

ORIGINAL ARTICLE

Enrichment of *Dioithonarigida* (Giesberch, 1896) with different microalgal diets and its effect on survival and growth of *Latescalcarifer* (Bloch, 1790) larvae

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Abstract

Copepods are vital key components of marine and freshwater ecosystems and play an important role in the transfer of energy from primary producers to secondary producers and consumers. The optimization of feeding copepods with the desired diet is essential to culture them in large quantities. Hence, this study aimed to investigate the high-density production of a marine copepod, *Dioithona rigida* using different microalgal diets and evaluate the growth and survival of marine finfish larvae (Asian seabass), *Latescalcarifer*. The high-density culture was observed in *D. rigida* fed a mixed diet (50:50) of *C. vulgaris* and *N. oculata*, which reached a population density of 22500±2523 ind./L on the 21st day culture period. Essential fatty acids like Linolenic acid and Docosahexaenoic acid show significantly higher percentages in copepods fed a mixed algal diet than those fed with mono algal diet. About the larval rearing experiment of *L. calcarifer*, the larvae fed *D. rigida* enriched with a 50:50 mixed diet of *C. vulgaris* and *N. oculata* showed faster growth (Length 17.2±0.34mm; Weight 25.4±0.38mg) and highest survival (90%) compared to the other experimental group. This indicates mixed algal diet is more suitable for culturing the aquaculture live feeds for copepods.

Keywords: Cyclopoid, Mixed algal diet, Fatty acid composition, Aquaculture.

INTRODUCTION

In the natural environment, copepods play an important role as primary consumers, feeding on available phytoplankton and providing themselves as a source of energy to the higher trophic level (Sivakumar et al. 2021; Nawaz et al. 2023). In recent years, copepods have been used as live feeds for finfish and shellfish larval rearing in the aquaculture industry because of their high nutritional value and high essential fatty acid content compared to other live feeds like artemia and rotifers (Santhanam & Perumal 2012; Palanichamy et al. 2022). Copepods, which belong to the order Cyclopoida, are the most commonly used live food among all other copepod groups because the nauplii of most Cyclopoida are about 100µm in size, and the nauplii hatch from the eggs in 2-3 days (Vijayaraj et al. 2022). *Dioithona rigida* is a small brackish water cyclopoid copepod that is abundant in Indian estuaries in terms of

abundance and biomass which fetch the attention of various researchers to use them as a live feed in aquaculture (Santhanam & Perumal 2013; Kumar et al. 2017).

The main objective of the present study is to investigate the effects of feeding *D. rigida* with the monoculture of two different algal diets (*Chlorella vulgaris* and *Nannocloropsis oculata*) and with the (50:50) mixture of both microalgae. The present study also intends to find whether *D. rigida* enriched with different diets has any effect on the larval rearing of *Latescalcarifer* (Asian sea bass). *N. oculata* and *C. vulgaris* are commonly available microalgae strains and are widely used in biodiesel production because of their high lipid content (Dianursanti et al. 2018). *L. calcarifer* is a commercially important aquaculture fish that is highly sought after for its delicious white flesh. They are being produced in large quantities in most



Fig.1. Microalgal culture setup.

South Asian countries. They have a fast growth rate and can be farmed in fresh or brackish water, which makes them suitable fish for aquaculture (Shanmugaarasu et al. 2018). Generally, in aquaculture, malnutrition is the main factor for the mortality of fish larvae, however, fish larvae fed copepods showed better growth and survival in various studies (Luis et al. 2010; Drillet et al. 2011; Santhanam & Perumal 2012). Zooplankton such as copepods play an important role in the aquatic environment by producing essential fatty acids from the food they ingest, which are necessary for the proper growth of fish (Kumar et al. 2014). Hence, this present study aimed to formulate a protocol for the high-density production of a marine copepod, *D. rigida* regarding feeding regimes and enhanced larval rearing of Asian sea bass, *L. calcarifer* larvae for better growth, and higher survival leading to a sustainable culture.

MATERIAL AND METHODS

Collection of marine microalgae and copepods: For the present study, the microalgal culture of *C. vulgaris* (OM337700) and *N. oculata* (OM337701) were obtained from Aquaculture Research Laboratory, Department of Marine Biotechnology, AMET (Deemed to be University),

Chennai, India. The microalgal cultures were subcultured using Conway media and maintained at 29°C with vigorous aeration and the photoperiod was set to a light/dark cycle of 12/12h with a light intensity of 8000 lux (Fig. 1).

Zooplankton samples were collected 5 nautical miles away from the coast by horizontally towing the plankton net during the month of August 2021 from Kovalam station in the early morning hours. Collected samples were transported immediately to the laboratory and *D. rigida* were isolated from zooplankton samples and classified up to species level using key descriptions (Radhika et al. 2016; Razouls et al. 2023).

Culture method of the copepod *D. rigida*: To acquire desirable stock, live *D. rigida* taken from the wild were optimized by growing ovigerous females in 500ml beakers for several generations. For the mass production experiment, *D. rigida* was fed *C. vulgaris* (approx. 30,000cells/mL), *N. oculata* (approx. 30,000cells/mL), and a 50:50 mix of *C. vulgaris*, and *N. oculata* (approx. 30,000cells/ml) diets in three different tanks of 100L and optimized the environmental parameter of the culture medium. Multiple generations of these species were produced to get the desired amount of stock and acclimatized the same to the laboratory condition (29°C temperature; 30psu salinity; 7.8 pH). The different microalgal cultures were added regularly (thrice a day) to *D. rigida* culture tanks to maintain the diet concentration of *D. rigida*. For counting the density of *D. rigida* in different culture tanks, random samples were collected from the culture tanks, and all life stages of the copepod were counted using the Sedgwick-Rafter chamber.

Biochemical analysis: Biochemical analysis of copepods was done following the standard methods. One microgram of *D. rigida* from each culture tank was used to estimate the proximate composition of carbohydrate, protein, and lipid. Carbohydrate in the sample were estimated following the protocol of Dubois et al. (1956), the protein was estimated following Lowry et al. (1951) and lipids were estimated following Folch et al. (1957). At the end of

Table 1. Total population of *D. rigida* at 21st day fed with different algal diets.

Diet	Density (ind./L)	Percentage of culture populations (%)			
		Nauplii	Copepodites	Adults	Ovigerous female
<i>C. vulgaris</i>	18033±1942	65	32	2	1
<i>N. oculata</i>	20120±1225	67	30	2	1
50:50 mix of <i>C. vulgaris</i> and <i>N. oculata</i>	22500±2523	68	28	2	2

the experiments, 5 grams of *D. rigida* were harvested from each culture tank, and the lipids were extracted as per Folch et al. (1957). The extracted lipids were then subjected to HCl derivatization for the analysis of fatty acid profiles, following the method described by Lepage & Roy (1984). Methanolic HCl was added to the dried lipid extract, and the solution was heated for a specific period. After derivatization, the samples were neutralized, and the fatty acid methyl esters (FAMES) were extracted using an organic solvent for further analysis using gas chromatography (Agilent 6890 - Agilent Technologies, Santa Clara, CA).

Larval rearing of *L. calcarifer* using *D. rigida*: For the present study, the 10 DPH (Day post hatch) *L. calcarifer* larvae were obtained from Rajiv Gandhi Centre for Aquaculture, Sirkali - 609109, India. Before the experiment, the larval fish were acclimatized to the laboratory condition for a period of 2 days. After acclimatizing the larval fish, the larval rearing experiment was conducted in three separate experimental tanks of 50 liters each filled with filtered seawater and 50 larval fish. The tanks were provided with continuous aeration. Larval fish in Tank A was fed *D. rigida* enriched with the monoculture of *N. oculata*. In Tank B, larvae fed *D. rigida* enriched with the monoculture of *C. vulgaris*. Tank C larvae were fed *D. rigida* enriched with a 50:50 mixed culture of *C. vulgaris* and *N. oculata*. The whole experiment was conducted until the 21st day post-hatch (DPH) of larval fish. The length and weight of larval fish were measured at the end of the experiment. 50% of the water in experimental tanks was regularly changed until the end of the experiment and the dead fish were collected during the period of water exchange and were counted for estimating the mortality and survival of the fish. The data was subjected to

statistical analysis. ANOVA was performed using JASP 0.16 (Love et al. 2019) to find out the significance differences between the groups.

RESULTS AND DISCUSSION

Intensive culture of *D. rigida*: In the present study, the *D. rigida* fed a 50:50 mixed diet of *N. oculata* and *C. vulgaris* reached a maximum density (22500±2523ind./L) on the 21st day. Whereas, the copepods fed a mono-algal diet of *C. vulgaris* and *N. oculata* showed comparatively less density on the 21st day with 18033±1942ind./L and 20120±1225ind./L, respectively. The percentage of *D. rigida* populations fed with a mixed algal diet was observed with nauplii at 68%, copepodites at 28%, adult copepods comprising of male and non-ovigerous females at 2%, and ovigerous females at 2% (Table 1). Whereas, a low percentage of nauplii was observed from the culture tank fed a mono-algal culture of *C. vulgaris*.

In a natural aquatic environment, microalgae are the main producers of fatty acids, while zooplankton-like copepods are the important link between them and the higher tropical levels (Nawaz et al. 2023). Zooplankton absorb, incorporate, and modify fatty acids from the microalgae they consume and synthesize additional fatty acids, which are essential for the growth of many aquatic organisms (Santhanam & Perumal 2012; Kumar et al. 2014). Previously, Kumar et al. (2014) reported high egg production in the cyclopoid copepod *Thermocyclops hyalinus* and *Mesocyclops aspericornis* fed a mixed algal diet of equal ratio. Similarly, a high percentage of ovigerous female *D. rigida* was observed in the culture tank fed mixed algal diets. Palanichamy et al. (2022) reported high growth parameters in *Macrobrachium rosenbergii* larvae fed a mixed diet of copepods and *Artemia*. This indicates mixed diet

Table 2. Composition of carbohydrate, protein, and lipid concentration of *D. rigida* enriched with different feeds. (mean±SD).

Diet	Carbohydrate (µg/ml)	Protein (µg/ml)	Lipid (µg/ml)
<i>N. oculata</i>	12.10±1.20 ^a	58.80±2.10 ^b	21.20±1.40 ^{ab}
<i>C. vulgaris</i>	11.40±0.90 ^{ab}	61.20±3.50 ^{ab}	20.00±0.20 ^b
50:50 mix of <i>C. vulgaris</i> and <i>N. oculata</i>	10.80±0.20 ^b	64.10±1.90 ^a	25.10±0.80 ^a

The values are represented as Mean±SD; ANOVA followed by DMRT's performed; Different superscripts in the same column show significantly different ($P<0.05$).

Table 3. Percentage of the fatty acid composition of *D. rigida* enriched with different microalgal diets.

Fatty acids		<i>N. oculata</i>	<i>C. vulgaris</i>	Mixed diet
Myristic acid	C14:0	9.79±0.23	8.19±0.16	8.24±0.22
Myristoleic acid	C14:1	2.13±0.21	3.22±0.24	1.12±0.21
Palmitic acid	C16:0	11.85±0.32	10.22±0.21	9.82±0.26
Palmitoleic acid	C16:1	5.41±0.51	5.87±0.59	3.21±0.16
Stearic acid	C18:0	21.2±0.21	23.43±0.65	20.12±0.21
Cis-9 Oleic acid	C18:1	31.19±0.43	32.54±0.34	29.31±0.54
Trans-9 Oleic acid	C18:1	2.52±0.11	5.23±0.72	2.98±0.21
Linoelaidic acid	C18:2	6.88±0.52	3.67±0.57	7.45±0.67
Linolenic acid	C18:3	1.33±0.12	1.02±0.08	2.76±0.34
Arachidic acid	C20:0	1±0.11	0.78±0.12	1.34±0.41
Eicosadienoic acid	C20:2	2.13±0.21	1.23±0.14	5.23±0.21
Cis- 11 Eicosatrienoic acid	C20:3	0.19±0.09	0.98±0.12	2.12±0.14
Docosadienoic acid	C22:2	0.82±0.19	0.54±0.12	1.34±0.15
Docosahecaenoic acid	C22:6	2.84±0.21	2.65±0.21	4.25±0.54

is always preferable to aquatic organisms, especially at the larval stage.

Biochemical analysis and fatty acid profiling of *D. rigida*: Biochemical analysis of *D. rigida* reveals that protein and lipid content were high in *D. rigida* fed a mixed algal diet compared to the mono algal diets (Table 2). However, more carbohydrate content was observed in the *D. rigida* fed the monoculture of *N. oculata*. Fatty acid profiling of *D. rigida* from different culture tanks shows that *D. rigida* fed a mixed algal diet shows a high percentage of Linoelaidic acid (C18:2), Linolenic acid C18:3), Arachidic acid (C20:0), Eicosadienoic acid (C20:2), Cis- 11 Eicosatrienoic acid (C20:3), Docosadienoic acid (C22:2), Docosahecaenoic acid (C22:6) compared to other 2 feeds (Table 3).

Throughout the experiment, no shooters (cannibalistic activity) were observed and the fish larvae showed good pigmentation from the start to

end of the experiment which indicates *D. rigida* fulfills the nutrient requirements of the fish. Stearic acid (C18:0), Cis-9 Oleic acid (C18:1), and Trans-9 Oleic acid (C18:1) were high in *D. rigida* fed *C. vulgaris*. The protein and lipid content of the copepods fed with mixed algal diets were high compared to the mono-algal diets. This could be because the microalgae *N. oculata* is smaller in size [2-5µm in diameter (Sukarni et al. 2014)] compared to the *C. vulgaris* [2-10µm in size (Coronado-reyes et al. 2022)]. Thus, providing the mixed microalgal culture to copepods will be suitable for copepods at different stages of their life (nauplii, copepodite, and adult) and provide enough nutrients to the copepod during their entire life cycle. This could also be the reason for the high content of essential unsaturated fatty acids in copepods fed mixed diets, as they receive different algal diets and can convert the lipid content to the required ratio compared to copepods

Table 4. Growth parameter of *L. calcarifer* fed with *D. rigida* enriched with different diets at the end of the experiment.

	Length (mm)	Weight (g)	Survival (%)
Initial	6.1±0.2	7.98±0.3	100
Tank A	16.10±0.12 ^a	22.16±0.21 ^a	85.67±2.08 ^a
Tank B	14.90±0.94 ^a	20.62±0.14 ^a	86.00±2.00 ^b
Tank C	17.20±0.34 ^b	25.40±0.38 ^b	89.67±2.51 ^b

The values are represented as Mean±SD; ANOVA followed by DMRT's performed; Different superscripts in the same column show significantly different ($P<0.05$).

fed mono algal diets accustomed to the same type of nutrients.

Larvae growth of *L. calcarifer*. Larval growth trial experiment reveals that sea bass (*L. calcarifer*) larva in experimental tank C showed high survival percentage and growth (Height and weight) at the end of the experiment (9th day of experiment or 21st DPH of larvae) compared to *D. rigida* of other two experimental tanks (Table 4). In between 15th-18th DPH, the larvae showed fast growth from all experimental groups. However, comparatively, the survival and growth rate of the fish larvae in experimental tank B were comparatively low. ANOVA between the length and weight of the larval fish fed different diets showed no significant differences with P -values of 0.9346 and 0.8494, respectively.

Valentin et al. (2016) showed better growth and development of *L. calcarifer*-fed rotifer enriched with a 50:50 diet of *N. oculata* and *C. vulgaris* compared to the larvae-fed rotifers enriched with a monoalgal diet. Fatty acids are necessary nutrients that play an important role in the growth and survival of most living organisms as they play an important role in the development of the immune system and aid in various physiological activities (Nguyen et al. 2022). Essential fatty acids are very crucial for larval development. A high percentage of essential fatty acids like Linolenic acid and Docosahexaenoic acid (DHA) were observed in the *D. rigida* fed a mixed algal diet. This could be the reason for better growth and survival of *L. calcarifer* larvae fed with *D. rigida* of a mixed diet. DHA is important for the development of nervous tissues and retina cells during larval development, thus low DHA results in high larval mortality in aquaculture (Tocher et al. 2008).

Similarly, Linolenic acid is also essential for the growth and survival of larval fish (Mejri et al. 2021). *D. rigida* has an adequate amount of DHA and linolenic acid in general which could be the reason for the high larval survival rate compared to the previous studies (Valentin et al. 2016; Santhanam&Perumal 2012). However, throughout the experiment, there were no cannibalistic activities of *L. calcarifer* larvae is observed and *L. calcarifer* larvae showed good pigmentation which indicates *D. rigida* provides all the necessary nutrients required. In general, the color of the fish indicates the health status of the fish (Das 2016). This indicates *D. rigida* could be the suitable live feed for larval rearing of *L. calcarifer*.

Microalgae are the main producers of fatty acids in the aquatic environment and zooplankton such as copepods act as an important link between them and higher trophic levels. The present study shows that the mixed algae diet provides better results in terms of density and essential fatty acid content of live feed like *Dioithonarigida* in aquaculture, which in turn promotes the growth and survival of fish larvae feeding on them. However, this is only a preliminary study of *D. rigida* and *L. calcarifer* under laboratory conditions at fixed physicochemical parameters. Therefore, copepod density and fish growth parameters may vary. Nevertheless, this study will provide aquatic biologists with much-needed information to select a suitable diet for the culturing of live feed for aquaculture practices.

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مقاله کامل

غنی‌سازی *Dioithona rigida* (Giesberch, 1896) با جیره‌های مختلف ریز جلبکی و تأثیر آن بر بقا و رشد لارو *Lates calcarifer* (Bloch, 1790)

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چکیده: کوپه‌پودها اجزای کلیدی حیاتی اکوسیستم‌های دریایی و آب شیرین هستند و نقش مهمی در انتقال انرژی از تولیدکنندگان اولیه به تولیدکنندگان ثانویه و مصرف‌کنندگان ایفا می‌کنند. بهینه‌سازی تغذیه کوپه‌پودها با رژیم مورد نیاز برای کشت آن‌ها در مقادیر زیاد ضروری است. از این‌رو، این مطالعه با هدف بررسی تولید تراکم بالا یک کوپه‌پود دریایی، *Dioithona rigida* با استفاده از جیره‌های مختلف ریزجلبکی و ارزیابی رشد و بقای لارو سی‌باس دریایی آسیایی، *Lates calcarifer* انجام شد. کشت با تراکم بالا در *D. rigida* که با رژیم غذایی مخلوط (۵۰:۵۰) *C. vulgaris* و *N. oculata* تغذیه شده بود، در روز ۲۱ دوره کشت به تراکم جمعیت 2523 ± 2250 ind/L رسید. اسیدهای چرب ضروری مانند لینولنیک اسید و دوکوزاهگزانوئیک اسید در کوپه‌پودهای تغذیه شده با جیره مخلوط جلبکی نسبت به آنهایی که با جیره تک جلبکی تغذیه می‌شوند، درصد بیشتری را نشان دادند. در بررسی پرورش لارو *L. calcarifer*، لاروهای تغذیه شده با *D. rigida* غنی‌شده با جیره مخلوط ۵۰:۵۰ *C. vulgaris* و *N. oculata* رشد سریع‌تر (طول $17/2 \pm 0/34$ میلی‌متر؛ وزن $25/4 \pm 0/38$ میلی‌گرم) و بالاترین بقا (۹۰٪) را نسبت به سایر گروه‌های آزمایشی نشان دادند. نتایج نشان داد که رژیم غذایی جلبکی مخلوط جهت پرورش غذاهای زنده برای کوپه‌پودها مناسب‌تر است.

کلمات کلیدی: سیکلوپوئید، رژیم غذایی جلبکی مخلوط، ترکیب اسیدهای چرب، آبی‌پروری.