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The Impact of Microalgae-Based Diet Enriched *Mesocyclopsaspericornis* Copepod on the Growth Performance and Biochemical Assessment of Ornamental Fish (*Brachydanio rerio*)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The cultivation of emerging species in aquaculture and the aquarium industry presents challenges, particularly during the sensitive larval stage, which often has a small mouth. Developing appropriate early feeding protocols is crucial. Copepods have gained recognition as a viable alternative to traditional live fish feeds, as they are natural prey for many fish species and do not necessarily

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require additional nutritional enrichment. This study aimed to assess the effect of enriching *Mesocyclopsaspericornis* with different microalgae (Spirulina, Azolla, Chlorella) when fed to *Brachydanio rerio*, focusing on growth, survival, and biochemical performance of the ornamental fish. Results revealed that *Brachydanio rerio* exhibited the highest specific growth rate (SGR) when fed with copepods enriched with Spirulina (1.49±0.022), and the highest survival rate was observed when copepods were enriched with Spirulina (93.4±2.549). Further analysis of the biochemical composition of enriched copepods indicated that Chlorella resulted in the highest protein and carbohydrate content, along with lower lipid content compared to other enrichment groups. This suggests that microalgae, particularly Spirulina and Chlorella can serve as a potential feed to enhance the nutritional status of copepods and support the early stages of fish development.

Keywords: Microalgae; copepods; growth Performance ornamental fish.

1. INTRODUCTION

A diminutive tropical freshwater cyprinid, the zebrafish is scientifically known as *Danio rerio* (Hamilton, 1822). The zebra fish is a vegetarian. Although phytoplankton, filamentous algae, spores, invertebrate eggs, debris, sand, and mud have also been documented from stomach content analysis, zooplankton and insects make up the majority of its natural diet [1]. Fish fed exclusively on prepared foods have been shown in numerous studies to have low survival rates and sluggish growth [2]. However, other researchers contend that, in comparison to live food alone, mixing live food with formulated diets during the early phases can result in higher growth rates and improved survival [3]. Early fish and crustacean stages in the natural world eat a diverse range of phytoplankton and zooplankton, which gives them a full and well-balanced diet.

Furthermore, live feeds—especially microalgae and zooplankton—are regarded as "living capsules of nutrition" that can support the development of larvae in a variety of aquaculture species [4], Rotifer and crustaceans, or cladocerans and copepods, are the dominant zooplankton groups in aquatic environments. Copepods stand out among them due to their remarkable diversity and abundance in a variety of environmental settings [5,6]. Copepods are also advantageous for their nutritional profile when compared to other live food species, and they are frequently utilized as feed for raising fish and shellfish larvae [7,8,9,10,11] Of the several copepods, Cyclops sp. from the Cyclopoida order and Diaptomus sp. from the Calanoida order show great potential for increasing the growth and survival rate of freshwater fish larvae.

There is now a convincing solution: feeding copepods and cladocerans a suitable microalgal diet will enhance their growth, fertility, and nutritional makeup. Several studies have been carried out to ascertain the impact of microalgal diets on Cyclopoida [12,13,14] and Calanoida [15,16] Since zooplankton is a natural food source for fish and prawn larvae, many hatcheries feed it to the larvae of ornamental fish [17]. The aim of this study was to evaluate the feeding efficiency of *Brachydanio rerio* using enriched *Mesocyclopsaspericornis*.

2. MATERIALS AND METHODS

2.1 Experimental Animal and Setup

An indoor experiment was conducted using four tubs arranged as a miniature model of intensive culture ponds, each capable of holding up to 50 liters of water. The larvae, type B. rerio fishes, were obtained from Beena Fish Farm in Coimbatore, Tamil Nadu. They were transported in oxygenated bags and acclimatized to laboratory conditions in a cement tank for two weeks. Throughout their maintenance period, the animals were fed commercial feed, and water renewal was carried out adequately.

In a laboratory setting, *M. aspericornis* copepods were enriched with *S. platensis*, *C. vulgaris*, and *A. pinnata* to feed the ornamental fish *B. rerio*. Adult nauplii of *M. aspericornis*, cultured for 48 hours, were fed with each type of food at the same concentration of 0.5 mg/ml/day. The powdered feeds were prepared by mixing them with distilled water at a concentration of 0.5 mg and vigorously stirred for 2-3 minutes. *M. aspericornis* (at a density of 50/ml) was introduced into 500ml culture flasks containing freshwater, and mild aeration was provided. After 6 hours of enrichment, adult nauplii of *M. aspericornis* were fed to the experimental B. rerio fishes twice a day. Daily observations were conducted.

The experimental setup involved dividing the fishes into four groups, each consisting of 50 individuals:

Control: *B. rerio* fed with *M. aspericornis* enriched by yeast

Treatment 1: *B. rerio* fed with *M. aspericornis* enriched by Azolla

Treatment 2: *B. rerio* fed with *M. aspericornis* enriched by Spirulina

Treatment 3: *B. rerio* fed with *M. aspericornis* enriched by Chlorella

2.2 Growth Parameters

The growth parameters were calculated by using the following equation according to [18].

$$\text{Weightgain(WG, \%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Specificgrowthrate(SGR, \%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Experiment period}} \times 100$$

$$\text{Feedconversionratio(FCR, \%)} = \frac{\text{Total feed intake}}{\text{Total weight gain}}$$

$$\text{Survivalrate(\%)} = \frac{\text{Number of fish survived}}{\text{Initial number of fish}} \times 100$$

2.3 Estimation of Protein

The protein content in different samples was estimated by employing the Folin-Ciocalteau method of [19].

Calculation:

$$\frac{\text{OD of Unknown}}{\text{OD of Known protein}} \times \text{Concentration of the standard}$$

2.4 Estimation of carbohydrate

Prepare the standard by taking 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard and make up the volumes to l ml in all the tubes including the sample tubes by adding the distilled water. Then add 4ml of anthrone reagent. Heat for 8 minutes in a boiling water bath, then cool rapidly and read the dark green colour at 630 nm followed by [20].

Calculation:

$$\frac{\text{OD of Unknown}}{\text{OD of Known}} \times \text{Concentration of standard}$$

2.5 Estimation of Lipid

The method of [21] was employed to extract the 500mg sample was homogenized in 2ml of chloroform: methanol, which is centrifuged at 3000 rpm for 10 minutes. The process is repeated thrice; the supernatant was pooled and concentrated at 40-45ºC in flask vacuum evaporator.

Calculation:

$$\frac{\text{OD of Unknown}}{\text{OD of Known}} \times \text{concentration of standard}$$

2.6 Statistical Analysis

Data obtained from biochemical studies subjected to one-way ANOVA. Duncan multiple range tests were used to test if the difference between the treatments were significant using SPSS 13 .0 software.

3. RESULTS AND DISCUSSION

3.1 Growth Parameters of *B. rerio* fed with Enriched *M. aspericornis*

The weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR) of *B. rerio* fish fed the experimental diets are detailed in Table 1. Fish fed Treatment 2 (Spirulina) demonstrated significantly higher WG, SGR, and SR compared to those fed Treatment 1 (Azolla), Treatment 3 (Chlorella), and the Control ($P<0.05$). Additionally, the FCR was lowest in the Treatment 2 group (1.78±0.052), indicating the most efficient feed utilization. The Control group had the highest FCR (2.51±0.065), followed by Treatment 3 (2.35±0.009) and Treatment 1 (1.94±0.032). The survival rate was also highest in the Spirulina group (95.7±0.36), followed by the Azolla (90.6±1.35), Chlorella (87.9±1.58), and Control (85.4±1.11) groups (Table 1). In summary, *B. rerio* fed with spirulina-enriched *M. aspericornis* showed superior growth parameters and survival rates compared to those fed with other enrichment sources, highlighting the potential efficacy of spirulina as a beneficial supplement for fish growth and overall health. Similar studies have indicated that microalgae can significantly enhance the growth of fish [22,23,24] and Copepod (*M.aspericornis*) can be utilized entirely as an alternative or supplementary feed for commercial *C. catla*seed production [25,26], provide numerous health benefits for aquatic animals, including immune enhancement [27] and anti-inflammatory or phagocytic properties [28].

3.2 Biochemical Analysis (Protein, Carbohydrates and Lipids)

In the present study, three different microalgae (Spirulina, Azolla,Chlorella, and yeast) were used enriched *M. aspericornis*fed to *B. rerio*. The protein, lipid and carbohydrate content of different algal sources were estimated (30- and 60-days experiments). Among the algal sources used to enriched *M. aspericornis*fed to *B. rerio* showed high amount of protein content were observed Treatment 2 (767.33±1.55[a] and 795.46±2.10[aa)] followed by Treatment 3 (640.6±0.11 [ca] and 681.71±1.60 [bb]), Treatment 1 (622.4±0.09 [c]and 653.86±0.09[ba]) and Control (586.70±0.09[ba]and 650.4±0.07[b]) in both 30- and 60-days trail. The above results are in similar to the findings of [29] who reported that Penaid larvae fed with *Chaetoceros sp*. showed high protein content. Therefore, it was important to standardize the microalgae culture conditions, in order to ensure the same FA enrichment day by day. In general, the biochemical composition of A*. tonsa* nauplii fed on I–R treatment was the richest in terms of protein, total LCPUFAs and EPA reported [30]. These results agreed with previous studies reporting the excellence of Rhodomonas spp. as a diet for calanoid copepods [31]. Early studies reported on the rearing of *M. rosenbergii* indicated that the optimal dietary protein level ranged from 15 to 80% [32]. This finding is corroborated by [33]. A study conducted on *Penaeus japonicus* found that diets containing less than 10% protein had low levels of highly unsaturated fatty acids (HUFA), which are crucial for the growth and survival of *Penaeus japonicus* larvae. Protein content below 15% could lead to mortality during the rearing of post-larvae, as protein is a primary consideration in formulating fish feed. However, an excess of protein in the diet may also higher growth [34].

After being fed *M. aspericornis* supplemented food, *B. rerio* showed a high concentration of carbohydrates were shown Treatment 3 (760.84±0.12 and 883.52±0.22ba), followed by Control (751.20±0.23 and 781.95±0.19), Treatment 1(759.03±0.10 ad and 782.60±0.20 ac) and Treatment 2 (754.23±0.16 and 770.31±0.21), finally Table 2, displays the significantly lower lipid content (310.5±0.85 and 396.58±0.39) in the Treatment 1 group as compared to the other group that fed *M. aspericornis* enriched food to *B. rerio* in both 30- and 60-day trials. Furthermore, research indicates that *P. monodon* larvae fed mix algae with high lipid content produce the highest carbohydrate content when analyzed up close [35,36]. According to a related study, lipids have the highest energy density of all the food classes and are essential components in both plants and animals. Because copepod consumption is outpaced by primary production in tropical conditions, phytoplankton food is always available, negating the need for lipid reserves. This could account for copepods' reported low lipid content [37,38]. Results from [39] show that there is a notable variation in the lipid content of tropical zooplankton compared to temperate zooplankton. This difference may be attributed to hydrological circumstances and kind of food available.

Table 1. Growth performance of *B. rerio* fed with enriched *M. aspericornis*by different feeds

Parameters	Control	Treatment 1	Treatment 2	Treatment 3
IW (g)	1.94±0.03 [a]	1.96±0.01 [a]	1.95±0.00 [a]	1.95±0.008[a]
FW (g)	2.95±0.04 [d]	3.80±0.00 [b]	4.33±0.04 [a]	3.43±0.04 [c]
WG(g)	1.01±0.008 [c]	1.84±0.006 [b]	2.35±0.04 [a]	1.48±0.032[bc]
SGR (%)	0.45±1.37 [d]	0.57±0.00 [b]	0.62±0.00 [a]	0.52±0.01[bc]
FCR	2.51±0.065 [a]	1.94±0.032 [b]	1.78±0.052[bc]	2.35±0.009 [ab]
SR (%)	85.4±1.11 [d]	90.6±1.35 [b]	95.7±0.36 [a]	87.9±1.58 [c]

The mean ± SD of three separate observations is shown for each value. Significant differences (P<0.05) exist between the mean values in the same row that share the same superscript.
Treatment 1: B.rerio fed with M. Aspericornis enriched by Spirulina, Treatment 2: B.rerio fed with M.aspericornis enriched by Azolla, Treatment 3: B.rerio fed with M. aspericornis enriched by Cholerlla, Control: B.rerio fed with M. aspericornis enriched by yeast.

Table 2. Biochemical analysis of *B.rerio* fed with enriched *M.aspericornis* by different algae

Days	Parameters(µg/ml)	Control	Treatment 1	Treatment 2	Treatment 3
1st day	Protein	454.16±2.09 b	454.16±2.09 b	454.16±2.09 b	454.16±2.09 b
	Carbohydrate	658.59±2.13 c	658.59±2.13 c	658.59±2.13 c	658.59±2.13 c
	Lipid	349.40±0.43 da	349.40±0.43 da	349.40±0.43 da	349.40±0.43 da
30th day	Protein	586.70±0.09 ba	622.4±0.09 c	767.33±1.55 a	640.6±0.11 ca
	Carbohydrate	751.20±0.23 ac	759.03±0.10 ad	754.23±0.16 ab	760.84±0.12 ae
	Lipid	477.09±0.60 c	310.5±0.85 ba	305.19±0.42 b	362.09±0.66 bd
60th day	Protein	650.4±0.07 b	653.86±0.09 ba	795.46±2.10 aa	681.71±1.60 bb
	Carbohydrate	781.95±0.19 ad	782.60±0.20 ac	770.31±0.21 aa	883.52±0.22 ba
	Lipid	531.63±0.42 c	396.58±0.39 ab	484.69±0.49 bd	476.16±0.20 bc

Each value is mean ± SD of three individual observations. Mean values within the same row sharing the same superscript are Significant different (P<0.05)
Exp 1: B.reriofed with M.aspericornisenriched by Spirulina, Exp 2: B.rerio fed with M.aspericornisenriched by Azolla, Exp 3: B.rerio fed with M.aspericornisenriched by Cholerlla, Control: B.rerio fed with M.aspericornisenriched by yeast.

4. CONCLUSION

In summary, our findings suggest that the specific growth rate and biochemical performance of the ornamental fish (*B. rerio*) were influenced by the enrichment of *M. aspericornis* copepods with different microalgae. Specifically, the highest specific growth rate in *B. rerio* was observed when fed copepods enriched with Spirulina. Additionally, *B. rerio* exhibited the highest survival rate when fed with Spirulina-enriched *M. aspericorni*s in our study. This suggests a potential for reducing hatchery costs by utilizing spirulina-based enrichment while simultaneously enhancing nutrient content. Overall, these findings highlight the importance of microalgae selection in enriching copepods for aquaculture, offering insights into optimizing both growth performance and nutritional quality in ornamental fish production.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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