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Evaluation of the suitability of microalgal and artificial diets for the mass production of harpacticoid copepod, *Euterpina acutifrons* (Dana, 1848)

K. S. Aneesh[1,2], B. Santhosh[1*], F. Muhammed Anzeer[1], Mijo V. Abraham[1,2], S. Darsana[1,2] and Ritty Maria Thomas[1]

[1]Regional Centre of ICAR-Central Marine Fisheries Research Institute, Vizhinjam-695 521, Kerala, India.
[2]University of Kerala, Thiruvananthapuram- 695 034, Kerala, India.

[*]Correspondence e-mail: santhoshars@gmail.com
ORCiD: https://orcid.org/0000-0003-4066-0073

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Original Article

Abstract

One of the major constraints in the marine aquaculture sector is the lack of sufficient fish seeds for farming. Live feeds are the critical factor influencing the health and survival of the finfish larvae. Copepods are nutritionally superior and more easily digestible live feed than the conventionally used *Artemia* and rotifers. The harpacticoid copepod, *Euterpina acutifrons,* is a promising species for finfish larviculture due to its hardy nature, adaptability to culture conditions, and small pelagic naupliar stages. This study evaluated the effect of different algal and non-algal diets on the development rate of naupliar and copepodite stages, time taken for maturity, average life span, egg production parameters, hatching success, survival rate of nauplius and copepodite stages, and population composition of *E. acutifrons.* The algal diets used were *Chaetoceros calcitrans* (CHA), *Chlorella marina* (CHL), *Pavlova lutheri* (PAV), and *Isochrysis galbana* (ISO), and the inert diets used included rice bran (RBN), groundnut oil cake (GNC), commercial shrimp feed (CSF), and carrot juice (CTJ). The diet CHA+CHL gave maximum copepodite survival (89%), faster naupliar development (2.7 days), faster copepodite development (4.5 days), early maturity (6.7 days), maximum number of egg sac production (7 nos.), better egg production frequency (1.7 days), maximum number of eggs per egg sac (22.5 nos.), maximum number of naupliar production (5368.25) and maximum adult number (479.25) in the culture population. Performance of all non-algal diets except RBN was inferior to that of the algal diets. In conclusion, the microalgal diets, especially those diatom-based ones and their combinations with other microalgae, remain superior for the optimum production of *E. acutifrons*.

Keywords: *Copepod, live feed, non-algal diet, harpacticoid, microalgae*

Introduction

Aquaculture is one of the world's most significant food-producing sectors, accounting for approximately half of the fish required for human consumption (FAO, 2025). In aquaculture, live feed provides essential nutrition for marine finfish larvae, particularly during their early developmental stages, and it is a major factor determining the survival and overall health of the larvae (Sun and Fleeger, 1995; Fleeger, 2005; Drillet, 2008; Olivotto, 2008). Factors such as size, nutritional factors, and acceptance are critical in determining the suitability of live feed for fish larval rearing. *Artemia* and rotifers are the popular live feeds used in aquaculture for rearing finfish and crustacean larvae. *Artemia* and rotifers are bigger than the mouth size of many fish larvae. These are also insufficient to meet all the essential nutritional requirements and often require enrichment (Shields *et al.*, 1999; Altaff and Vijayaraj, 2021; Olivotto, 2008, 2010).

In contrast, the copepods are available in varying size ranges, are nutritionally superior and are easily digestible compared to *Artemia* and rotifers. Copepods are a rich source of proteins, essential fatty acids, enzymes, and vitamins required for the optimum survival and growth of marine finfish larvae (Shields, 1999; van der Meeren, 2008). Copepods are the natural feed of marine fish larvae in the wild (Llopiz, 2013; Robert *et al.*, 2013). The distinctive 'stop and swim' movement patterns stimulate first feeding, even in weak larvae with poor vision (Støttrup, 2000; von Herbing and Gallagher, 2000; Drillet, 2011).

Copepods naturally feed on microalgae and other suspended organic particles (Sautour and Castel, 1993), however, under hatchery conditions, non-living organic particles can considerably deteriorate the water quality of the culture system. Copepods belonging to the Orders Calanoida, Cyclopoida, and Harpacticoida are popularly used as live feeds in hatchery for early stages of fish larvae. Calanoids are the most studied group as a live feed because of their abundance in pelagic waters and suitability for large-scale production. Generally, copepods are cultured using microalgae as their primary food source. Although there are many reports on the culture of harpacticoid copepods using inert feed materials (Støttrup, 2003, 2006; Fleeger, 2005; Ribeiro and Souza-Santos, 2011), it remains challenging to culture both calanoids and cyclopoids with low-cost artificial feeds.

Harpacticoids are generally epibenthic copepods that graze on organic debris, and there is a possibility of culturing this group using inert feeds. Harpacticoids are naturally found in many habitats, including the open sea, the deep sea, brackish water, and freshwater environments (Huys and Boxhall, 1991). *Tisbe* spp., *Tigriopus* sp., and *Euterpina acutifrons* are the major harpacticoid copepods commonly used in aquaculture trials. *E. acutifrons* have more pelagic naupliar stages and are reported to be suitable as a live feed for marine finfish larviculture (Kraul, 1989; Støttrup, 1997). The present study mainly focuses on the practical evaluation of the effect of selected algal and non-algal diets on the important production parameters such as egg production, egg hatching, development rate of different life stages and population of *E. acutifrons*.

Material and methods

Collection and isolation of copepods

Zooplankton samples were collected in the early morning from the near-shore area of Vizhinjam coast of Kerala, India (8°20'45.60" N, 76°69'2.40" E). The samples were collected using a 150 µm plankton net (Gallienne and Robins, 2001) and transferred immediately to the laboratory with sufficient aeration. In the laboratory, collected samples were filtered through a 500 µm sieve to remove the non-targeted large organisms and then diluted with fresh seawater. From the diluted samples, *E. acutifrons* were isolated and stocked into 1000 ml glass beakers containing 800 ml of fresh seawater (Santhosh *et al*., 2018).

Development of stock culture

Samples from each glass beaker were thoroughly examined under a stereo-zoom microscope (Leica S8APO) for mature adult females of *E. acutifrons* with egg sacs and were isolated with the help of Pasteur pipettes. Separated organisms (n=50) were washed thoroughly several times to avoid any possible contamination and transferred to new 1000 ml glass beakers containing 800 ml filtered, fresh seawater of salinity 35 ppt and mixed microalgae (Norsker, 1997; Støttrup and Norsker, 1997; Rajthilak *et al.,* 2014; Santhosh *et al*., 2018). The glass beakers were incubated in sufficient light and aeration, and partial water exchange was carried out on alternate days. The culture was frequently monitored for population growth and any possible contamination (Santhosh *et al*., 2018). Faecal pellets and algal debris were removed routinely with 10-20% water replacement. The samples were serially transferred to larger containers of varying capacities (2 l, 5 l, and 10 l) with a minimum density of 0.5/ml. Upon achieving a density of 1/ml in 10 l containers, the culture was upscaled to 300 l capacity round high-density polyethene (HDPE) tanks, filled with 150 to 180 l of fresh seawater. The cultures were thoroughly screened for contamination during the scale-up process (Santhosh *et al*., 2018).

Algal stock and mass culture

From the algal stock culture facility of the Regional Centre of ICAR-CMFRI, Vizhinjam, the microalgal species *Chaetoceros calcitrans, Chlorella marina, Pavlova lutheri*, and *Isochrysis galbana* were collected. Stock and mass cultures of adequate quantity were prepared and maintained using standard methods, using Walne's medium (Walne, 1970) for feeding the copepods (Santhosh *et al*., 2018). The axenic algal cultures were harvested and fed to copepods when the culture was in the exponential growth phase.

Preparation of experimental diets

The diets used for the feeding trials were prepared using microalgae and inert feeds according to standard methods (El-khodary *et al*., 2020; Magouz *et al*., 2021). Inert feeds were purchased from the local market. The microalgae and prepared inert feeds were fed to copepods at a carbon equivalency of 1500 µg l^{-1} during the experiment (Strathmann, 1967). The algal density was maintained by adding sufficient quantities of algae as and when required. The microalgae, *C. calcitrans* (CHA), *C. marina* (CHL), *P. lutheri* (PAV), and *I. galbana* (ISO) were used for the trials. The algal diet was prepared as a mixture of two species at a ratio of 1:1 (A'tirah *et al*., 2016).

Inert feeds used for feeding trials included rice bran (RBN), groundnut oil cake (GNC), commercial shrimp feed (CSF), and carrot juice (CTJ) (Kahan, 1979; Phatarpekar *et al*., 2000). The GNC (5 g) was soaked in 100 ml of sterile seawater for 30 minutes and then thoroughly mixed using a kitchen blender

to break up the clumps (Rhodes, 2007; El-khodary *et al.*, 2020; Magouz *et al.*, 2021). The water containing suspended GNC particles was filtered through a 20 μm sieve, and the filtrate was used as the diet for the experiment. Similarly, freshly prepared carrot juice in seawater was sieved through a 20 μm filter mesh, and the filtrate was used to feed the copepod (Perumal *et al.*, 2008; Rajthilak *et al.*, 2014). Approximately 10% of the water was replaced every 24 hours, and a sufficient amount of feed was added to maintain adequate feed density.

Isolation of nauplii of E. acutifrons

Adult copepods from stock culture were filtered through a 200 μm filter mesh and stocked in tanks containing 200 l of chlorinated, dechlorinated, and filtered seawater at room temperature (28 °C). After 24 hours, the whole culture was filtered using a 125 μm filter mesh by gentle siphoning, and all the newly hatched nauplii (N1) were separated. Furthermore, the nauplii were transferred into another container (50 l) and allowed to grow until maturity, after which the adult copepods were used for all subsequent trials.

Estimation of development time with different diets

A total of 500 nauplii (N1) were inoculated in 1000 ml polypropylene transparent beakers filled with 500 ml of seawater and designated diet. Four replicates were kept for each treatment. A gentle aeration of one to five bubbles per second was supplied to all experimental containers to ensure sufficient oxygen and prevent food particles from settling (Rhodes, 2003; Miller and Roman, 2008). Every 24 hours, 10 individuals were randomly collected from each beaker to estimate the development until 50% of the culture attained maturity (Kiorboe and Sabatini, 1995; Landry, 1983). Nauplius I to nauplius VI stages were counted as nauplii, copepodite I to copepodite V stages as copepodites, and copepodite VI as adult.

Egg and naupliar production with different diets

Five newly moulted, mature females of *E. acutifrons* with their first egg sac (Zurlini *et al.*, 1978) were introduced into a custom-made two-inch diameter PVC coupling having a length of 5 cm, and a 125 μm mesh was attached to the bottom (Darsana *et al.*, 2022; Chintada *et al.,* 2022). The couplings were immersed in a 500 ml polypropylene beaker containing 350 ml of seawater and the designated diets. In this setup, the nauplii can pass through the mesh and can be retrieved by filtering the water from the beaker through a 50 μm mesh. Every 24 hours, the PVC couplings were removed from the beaker and examined under a stereo-zoom microscope for any mortality and/or development of new egg sacs and nauplii. The nauplii that were retrieved from the beaker were enumerated, and naupliar production was assessed. The experiment continued until the death of all initially stocked females in the treatment and estimated the total naupliar production.

Naupliar and copepodite survival and total life span

After enumeration, the nauplii retrieved on the first day from the previous experiment were transferred to a 125 ml specimen container with 100 ml of seawater and a designated diet. Every 24 hours, the development and survival of the stocked nauplii were examined under the stereo-zoom microscope. Any copepodite (C1) observed was transferred to another container with the same volume of water and feed. The survival from nauplii (N1) to copepodite (C1), C1 to adult, and the total life span were also assessed.

Total population and population composition

Newly moulted mature females and males of *E. acutifrons* were identified (Razouls *et al.*, 2025) and selected for the experiment. The adults (female: n = eight; male: n = two) were stocked in 500 ml seawater with different feed treatments in 1 l polypropylene beakers. After 10 days, the treatments were filtered through a 50 μm mesh, fixed using 5% formalin, and enumerated to assess total population and population composition.

Statistical analysis

All the data were analysed using one-way analysis of variance (ANOVA). Tukey's multiple comparisons test was used to determine the significant differences ($p < 0.05$) between each diet treatment. All statistical analyses were conducted using the SPSS program, version 22. Data are presented as mean±standard deviation (mean±SD).

Results and discussion

Estimation of development time in different diets

The mean development time (days) of *E. acutifrons* from N1 to C1 (Fig. 1) varied from 2.7±0.5 to 6.0±0.82 days in different feed treatments. Faster naupliar development was observed in the feed combination of microalgae CHA+CHL (2.7±0.5) and CHL (2.7±0.5). Slowest naupliar development in feed

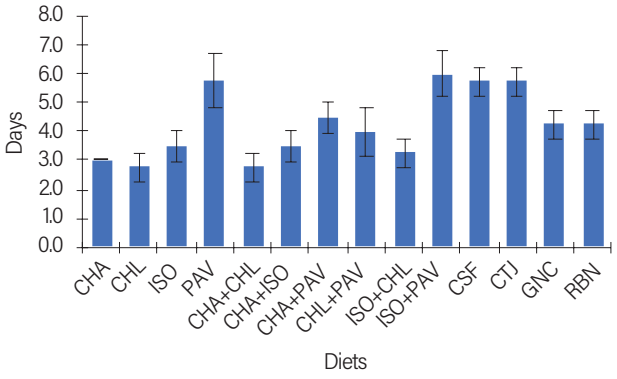

Fig. 1. Mean development time of N1-C1 in *E. acutifrons* when fed with different natural and artificial diets

combination ISO+PAV (6±0.82). The development rates were comparatively better in RBN and GNC than in other non-algal feeds, with a mean development time (from N1 to C1) of 4.2±0.50 in both cases.

The mean development time (days) from C1 to adult varied from 4.5±0.58 to 7.5±0.58. The mean development time from C1 to adult (Fig. 2) was shortest in the feed treatment CHA+CHL (4.5±0.58), and it was not statistically different in the second-best-performed diet CHA (4.7±0.50). The mean development time from C1 to adult was highest (7.5±0.0) in the treatments fed with CTJ, CSF, and ISO+PAV, which were statistically similar to the samples fed with CHL+PAV (7.2±0.50) and PAV (7±0.0). The non-algal feed RBN (5.2±0.5) and GNC (5.5±0.58) performed better than the other non-algal diets.

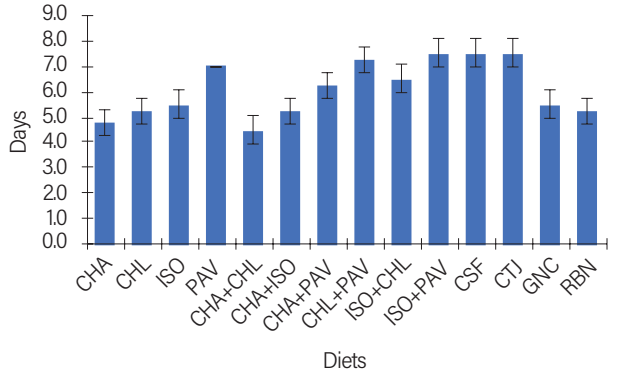

Fig. 2. Mean development time of C1-Adults in *E. acutifrons* when fed with different natural and artificial diets

In the trials, the mean time (days) to first maturity (Fig. 3) of *E. acutifrons* was shortest in the treatment CHA+CHL (6.7±0.50), and it was delayed by up to 9.7±0.96 days in the samples treated with CTJ. The non-algal feeds used were not stable for attaining first maturity in *E. acutifrons,* and all inert feeds delayed the first maturity compared to the algal treatments. 50% of the *E. acutifrons* population attained their maturity within 8±0.82 to 10.7±0.5 days in different

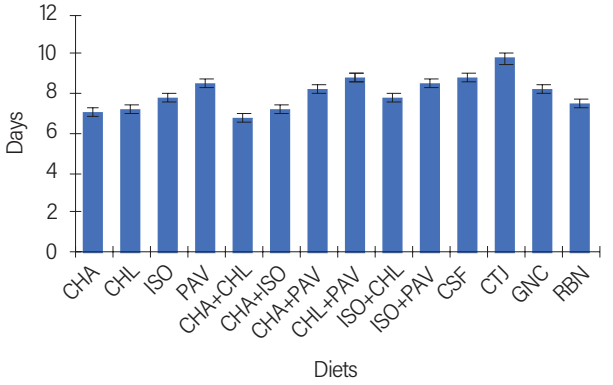

Fig. 3. Mean time to first maturity of *E. acutifrons* when fed with different microalgal and artificial diets

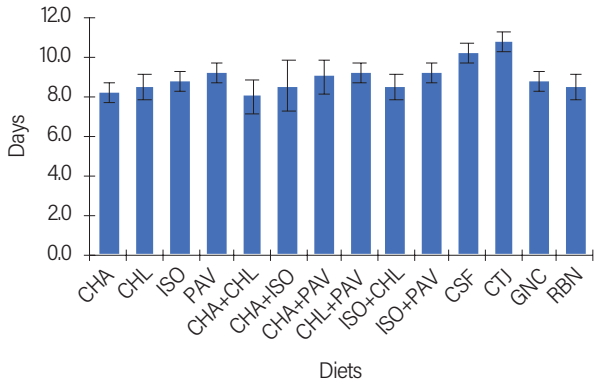

Fig. 4. Mean time to >50% maturity in total individuals of *E. acutifrons* when fed with different microalgal and artificial diets

feed treatments (Fig. 4). The results were statistically not different (P>0.05) in the case of microalgal diets CHA+CHL (8±0.82 days), CHA (8.2±0.50), CHA+ISO (8.5±1.29), and the inert diet RBN (8.5±0.5). *E. acutifrons* fed with CTJ took a maximum period (10.7±0.50 days) to attain >50% maturity in the population. It was evident that the utilisation of non-algal diets, except RBN, is inferior to the algal diets in *E. acutifrons*. Previous studies have reported that *E. acutifrons* prefers diatomaceous algae mostly (Santos, 1999; De Troche, 2006; Wyckmans, 2007). It is also evident that *E. acutifrons* culture performed better when fed mixed microalgal cultures because of their stage-specific feed preference (Nassogne, 1970; Koski *et al.,* 2006).

Egg production

Number of egg sacs per female: The total egg sacs produced per female during the life span of *E. acutifrons* (Fig. 5) in different treatments varied between 7±0.82 and 0.75±0.5. *E. acutifrons* fed with CHA+CHL yielded the maximum number of egg sacs (7±0.82), and CTJ produced the minimum number of egg sacs (0.75±0.5) in the life span. The number of egg sacs produced was very low for the non-algal diets in the

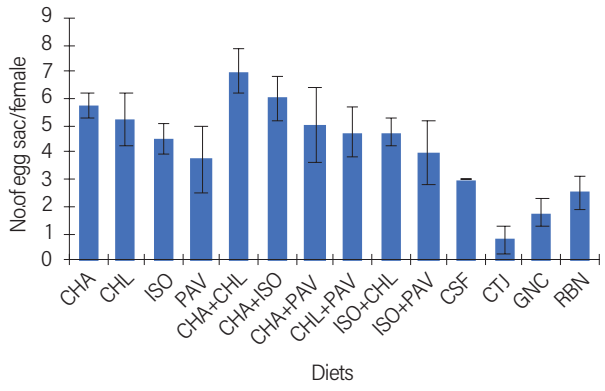

Fig. 5. Number of egg sacs produced per female during their life span by *E. acutifrons* when fed with different natural and artificial diets

order of CSF (3±0.00) >RBN (2.5±0.58) >GNC (1.75±0.50) >CTJ (0.75±0.50). In laboratory conditions, *E. acutifrons* collected from Menai Straits, Anglesey, England, produced a maximum of 5 egg clutches per female (Haq, 1972). In the present study, the maximum egg clutch produced was 7±0.82 per female, and the variations in the egg production of harpacticoids may be due to the differences in the strain (Haq 1972), temperature (Rajthilak, 2014; Barth Jensen *et al.*, 2020) and feed (Castellani *et al.,* 2007; Halsband and Hirche, 2001; Camus and Zeng, 2012). *E. acutifrons* fed with microalgal diets produced new egg clutches immediately after a few hours of hatching the previous egg clutch, but in the case of all non-algal diets, it was delayed up to three or more days. Similarly, a delay of 3 days for the production of new egg sacs in *E. acutifrons* was recorded when fed with the diatom *Phaeodactylum tricornutum* (Haq, 1972).

Number of eggs per egg sac: The number of eggs in each sac (Fig. 6) varied from 6.25±0.5 to 22.5±0.58 among different trials. *E. acutifrons* fed with CHA+ISO (21.75±0.96) and CHA (21.25±0.50) produced maximum eggs/sac; the results were statistically similar to the diet CHA+CHL. *E. acutifrons* can

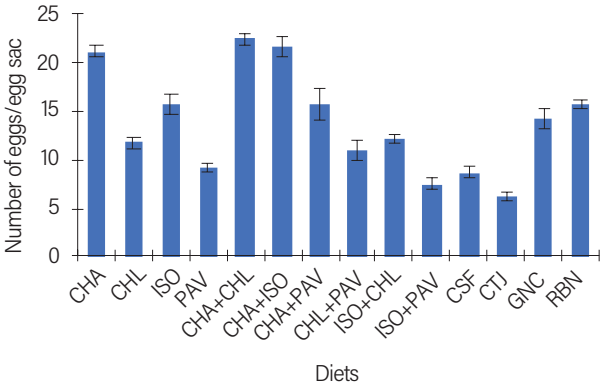

Fig. 6. Number of eggs per egg sac in *E. acutifrons* when fed with different natural and artificial diets

effectively utilise most microalgal feeds, resulting in optimum egg production (Boxshall and Hulsey, 2004; Camus and Zeng, 2009, 2012). Among the non-algal diets, feeding with RBN produced the highest number of eggs/sac (15.75±0.50), while CTJ treatment produced the lowest (6.25±0.50). The number of eggs in the case of a non-algal diet may be lower, as providing a higher concentration of non-algal diets forms clumps that attach to the appendages of copepods, making their movement difficult (D'Apolito, 1979), ultimately resulting in increased mortality. Guisande *et al.* (1996) reported that *E. acutifrons* produced comparatively large eggs with fewer numbers in low food concentration and *vice versa*. Providing surplus feed in case of non-algal feeds is not applicable due to fouling and contamination (Zurlini, 1978; Kahan, 1979; Støttrup, 2006).

Egg sac production frequency: In the present study, the frequency of egg sac production (in days) in *E. acutifrons* (Table 1) varied according to different diets. The average period between egg sac production varied from 1.7±0.5 to 3.7±0.5 days. Copepods fed with the diets CHA+ISO (1.7±0.5 days) and CHA+CHL (1.7±0.5 days) produced new egg sacs more frequently than any other treatments. The egg production frequency was lower in *E. acutifrons* when fed with non-algal diets like CTJ (3.7±0.50), GNC (3.5±0.58), RBN (3±0.00) and CSF (3±0.82).

Feeding strategy can alter the quality and quantity of eggs, as well as the frequency of egg production, in cultured

Table 1. Egg production frequency (days) in *E. acutifrons* when fed with different natural and artificial diets

Diets	Frequency (days) of egg sac production (Mean±SD)
CHA	2 ± 0.0^{ab}
CHL	2 ± 0.0^{ab}
ISO	2 ± 0.0^{ab}
PAV	2.5 ± 0.58^{abc}
CHA+CHL	1.75 ± 0.5^{a}
CHA+ISO	1.75 ± 0.5^{a}
CHA+PAV	2 ± 0.0^{ab}
CHL+PAV	2.25 ± 0.5^{ab}
ISO+CHL	2 ± 0.0^{ab}
ISO+PAV	2.25 ± 0.5^{ab}
CSF	3 ± 0.82^{bcd}
CTJ	3.75 ± 0.5^{d}
GNC	3.5 ± 0.58^{cd}
RBN	3 ± 0.0^{bcd}

copepods (Kleppel, 1993; Alajmi and Zeng, 2015; Dayras, 2021). In the present study, although survival and egg production were observed in all diets, both survival and egg production frequencies were lower in the non-algal diets, which may be due to the differences in the nutritional quality of the microalgal and artificial diets. Goswami (1976) and Zurlini *et al.* (1978) reported that the quality and quantity of food influence the total egg production and egg production frequency in *E. acutifrons*.

Egg hatching success: Maximum egg hatching success (Fig. 7) was observed in the diet treatments with CHA+ISO (95.5±4.04%) and is statistically similar to the treatments CHA (94.25±3.86%) and ISO (94.5±4.36%). Jasmine *et al.* (2016) also reported maximum egg hatching success in the combination of CHA+ISO in *E. acutifrons*. The egg hatching success obtained using the non-algal diet RBN was 87.25±2.5%, which was similar to that of other non-algal diets, such as GNC (86±8.16%) and CSF (81.5±5.51%).

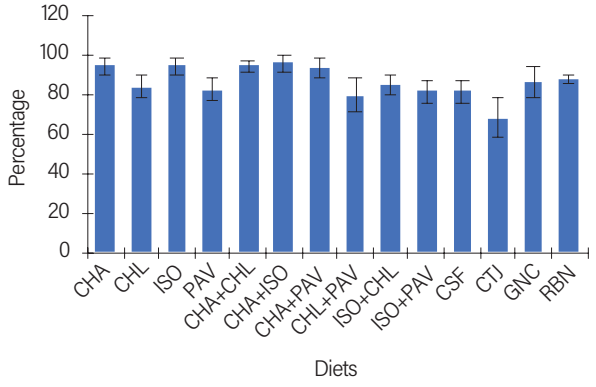

Fig. 7. Egg hatching success in *E. acutifrons* fed with different natural and artificial diets

Naupliar and copepodite survival

Naupliar survival percentage (Fig. 8) of the copepod *E. acutifrons* fed with different feeds showed significant variations between treatments. In the treatment, CHA+ISO, 91.75±0.50% of the total nauplii survived up to copepodites. The least naupliar survival was observed in the feed treatment PAV (30±5.89%). Among the non-algal feeds, RBN (60.5±3.70) supported the highest naupliar survival, and CTJ (34.25±7.14%) was the lowest. The result correlates with that of Jasmine *et al.* (2016), which reported the maximum naupliar survival for the combination of CHA+ISO. In general, harpacticoids prefer diatomaceous algae (Santos, 1999; De Troche, 2006; Wyckmans, 2007).

Maximum survival (89±5.77%) (Fig. 9) of *E. acutifrons* from copepodites to adults was observed in the treatment

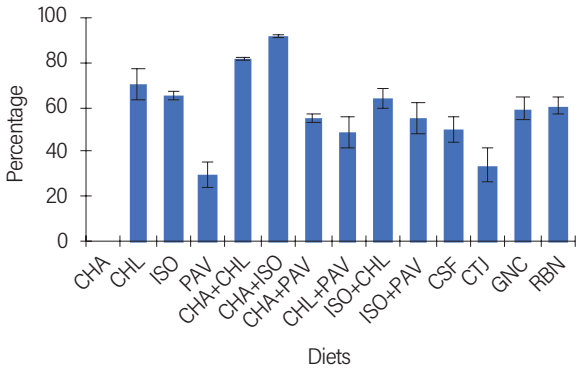

Fig. 8. Naupliar survival (%) of *E. acutifrons* fed with different natural and artificial diets

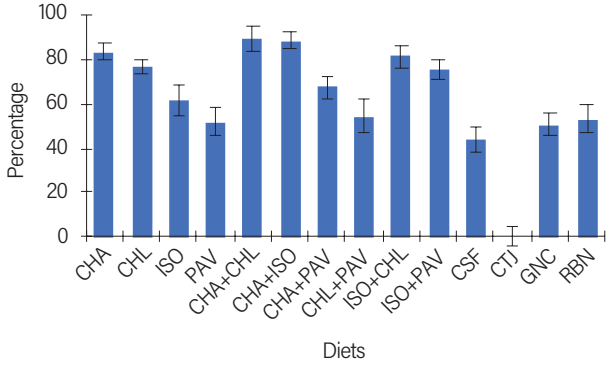

Fig. 9. Copepodite survival (%) of *E. acutifrons* fed with different natural and artificial diets

CHA+CHL. This result was statistically similar (P>0.05) to those of the treatment CHA+ISO (88.5±3.87%) and CHA (83.5±4.20%). In *E. acutifrons*, the highest naupliar and copepodite survival was found in the mixed diet of *Tetraselmis* and *Chaetoceros* (Camus and Zeng, 2012). In *Nitokra affinis*, the maximum population growth was observed in *C. marina* (Rajthilak *et al.*, 2014), and in *Acartia bilobata*, maximum nauplii and copepodite survival was observed in *Isochrysis galbana* (Chintada *et al.,* 2022). In the present study, RBN performed better (53.25±6.08 %) among the non-algal diets, and CTJ showed the least survival in the copepodite stage (37.25±11.32%). Similarly, carrot juice was an inferior diet for *N. affinis* (Rajthilak *et al.*, 2014).

Life span

The average lifespan (days) (Fig. 10) of the copepod *E. acutifrons* varied significantly with different feed treatments. The longest life span (30.5±0.58) was observed in the trials with CHA+CHL, and the shortest was with CTJ (13.5±0.58). The RBN (21.5±0.58) and CSF (21± 1.15) resulted in a better average lifespan among non-algal diets.

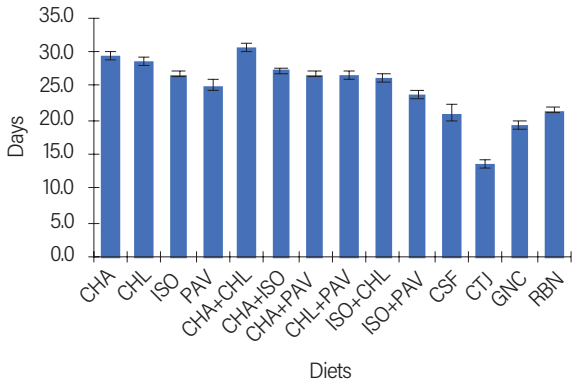

Fig. 10. Lifespan (days) of *E. acutifrons* fed with different natural and artificial diets

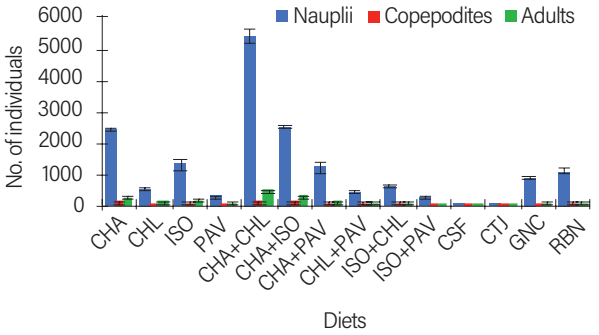

Fig. 11. Population composition of *E. acutifrons* fed with different natural and artificial diets

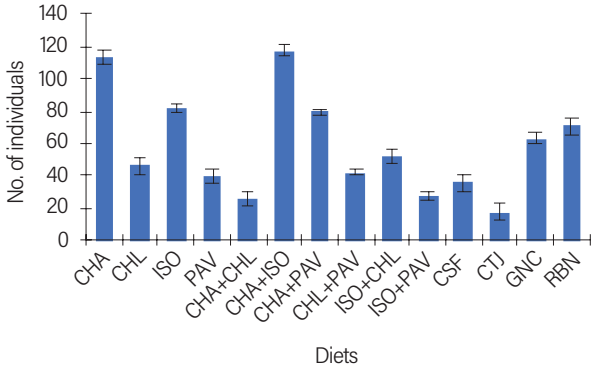

Fig. 12. Number of egg-bearing females of *E. acutifrons* fed with different natural and artificial diets

In the present study, a positive, combined effect of CHA+ISO was observed on sexual maturity, egg production frequency, number of eggs per egg sac, egg hatching success, naupliar survival, and copepodite survival. This finding also aligns with the results of Jasmine *et al.* (2016), who reported that CHA+ISO yielded the maximum survival, faster maturity, higher egg production, and hatching success. The effect of ISO on naupliar development was reflected in the population growth experiment, where the maximum number of nauplii developed into copepodites in samples fed ISO or its combinations. Payne and Rippingale (2001) reported that the fatty acid profile of *I. galbana* has an increased DHA: EPA ratio, a desirable quality of live feed, which helps copepods for better egg production.

Total population and population composition

The feed utilised directly influenced the population growth of *E. acutifrons*. A maximum number of nauplii (Fig. 11) was present in the samples fed with CHA+CHL (5368.25±208.79) and a minimum in the samples fed with CTJ (1.5 ±2.60^a). Among the artificial feeds, RBN (1087.25 ±79.52) performed better. The number of copepodites (Fig. 11) was maximum in *E. acutifrons* fed with CHA+ISO (141.5±10.43) and was not statistically much different from the number of copepodites present in the samples fed with CHA (131.5 ±19.86) and CHA+CHL (129.75±12.19). Among the non-algal feeds, RBN (67 ±9.64) performed better, and the least output was observed in the samples fed with CTJ (0.25±0.43). The maximum number of adults (Fig. 11) was observed in the treatment CHA+CHL (479.25±4.32), and the minimum number was in CTJ (31±2.55). Among non-algal diets, RBN performed better than all other non-algal feeds (108±6.75). The number of egg-bearing females (Fig. 12) was higher in the samples fed with CHA+ISO (117.5±3.77) and CHA (113.75±4.87) and was lower in samples fed with CTJ (17.75±4.71). Among the non-algal feeds, the performance of RBN-fed (70.75±4.76) was better.

In contrast to the findings of Jasmin *et al.* (2016), the present study yielded promising results from the microalgae CHL, particularly when combined with ISO. In general, combinations of microalgae CHA+ ISO gave better output for many parameters observed in the present study. It may be assumed that small-sized algal feeds favour the survival and development of lower developmental stages, while adult copepods prefer larger cells (Wilson, 1973; Jasmin *et al.*, 2016). This size preference for different developmental stages of copepods in large laboratory cultures is also recommended by many researchers (Murray and Marcus, 2002; McKinnon, 2003). Similarly, Berggreen *et al.* (1988) reported that in the copepod *Acartia tonsa,* the size of microalgae directly influences the feeding efficiency in various developmental stages. Findings of Nassogne (1970) concluded that the minimum size of the algal cell that can be handled and retained by adult *E. acutifrons* in its setae is 6-7 µm. This study also suggested that the mixed feeding strategy is one of the most successful modes of feeding *E. acutifrons* cultures, which supports all developmental stages equally.

The present study confirmed that *E. acutifrons* can survive on all the algal and inert diets used, but the performance of non-algal diets was very poor. It was also observed that the diet with the diatom, CHA, and its combinations with other selected microalgae were better for maximum population growth in *E. acutifrons*. In contrast, poor performance of calanoid copepods fed with diatoms has been reported by Miralto *et al.* (1999), Buttino *et al.* (2009) and Turner *et al.* (2001). However, in harpacticoids, diatomaceous algae are more popularly recommended (Santos, 1999; De Troche, 2006; Wyckmans, 2007). Poor performance was observed in the algal diets with PAV and its combinations. For *E. acutifrons,* PAV is the least preferred diet and results in developmental abnormalities (Camus and Zeng, 2012).

Conclusion

The feed offered to the copepods directly influences the overall quality of the culture medium physically and biologically. Even though *E. acutifrons* accept a wide variety of phytoplankton, inert particles, and microorganisms; however, the non-algal feeds affected the performance of copepods in all trials. This confirms that the non-algal diets tried here are not suitable for the culture of *E. acutifrons*. In general, microalgae are the natural food source of *E. acutifrons* in the wild, and artificial feed may lack the diversity of nutrients in both quality and quantity. Moreover, the non-algal feeds easily form larger clumps in the culture medium, which eventually attach to the appendages of copepods, increasing mortality and making harvesting more difficult. Low-cost, non-conventional diets can be utilised only when the output is sufficiently high in terms of both quantity and quality, and they should also be produced economically. Unfortunately, all the non-conventional diets tried here, especially the vegetable juices and commercial fish feeds, considerably deteriorated the water quality and promoted the growth of ciliates and other contaminants. Non-conventional feed, like vegetable juices and other inert feeds, is not advisable for *E. acutifrons* culture. In conclusion, better nutrient quality and digestibility of microalgae make them more efficient as an ideal feed for *E. acutifrons,* both for short-term and long-term culture. It is also concluded that among all the feeds tested here, overall performance was better in the combination of *Chaetoceros calcitrarns* and *Chlorella marina* and in general, the *C. calcitrans* and its combination are the ideal feed for the hatchery production of *E. acutifrons.*

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Author contributions

Conceptualisation: BS; Methodology: KSA, BS; Data Collection: KSA, FMA, MVA, SD, RMT; Data Analysis: KSA; Writing Original Draft: KSA; Writing, Review, and Editing: KSA, BS, MAF; Supervision: BS.

Data availability

The data are available and can be requested from the corresponding author.

Conflict of interest

The authors declare that they have no conflict of financial or non-financial interests that could have influenced the outcome or interpretation of the results.

Ethical statement

No ethical approval is required, as the study does not involve activities that necessitate ethical approval or involve protected organisms/human subjects, or the collection of samples from protected environments.

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