comparisons test was used to determine specific difference among treatments.

RESULTS

E. acutifrons consumed all the treatment diets including *I. galbana*, *T. suecica*, *Dunaliella* sp. and baker's yeast (*S. cerevisiae*) without a clear preference (Figure 1).

The ingestion rate of *E. acutifrons* significantly increased with an increase in all diet concentrations ($P< 0.05$) (Figures 1 and 2). At 20,000 cells ml^{-1} of prey, the average lowest ingestion rate ($1.00\times10^4\pm1.61\times10^2$ cells *E. acutifrons*$^{-1}$ day^{-1}) of *E. acutifrons* was observed when a combination of *I. galbana* and *T. suecica* was provided, while the highest ingestion rate ($1.55\times10^4\pm2.83\times10^3$ cells *E. acutifrons*$^{-1}$ day^{-1}) was found when *E. acutifrons* was fed with a combination of *I. galbana* and *Dunaliella* sp.

The sex of *E. acutifrons* showed no significant influence on ingestion rates ($P>0.05$). Male *E. acutifrons* showed ingestion rates varying from 4.45×10^2 to 1.53×10^4 cells *E. acutifrons*$^{-1}$ day^{-1} while female *E. acutifrons* showed ingestion rates varying from 5.08×10^2 to 1.54×10^4 when fed on *I. galbana* at different densities (Figure 2a). Ingestion rates of male and female *E. acutifrons* fed on a combination of *I. galbana* and *T. suecica* varied from 2.68×10^2 to 1.02×10^4 cells *E. acutifrons*$^{-1}$ day^{-1} and from 2.41×10^2 to 9.78×10^3 cells *E. acutifrons*$^{-1}$ day^{-1}, respectively (Figure 2b). Male *E. acutifrons* fed on the combination of *I. galbana* and *Dunaliella* sp. showed ingestion rates varying from 3.79×10^2 to 1.66×10^4 cells *E. acutifrons*$^{-1}$ day^{-1} and female *E. acutifrons* showed ingestion rates varying from 3.50×10^2 to 1.40×10^4 cells *E. acutifrons*$^{-1}$ day^{-1} (Figure 2c). Ingestion rates of male and female

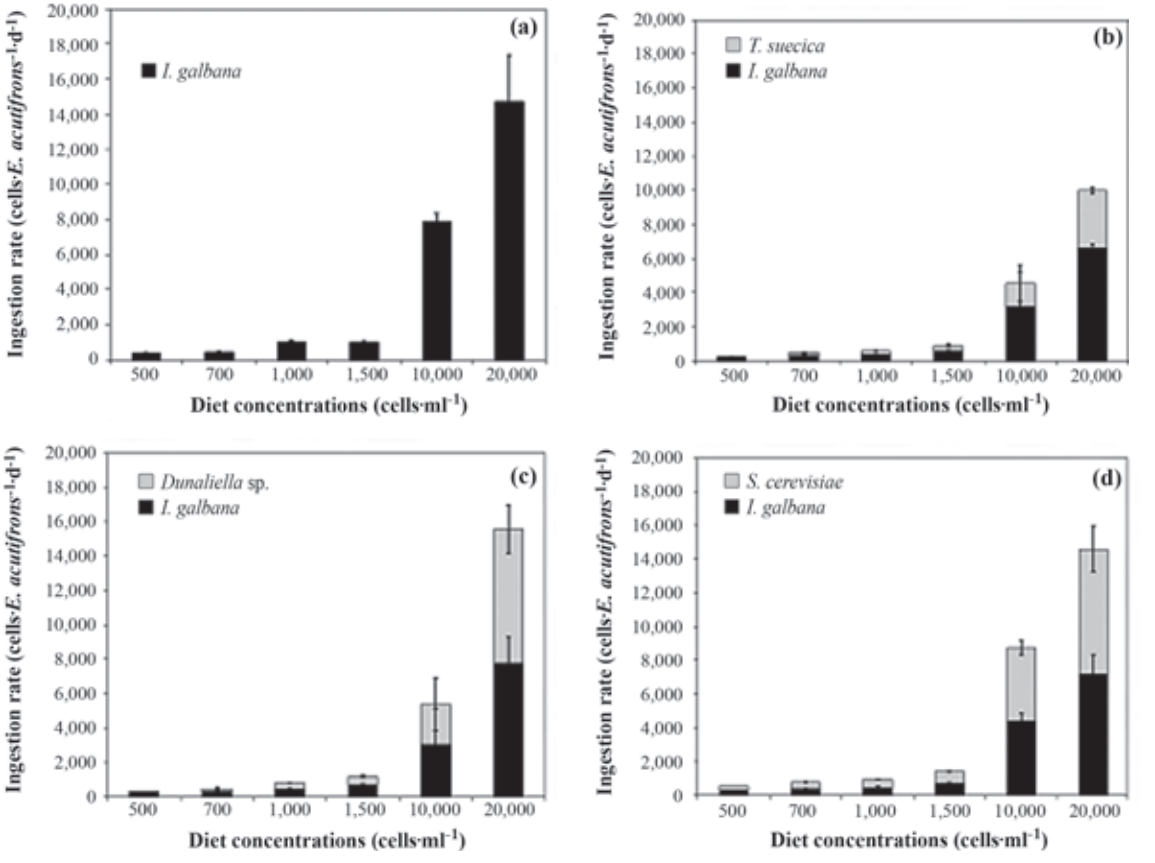

Figure 1. The ingestion rate of *E. acutifrons* fed with different diets and densities: (a) *I. galbana*, (b) *I. galbana* and *T. suecica*, (c) *I. galbana* and *Dunaliella* sp., and, (d) *I. galbana* and baker's yeast (*S. cerevisiae*).

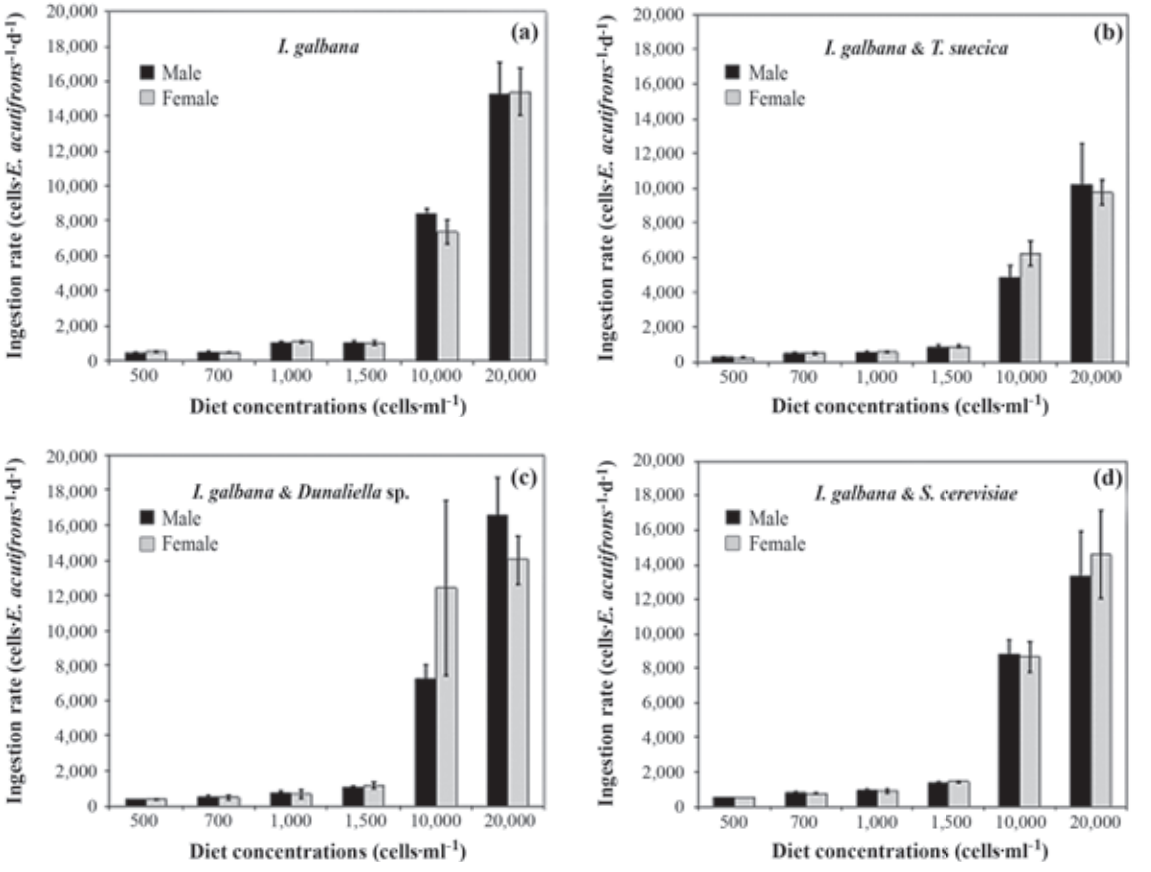

Figure 2. Ingestion rates of male and female *E. acutifrons* fed with different diets and densities: (a) *I. galbana*, (b) *I. galbana* and *T. suecica*, (c) *I.galbana* and *Dunaliella* sp., and (d) *I. galbana* and baker's yeast (*S. cerevisiae*)

E. acutifrons fed on a combination of *I. galbana* and baker's yeast (*S. cerevisiae*) varied from 5.28×10^2 to 1.33×10^4 cells *E. acutifrons*$^{-1}$ day^{-1} and from 5.20×10^2 to 1.46×10^4 cells *E. acutifrons*$^{-1}$ day^{-1}, respectively (Figure 2d).

DISCUSSION

One of the most important questions in sustainable copepod culture is the kind and amount of food needed for optimum growth (Matias-Peralta *et al*., 2012). Our results show that *E. acutifrons* could consume all the assigned diets. These findings confirm Sautour and Castel's (1993) results, which showed that *E. acutifrons* is a passive filter feeder when small diets are presented. Our results also indicate that baker's yeast can be used as a source of food for *E. acutifrons*, reducing the cost and time to maintain *E. acutifrons* in culture, compared to microaglae. Tanyaros *et al*. (2016) have recently shown that tropical juvenile oysters (*Crassostrea belcheri*) could be reared with microalgae and a 25% substitution of untreated yeast. However, further experiments on the effect of a baker's yeast - mixed diet on reproductive performance of *E. acutifrons* is recommended to determine its potential use in the mass culture of *E. acutifrons*.

The ingestion rate of *E. acutifrons* increased with an increase in all diet concentrations. However,

the ingestion rates of *E. acutifrons* did not remain constant when the highest diet concentration was provided. This indicates that our results did not reach the maximum ingestion rate for *E. acutifrons*. These are in agreement with previous studies on *E. acutifrons* and other copepods (Sautour and Castel, 1993; Abu-Rezq *et al*., 1997; Oliveira Lemos *et al*., 2006). Thus, Oliveira Lemos *et al*., (2006) found that ingestion rates of *E. acutifrons* fed with *Thalassiosira weissflogii* varied between 4.32×10^3 and 1.95×10^5 cells copepod^{-1} d^{-1}. Our results also showed no difference in ingestion rates among varying diets and between male and female *E. acutifrons*. This may be because the size ranges of the diets used in our study are within those reported previously for *E. acutifrons* (6-16 µm), and it has been shown that it can feed on particles other than phytoplankton (Kinne, 1977, cited in Guisande *et al*., 1996; Sautour and Castel, 1993; Díaz *et al*., 2003). Interestingly, while the ingestion rate was not significantly influenced by sex, this has been the case in other copepods (Dhanker and Hwang, 2013). The effect on culture productivity and biochemical composition of *E. acutifrons* when fed different diets at higher diet concentrations are critical in determining if *E. acutifrons* is a good candidate for feed for marine fish larvae commercially.

CONCLUSION

E. acutifrons can eat a variety of diets from microalgae to yeast. Diet concentrations influenced ingestion rates of *E. acutifrons*. The highest ingestion rate of *E. acutifrons* was observed when fed a combination of *I. galbana* and *Dunaliella* sp.

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