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Standardization of culture aspects of *Apocyclops cmfri* sp. nov. a native cyclopoid copepod

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Copepods are considered as ideal larval feed, due to their smaller size (<100µm), high protein and favourable fatty acid profiles. There are three types of copepods viz., harpacticoid, calanoid and cyclopoid, which can be cultured on a large scale under controlled conditions as live feed for the initial stages of fish and shellfish larvae. Karwar Research Centre of ICAR- CMFRI made a significant contribution in standardizing the protocols for the culture of a new cyclopoid copepod, *Apocyclops cmfri* sp. nov. a species native to Karwar (Loka *et al.*, 2017, *Indian J. Fish.*, 64(2): 1-9).

Copepods were collected off Karwar (14° 49' 914" N; 74% 06' 002" E) near the cage culture site, using plankton net (100 µm mesh size) and transported to the laboratory for further isolation and culture. The cyclopoid copepod, Apocyclops cmfri sp. nov. was isolated using standard methods and was cultured in laboratory by feeding with Chaetoceros calcitrans and successive generations were maintained. Length and width of the newly hatched nauplii and adults were measured (µm unit) under microscope. By following the standard methods, life cycle, hatching and survival rates were assessed. To study the variations in size and density of copepods fed with different microalgal diets (1.2x10⁷ cells /ml) and salinities (0, 10, 20, 30 and 40 ppt), density trials were carried out for a period of one month. Experimental trials were carried out on suitability of cyclopoid copepod as live feed in hatcheries. 1500 numbers of Penaeus vannamei larvae (Average weight: $20 \text{ mg} \pm 0.5 \text{ mg}$) and Chanos chanos (10 mg \pm 0.1 mg) were fed with nauplii and

copepodites (@ 10 numbers/fish respectively) for twenty days. Water exchange was carried out with filtered seawater daily. The survival and growth rates of the fish were calculated at the end of the experiment.

Life cycle of hatchery reared *Apocyclops cmfri* sp. nov. was studied in the live feed laboratory of Karwar Research Centre. The shortest life cycle recorded was 8 days, when fed with *Chaetoceros*, whereas it was 15 days when given *Chlorella vulgaris*. Fecundity was 175 eggs in its life span. Hatching rate of eggs was 90% while survival rates nauplii and adult copepod sages were 90 and 95% respectively. Hatching rate and survival of naupliar stages and copepodites varied significantly (P<0.05) with salinity and microalgal diets provided during rearing.



Different life stages of the copepod

Pure isolates of cyclopoid copepods were cultured in batch and mass culture tanks by feeding with different microalgal diets viz., Chaetoceros calcitrans, Isochrysis galbana, Nannochloropsis oculata, Chlorella vulgaris and also by varying the salinities. Salinity experiments revealed that it tolerates the salinity range between 0 to 40 ppt with an optimum of 30 ppt. A significant variation in the size and density of nauplii and copepodites fed with different microalgal diets at different salinities was recorded (Table 1, Fig. 1). The size of the nauplii was minimum when fed with Chlorella vulgaris and maximum when fed with Chaetoceros calcitrans. Size of the copepod nauplii ranged between 70 - 110 µm whereas the copepodite size varied between 220 - 650 μ m and for the adults it varied between 850 μ m - 1.2 mm. It was observed that the size of nauplii was minimum when fed with C. vulgaris. The minimum size of nauplii fed with C. calcitrans was 80 µm. Thus the naupliar and copepodite stages are suitable as live feed in fish and shrimp hatcheries.







Fig. 2. Weight gain (mg/day) of fish and shrimp larvae fed with *Apocyclops cmfri* sp. nov.

The density trials with different microalgae, *N.oculata*, *C. calcitrans*, *C. vulgaris* and *I. galbana* resulted in a significant variation (P<0.05) between the diets, with a maximum density of 50 numbers per ml when fed with *C. calcitrans*. The density of this species was more when fed with *C. calcitrans* in all the salinities with a maximum at 30 ppt.

Experimental trials examined revealed it is suitable as live feed for both fish and shrimp. The survival rate was 90% for shrimp (*P. vannamei*) and milkfish larvae. The study also revealed that shrimp larvae showed a significant increase in growth rate of 30.2 ± 0.5 mg per day, whereas, in milk fish weight gain was recorded as 14.5 ± 1.5 mg/day after 20 days of the experiment. It was observed that there is a significant variation (P<0.05) in the weight gain of both the fish and shrimp, with a maximum weight gain on 14th and 20th day of experiment respectively.

In conclusion, the study indicated that cyclopoid copepod, *Apocyclops cmfri* sp. nov. isolated from

Table 1.	Size	variations	of nau	olii,	cope	podites a	and adult	cope	pods	fed	with	different	microalg	gal di	ets

Microalgae		Size (µm)	Size (µm)					
	Nauplii	Copepodites	Adult copepods					
Nannochloropsis oculata	85-105	320-520	850-1200					
Chaetoceros calcitrans	80-110	380-650	950-1200					
Chlorella vulgaris	70-85	220-450	825-950					
Isochrysis galbana	75-90	250-560	900-1125					

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Karwar waters, can tolerate a wide range of salinities with an optimum of 30 ppt and minimum naupliar size and maximum density occurs when the salinity was 30 ppt. Culture protocols for this species were standardized in Hatchery complex of ICAR-CMFRI at Karwar with 1.2. million naupliar production at every three days interval. The lifecycle was 8 days with high fecundity and survival rate of 90%. It is suggested that *C. calcitrans* is the most suitable diet for rearing *Apocyclops cmfri* sp. nov. a potential candidate species for rearing of shrimp larvae and can contribute to the aquaculture feed industry.