

The consumption rate of three zooplankton species of different size fed on the green microalgae *Chlorella vulgaris*

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ABSTRACT

The live food (phytoplankton and zooplankton) is a very important food source in fish farming, particularly for early larval stages. Therefore, the present study was carried out to determine the consumption rate (grazing (G) and ingestion (I) rates) of three zooplankton species of different size, *Brachionus plicatilis*, *Acanthocyclops trajani*, and *Heterocypris salina*. Five different concentrations (10, 20, 30, 40 and 50 $\times 10^4$ cells/ml) of the most common cultured phytoplankton species (*Chlorella vulgaris*) were utilized as fed for these species. The optimal concentration of the green algae that nourish the zooplankton species with minimum leftover that could affect the water quality was determined. The results revealed that grazing and ingestion rates of *B. Plicatilis* and *H. salina* showed linear regression with algal concentrations ($p < 0.008$; $R^2=0.72$ and 0.82 ,) and ($p < 0.001$; $R^2= 0.969$ and 0.964), respectively. Their consumption rates values increased markedly with increasing the concentration of *C. vulgaris*. On the other hand, the consumption rate of *A. trajani* were independent of algal concentrations ($p \geq 0.09$ and 0.07 ; $R^2= 0.07$ and 0.05 , respectively), and reached their maximum values at 30×10^4 cell /ml. The study concluded that *C. vulgaris* is a suitable food for *B. Plicatilis* and *H. salina* while it is not for *A. trajani*, where its feeding behaviour still needs more in-depth studies.

INTRODUCTION

Live feeds are the main item in the diet of cultured fish larvae and they are of particular importance when rearing marine fish larvae of the altricial type (Conceição *et al.*, 2010). At first-feeding of altricial larvae, the digestive system is still embryonic with undeveloped stomach, so the protein digestion takes place in hindgut epithelial cells (Govoni *et al.*, 1986). Feeding of most importance species to aquaculture is still dependent on live feeds during the early stages of life (Yousef and Hegab, 2017). Also, Live preys are swimmers, thus they are constantly available to the larvae in the water column, while the artificial diets tend to aggregate on the water surface or sink within a few minutes to the bottom. Thus, small and swimming prey is more acceptable to fish larvae (Herbing, 2001). Moreover, live preys are characterized by their thin exoskeleton and high water content, that may be more suitable to the larvae compared to the hard, dry artificial feeds (Conceição *et al.*, 2010).

Further more, in the management of larval fish culture of various species, zooplankton as natural feed is a critical point to successful transition of larvae to the fingerlings stage.

In addition, information about the relative status of plankton (zooplankton and phytoplankton) communities gives insight into water quality parameters and the possible success or failure of the culture season (Morris and Mischke, 1999).

Most hatcheries grow a variety of planktonic species that meet the different needs during the production cycle with respect to larval size, digestibility, culture characteristics, nutritional requirements (Muller-Feuga *et al.*, 2003). Thus, it is a necessary to supply fish larval culture with a various species of zooplankton with different size that correspond to growth of these larvae and the development of their mouth opening.

Brachionus plicatilis (rotifers species) has the shortest life span (12 days), reach its high reproductive peak in about 3.5 days (Allan, 1976). More than 60 marine finfish and 18 crustaceans' species are fed on *B. plicatilis* (Abu-Rezq *et al.*, 2002). It is the smallest among studied species where its lorica length ranged from 123 to 292 μm (Snell and Carrillo, 1984). Many studies applied *Chlorella* spp. as feed for culturing *B. plicatilis* (eg; James and Abu-Rezeq 1988; Maruyama and Hirayama, 1993; Maruyama *et al.*, 1997; Alam and Shah, 2004; Viayeh and Mohammadi, 2012; Kim *et al.*, 2014). However, the studies conducted to calculate its grazing and ingestion rates on *Chlorella* spp. are still scarce (e.g; Hotos, 2003; Savas and Guclu, 2006).

Acanthocyclops trajani (copepod species), has only sexual reproduction, require longer periods to increase its population levels. However, copepods able to keep up their populations during the later stages of a culture season, because they are fast and powerful swimmers (Geiger and Turner 1990). The mean body length of *A. trajani* is about $398 \pm 54 \mu\text{m}$ (Bláha, 2010). Although *A. trajani* has become dominant in the northern delta lakes (Dumont, 2009) and fish ponds in Egypt, there were no attempts to cultivate it and calculate its grazing and ingestion rates on phytoplankton species.

Heterocypris salina (ostracod species). As ostracod species, is important as a natural food item for fish and invertebrates (Chakrapani *et al.*, 1996). *H. salina* is the largest among the three studied species, it has carapace length of about 1.2 – 123 mm (Ali *et al.*, 2018). *H. salina* is well-known for its high egg productivity and it can tolerate the hard environmental conditions (Kubanc *et al.*, 2007). While it is easy to culture *H. salina*, the data on its culturing, grazing and ingestion rates on phytoplankton species are still rare (Yousef and Hegab, 2017).

Chlorella vulgaris is one of well known green unicellular microalgae characterized with biological and pharmacological properties. The chloroplast of *Chlorella* contains the green pigments chlorophyll *a* and *b*. it doing multiplication through the photosynthesis process. *C. vulgaris* used extensively as a food source due to presence of unique functional nutrients like, polysaccharides, proteins, omega-3 polyunsaturated fatty acids, vitamins and minerals. (Panahi *et al.*, 2016).

In order to promote optimal mass production of zooplankton species to be used in rearing fish larvae, there is a need for the standard grazing (G) and ingestion rates (I) of these species fed on phytoplankton. This information is very important and required for zooplankton culture in the advanced fish culture patterns. Therefore, the present study was carried out to determine the grazing and ingestion rates (consumption rate) of three zooplankton species of different size, *Brachionus plicatilis*, *Acanthocyclops trajani*, and *Heterocypris salina*. In this study, the most

common phytoplankton cultured species (*Chlorella vulgaris*) was utilized as fed for these species.

MATERIALS AND METHODS

The three zooplankton species with different size (*Brachionus plicatilis*, *Acanthocyclops trajani* and *Heterocypris salina*) were used in three separated experiments to calculate their grazing and ingestion rates (consumption rate) on the green algae *Chlorella vulgaris*.

The green algae *Chlorella vulgaris* was grown under sterilized laboratory conditions in modified BG11 medium (Rippka *et al.*, 1979) at controlled conditions of temperature $25 \pm 1^\circ\text{C}$ and light intensity of $30\mu\text{Em}^{-2}\text{s}^{-1}$ under a day / night program of 14 h light followed by 10 h darkness. Growth of microalgae culture was determined by using Neubauer counting chamber each day. The algae was harvested in its exponential growth phase to conduct the grazing and ingestion rates experiments. Nutrient solution of modified BG11 medium (5ml) was added to avoid nutrient deficiency in the experimental flasks, also to compensate the growth of algae. Grazing and ingestion rates of each zooplankton species were compared using five different concentrations of *Chlorella vulgaris* (10, 20, 30, 40 and 50×10^4 cells/ml) to determine the optimal concentration of the green algae that nourish the zooplankton species with minimum leftover that could affect the water quality.

Before the start of experiments, zooplankton species were starved in clear water (without algae) for 24 h. The experiments were conducted in three replications in transparent glass culture flasks (250 ml).

Experiment (1) Grazing and ingestion rates of *Brachionus plicatilis*

The species was derived from preparatory culture of *B. plicatilis* at the National Institute of Oceanography and Fisheries, Inland water branch, Egypt. To calculate grazing and ingestion rates, *B. plicatilis* was cultured with the different concentrations of *Chlorella vulgaris* in 100 ml saline water (20 ‰) at 25°C for 3 hours. The experiment was started with fixed initial *B. plicatilis* (10 ind./ml) of each algal concentration.

Experiment (2) Grazing and ingestion rates of *Acanthocyclops trajani*

The species was collected from fish pond at the National Institute of Oceanography and Fisheries, Inland water branch, Egypt. Adult individuals were isolated under binocular microscope to be used in the experiment. The experiment was conducted in 100 ml freshwater at 25°C for 3 hours.

The experiment was started with fixed number of *A. trajani* (2 ind./ml) of each algal concentration.

Experiment (3) Grazing and ingestion rates of *Heterocypris salina*

The adult individuals of *H. salina* were collected from its preparatory culture at the National Institute of Oceanography and Fisheries, Inland water branch, Egypt. The experiment was conducted in 100 ml freshwater at 25°C for 3 hours.

The experiment was started with fixed number of *H. salina* (1 ind./ml) of each algal concentration.

Grazing and ingestion rates calculation:

Grazing rate (G) is the volume of water filtered or cleared by a single individual of zooplankton to remove all food suspension cells in a unit of time. While, ingestion rate (I), is the measure of mass (total number of cells) flow into an individual of zooplankton per time (Peters, 1984).

Grazing rate (G) and ingestion rate (I) were calculated according to the equation:

$$G = V(\ln C_0 - \ln C_t) / nt; I = G \sqrt{C_0 C_t} \text{ (Schlosser \& Anger 1982),}$$

where C_0 is the initial concentration of *Chlorella vulgaris*, C_t is the final concentration of *Chlorella vulgaris* at time t (minutes), n is the number of zooplankton species in volume v (ml).

t-test and regression analysis was applied to study the relationship between grazing, ingestion rates of each zooplankton species and the concentrations of *Chlorella vulgaris* using XLstat 2016 software.

RESULTS

Grazing and ingestion rates of *Brachionus plicatilis*

Grazing and ingestion rates of *B. plicatilis*, of density of 10 ind. ml⁻¹, at different concentrations of *Chlorella vulgaris* are shown in Fig. 1. There was significant regression between grazing, ingestion rates of *B. plicatilis* and algal concentrations ($p < 0.002$ and 0.008 ; $R^2 = 0.725$ and 0.825 , respectively). Where, the optimal values of grazing and ingestion rates (0.33 ml/ind.min⁻¹ and 1.14 cells ind.⁻¹ min⁻¹) were recorded at the highest concentration of *C. vulgaris* (50 x 10⁴ cell ml⁻¹). Grazing and ingestion rates values decreased markedly with decreasing the densities of *C. vulgaris* to reach the lowest values of 0.16 ml/ind.min⁻¹ and 0.35 cells ind.⁻¹ min⁻¹ with algal concentration of 20 x 10⁴ cell ml⁻¹. However, grazing and ingestion rates slightly increased (0.2 ml/ind.min⁻¹ and 0.56 cells ind.⁻¹ min⁻¹) with the lowest algal concentration of 10 x 10⁴ cell ml⁻¹.

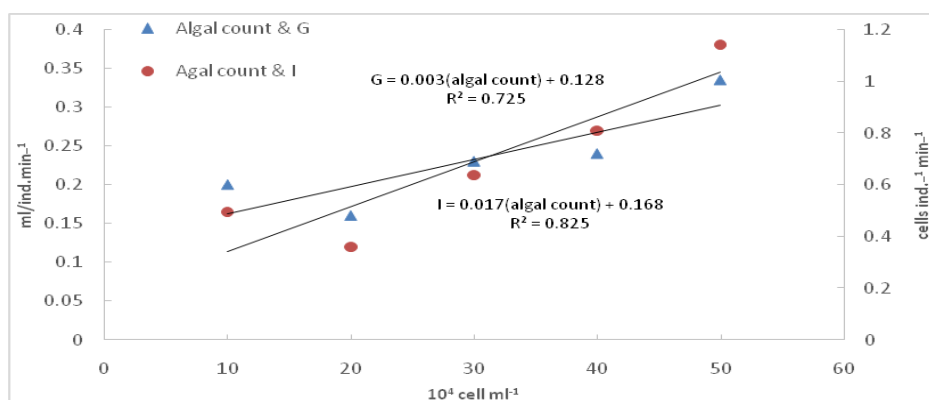


Fig.1. grazing and ingestion rates of *B. plicatilis* with different concentrations of *C. vulgaris*.

Grazing and ingestion rates of *Acanthocyclops trajani*

Figure 2 shows that, grazing and ingestion rates of *A. trajani* were independent of algal concentrations in the range observed ($p \geq 0.09$ and 0.07 ; $R^2 = 0.07$ and 0.05 , respectively). The maximum grazing and ingestion rates were calculated at the algal concentration of 30 x 10⁴ cell ml⁻¹ with values of 2.5 ml/ind. min⁻¹ and 8.6 cells ind.⁻¹ min⁻¹, respectively. *A. trajani* attained the minimum values of grazing and ingestion rates (0.1 ml/ind.min⁻¹ and 0.6 cells ind.⁻¹ min⁻¹) at algal concentration of 20 x 10⁴ and 40 x 10⁴ cell ml⁻¹, respectively.

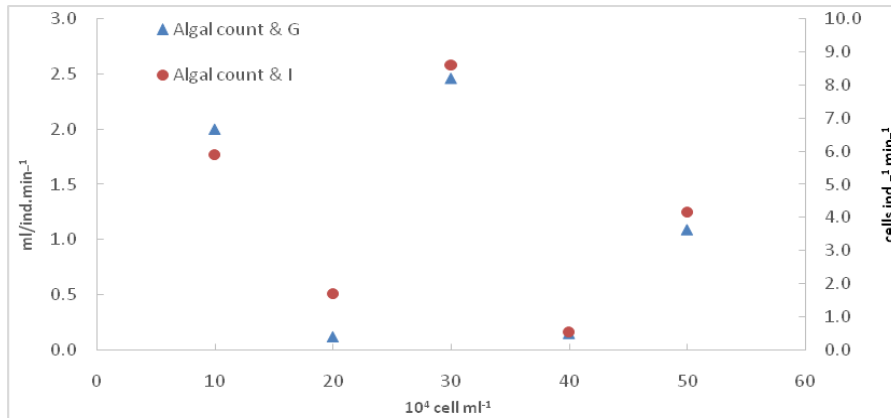


Fig. 2: Grazing and ingestion rates of *A. trajani* with different densities of *C. vulgaris*

Grazing and ingestion rates of *Heterocypris salina*

Grazing and ingestion rates of *H. salina* with different densities of *C. vulgaris* are shown in Fig. 3. Grazing and ingestion rate were significantly related to algal concentrations ($p < 0.001$; $R^2 = 0.969$ and 0.964 , respectively). Where, the highest values of grazing and ingestion rates ($9 \text{ ml/ind.min}^{-1}$ and $35 \text{ cells ind.}^{-1} \text{ min}^{-1}$, respectively) were calculated at the high algal concentration ($50 \times 10^4 \text{ cell ml}^{-1}$), while the lowest values of $1.2 \text{ ml/ind.min}^{-1}$ and $3.1 \text{ cells ind.}^{-1} \text{ min}^{-1}$, respectively were calculated at the lowest algal concentration ($10 \times 10^4 \text{ cell ml}^{-1}$).

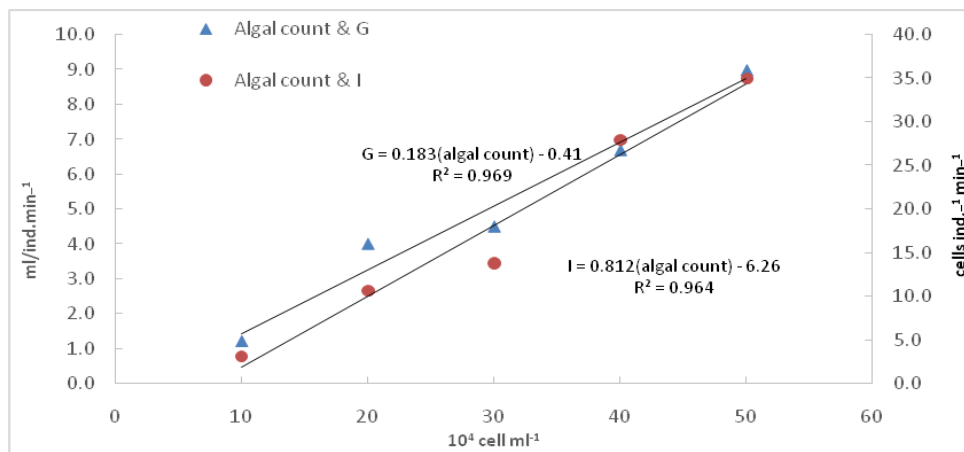


Fig. 3: Grazing and ingestion rates of *H. salina* with different densities of *C. vulgaris*.

DISCUSSION

The utilization of zooplankton species on any algal species is expressed by the ingestion rate that is depended on the grazing rate (Hotos, 2003). Grazing and ingestion rates are affected by several factors: (a) food type (Lubian 1982); (b) temperature (Pourriot 1990); (c) food concentration (Hotos, 2003); (d) cell size (Hotos, 2003) and (f) zooplankton species (Yufera and Pascual 1985). There is a need to calculate the standard grazing and ingestion rates of zooplankton on phytoplankton. This information is very important and required for zooplankton culture in the advanced aquaculture.

Selection of *Chlorella vulgaris* because of its suitable size ($3 \mu\text{m}$), culturing easy, non toxicity and its significant contents of proteins, long-chain poly unsaturated fatty acids, vitamins and sterols making this algae very unique with its health

benefits (Belasco, 1997). Also digestible cell wall to gain its nutrients (Raja *et al.*, 2008; Patil *et al.*, 2007).

The present experiments were conducted at water temperature of 25 °C. Temperature is known to influence feeding rates of zooplankton, where grazing rates of *B. plicatilis* enhanced to 42 % when water temperature increased from 18 °C to 23.5°C (Schlosser and Anger, 1982). Yousef and Hegab (2017) mentioned that, the optimum water temperature to grow *H. salina* is $25 \pm 1^\circ\text{C}$. Also, *A. trajani* was abundant at the same water temperature of 25°C (the time of species collection and isolation) from fish pond.

The results revealed that grazing and ingestion rates of *B. plicatilis* and *H. salina* were increased with increasing algal concentration, where the highest values recorded at the highest concentration of *Chlorella vulgaris* ($50 \times 10^4 \text{ cell ml}^{-1}$). On the other hand, grazing and ingestion rates of *A. trajani* were independent of algal concentrations, and reached their maximum values at $30 \times 10^4 \text{ cell ml}^{-1}$. The positive linear correlation between algal concentration and G, I of both *B. plicatilis* and *H. salina* can be explained on the basis of that the high food concentration stimulate zooplankton to consume a large amount of food, that may enhance their vital processes including growth and reproductive rates. Nogrady *et al.*, (1993) mentioned that rotifers are opportunistic species; respond more quickly to the changes in food concentrations. The increase in food concentration results in increased egg output and population growth rate of rotifers (Dumont *et al.*, 1995; Nandini and Sarma, 2003). Furthermore, the high food concentration improves the production dynamics and biochemical composition of *B. plicatilis* (James and Abu-Rezeq 1988). Espinosa-Rodríguez (2014) reported that the population growth of four rotifer species (*Brachionus angularis*, *B. havanaensis*, *B. rubens*, and *Plationus patulus*) increased with increasing algal concentrations. The reproductive rate of zooplankton is affected by the change in food concentrations (Donelson *et al.*, 2010). All this could explain the increase of grazing and ingestion of *B. plicatilis* and *H. salina* with increasing algal concentration. while some studies mentioned that the grazing and ingestion rates of *B. plicatilis* decreased with increasing *Chlorella* concentrations (Hotos, 2003; Savas and Guclu, 2006), these studies were investigating algal concentration more than the highest algal concentration of this study. This inconsistency may be explained by the hypothesis that grazing and ingestion rates increase with increasing food concentrations to the limit, that sufficient to maintain the biological activities of individuals in optimum condition, after which, the grazing and ingestion rates decrease at the higher cell concentrations (Yúfera and Pascual, 1985). Navarro (1999) concluded that, as microalgal concentration increases from very low concentration, both grazing and ingestion rates of *B. plicatilis* rise until reached to a reduction point. Above this point, the maximum food processing rate could be kept with grazing and ingestion rates that decrease progressively.

While *B. plicatilis* and *H. salina* are completely dependent on algae as the only source of food (Loka *et al.*, 2016; Yousef and Hegab, 2017), *A. trajani* feeding behavior has not been fully studied yet. However, the cyclopoid copepods are, generally, well known of their dependency on both animal or algal food sources (Adrian and Frost, 1993). This may explain the difference in grazing and ingestion pattern of *B. plicatilis* and *H. salina* in one side and *A. trajani* in the other. *A. trajani* may be not completely dependent on algae in its natural feeding habit. Therefore, under this study experimental condition, where no animal food source was available, the only food source was the algae, which lead to irregularly in the correlation between grazing, ingestion and the algal concentration.

CONCLUSION

Chlorella vulgaris is a suitable food for *Brachionus plicatilis* and *Heterocypris salina*. Their grazing and ingestion rates were dependent on the algal densities, where 50×10^4 cell ml⁻¹ of *C. vulgaris* was an adequate density to raise the consumption rate of *B. plicatilis* and *H. salina* to the highest values. On the other hand, the grazing and ingestion rates of *A. trajani* were independent of algal density, where its feeding behaviour still needs more in-depth studies.

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ARABIC SUMMARY

معدل استهلاك ثلاثة أنواع من العوالق الحيوانية ذات أحجام مختلفة تتغذى على الطحالب الخضراء *Chlorella vulgaris*

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تعتبر الأغذية الحية (العوالق النباتية والحيوانية) مصدرًا غذائيًا مهمًا للغاية في تربية الأسماك ، لا سيما في مراحل اليرقات المبكرة. لذلك ، أجريت هذه الدراسة لتحديد معدلات الاستهلاك (معدلات الرعي (G) والابتلاع (I)) لثلاثة أنواع من العوالق الحيوانية ذات أحجام مختلفة ، *Brachionus plicatilis* ، و *Acanthocyclops trajani* ، و *Heterocypris salina*. في هذه الدراسة ، تم استخدام خمسة تركيزات مختلفة (١٠ و ٢٠ و ٣٠ و ٤٠ و ٥٠ × ١٠^٤ خلية / مل) من النوع الأكثر شيوعًا من العوالق النباتية المستزرعة (*Chlorella vulgaris*) لتغذية هذه الأنواع وذلك لتحديد التركيز الأمثل للطحالب الخضراء التي تغذي أنواع العوالق الحيوانية بأقل بقايا يمكن أن تؤثر على جودة المياه. أظهرت النتائج أن معدلات الرعي والابتلاع لـ *B. Plicatilis* و *H. salina* أظهرت انحدار خطي بتركيزات الطحالب ($P > 0.008$ ؛ $R^2 = 0.72$ و 0.82) ، و ($P > 0.001$ ؛ $R^2 = 0.969$ و 0.964) ، على التوالي. كما زادت قيم معدلات الاستهلاك بشكل ملحوظ مع زيادة تركيز *C. vulgaris*. من ناحية أخرى ، كانت معدلات استهلاك *A. trajani* مستقلة عن تركيزات الطحالب ($P \geq 0.09$ و 0.07 ؛ $R^2 = 0.07$ و 0.05 ، على التوالي) ، ووصلت إلى الحد الأقصى القيم في 10×30^4 خلية/ مل . وخلصت الدراسة إلى أن *C. vulgaris* هو غذاء ملائم لـ *B. Plicatilis* و *H. salina* ولكن ليس كذلك لـ *A. trajani* ، حيث لا يزال سلوك التغذية لهذا النوع يحتاج إلى مزيد من الدراسات المتعمقة.