



The Influence of Different Dietary Categories on Life-History of *Daphnia pulex*



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Abstract

The type and amount of food that Cladocera species eat is known to affect their growth, development, capacity for reproduction, and ultimately the size of their populations. This study examines the influence of different diet types and mixtures on the *Daphnia pulex* (Leydig, 1860) life-history characteristics such as development, growth, and reproduction in a lab setting. *D. pulex* was fed with a yeast diet (*Saccharomyces cerevisiae*), a fresh green algal diet (*Scenedesmus acutus*), an aquarium fish feed diet (*Arthrospira platensis*), and a mixture diet (yeast, algae and fish feed) in a controlled static experiment for 21 days in the study. When *D. pulex* was fed with only one type of food, body weight, body size, somatic, and population growth rates decreased compared to the mixed diet but continued to increase compared to the control group throughout the 21-day experimental duration ($P \leq 0.05$). In contrast, according to morphometric measurements, the effect of a diet containing only cyanobacteria caused a stagnation in growth rates, while growth rates increased in those fed with cyanobacteria-containing feeds added to the mixed diet ($P \leq 0.05$). Our results show that the type of food and its mixtures significantly affect the vital characteristics of the *D. pulex* population.

Keywords: *Daphnia pulex*; Diet types; Growth; Mixture; Reproduction.

1. Introduction

Daphnids are a genus of small planktonic crustaceans (Order: Anomopoda; Family: Daphniidae), are key consumers affecting abundant and widespread algal communities in ponds and lakes, and play a major role in the aquatic food webs [1]. Daphnids are the natural balancers of increased phytoplankton in the aquatic ecosystem [1]. They also feed on algae, bacterial flora, and other small plankton organisms smaller than themselves in their natural pond habitats [2]. Another ecological importance of these crustacean species is to ensure the transmission of phytoplankton, bacteria, and natural particles as energy for native freshwater fish, which are important at high trophic levels [3]. Daphnids have a high protein content, essential fatty acids, and a distinguished density of vitamins A and B. Therefore, they are a high-quality and nutritious food type for fish [4]. The most significant primary food source for zooplankton life in the aquatic environment is phytoplankton. Recently, attention has been drawn to research that examines how the quality of phytoplankton nutrients affects the morphological traits and biochemical nutrient requirements of Cladocera species [2, 5, 6]. Specifically, research has demonstrated that zooplankton can be identified in water bodies with varying nutrient concentrations [5, 7]. On the other hand, a variety of negative circumstances have been encountered by these organisms, such as the deterioration of their habitat, the disappearance of regions where they are able to sustain a particular diet, the inadequacy of their nesting sites, and more [8, 9]. In addition, changes in the population characteristics of organisms have been observed due to climatic changes and the emergence of environmental pollution situations [8, 10]. Determining the population densities of organisms based on their dietary preferences is crucial to supporting the sustainability of those treatments in the aftermath of potential environmental catastrophes. Accordingly, this study aimed to determine the effect of diet type and mixture on various metabolic states such as growth rates, fertility, lifespan, and life expectancy within the life-history characteristics of *Daphnia pulex*.

2. Materials and Methods

2.1. Experimental Animals

Daphnia pulex was selected as the experimental animal in the research. Due to its small size, global distribution, and uniqueness as the first species of crustacean with a sequenced genome, it has become a valuable model species for studies conducted in lab and environmental settings [11]. *D. pulex* was isolated from ponds in aquatic environments in Northern Anatolia, and green water culture food was used to maintain a stock culture of parthenogenetic females carrying eggs. In the experiments, during both culture and stock tank maintenance, dead individuals harmful to other organisms and structures such as shell pieces related to growth were cleaned and monitored daily. Third-generation zooplanktons were preferred to ensure the availability of parental characteristics and phenotypic traits of the species in stock culture [12]. The culture tank was maintained at a consistent temperature of roughly $20.10 \pm 0.91^\circ\text{C}$, and the aquariums were replenished with spring water and aerated (VS-6003f Venusaqua, 20-Watt, 220-240 V/50Hz, India) in compliance with the references' recommendations [13].

2.2. Experimental Design

After the adaptation period with fertile individuals, a total of 200 neonate's samples were collected, and 40 samples were transferred to control and treatment tanks to determine both somatic and population growth rates. From fresh plankton samples in a fishpond, the green alga *Scenedesmus acutus* was isolated and sterilized in Jaworski's medium [14]. The serial sequential dilution (SSD) method was used to dilute the media [15] of 1 ml volumes from the original stock nutrient solutions, and they were investigated on 21 days. The bacteria and algae concentrations were determined using a hemocytometer under a dissecting microscope [16]. A 1000-ml volume tank was created by adding 500 ml of spring water and 500 ml of water taken from the organisms' own habitats for the control group, and *D. pulex* was fed only with green water culture on 21 days. Four experimental tanks were used in this research on the life-history of *D. pulex* and the food categories to be examined in the experiments. The diets of volume 1×10^5 cells ml^{-1} that were given to *D. pulex* were: baker's yeast (T1: *Saccharomyces cerevisiae*), fresh green algae (T2: *Scenedesmus acutus*), fish feed with cyanobacterium (T3: *Arthrospira platensis* containing fish food), and a mixture diet (T4: yeast, green algae, and cyanobacterium). Body weight (BW, μg) and carapace length (CL, mm) of *D. pulex* fed with different diet types for 21 days were measured weekly. The carapace length was measured using the ToupView imaging program that was built into the light microscope (Omax Microscope, USA), and the total body weight was calculated using an ultra-micro balance (Mettler Toledo, USA). The ambient water hardness of the treatment tanks was determined as 92 ± 1.15 mg/l CaCO_3 by the volumetric method, while the dissolved oxygen level was determined as 7.30 ± 0.07 mg/l by the Winkler method [17]. The temperature of both the culture and experimental quarium environments was kept constant with an electronic adhesive aquarium thermometer. Some physical and chemical properties of spring water used in the preparation of both culture and treatment tank environments are given in Table 1.

Table-1. Water quality characteristics of spring water

Parameters	Units	Values
pH	-	7.64
Conductivity (EC)	$\mu\text{S}/\text{cm}$	255.2
Total organic carbon (TOC)	$\mu\text{g}/\text{l}$	0.86
Sulfate (SO_4^{+})	$\mu\text{g}/\text{l}$	5.77
Sodium (Na^{+})	$\mu\text{g}/\text{l}$	8.07
Chloride (Cl^{-})	$\mu\text{g}/\text{l}$	10.47
Iron (Fe^{+2})	$\mu\text{g}/\text{l}$	5.57

2.3. Determination of Changes of Life-History and Somatic Growth Rate

For each diet category, 20 daphnia hatchlings left in the aquariums were kept in the relevant diet treatments until the spawning period. The number of surviving offspring was determined in each successive reproductive cycle. Population growth rates (r) were determined according to the calculation in Equation 1 [18]. In the equation, notations l_x , m_x , x , and T are age-specific survival rate, age-specific fecundity, age class, and generation time (in days), respectively [18]. To determine the changes in the somatic growth rates (g) of Cladocera's in each treatment control group according to feeding types, firstly, the morphological measurements of 20 samples were determined over a period of 21 days and calculated according to the relationship in Equation 2 [18].

$$r \cong \frac{\ln(\sum l_x m_x)}{T} \quad (1)$$

$$g = \frac{\ln(W_t) - \ln(W_0)}{t} \quad (2)$$

2.4. Statistical Analysis

To compare the morphometric characteristics, somatic, and population growth rates of *D. pulex* fed with different diet types, a Newman-Keuls multiple comparison test and an analysis of variance test in SPSS statistical

software (SPSS Inc., Chicago, IL, ABD) were used. The probability value was taken as the significance value for dietary practices as $P \leq 0.05$, and all data were expressed as mean \pm standard error [19].

3. Results

The effects of four diet types on morphometric measurements, somatic growth rates, population growth rates, and population density of *D. pulex* on days 1, 7, 14, and 21 were investigated in this study (Figures 1, 2). The body weight of *D. pulex*, especially at T2, T3, and T4, showed a significant increase compared to the control group ($P \leq 0.05$), whereas body size was determined to be the highest at T2 and T4 on the first day of the experimental duration (Figure 1). In general, the T2 and T4 diets caused a significant increase in the mean of body weight and body size for *D. pulex* at day 7 relative to the other diet types ($P \leq 0.05$). The body weight of *D. pulex* increased on day 14 of the T2 and T4 diets compared to the control group ($P \leq 0.05$), whereas body size showed significant changes in all groups compared to the control group ($P \leq 0.05$). At the end of the 21 day experiment in which all diet groups were examined compared to the control group, the body weight of *D. pulex* increased under the influence of T1, T2, and T4, but body size increased in all groups compared to the control group ($P \leq 0.05$).

The population densities and growth rates of *D. pulex* fed with the mixed diet (T4) and fresh algae diet (T2) increased significantly compared to those fed with yeast (T1) and cyanobacterium (T3). *D. pulex* fed with a mixture of yeast, green algae, and cyanobacterium for 21 days had the highest population density compared to the other diets (1361.00 individuals l^{-1}). On the other hand, the population density of *D. pulex* fed with fish feed containing cyanobacterium (T3) was observed to decrease on the 14th day (Figure 2). Population densities were determined in order from most to least as $T4 > T2 > T1 > Control > T3$. When *D. pulex* was fed with baker's yeast and fresh green algae species, significant differences were determined in somatic and population growth rates ($P \leq 0.05$); nevertheless, these values did not affect the growth rates of *D. pulex* population as much as the mixture effect ($P \leq 0.05$). The highest increase in all experimental periods was determined in the yeast, green algae, and cyanobacterium treatment effect, indicating the mixture diet triad ($P \leq 0.05$). In contrast, the least increase in growth rates was determined in *D. pulex* fed only with cyanobacterium as negative growth. The PGR of *D. pulex* fed with the mixture diet was 3.62 times higher than that of those fed with cyanobacterium (Figure 2).

4. Discussion

Different diets have been used in the studies for the water flea culture tanks. *S. acutus*, *S. dimorphus*, *S. obliquus*, *Ankistrodesmus falcatus*, *Selenastrum capricornutum*, and *Chlamydomonas reinhardi* have been studied among the single-celled green algae. Furthermore, a variety of diet types have been shown to be advantageous, including farm manure, dried *Chlorella vulgaris*, baker's yeast, and some samples of protozoa [20]. Another study found that the offspring production and size of *Daphnia magna* fed with *C. vulgaris* that had lost its freshness were significantly lower than those fed with fresh *C. vulgaris* [21]. In this study, in which the effects of different diets on morphological characteristics, somatic growth, and population growth rates of *D. pulex* were investigated, it was determined that fresh diets provided positive growth on *D. pulex*. Especially, it was determined that baker's yeast (T1) and fresh green algae (T2) diets increased the growth in populations 2.41 and 3.03 times more than non-fresh fish feed with cyanobacterium (T3). In another study to determine the most suitable nutrient medium for *D. pulex* culture under laboratory conditions, the organisms were fed with three different diets: single-celled green algae (*S. disciformis*), commercial trout fry feed, alfalfa meal mixture, and dry baker's yeast [21]. The highest reproduction efficiency in the culture of *D. pulex* was obtained from single-celled green algae nutrient medium, followed by trout fry feed, alfalfa meal mixture and dry baker's yeast, respectively [21]. In our study, green algae nutrient medium (T2) was parallel to that study as it provided the highest growth and fertility after the mixed diet (T4). In addition, the diet type with the least determined effect on morphological development, somatic growth rate, and population growth rate of *D. pulex* was fish food (T3) containing a non-fresh cyanobacterium diet. *A. platensis* is known to be an important dietary product consumed as a food supplement in human and animal nutrition. It is a cyanobacterium rich in proteins and bioactive compounds [22, 23]. In one study, it was found that *A. platensis* alone was not effective in both survival and growth rate of *D. magna*, but increased survival rate after 20% *C. reinhardtii* was added. When *A. platensis* diet was mixed with 50% or more *C. reinhardtii*, the growth rate of the organism was found to be higher than when fed with *C. reinhardtii* alone [24]. It was observed that the aquarium T3 diet (*A. platensis* containing fish food) alone was not sufficient for both the survival and growth rate of *D. pulex*, but it provided significant benefits in the mixed diet (T4: yeast, algae, and *A. platensis* containing fish food) in our study. This situation led us to conclude that there are some nutritional limitations required for the survival and growth of daphnids, such as *A. platensis* being less fresh for *D. pulex*, the food size not being suitable for feeding fry and juvenile individuals, metabolic digestibility deficiency, and for reasons yet unknown.

5. Conclusion

In this study, where the effects of different diets on the growth rates and welfare of *Daphnia pulex* were examined, the best result was determined by the mixture effect, followed by the group obtained from the unicellular green algae nutrient medium. However, the reproductive performance of *D. pulex* was low in the nutrient medium prepared only with a non-fresh cyanobacterium diet and only with dry baker's yeast. This situation shows that algae culture is important in *Daphnia* feeding, as stated in the current literature. Algae production for habitat destruction observed in natural ecosystems provides convenience in studies since they are practically obtainable nutrients in terms of time and cost. In this direction, it will be beneficial to consider the food types and their rations in future studies on the production of this species.

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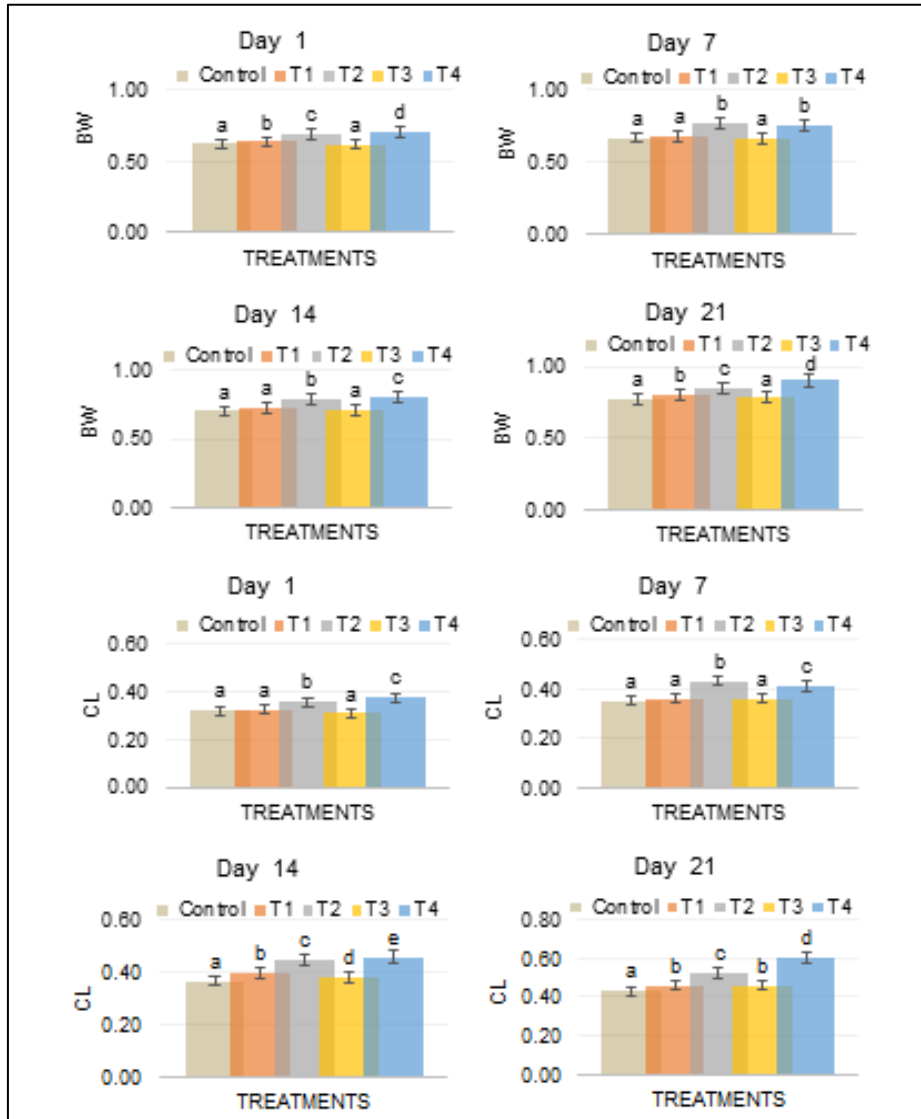


Figure-1. The influence of different dietary types on body weight (BW, µg) and carapace length (CL, mm) of *D. pulex*. The distinction between treatments is significant at $P \leq 0.05$ and is indicated by the letters a, b, c, d, and e

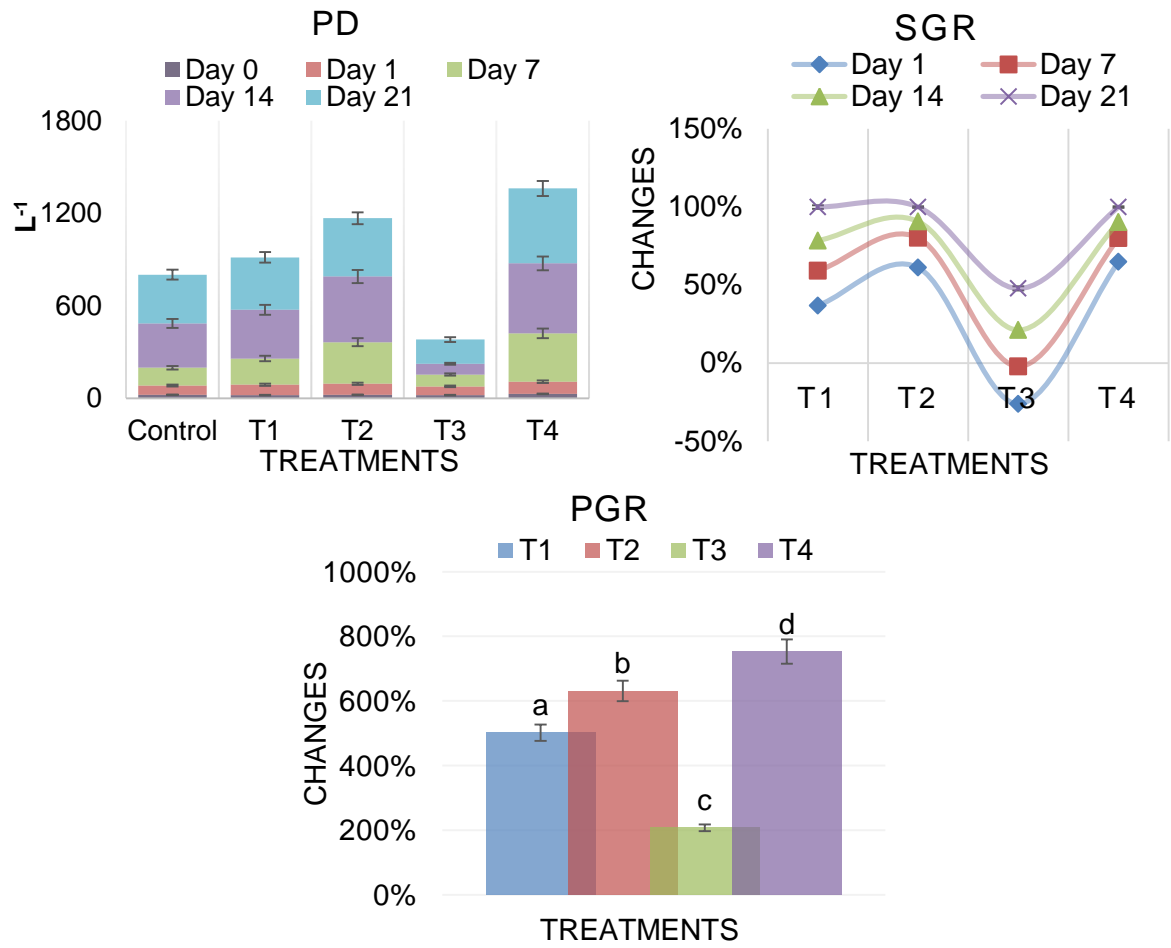


Figure-2. The influence of different dietary types on population density (PD) of *D. pulex* and change in somatic growth rates (SGR), population growth rates (PGR) compared to control.