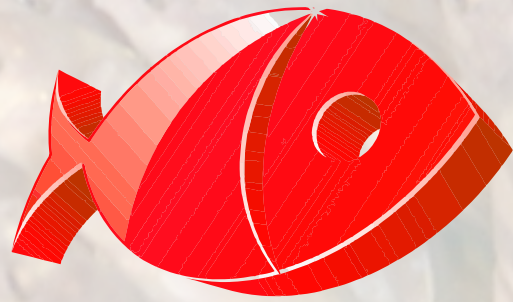
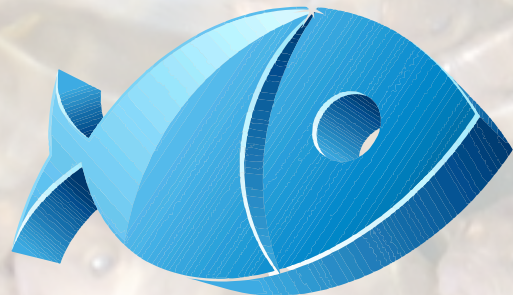


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**FISH LARVAL PERFORMANCE FED WITH COPEPODS (*ACARTIA GRAN*) AND THE DINOFLAGELLATE (*OXYRRHIS MARINA*) AS SUPPLEMENT: THE CASE OF DUSKY GROUPEL (*EPINEPHELUS MARGINATUS*)**

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### **Introduction**

Early life stages of dusky grouper, *Epinephelus marginatus* (Lowe 1834) are still a bottleneck in larval rearing due to their high mortality. Diet and low disturbance of the water column during larval rearing affect survival because the species is fragile and needs small and nutritious live feed (Cunha et al., 2009). Copepods are the natural food items in the wild possessing 2 to 3 times more highly unsaturated fatty acids than rotifers. The use of large tanks like the ones used in semi-intensive systems promotes stability of the water column and reduces interference with larvae.

The aim of this work is to compare the effect of the introduction of copepods in the diet of dusky grouper larvae and the use of the semi-intensive systems on their development and quality.

### **Materials and methods**

Oocytes from hormonally induced spawning of captive females were fertilized with milt from fluent males, both kept at indoor tanks (10.6m<sup>3</sup>) at 22.5±0.5°C. The eggs were incubated directly in the tanks used for comparison of the effect of diets, volumes, and densities on larval development and quality (Table 1). The parameters used in the comparisons were growth (length and weight), survival, activity of digestive enzymes, and skeletal malformations

Two types of diets were compared. One was the generalized diet use in aquaculture composed by small rotifers (*Brachionus* sp.), artemia (*Artemia* sp.) and dry feed, and the second by a mixed diet of small rotifers (*Brachionus* sp.) and copepod (*Paracartia grani*) nauplii followed by artemia (*Artemia* sp.) and dry feed. These comparisons were carried out on two large volume (18m<sup>3</sup>) tanks at low larva (3 larvae.l<sup>-1</sup>) densities and named as semi-intensive system (SIS) with and without copepods (Fig.1). *Acartia grani* eggs were obtained from an adult population fed on a mixture of *Rhodomonas salina* and *Oxyrrhis marina*. Rotifers and *Artemia* instar II/III nauplii were enriched to increase levels of protein and the dry feed contained also high levels of protein.

Table I. Experimental settings for dusky grouper egg incubation and larval rearing. (I. – *Isochrysis*; N. – *Nannochloropsis*; O. – *Oxyrrhis*; B. – *Brachionus*; A. – *Artemia*; P. - *Paracartia*).

Rearing Systems	Intensive	Semi-intensive with- out copepods	Semi-intensive with copepods
Location	Indoor	Outdoor	Outdoor
Volume (m <sup>3</sup> )	1.5	18	18
Dissolved Oxygen (mg.l <sup>-1</sup> )	5.5±0.90	5.9±1.42	5.7±1.35
Temperature (°C)	23.3±0.87	22.9±1.01	22.9±1.01
Photoperiod	Artificial (14L/10D)	Natural (15L/9D)	Natural (15L/9D)
Phytoplankton	<i>I. galbana</i> + <i>N. oculata</i>	<i>I. galbana</i> + <i>N. oculata</i>	<i>I. galbana</i> + <i>N. oculata</i> + <i>O. marina</i>
Zooplankton (N.ml <sup>-1</sup> )	5 <i>B. spp.</i> + 1 <i>A. sp.</i>	5 <i>B. spp.</i> + 1 <i>A. sp.</i>	5 <i>B. spp.</i> + 1 <i>A. sp.</i> + 2 <i>P. grani</i>
Larval Density (N.l <sup>-1</sup> )	40	3	3

The effect of larval density/rearing volume was studied comparing the results obtained with the 1.5m<sup>3</sup> and 18m<sup>3</sup> tanks using the same rotifers (*Brachionus plicatilis*), artemia (*Artemia* sp.), and dry feed diet. The treatment in the smaller volume tank is referred as intensive system (IS).

10 to 25 larvae were sampled for growth (dry weight and total length) at 2, 6, 11, 16, 20, 25, 30, 62 days after hatch (DAH). Trypsin and pepsin activities were determined in 10 larvae at 30 and 62DAH. Skeletal malformations were analysed at 62DAH (n=60) using radiography, with Kodak X-ray DXS System for 4000 capture the images with software Carestream DXS.

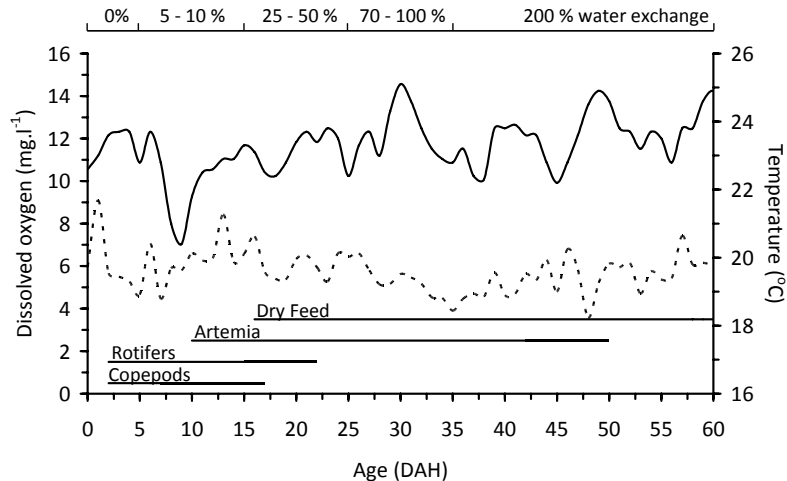


Fig 1. Daily water exchange, mean dissolved oxygen (dashed line) and temperature (solid line) in the rearing tanks, and feeding schedule during the experimental period.

Analyses of variance (ANOVA) were used to compare treatments and data was log normalized when necessary.

## Results and discussion

Comparison between the effect of diets (SIS with and without copepods) show that, at the end of the live feed period (30DAH), fish were significantly longer ( $P > 0.001$ ) in SIS fed with copepods (Table II). No significant differences were obtained in relation to weight. At the end of the experiment (62DAH) when fish was fed only with dry food for 1 month there were significant differences in fish length and weight with the fish from SIS without copepods showing significantly larger and heavier benthic juveniles. Comparison between the effect of larval densities/rearing volume (IS and SIS without copepods) showed no significant differences in fish length and weigh at 30DAH. However at 62DAH the fish were significantly larger and heavier in the SIS without copepods.

At 30DAH dusky grouper from semi-intensive system without copepods exhibited significant ( $P < 0.001$ ) higher values of trypsin activity and lower values of pepsin activity when compared to larvae from other systems. At 62DAH no differences were observed among treatments. Differences at 30DAH reflect the delay of the larvae from the semi-intensive system without copepods, more dependent on pancreatic enzymes whereas the other treatments exhibited already an acidic digestion.

Table II. Mean and standard deviation of main growth parameters and enzymes, final number of harvest fish and degree of total skeletal malformation in dusky grouper juveniles.

Rearing systems:	Intensive		Semi-intensive without copepods		Semi-intensive with copepods	
	30	62	30	62	30	62
Age (DAH):						
Length (mm)	9.9±4.51	42.2 <sup>a</sup> ±5.51	10.3 <sup>b</sup> ±5.98	50.4 <sup>ab</sup> ±5.78	11.6 <sup>b</sup> ±4.97	46.1 <sup>b</sup> ±7.93
Weight (g)	0.04±0.20	1.08 <sup>a</sup> ±0.32	0.05±0.23	1.80 <sup>ab</sup> ±0.60	0.05±0.23	1.43 <sup>b</sup> ±0.69
Trypsin (mU.mg prot <sup>-1</sup> )	0.1 <sup>a</sup> ±0.03	0.2±0.16	2.3 <sup>ab</sup> ±0.22	0.1±0.07	0.7 <sup>b</sup> ±0.49	0.1±0.02
Pepsin (mU.mg prot <sup>-1</sup> )	0.3±0.15	1.3±0.24	0.1±0.03	1.2±0.15	0.3±0.23	1.4±0.39
Final harvest (number)		175		170		351
Malformations (%)		43		40		23

Superscript notations denote significant differences between comparisons: a – Intensive system and Semi-intensive without copepods; b – Semi-intensive without and with copepods)

Number of harvest fish at the end of the experiment was similar between the intensive and semi-intensive systems without copepods but at the semi-intensive systems with copepods survival was two times higher. The higher final density in SIS with copepods probably explains the smaller mean size of fish when compared with SIS without copepods due to the aggressive and territorial behaviour of grouper.

Skeletal malformations occurred with lower incidence in the semi-intensive systems fed with copepods and were almost two times lower than in the semi-intensive without copepods.

### Conclusions

The semi-intensive systems where the larvae were fed with copepods seem to be the most suitable system in dusky grouper larval production, since there was higher survival, lower rate of malformations, with no significant differences in growth. In general, juveniles were of better quality.

### References

Cunha M.E, H. Quental, A. Barradas, P. Pousão-Ferreira, E. Cabrita, and S. En-grola. 2009. *Scientia Marina* 73S1:201-212.