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Enhancing and Shifting the Reproduction Mode in *Daphnia Carinata* (King, 1853) Fed on Different Types of Powdered Food

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Abstract

The purpose of this study is to test the effect of four different powdered food such as Rice (R), Wheat (W), Chickpeas/Hummus (C), *Spirulina* (S) and a mixture of the previous feed (R+W+C/H+S) on parthenogenesis and resting eggs (*Ephippia*) production in *Daphnia carinata* (King, 1853) hatched from the desert of Abu Dhabi, UAE. The study continued for 21-days at temperature 19°C ± 2°C and under 12:12 hours light/dark conditions. The analysis of the experimental results has shown that test animal fed on a mixture of food resulted in significant ($p<0.05$) highest production of resting eggs and lowest ($p>0.05$) production in parthenogenesis in comparison to other food. Meanwhile, Chickpeas/Hummus and Wheat respond similarly and produce nearly the same number ($p>0.05$) of *Ephippia* and parthenogenesis eggs in comparison to the *Spirulina* powder. However, the least number of resting eggs and parthenogenesis ($p<0.05$) has produced by *Daphnia carinata* fed on Rice powder. Therefore, we concluded that a mixture of highly nutritive feeds strongly induces *Ephippia* production of the *Daphnia carinata* and shifts its life cycle from asexual to sexual production, which may be useful in the production of *Daphnia* egg bank. On the other hand, Chickpeas/Hummus, Wheat, or *Spirulina* powder can trigger the cyclic parthenogenesis and each of them can be used for the production of *Daphnia carinata* as live inexpensive food in aqua hatcheries. Finally, Rice powder did not support *Daphnia carinata* life cycle and was not a force requirement of shifting from parthenogenesis to *Ephippia* productions.

Keywords

Daphnia; Food; Reproduction; Parthenogenesis; *Ephippia*; Aquaculture

Introduction

The microcrustacean *Daphnia* play an important role in carbon transferring from primary producers to higher trophic levels [1]. It is the primary consumer feed on bacteria, algae, protozoa, detritus, and yeast and principal prey of many fish species [2]. Therefore, the changing of its population can cause a significant effect on freshwater food webs [3, 4]. Also, *Daphnia* has been considered an important representative of *Cladocerans* in both basic and applied aspects of research due to the short life cycle, small size, and easy to maintain in the laboratory [5,6].

One of the interesting characteristics of *Daphnia* (*Cladocera*) is cyclical parthenogenesis, i.e., they have two different types of reproduction including sexual and asexual (parthenogenetic) reproduction modes in its life cycle. During the parthenogenetic cycle, females produce diploid eggs that develop directly into daughters. Sexual Reproduction in which female *Daphnia* produces haploid eggs that require fertilization by males. Then, these eggs are enclosed in a protective shell (*Ephippia*) and need to undergo a diapause before female offspring will hatch from them [6-8].

Ephippia play an important role in maintaining the *Daphnia* population during harsh environmental conditions such as high population density, low food, too high or too low temperature, drought [9,10], co-existing of predators [11]. The dormant eggs, diapausing or resting eggs, cysts or statoblasts that often sink and accumulate in sediments, forming egg banks [12] which have proved an important tool for biodiversity studies, understanding the environmental cues that induce *Ephippia* hatching, ecological biogeography, paleolimnology, nature conservation, evolutionary ecology, and community population ecology, and taxonomy [13]. One another function of *Ephippia* is to invade new aquatic areas through transporting by water, animals, or human transport [14]. Also, *Daphnia Ephippia* morphology was used as a key for the identification of some *Daphnia* species [15].

Also, *Cladocerans* are used in aquaculture industry due to their abundance, tolerance to environmental conditions, high nutritional quality, ease of handling and sorting from other zooplanktons, suitable sizes (0.2 mm-6 mm), parthenogenetic reproduction, short generation time, richness in digestive enzymes, and high caloric value [16,17]. Moreover, *Cladocerans* as a potential live feed available in small ponds and lakes [18] have gained certain economic importance as they are widely used in larvae aquaculture, and large filter-feeding planktonic species have an indirect economic impact as important fish food or phytoplankton-controlling group [5]. Another research has reported high levels of protein, free amino acids, fats, and carbohydrates in *Cladocerans* like *Daphnia carinata, D. longispina, D. magna, and D. pulex*, which are considered valuable live feeds [19]. Therefore, *Daphnia* species are excellent food sources, which could provide quality first feed for fish and crustaceans [20,21]. The *Cladoceran* like *Moina* sp, *Daphnia* sp, etc. have already been explored as a living capsule of nutrition for many cultivable fishes [22]. On the other hand, *Caldocerans* as intermediate hosts of some parasites may potentially pose a threat to human health [5].

Despite a large number of studies on the mechanisms behind the reproductive switch in *Cladocerans* [8], the exact environmental stimuli needed for sexual reproduction are still uncertain because zooplankton communities are very sensitive and react to a wide variety of environmental stress [23]. Several environmental factors have been reported as stimuli for switching between reproductive modes and *Ephippia* formation including ultraviolet light and storage

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time in darkness, temperature, pH, population density, food quantity and quality, photoperiod, fish kairomones, population dynamics and fecundity [8, 23-30].

On the other hand, laboratory and field studies on nutritional deficiency in *Cladocerans* resulted in a negative effect on growth and reproduction. In extreme conditions, *Cladocerans* produce resting eggs and disappear from the plankton [31-34]. Also, most of the environmental factors research on the reproduction in *Cladocerans* was done with species of temperate origin including *D. magna* [8,28,35] and *D. pulex* [36,37], and very few have been done for the tropical species such as *D. carinata* [6,38].

Therefore, we performed this study to extend the experimental research on the effect of food quality on *Daphnia* reproduction and to present further evidence for the importance of food type on its reproduction mode. Consequently, we tested the effect of four different types of powdered food such as Rice, Wheat, Chickpea/Hummus, and *Spirulina* on the parthenogenesis and *Ephippia* production of the tropical *D. carinata*. Besides, a mixture of the previous feeds was also tested to alleviate any nutritional deficiencies and *Daphnia* can benefit from its elements. Moreover, this study, up to our knowledge, is the first to test different powdered food on the reproductive mode in the tropical *D. carinata* and it could be important basics for further studies of *D. carinata* life cycle as a portion of live food in aquaculture and its important role in carbon transferring from primary producers to higher trophic level.

Material and Methods

Daphnia carinata origin

D. carinata used in the present study was hatched from Alshwaib-Dam within Abu Dhabi Emirate-UAE [39] and its life cycle (Figure 1) was maintained nearly for two years in the marine biology laboratory, United Arab Emirates University. Cultures were grown in desalinated commercial water (Oasis Company-AlAin, UAE) at 19°C ± 2°C and fed on a mixture of food (R, W, C, and S) under 12:12 hours light/dark conditions.

Animals isolation

About 150 no. of *D. carinata*, resting and parthenogenetic eggs-free females, were isolated from the maintained cultures in separate jars by a plastic dropper under the stereomicroscope (Leica, 200M 2000) and used in this research. Isolated *D. carinata* females were left for 48 hours in the laboratory to ensure that males did not fertilize them. The water used in stock and experimental cultures were filtered through a 0.22 µ micropore filter system before using in *Daphnia* cultures and salinity was checked by salinometer and was zero ppt.

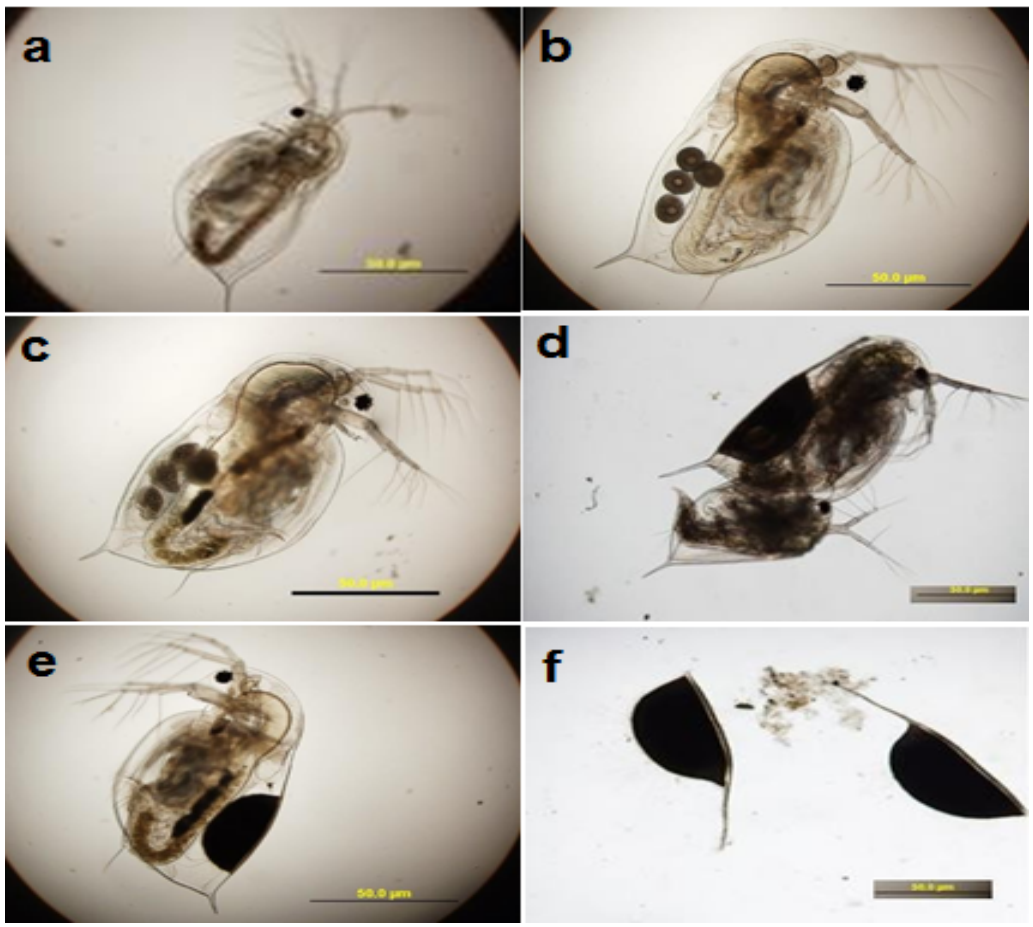

Figure 1: Reproductive cycle of *Daphnia carinata*; (a) Parthenogenetic and *ephippia* free female; (b) Parthenogenetic female carrying four eggs; (c) Parthenogenetic female with four embryos; (d) A female and male coupling; (e) Ephippial female; (f) Ephippium (resting egg) X100.

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Laboratory food-experiments on *Daphnia* reproduction

On starting, 120 females *Daphnia* resting and parthenogenetic eggs-free were divided into 5 groups, each consists of 24 animals. Every group was run in three replicates each containing eight animals. All animals were allowed to grow in 250 ml jars containing 150 ml of desalinated water at 19°C ± 2°C and under 12:12 hours light/dark cycle (Figure 2) and fed daily 1ml of 60 mg/l powdered food for 21 days. Powdered organic food was selected because it consists of fine granules that enable *Daphnia* to filter and feed it from the water without the blockage of its filtration system. Food was prepared daily in separate bottles. Group one replicates were fed on Rice flour (Safeer, AlAin, UAE), group two replicates were fed on Wheat flour (Safeer, AlAin, UAE), group three replicates were fed on Chickpeas/Hummus flour (Safeer, AlAin, UAE), group 4 replicates were fed on grinded *Spirulina* tablets (now-USDA-Organic-Code 26988 VB), and group 5 replicates were fed on a mixture of the previous feeds. For testing the effect of food type on the reproduction mode, the number of *Ephippia* and parthenogenetic females was determined by counting under the Stereomicroscope (Leica 200M 2000) for 21 days.

Statistical analysis

Data were statistically analyzed using SPSS, 17.0 for windows (SPSS, Chicago, IL, USA). Standard error was calculated and Analysis of Variance (ANOVA) was performed on the data to determine the Least Significant Difference (LSD) between treatment means with the level of significance at $P \leq 0.05$. The average followed by the same letter does not differ, according to the t-test or one-way ANOVA ($p \leq 0.05$).

Results

Effect of food type on Parthenogenetic females production (Asexual reproduction)

The results showed that there were evident differences and clear interactions between animal groups and food type in the number of parthenogenetic-females production during the experiment. Animals fed with Wheat, Chickpeas/Hummus, and *Spirulina* produced the maximum number of parthenogenetic females, whereas animals fed on Rice, and a mixture of food (R, W, C, and S) produced the minimum number of parthenogenetic females (Figure 3).

Effect of food type on *Ephippia* production (Sexual reproduction)

The results revealed that the total number of resting eggs produced by *D. carinata* during the 21 days of the experiment was affected by the type of food. *Daphnia* fed on a mixture of food consists of (R, W, C, and S) produced the maximum significant number of resting eggs among the other groups which fed only on Rice, Wheat, Chickpeas/Hummus or *Spirulina*. On the other hand, there was no significant difference in the resting eggs production between *D. carinata* fed on Wheat, Bean, or *Spirulina*. Whereas, Rice produced the minimum

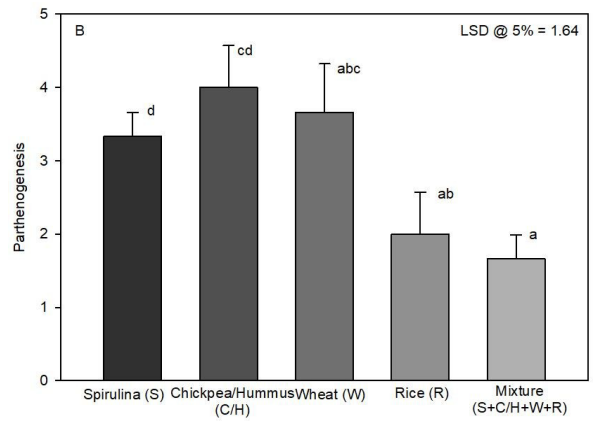

Figure 3: Shows the Parthenogenetic female production in *Daphnia carinata* when fed on different types of food after 21 days of growth. The bars represent the standard error of average of three replicates. The averages followed by the same letter do not differ, according with the test of t-test or one-way ANOVA ($p \leq 0.05$).

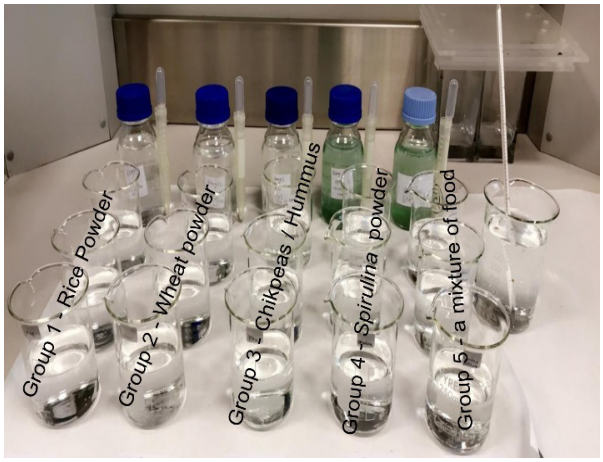

Figure 2: Shows the five *Daphnia* cultures growing in desalinated commercial water in the laboratory and fed on Rice powder; Group 1: Wheat powder; Group 2: Chickpeas/Hummus flour; Group 3: *Spirulina* tablets powder; Group 4: a mixture of the previous feeds; Group 5: at 19°C ± 2°C, under 12:12 hours light/dark conditions.

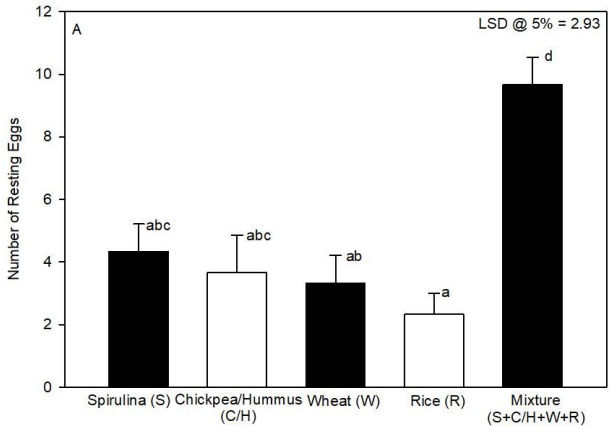

Figure 4: Shows the *ephippia* production in *Daphnia carinata* when fed on different types of food after 21 days of growth. The bars represent the standard error of average of three replicates. The averages followed by the same letter do not differ, according with the test of t-test or one-way ANOVA ($p \leq 0.05$)

significant number of resting eggs among all experimental groups (Figure 4).

Discussion

The enormous success of the genus *Daphnia* in freshwater ecosystems is at least partially due to their cyclical parthenogenetic life cycle, in which asexual and sexual reproduction alternate periodically. This temporal change between reproductive strategies allows for (1) rapid population growth via subitaneously (parthenogenetic) developing eggs when environmental conditions are appropriate and (2) the maintenance of genetic diversity via sexual reproduction and the production of resting eggs when environmental conditions deteriorate [40]. The success of this reproduction mode is reflected in the known 620 species that radiate within *Cladocera* and the genus *Daphnia*, within *Cladocera*, approximately 150 species-rich [41]. Besides, the reproduction in cladocerans can either be parthenogenetic (asexual) or maybe intermixed with periods of gamogenetic (sexual) reproduction. Two female morphotypes are thus recognized in the populations, which include the parthenogenetic individuals and ephippial forms. Although, parthenogenesis is the predominant mode of reproduction [42].

The present study found that Wheat or Chickpeas/Hummus or *Spirulina* flours has increased the number of parthenogenetic females of *D. carinata* in comparison to Rice and a food mixture, and they also produced the considerable number of ephippia which may indicate that crowding is on start and triggers the production of males [43,44]. The powdered food used in this study especially (W, C/H, and S) are good sources of, proteins, saturated and non-saturated fatty acids, carbohydrates, minerals, trace minerals, fibers, and many vitamins [45-49]. This finding is in agreement with the previous reports which indicated that the growth and reproduction of *Daphnia* need important biochemical components such as essential lipids, sterols, and minerals [50,51], polyunsaturated fatty acids [52,53], the availability of dietary phosphorous [54]. Moreover, the switch from parthenogenetic to sexual reproduction in a cyclical parthenogenetic organism can be influenced by the chemical composition of food [36]. Another report showed that dietary proteins or specific dietary amino acids like arginine and histidine are involved in triggering the switch between reproductive modes in *Daphina galeata* and *Daphnia pulex* by enhancing food quality and subitaneous (parthenogenetic) reproduction and avert crowding by inducing resting egg production. As a combined effect of both processes, the population growth rate has increased [36,40]. The powdered food used in this study especially (W, C/H, and S) is good sources of, proteins, saturated and non-saturated fatty acids, carbohydrates, minerals, trace minerals, fibers, and many vitamins [45-49].

Also, this study proved that there was a relationship between the type of powdered food (R, W, C/H, S, and a food mixture) and the *Ephippia* production of the tropical *D. carinata*. This was evident because the animal group fed on a mixture of food (R, W, C/H, and S) has produced a significant number of *Ephippia* in comparison to (R or W, or S) groups. So, the results of the present research agree with the previous studies, which reported that the life history traits of *Cladocera* change depending on the quality and quantity of the resource [55, 43]. Besides, a mixture of food (R, W, C/H, and S) which contains a combined high concentration of all ingredients found in these feeds, may increase and block the filtration system of *Daphnia* and switch its reproduction mode as an unfavorable condition. Moreover, minerals in a mixture of food have increased and it may be another stimulus for the *Ephippia* production because the elemental characterization of *Daphnia* resting eggs by X-ray analytical microscopy demonstrated that phosphorus, sulfur, potassium, and calcium were detected as elemental components of the resting egg [56,57].

On the other hand, it was reported that food limitation is not an obligate requirement of shifting from parthenogenesis to sexual *Ephippia* production in *D. carinata* [6]. This research showed that Rice alone produced the least number of *Ephippia* between all groups because there was a significant decrease in its formation in comparison to (W, C, S, or a food mixture). This may be because Rice does not contain essential lipids, sterols, minerals, and especially amino acids like arginine, histidine, and eicosapentaenoic, which are important components needed for the formation of *Ephippia* in *Daphnia* [36,40,58]. Also, the number of parthenogenetic females was low in comparison to (S, C/H, and W), and this is in agreement with the previous reports which found that food limitation/deficiency is a major cause of reduced fertility in *Simocephalus expinosus* (*Cladocera*: *Daphinidae*) reproducing smaller-sized females which matured slowly and generate a lesser number of neonates [59,60]. Moreover, mineral limitation such as calcium was found to affect reproduction, molting, and population growth when food quality and quantity is low [61].

Conclusion

The results of this study have revealed that Wheat, Chickpeas/Hummus or *Spirulina* powder has triggered the parthenogenesis in *D. carinata* and can be used in aquaculture of *D. carinata* as a highly nutritive and cheap live food. Moreover, sexual *Ephippia* production in *D. carinata* could be promoted when fed with a mixture of highly nutritive powdered food consists of Rice, Wheat, Chickpeas/Hummus, and *Spirulina,* which may be helpful for the production of an egg bank of this species. Indeed, the produced egg bank can be used in some invertebrate aquaculture or toxicological experiments for further understanding of the reproductive cycle of the tropical *D. carinata*. On the other hand, low-value nutritive food such as Rice was not an obligate requirement to change the mode of reproduction in the tropical *D. carinata* but it affects negatively on its life cycle by producing the lesser numbers of resting eggs and parthenogenetic females.

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