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Effects of diets on structure and population size of *Apocyclops royi* (Copepoda: Cyclopoida)

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Abstract. This paper investigates the effects of different diets on the growth and development of *Apocyclops royi* (*A. royi*) in the laboratory. *A. royi* individuals were acclimatised in a laboratory culture environment, and newly hatched larvae of the same age were collected and raised until adulthood for the experiment. Different diets were arranged and evaluated to select the optimal food for the growth and development of *A. roy*i. The experiments included the following treatments: the green alga *Chlorella vulgaris (C. vulgaris)*, microbially fermented rice bran combined with *C. vulgaris* algae, and yeast combined with *C. vulgaris* algae. The results show that the diet containing microbially fermented rice bran combined with *C. vulgaris* algae yielded the best performance for the *A. royi* population, evidenced by the highest density of 9,022.22 ± 1,066 ind·L^{-1} at day 12 and the highest larval yield compared with the other treatments ($p < 0.05$). This study provided a scientific foundation for selecting the optimal diet to enhance the biomass production efficiency of copepods, a nutrient-rich natural food source for fish and marine crustacean larvae. Consequently, it contributed to the optimisation of the food chain in sustainable aquaculture.

Keywords: aquaculture, *Apocyclops royi*, copepoda, cyclopoida, optimal diet

1 Introduction

Copepods are a natural prey item for numerous marine organisms and are considered an ideal food source for various marine fish larvae [1–3]. This is due to the nutritional composition, size, and swimming style of marine crustaceans [4, 5]. Copepods have a higher nutritional value than rotifers and *Artemia* [6, 7], which is primarily because of their significant content of essential fatty acids (EFA), such as arachidonic acid (C20:4n-6, ARA), eicosapentaenoic acid (C20:5n-3, EPA), and docosahexaenoic acid (C22:6n-3, DHA). These EFAs are essential for ichthyoplankton [8]. Copepods are also a rich source of other micronutrients, including vitamin A [9], vitamin C, vitamin E [10], and various minerals [11]. Therefore, copepods are suitable as the primary natural food source for animals.

A. royi is commonly found in estuaries, lagoons, and brackish water aquaculture ponds, and is often used as supplementary food for larvae or juvenile groupers in large-scale commercial hatcheries [12, 13]. *A. royi* has been cultivated and produced commercially in several countries, such as Taiwan and Denmark; however, the intensive cultivation of *A. royi* is limited. Besides, research on the effectiveness of food sources, as well as the innovation of food sources in aquaculture, has not been conducted comprehensively. Today, the primary food source used to cultivate *A. royi* is microalgae. This is costly for *A. royi* intensive farming, leading to the increasing prices of products. Therefore, this study aims to identify alternative food sources for microalgae to reduce costs while maintaining the efficiency of *A. royi* farming.

2 Materials and methods

2.1 *A. royi* stock culture

A. royi species was isolated in Lang Co Lagoon, Thua Thien Hue Province. The copepod has been cultivated in the Laboratory of Environment Technology, University of Science and Education, The University of Danang. Healthy ovigerous copepods were then transferred to 500 mL beakers filled with 100 mL seawater. When the eggs hatched, the nauplii were collected by filtering the culture through a 50 μm mesh. All the nauplii were maintained in a controlled environment: temperature, 26–28 °C; light conditions, a 12:12h light/dark cycle at 1,500 lux; and salinity, 15 ppt. The food for *A. royi* was *Chlorella vulgaris* (*C. vulgaris*) microalgae, obtained from the Lab for Algae Research – Faculty of Biology and Environmental Science, Danang University of Science and Education, The University of Danang. Algae were added at a density of 7×10^4 to 8×10^4 cells·mL^{-1}, three times a day. When 100% of the nauplii had become adult copepods, they were immediately used for the experiments. Fig. 1 presents the images of female and male adults of *A. royi*.

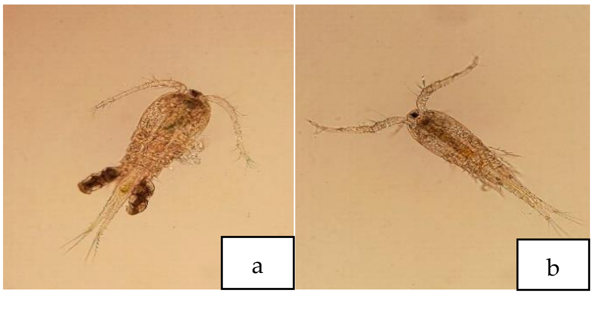

Fig. 1. Adult of the species *A. royi*. a. female; b. male

2.2 Experimental design

The copepods were divided into 3 groups that were fed with 3 different types of diets: microbially fermented rice bran + *C. vulgaris* (Trial 1), bread yeast + *C. vulgaris* (Trial 2), and *C. vulgaris* only (Trial 3), with a concentration of 0.001 g/10 individuals over 24 h. The initial density of each trial was 400 individuals per litre.

The formula of rice bran incubated with microbial product EM was: 10 mL EM product + 50 mL molasses + 250 mL water + 10 kg of rice bran. This mixture was thoroughly blended, and water was added to achieve a humidity level of 30–40%, if necessary. The mixture was tightly covered and incubated for 2–4 days. The feeding frequency was twice per day. The environmental conditions for the experimental setup were controlled in the same manner as those used for culturing the copepod stock. Ecological factors, namely salinity, pH, and dissolved oxygen concentration, were measured every 2 days for control.

Three trials were conducted in 1000 mL culture flasks with three replicates for each diet. The experiments lasted for 16 days. The parameters monitored throughout the experiment included the density of individuals, population structure, and the growth rate, with a monitoring frequency of twice a day. All these parameters can be observed under a stereomicroscope.

2.3 Statistical analysis

The population growth rate (r) was calculated according to Yin et al. [14], following Equation (1)

$$R = \frac{\ln N_t - \ln N_0}{t} \quad (1)$$

where N_0 and N_t are the initial and final population densities, and t is the incubation time in days.

Data on density, sex ratio, and population structure were presented as mean values ± standard errors (Mean ± SE). The comparison of mean values between sample groups was

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conducted with the ANOVA method implemented on IBM SPSS Statistics software (Version 27.0), followed by Tukey's HSD tests to determine which pairs of group means were statistically different. A probability $p \leq 0.05$ level was accepted as significant.

3 Results and discussion

3.1 The population size of *A. royi*

The population density regarding the three diets (*C. vulgaris* algae only, microbially fermented rice bran combined with *C. vulgaris* algae, and yeast combined with *C. vulgaris* algae) is presented in Fig. 2.

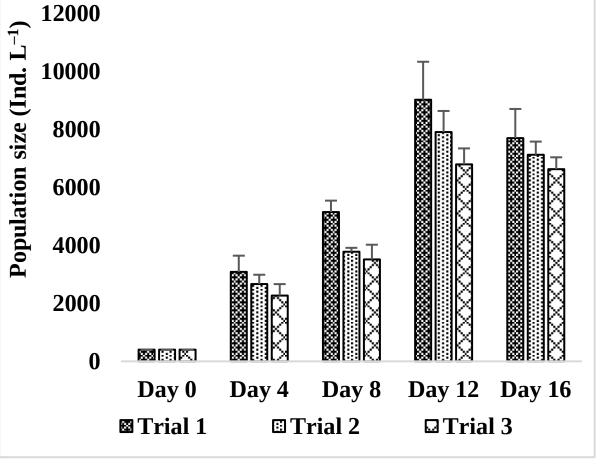

Fig. 2. Effects of diets on population density of *A. royi*. Data are presented as Mean ± SE ($n = 3$)

The population density in all trials increased from day 1 to day 12 but decreased by day 16.

In particular, Trial 1 yielded the highest density when its population density was significantly higher than that in the other trials ($p < 0.05$), ranging from 3,078 ± 460.54 individuals per litre (ind·L^{-1}) to 9,022.22 ± 1,066 ind·L^{-1}, peaking on day 12 at 9,022.22 ± 1,066 ind·L^{-1}. In contrast, Trial 3 demonstrated the lowest density compared with the other trials, with values ranging from 2,700 ± 319.76 ind·L^{-1} to 7,366.66 ± 450.78 ind·L^{-1}, also reaching its maximum on day 12.

The difference in total *A. royi* population density between treatments became more pronounced from day 8 to day 12. On day 8, the rice bran and algae diet (Trial 1) yielded the highest total density among all treatments at 5,144.44 ± 509.1 ind·L^{-1} ($p < 0.05$). However, by day 12, Trial 3 (algae-only diet) resulted in the lowest total density compared with the other treatments, at 3,511 ± 134.71 ind·L^{-1} ($p < 0.05$). These findings indicate that the addition of probiotic groups from rice bran fermented with EM1 or *Saccharomyces cerevisiae* (baker's yeast) is crucial for enhancing the digestion of microalgae. These probiotics provide essential enzymes and vitamins B while also maintaining a cleaner culture environment. Together, these factors contribute to improved vitality and growth rates of the *A. royi* population.

These observations suggest that combined feeding diversifies the food source for *A. royi*. Copepoda are inherently omnivorous [15, 16], so the combination of microbially fermented rice bran with *C. vulgaris* algae provides them with more options for optimal nutrition [17].

Nielsen et al. [18] reported that *D. tertiolecta* algae were considered the most suitable feeding mode when recording the maximum *A. royi* population density at 2,175 ± 1,269 ind·L^{-1}. However, with Trial 1 (*C. vulgaris* algae combined with microbially fermented rice bran), we recorded up to 9,022.22 ± 1,066 ind·L^{-1}, about 4 times that of Nielsen et al. This may be due to the difference in feeding regimes and the suitability of *C. vulgaris* algae as the primary food source for *A. royi*. Biomass production of *A. royi* species can be more effective when combined with other foods, such as bread yeast or microbially fermented rice bran.

3.2 Structure of *Apocyclops royi* population

The structure of the *A. royi* population was evaluated on the 12th day of the experiment, when the population density was at its highest, and the results are illustrated in Fig. 3.

In general, across all treatment groups, there was a higher proportion of nauplii than the rest of the instars throughout the experimental period (68.65 ± 4.33% to 70.13 ± 4.46%). The percentage of males was the lowest of the total population at all trials (2.79 ± 1.23% to 3.36 ± 0.47%). The results from the statistical test indicate that there was no significant difference in gender and age composition amongst trials ($p > 0.05$). It can be inferred that different diets had no effect on the *A. royi* population structure over time. Therefore, replacing part of the microalgae with microbially fermented rice bran and bread yeast had no effect on the structure of the *A.royi* population.

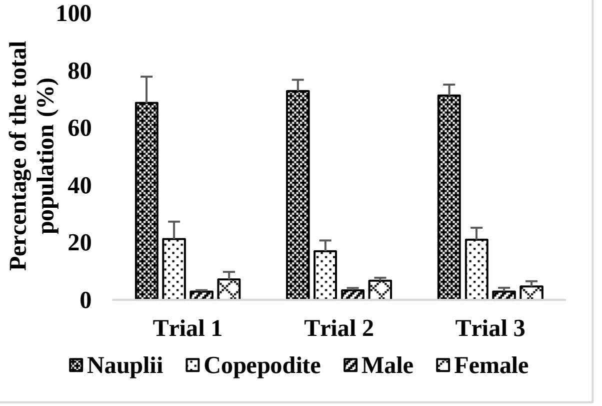

Fig. 3. Effects of different diets on structure of *A. royi*. Data are presented as Mean ± SE ($n = 3$)

Magoz et al. [19] studied the effects of different diets on the growth of *Oithona nana* (Cyclopoida). They found that nauplii accounted for a high proportion of the population across all treatments, a finding similar to ours. However, while the number of males and females was nearly equal in Magoz et al.'s study, the number of females of *A. royi* in our study was higher than the male population in all trials. The consistent observation of a greater quantity of females compared with males across all nutritional treatments suggests that the sex-specific difference in survival is likely governed by an intrinsic biological mechanism. The inherent fragility of males, which exhibits lower tolerance to starvation and physiological stress compared with females [20], provides a possible underlying explanation. Males typically have a shorter lifespan and accumulate more oxidative damage with age [21] because of high energy consumption associated with mate-seeking [22]. Therefore, even the best treatment is not stress-free enough to equalise the sexes, as males are biologically programmed for a higher baseline mortality rate.

3.3 Growth rate of *A. royi* population

Fig. 4 presents the growth rate of the *A. royi* population with different diets.

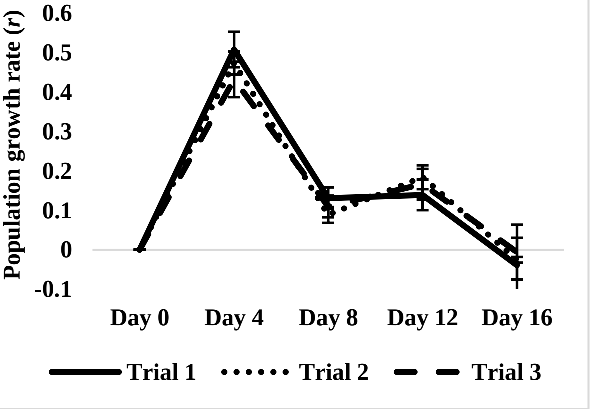

Fig. 4. Effect of different diets on population growth rate (r) of *A. royi*. Data are presented as Mean ± SE ($n = 3$)

Fig. 4 illustrates that different diets have a significant impact on the population growth rate. The *A. royi* population growth rate in all trials increased rapidly during the first 4 days. After that, it decreased sharply over the next four days, followed by a slow fall until day 16 when the growth rates of the three trials were

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approximately equal (ranging from 0.175 ± 0.003 to 0.184 ± 0.008).

Among the different diets, the microbially fermented rice bran combined with *C. vulgaris* algae (Trial 1) yielded a higher growth rate of *A. royi* than the remaining trials from the first day to the 8th day. The growth rate values from day 1 to day 4 and from day 4 to day 8 were 0.507 ± 0.045 and 0.131 ± 0.027, respectively. It can be seen that although the microalgae *C. vulgaris* was a crucial food source for the growth of *A. royi*, the addition of rice bran fermented with microorganisms was necessary to improve the efficiency of biomass production of *A. royi*, thereby providing a better food source for shrimp and fish larvae. Fermented rice bran is an economical and highly efficient substrate for culturing beneficial probiotic strains [23]. The fermentation process is critical for synthesising significant amounts of extracellular enzymes, such as proteases, amylases, and other bioactive compounds, while also degrading anti-nutritional factors, thereby substantially enhancing substrate digestibility [24, 25]. The nutritious source from rice bran fermented with bacteria significantly boosted its highly unsaturated fatty acid profile, enzyme activity, and growth performance, thereby delivering a premium live feed that enhances the survival and disease resistance of aquaculture larvae [26].

4 Conclusion

The results show that different diets affected the growth and density of the *A. royi* population. However, the *A. royi* population structure was not influenced by diet. The bran diet combined with *C. vulgaris* algae was the most effective food for *A. royi*, as evidenced by the maximum density of 9,022 ± 1,066 ind·L^{-1} on day 12 in the study. Furthermore, the number of ovigerous females and nauplii was higher with this diet than in the other treatments throughout the experimental period. The least effective diet for *A. royi* was *C. vulgaris* algae, as evidenced by lower values in the population density, the percentage of ovigerous females, and the growth rate.

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