Journal of Zoological Research
Volume 1, Issue 1, 2017, PP 10-15

Evaluation of Two Novel Feeding Protocols Utilizing Alive and Dried Chlorella *vulgaris* to Grow *Heterocypris salina* (Ostracoda: Crustacea)

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ABSTRACT

Heterocyprissalinais well-known for its high egg production and it can adaptto livein different environments. However, the data on H.salina culturing are still scarce, so it is necessary to study the best environmental conditions and food items to culture ostracod species. The current study was carried out to generate baseline information on the food and feeding protocols of H. salina by evaluating living and dried Chlorella vulgaris as a food source for this species .Gravid females of H.salina with the same length and age were separated to start the experiment, which divided into two treatments based on alive and dried Chlorella vulgaris. The current study concluded that, the alive and dried C. vulgaris is a suitable diet for H. salina because it led to enhance its growth rate, increasing its individual number and shortened the time to reach adulthood.

Keywords: *Heterocyprissalina- Freshwater ostracods- zooplankton Culturing - live food for fish;*

INTRODUCTION

Aquaculture is one of the fastest-growing animal food-producing sectors; it contributed 44% of the yield from wild fisheries in the year 2014 and is expected to surpass the yield of wild fisheries by2020–2025 (FAO, 2016). However, culture of fish and crustaceans is facing two major challenges. The first challenge is the high price of the industrial diet while the second one is the acceptance of industrial diets for fish larvae, where they have non-functional stomach with weak enzymatic activity (Pedersen and Hjelme land, 1988). Generally, food for fish larvae is usually depending on fish mouth size, prey size and fish ability to prey, which suggests that small prey with a slow swimming would be more suited to fish larvae (Hunt Von Herbing et al., 2001).Thus, the live food (Phyto and zooplanktons) is a very important food source in fish farming, particularly for early larval stages.

Zooplanktons play an important role in any aquatic ecosystem, where theyare occupying an intermediate position in the food chain (Xie et al., 2008).They are very important as food for many fishes, especially in their early life phases (Szlauer and Szlauer,1980).Ostracods, as one of zooplankton groups, are important food for fish and benthic macro invertebrates (Chakrapani et al., 1996).

Ostracods are small crustaceans that have a bivalve carapace covering their soft parts and appendages. They live in nearly all aquatic habitats, including marine, brackish, freshwater, and underground water (Moore, 1961). Ostracods feed principally on detritus, bacteria and some of them sucking plant juices by their mandibles (Grigg, 1985).They can be used as bio indicators for environmental changes and pollution due to their sensitivity to certain changes in environmental conditions and pollution (Triantaphyllou et al., 2005; Yasuhara and Yamazaki, 2005; Bergin et al., 2006; Boomer and Attwood, 2007).Living Ostracoda is divided into two subclasses; Myodocopa and Podocopa. The first subclass is marine while the second have both marine and non-marine groups (Horne et al., 2002).

Heterocypris salina belongs to sub class Podocopa, it prefers small, slightly salty coastal and inland waters. Nevertheless, it was also recorded and could be cultured in pure freshwater

(Meisch, 2000).Hence, the culturing of *H. salina*in small and slightly salty (brackish water) will provide the live food for fish farming without freshwater consuming.

*H.salina*is well-known for its high egg productivity, which moults eight times to reach the adult stage and it isadapted to the hard environmental conditions (Kubanc et al., 2007). On the other hand, the data on *H. salina* culturing are still rare, Thus the current study was carried out to evaluate the alive and dried *Chlorellavulgaris* (which characterized by higher nutritional value and easily culturing) as a food source for growing and flourishing of *H. salina* to increase its individual number and using it as a food item in a fish farm.

MATERIAL AND METHODS

Samples Collection and Isolation

Samples of ostracod species *Heterocyprissalina* were collected from Darwa Canal (freshwater) (Fig. 1), which connected to the Damietta Branch of River Nile at El-Kanater El-Khyria region in June 2016 by washing stems and roots of higher aquatic plants through plankton net (55 µm mesh size). The collected samples were maintained for five hoursin an open plastic jar (5 liter) which full of underground water. Then, gravid females of *H.salina* with the same length and age were separated to conduct the experiment.

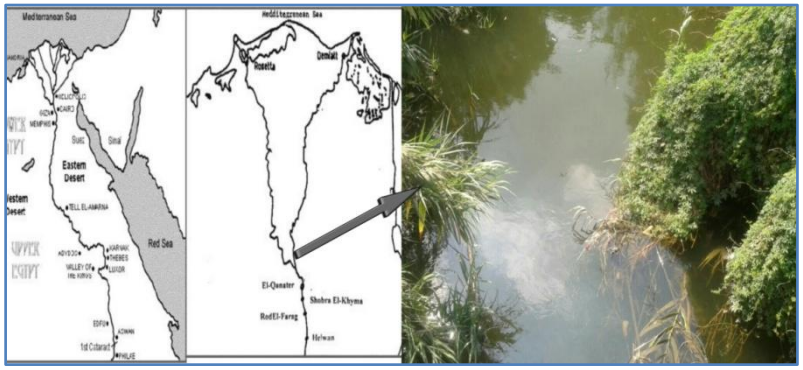

Figure1. *A map and photograph showing the collecting site on the Damietta branch of the Nile River, Egypt.*

Experimental Conditions

The experiment was conducted at the same conditions of sample collection, where water salinity was 1%**o**, dissolved oxygen levels were maintained between 5.5 to 7.0 mg l^{-1}, water temperature varied between 24 – 25 C° and pH ranged from 7.6 to 8.1.

Feeding Protocols

The experiment was divided into two treatments based on food type resource and a control as the following:

Treatment (A)

1ml of alive *Chlorella vulgaris* with a density of 10×10^6 cells / ml was daily added during the experiment period.

Treatment (B)

0.1gm of dried *Chlorella vulgaris* was daily added during the culturing period.

Control

Three replicates of two individuals of the species were cultured without any additive food source.

Experimental Procedure

Each treatment conducted in three replications, each one was started by culturing two individuals of *H. salina* at the same length and age in a Petri dish. The water level was maintained constant along the period of the experiment.

The moulted carapaces were collected two times every day (every 12 hours) to measure the length and height of their valves in order to obtain information about the size, moulting time and daily growth rate of each instar. Also, the period between each in star and the subsequent one, and the time from hatching to adult stage were calculated. The individual survival was checked daily until adulthood during the experiment period.

Data Analysis

Growth Rate (GR)

The growth rate is calculated by measuring the length and height of the moulted carapaces from first in star (A-8) until attaining the adult stage.

The growth rate was calculated daily according to the following equation:

μ (GR) = (ln N_t - lnN_0) / t,

Where,

μ = Growth Rate (GR)

N_0= Initial growth

N_t= Final growth at day-t of culture period

t = Culture period (day)

A two-way analysis of variance (ANOVA) was used to find out the significance of the differences in the daily growth rate and number of individuals among the two treatments.

RESULTS

In current study, the gravid females of *H. salina* of each treatment have parthenogenetic eggs, where no males were observed. On the other hand, the all individuals of control (no external food sources were supplied) died during the first 24 hours. In treatment (A) the gravid females laid about 68 eggs after two days, then eggs hatched to first instars (A-8) after another two days. At the same time, the gravid females of treatment B laid about 66 eggs after two days too, which hatched to first instar (A-8) after two days else. Subsequently, the A-8 instars moulted seven times to become adults in treatment A and B. Each instar is distinguishable by the length and height of its carapace (Table 1 and Figure 2).

Development Time and Growth Rate

The development time of each in star was nearly changeable, where the earlier instars (A-8, A-7 and A-6) were moulted rapidly than the later ones. Generally, the in star A-8 was transferred to in star A-7 then in star A-6 on the first day after egg hatching. On the other hand, the development time of the later instars was nearly constant, where each in star spent one day to transfer to the next one in the both treatments (Table 2). The growth rate showed the same pattern, where the highest growth rate (1.1/ day for carapace length and 1/day for height) was calculated at the first day. The growth rate of *H. salina* was gradually decreased to attain the lowest rate (0.1/day for carapace length and height) at the seventh day (Figure 3). Furthermore, the growing of *H. salina* stopped after ultimate moult and egg production initiated after 8 and 10 days for treatments A and B, respectively. Thus, the time spent from egg hatching to attain adults have parthenogenetic eggs was 14 and 16 days for treatments A and B, respectively.

The variance analysis (ANOVA) appeared non-significant difference ($p > 0.05$) in the number of individuals and growth rate between treatments A and B.

The Survival Rate

During the two treatments, all hatched individuals (68 and 66 of treatment A and B) reached to adulthood without any mortality observed through the culturing period.

Table1. *Length and height of all instars of H. Salina from A-8 to A in two treatments (A and B).*

Instars	Length (µm)	Height(µm)
First instar (A-8)	131- 169	75 – 114
Second instar (A-7)	215 – 266	140 – 163
Third instar (A-6)	329 – 378	188 – 214
Fourth instar (A-5)	440- 492	245 – 297
Fifth instar (A-4)	548 – 593	342- 385
Sixth instar (A-3)	662 – 704	499 – 525
Seventh instar (A-2)	759- 812	556- 602
Eighth instar (A-1)	894- 977	638- 674
Adult (A)	1089- 1200	700- 768

Table2. *Development time of each in star of H. salina in Treatment A and B.*

Instars		(A-8)	(A-7)	(A-6)	(A-5)	(A-4)	(A-3)	(A-2)	(A-1)	(A)
T (A)	Time (days)	1	1	1	2	2	2	2	3	
T (B)		1	1	2	2	2	2	3	3	

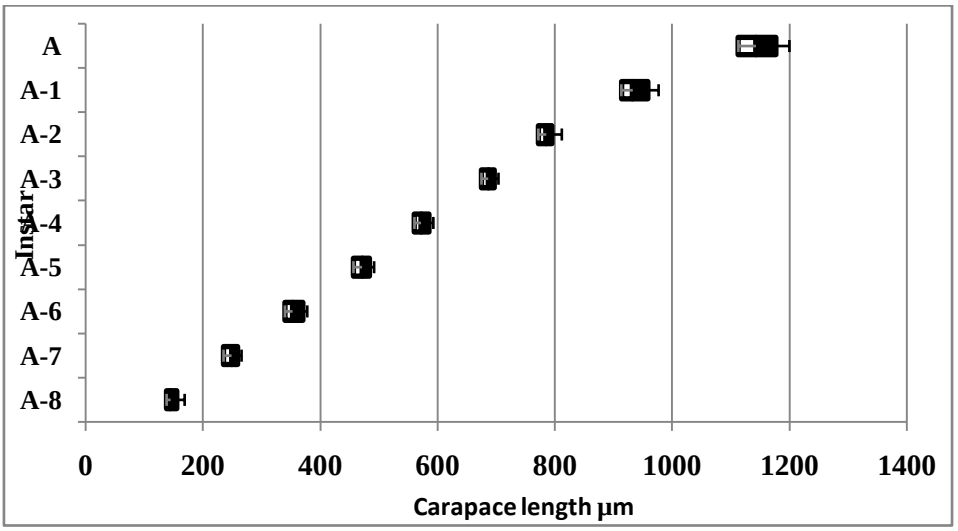

Figure2. *Box plots showing the carapace length for the nine instars attained fromculturing H. salina in the laboratory (Treatment A and B).*

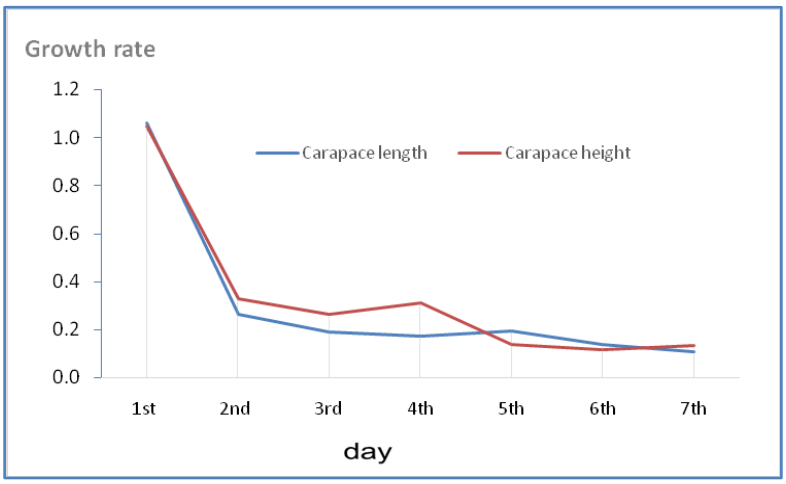

Figure3. *The daily growth rate of H. Salina from instar A-8 to instar A in the two treatments.*

DISCUSSION

In this study, we selected *Chlorellavulgaris* as a natural food for *H. salina*, because *Chlorella* is one of the most widely used foods for planktonic culturing (Pourriot and Rougier, 1997). Where, it contains 40% protein, 20% fat, 20% carbohydrate, 5% fiber, and 10% minerals and vitamins, as well as its easy to culture (Belasco, 1997).

The growth pattern of ostracod species *H. salina* (*Cypridoidae*) displayed nine instars from the egg to the adult stage in the two treatments. This result agrees with Kubanç et al. (2007) who studied the ontogeny of *H. Salina* from Büyükcekmece Lake and is the same findings, with most other podocopidostracods in the number of juvenile instars (Cohen and Morin, 1990; Kesling, 1961).On the other hand, some podocopidostracods such as, *Xestoleberishanaii* (Ikeya and Kato 2000), *Neonesideaoligodentata* (Smith &Kamiya, 2002) and *Neonesideaschulzi* (Yousef and Moustafa, 2017) with only seven instars from the egg to the adult stage.

In our results, we introduced differences in the duration of development time of instars of *Heterocyprissalina*, the earliest instars moulted after a shorter period than the latest ones. This result accords with that of some authors (Ikeya and Kato, 2000; Aguilar-Alberola and Mezquita, 2008).

Ostracods show a wide degree of variation in their rate of maturation, the present studied species *Heterocyprissalina* reached to adult stage in 14 and 16 days in treatment A and B, respectively. Aguilar-Alberola and Mesquita (2008) showed that A-7 instar of *H. bosniaca* reached to adult stage at 17 days at temperatures above 20ºC.Also, Latifa (1987) obtained similar outcomes for *H. incongruens*, where its larval development reached adulthood at about 16.7

days at 20ºC and 12.5 days at 25ºC, respectively. However, the marine myodocopid *Skogsbergialerneri* reached to maturity in 65-93 days at 25 ° C (Cohen, 1983).

Passano, 1960 concluded that a high growth rate would determine by a reduction in the duration of the development time of each instar and an increase in the size of animal after each moult. Our results concluded that, the growth rate was toughly influenced by *chlorella* as a food for *H. salina* produces a large number of individuals. On the other hand, Schmit, 2007 recorded that the algal *Tolypothrixtenuis* is suitable diet for*Eucyprisvirens* with other food items. Also, Nandini and Rao (1998) noted that the growth rates of *Simocephalusvetulus*on*Microcystis* sp. was significantly and higher (0.13–0.32 day 21) than that of *Chlorella vulgaris*(0.06–0.17 day 21).Havel and Talbott (1995) recorded that *H. incongruens* displays a higher growth rate and short generation time and high densities on diets of *Selenastrum*in comparison to other algae. Ganning, 1971 recorded that *Heterocyprissalinus* and *Cypridopsisaculeata* are restricted to the green algae of the pools such as the desmids and did not find any animals in their stomach. As well as Ganning, 1971 recorded that *Chlorella* constitute 50 to 90% of the stomach contents of *H. salinus*, and it required 35 days to complete its life cycle.

In the current culture, all individuals showed high survival values, no mortality was recorded along the duration of the experiment. Havel and Talbott(1995) reported that *H. incongruens* have high fecundity and adaptable time of egg hatching producing sufficient individuals in a longer time period of 2–4 months. On the other hand, neither survived nor reproduced well on the filamentous *Planktothrix* sp., but had similar survivorship and fecundity patterns on diets of *Microcystis* sp. when compared with the green algal diet.

The geographical parthenogenesis is a common pattern in cyprididostracods and in the genus *Heterocypris* (Horne et al., 1998). In our study, we observed the absence of males in the study area and in culture so the current studied species is parthenogenetic species. Our results are in accordance with Baltanàs et al. (1990) who recorded that males of *H. salina* are not known in Europe nor in North Africa. Also, (Meisch, 2000)observed that *Heterocyprisincongruens* is a cosmopolitan geographic parthenogenetic species inhabiting shallow seasonal pools.

From our observations, ostracods are relatively common in most Egyptian water bodies and our results indicate that *Chlorella vulgaris* is a suitable food for *H. salina* and increasing its fecundity in short time. In this way *H. salina* can be used as a natural food source in fish farms. Where, Fernando (1994) stated that zooplankton is considered universal food for young fishes, and planktivorous fish may consume zooplankton. Furthermore, Engdaw (2014) observed that the occurrence of ostracods in the gut fish constituting 42.1% (4.12% of the total volume).

The present study concluded that, the alive and dried *C. vulgaris* is a suitable diet for *H.Salina* because it led to enhanced its growth rate, increasing its individual number and shortened the time to reach adulthood (14-16 days)

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