

## **Short communication**

# **Efficacy of *Apocyclops royi* (Cyclopoida, Copepoda) nauplii as live prey for the first feed larvae of silver pompano, *Trachinotus blochii***

### **ABSTRACT**

In the rearing of first exogenous feeding marine finfish larvae availability of live prey with suitable size and nutritive content is vital for their growth and survival. Copepods represent an important alternative food to present traditional live feed organisms used in marine fish hatcheries. Use of copepods is known to improve survival, growth, and development of fish larvae. The silver pompano, *Trachinotus blochii* is one of the suitable candidate species for aquaculture due to its fast growth, good meat quality and high market demand. The present study aimed to investigate the efficacy of *Apocyclops royi* nauplii in the rearing of first feeding larvae of *T. blochii*. The feeding experiments were conducted from 3 to 7 Day Post Hatch (DPH) using *A. royi* nauplii and *Brachionus plicatilis* (s type) diets. Higher growth of *T. blochii* larvae was evident with *A. royi* nauplii than that of rotifer diet. The *A. royi* nauplii fed experimental group of *T. blochii* larvae showed significantly different length ( $p < 0.05$ ) compared to rotifer fed larvae and the specific growth rate recorded was 29.42% and 24.28% in *A. royi* and Rotifers fed experimental groups, respectively. On 7 DPH, *A. royi* nauplii fed *T. blochii* larvae showed higher mortality than rotifer fed group. With *A. royi* feed *T. blochii* larvae produced higher pigmentation which gradually started on the head and dorsal surface of the body from 3 DPH onwards and spread all over the body and became intense as the larvae grew to 7 DPH. Our results suggest the *A. royi* nauplii are suitable live prey for the *T. blochii* larvae during the initial feeding phase for better growth and pigmentation.

*Keywords: Fish seed production, silver pompano, Trachinotus blochii, Copepod, Apocyclops royi*

### **1. INTRODUCTION**

Development of seed production and culture technologies for many commercially important and high value marine fish species is needed to expand mariculture [1]. Aquaculture of silver pompano (*Trachinotus blochii*) is being successfully undertaken in many Asian countries [2]. In India, the silver pompano is one of the most promising species as its growth rate is high, meat quality is good and it fetches high price in the market. Body shape, colouration and meat quality of this fish is like that of highly priced silver Pomfret (*Pampus argenteus*) [2,3,4]. Still there is need to upscale the seed production technology and raising fingerlings to promote their farming on a large scale [3]. In addition, silver pompano is a fast swimming

marine fish with darting movements and it requires highly nutritive feed to meet its energy requirements [3]. The copepod nauplii are a nutritious food item for first feeding marine fish larvae. Copepods are the most important group of zooplankton which forms the natural food for many fishes and invertebrates [1]. One of the important problems in the marine fish hatchery is the lack of complete balanced larval feed. Copepods, even the newly hatched nauplii are nutritious, rich in PUFA, DHA and EPA, in most desirable ratios, easily digestible and rich in antioxidants and vitamins [5]. The copepod species belonging to the orders Calanoida, Cyclopoida and Harpacticoida are popular for hatchery production as live feed [1]. Among these, the Cyclopoida is potential for live feed in marine finfish rearing. Cyclopoid nauplii are mostly less than 100 µm in length [6]. In addition, the Nauplii of the cyclopoid copepod *Apocyclops royi* develop from eggs in 2 to 3 days and are used for first feeding practices. Likewise, Cassiano *et al.*, [7] assessed the calanoid copepod, *Pseudodiaptomus pelagicus*, nauplii as initial prey for first-feeding finfish larvae. Recently Weirich *et al.*, [ ] reported that the pompano larvae require exogenous feeding as yolk reserves are diminished at 2 - 3 Days Post Hatch (DPH) and require small rotifers or copepod nauplii initially before they can be transitioned to larger prey/feed. Nonetheless, seed production technology of silver pompano *T. blochii* using copepod nauplii are still not clear in this species. Hence, present study attempted to investigate the efficacy of *A. royi* nauplii as first feeding live prey in the hatchery rearing of first feeding larvae of *T. blochii*.

## 2. METHODOLOGY

A stock of fertilized eggs of *T. blochii* was obtained from Central Marine Fisheries Research Institute (CMFRI), Kochi, Kerala - 682018, India and were carefully transported in sealed plastic bags containing seawater and inflated with oxygen. They were acclimatized to the laboratory tank conditions by maintain them in filtered sea water at 28°C temperature, 30 psu salinity, 7.8 pH and 5.5 mg/L dissolve oxygen for further experiment. The newly hatched larvae were stocked in the FRP tanks of 100 L capacity at a density of 20 individuals per liter. During the experiments the aeration in the tanks was increased slightly. In about 48 hrs. Yolk was utilized by the larvae and exogenous feeding commenced. Experimental tanks were provided with green water, feeding was initiated using *Nannochloropsis oculata* and the detailed protocol is given in table-1.

**Table -1: Larval feeding protocol for *T. blochii***

Green water / Feed	Days Post Hatch (DPH)							Density	Feeding Frequency	Water exchange
	1	2	3	4	5	6	7			
Green water ( <i>N. oculata</i> )	**							30,000 cells/mL	Nil	0%
Green water ( <i>A. royi</i> nauplii)			*****					10-12 ind./Lit.	2 times per day	20%
Green water ( <i>Rotifer</i> )			*****					10-12 ind./Lit.	2 times per day	20%

First feeding trials were conducted in triplicate up to 7 DPH in fiberglass tank under optimized conditions (28°C temperature, 30 psu salinity, 7.8 pH and 5.5 mg/L dissolve oxygen). The temperature, salinity, pH and Dissolved oxygen were recorded in each tank daily using standard methods. The dead fish larvae were collected during the period of water exchange and were counted for estimating the mortality and survival was quantified. The lengths of fish larvae (20 nos.) were measured daily. They were placed on glass slides and photographed using Magnus Trinocular Zoom Stereo Microscope (MSZ-TR) and High-resolution CMOS camera with 5 mega pixel (D - series). Specific growth rate (SGR) in mm was calculated as follows, to estimate the percentage length increase per day.

$$\text{SGR} = \frac{\ln \text{ Final length (mm)} - \ln \text{ Initial length (mm)}}{\text{Duration of the experiment (Days)}} \times 100$$

The data on length of *T. blochii* larvae fed on *A. royi* nauplii and rotifer regimes was statistically analyzed One-Way ANOVA test using GraphPad Prism 8 Software. A value of 0.05 was considered to indicate statistical significance and results are expressed as mean  $\pm$  SD.

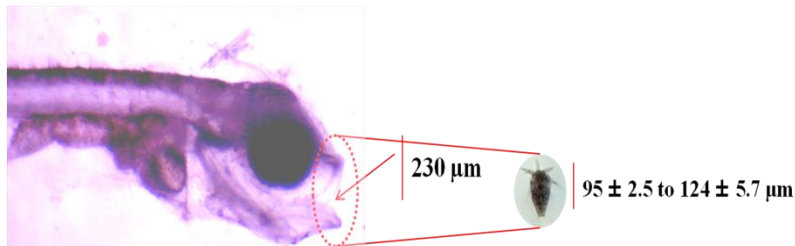
### 3. RESULTS AND DISCUSSION

In the hatchery rearing of marine finfish larvae, diet consisting of high nutritive quality, efficacy, and digestibility is essential for better growth and production [8]. The appropriate live feed with suitable size and nutritional requirements is the key for successful larval rearing in hatcheries, failing these factors can cause high mortality [9]. This is particularly evident during the transition period from endogenous to exogenous feeding of larvae. Understanding the prey (copepod/rotifer) size and predator (fish larvae) mouth size for providing appropriate feed especially during early feeding is important to achieve successful seed production. In the present study, the larval feeding protocol developed for successful seed production using copepod nauplii is summarized in table 1. Various larval stages development of silver pompano is presented in Figure-1. The newly hatched larvae lack mouth opening up to 2 DPH (Figure.1 a-b).



**Figure -1: Endogenous feeding stages of larvae of silver pompano**

They have large and elongated yolk sac extending beyond the head and along the ventral region of the head and alimentary canal. The yolk reserves generally last for two to three days depending on water temperature and it coincides with eye pigmentation, opening of mouth and first feeding. In the present study mouth of the *T. blochii* larvae opened on 3 DPH and the mouth size is around 230  $\mu\text{m}$  and the *A. royi* nauplii size used in the present experiment ranged between 95  $\pm$  2.5 to 124  $\pm$  5.7  $\mu\text{m}$ . The active feeding and occurrence of *A. royi* prey in the gut of the *T. blochii* larvae indicated its size compatibility with the first feeding larvae of *T. blochii* (Figure-2).



**Figure 2: 3DPH Silver pompano larva showing larval mouth size and *A. royi* nauplii size**

In *A. royi* fed *T. blochii* larvae pigmentation gradually started on the head and dorsal surface of the body from 3 DPH onwards which spread all over the body and became intense as the larvae grew and thereafter, the larvae looked darker in colour compared to rotifer fed larvae till the end of the experiment (7 DPH). During the experimental period, the fins and scales have developed distinctly in *A. royi* nauplii when compared to rotifer fed fish larvae and the larval growth pattern recorded during the experiment period is presented in figure 3 a-d.

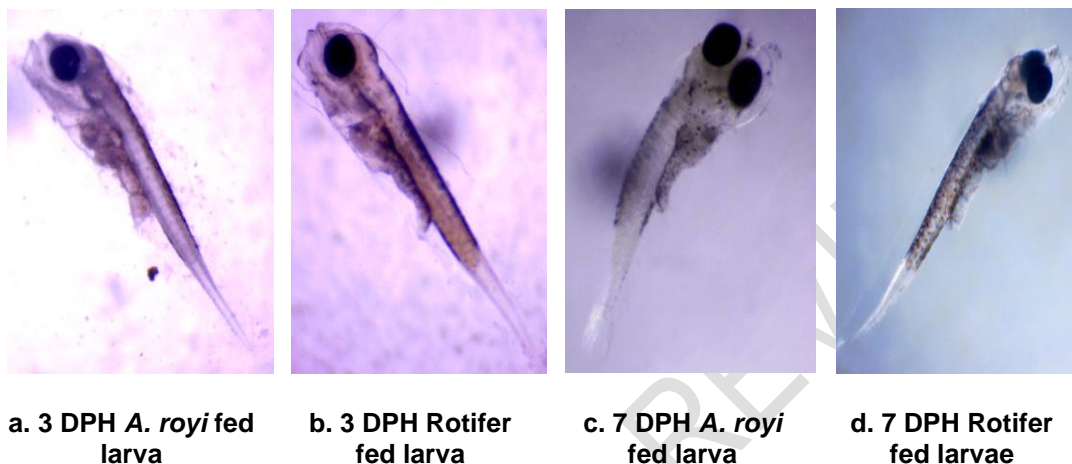


Figure -3: Exogenous feeding stages of larvae of silver pompano

With reference to the growth and survival of *T. blochii* larvae, the length of the *T. blochii* larvae were significantly high ( $p < 0.05$ ) in *A. royi* fed experimental group when compared to rotifer fed *T. blochii* larvae and the specific growth rate was 29.42% and 24.28% in *A. royi* and rotifers experimental group, respectively. The larvae do not swim actively in rotifer feeding regime while active and faster swimming was observed in *A. royi* nauplii fed ones. Overfeeding resulted higher mortality in both *A. royi* and rotifer fed larvae. There is no cannibalistic activity observed in *T. blochii* larvae. During the experiment of *T. blochii* larvae, critical stages is 7 DPH at which high mortality was recorded.

Table-2: Survival and growth performance of *A. royi* and Rotifer fed *T. blochii* larvae

Days Post Hatch (DPH)	Mortality (%)		Survival (%)		Length (mm)		SGR (%)	
	<i>A. royi</i> feed	Rotifers feed	<i>A. royi</i> feed	Rotifers feed	<i>A. royi</i> feed	Rotifers feed	<i>A. royi</i> feed	Rotifers feed
1	0	0	100	100	0.5±0.05*	0.5±0.05*		
2	0	0	100	100	0.83±0.07*	0.79±0.13*		
3	0	0	100	100	1.0±0.15*	0.9±0.1*		
4	10±2	0	90±10	100	1.9±0.02*	1.26±0.11*	29.42	24.28
5	30±2	0	70±5	100	2.23±0.23*	1.8±0.26*		
6	45±5	20±2	50±5	80±2	2.23±0.05*	2.16±0.28*		
7	65±6	50±5	34±4	50±5	2.56 ±0.23*	2.2 ±0.2*		

\* The values are represented in Mean ± SD. The results are not statistically significant at  $p < 0.05$  when compared to rotifer fed fish larvae; \*p-value = 0.8329.

Feeding copepod nauplii has proven beneficial for the better seed production of marine fish larvae. Toledo *et al.* [10] fed nauplii of *Acartia tsuensis* to larvae of *Epinephelus coioides* from 2 to 6 DPH, this experiment has indicated that the copepod nauplii has improved the feeding incidence, growth, and survival compared with rotifers fed larvae. Similarly, the first feeding larvae of turbot *Scophthalmus maximus* has provide the better growth and survival when fed *Tisbe holothuriae* nauplii from 3 to 6 DPH [11]. The *Pagrus auratus* larvae have fed on nauplii of *Gladioferens imparipes* from 4 to 10 DPH. On 6 DPH, the *G. imparipes* fed *P. auratus* larvae showed better growth when compared to rotifers feed experimental group [12]. Hence, our study also indicated that the *A. royi* nauplii fed to silver pompano larvae showed better growth and pigmentation during 1-7 DPH. Nevertheless, further investigation is required to identify the feed concentration for larval rearing of silver pompano, *T. blochii*.

## CONCLUSION

This is the first report on the silver pompano first larval rearing using *A. royi* nauplii. Our study indicated that, the *A. royi* nauplii feeding regime for *T. blochii* resulted in increased growth, and pigmentation compared to traditional rotifers live feed. With reference to the survival, the rotifers fed *T. blochii* have shown higher survival than the *A. royi* nauplii. Our results suggest that the *A. royi* nauplii are suitable for the silver pompano larvae during the initial feeding phase as a suitable diet for better growth and pigmentation. Further experiments needed to reduce the mortality of the larvae for successful seed production of silver pompano in the hatchery.

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## REFERENCES

1. Altaff, K. (2020). Indigenous live feed for aqua hatchery larval rearing of finfish and shellfish: A review. *International Journal of Zoological Investigations*, 6(1), 162-173.
2. Santhiya, A. A. V., Kumar, M., & Chrispin, C. L. (2021). Current Scenario and Culture Techniques of Silver Pompano Fish, *Trachinotus blochii*. *Biotica Research Today*, 3(10), 834-835.
3. Jayakumar, R., Sakthivel, M., Nazar, A. A., Tamilmani, G., Ramesh Kumar, P., Samal, A. K., ... & Gopakumar, G. (2018). Impact of increase in temperature and light intensity on development and metamorphosis of hatchery reared silver pompano *Trachinotus blochii* (Lacepede, 1801) larvae. *Indian Journal of Fisheries*, 65(2), 133-137.
4. Ranjan, R., Megarajan, S., Xavier, B., Ghosh, S., Santhosh, B., & Gopalakrishnan, A. (2018). Broodstock development, induced breeding and larval rearing of Indian pompano, *Trachinotus mookalee*, (Cuvier, 1832)—A new candidate species for aquaculture. *Aquaculture*, 495, 550-557.
5. Altaff, K., & Vijayaraj, R. (2021). Micro-Algal Diet for Copepod Culture with Reference to Their Nutritive Value—A Review. *Int J Cur Res Rev| Vol*, 13(07), 86.
6. Muthupriya, P., Altaff, K., 2009. Effect of salinity and temperature on the reproduction of the estuarine copepod *Apocyclops royi* (Lindberg, 1940). *J. Exp. Zool.* 12(1), 103-106.
7. Cassiano, E. J., Ohs, C. L., Weirich, C. R., Breen, N. E., & Rhyne, A. L. (2011). Performance of larval Florida Pompano fed nauplii of the calanoid copepod *Pseudodiaptomus pelagicus*. *North American Journal of Aquaculture*, 73(2), 114-123.
8. Weirich, C. R., Riley, K. L., Riche, M., Main, K. L., Wills, P. S., Illán, G., ... & Pfeiffer, T. J. (2021). The status of Florida pompano, *Trachinotus carolinus*, as a commercially ready species for US marine aquaculture. *Journal of the World Aquaculture Society*, 52(3), 731-763.

9. Chen, J. Y., Zeng, C., Jerry, D. R., & Cobcroft, J. M. (2020). Recent advances of marine ornamental fish larviculture: broodstock reproduction, live prey and feeding regimes, and comparison between demersal and pelagic spawners. *Reviews in Aquaculture*, 12(3), 1518-1541.
10. Toledo, J. D., Golez, M. S., Doi, M., & Ohno, A. (1999). Use of copepod nauplii during early feeding stage of grouper *Epinephelus coioides*. *Fisheries Science*, 65(3), 390-397.
11. Stottrup, J. G., and N. H. Norsker. 1997. Production and use of copepods in marine fish larviculture. *Aquaculture* 155:231–247.
12. Payne, M. F., and R. J. Rippingale. 2001. Intensive cultivation of the calanoid copepod *Gladioferens imparipes*. *Aquaculture* 201:329–342.

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