RED PORGY, *Pagrus pagrus* L., (PISCES: SPARIDAE) LARVAE CULTURE AND LIVE FOOD DENSITY UNDER MESOCOSM CULTURE CONDITIONS

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With 3 figures and 1 table

ABSTRACT. Red porgy (*Pagrus pagrus* Linnaeus, 1758) larvae were produced under mesocosm semi-intensive culture conditions provided with adaptations from methodology previously used for rearing other sparids. A shift from 24 to 12 hours light photoperiod was introduced at 19 DAH, simultaneously with a late and low density supply of *Artemia salina* (Leach, 1819). Also, new methods were applied to improve control over larvae populations and culture conditions.

Results indicated high egg hatching rate (72 ± 15.52%) and good larvae performance, as determined by growth of the majority of the population ($TL = 3.208e^{0.035\ DAH}$; $r^2 = 0.9756$; $n = 200$), survival (16%) and functional swimbladder (90%). Rotifers density in the rearing tank was evaluated throughout the culture trial and pointed to persistent higher food availability than larvae consumption rate. This represents a relevant tool to provide larvae with adequate live feed supply in culture conditions. Further studies on the biology and ecology of the species can be carried out without larval food limitations.

Key words: *Pagrus pagrus* larvae, red porgy larvae, mesocosm culture, live food density.

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RESUMO. Cultivaram-se larvas de pargo (*Pagrus pagrus* Linnaeus, 1758) em mesocosmos de metodologias semi-intensivas, testando diferentes modificações aos métodos previamente utilizados na cultura de outros esparideos. Aos 19 dias após-eclosão alterou-se o fotoperíodo de 24 para 12 horas de luz, em simultâneo com um fornecimento tardio e de baixa densidade de *Artemia salina* (Leach, 1819). Foram também aplicados novos métodos de controlo das populações larvares e das condições de cultura.

Obteve-se uma taxa de eclosão elevada (72 ± 15.52%) e uma boa performance larvar, determinada pelo crescimento populacional ($TL = 3.208e^{0.035\ DAH}$; $r^2 = 0.9756$; n = 200), sobrevivência (16%) e bexiga-natatória funcional (90%). A densidade dos rotíferos no tanque de cultura, avaliada ao longo de todo o período experimental, indicou maior disponibilidade de alimento do que o requerido para consumo larvar. Os resultados encontrados representam uma ferramenta importante para um adequado fornecimento de alimento vivo às larvas de pargo em condições de cultura. A adopção das novas metodologias permitirá que estudos posteriores da biologia e ecologia da espécie possam ser efectuados sem limitações de alimento larvar.

PALAVRAS CHAVE: *Pagrus pagrus*, cultura larvar, mesocosmos, densidade de alimentos vivos.

INTRODUCTION

Most marine hatcheries make use of intensive technologies for mass production of fish larvae, with larval rearing tanks from 0.5 to 10 m^3 in volume and larval densities up to 150 individuals *per* litre.

In recent years mesocosm semi-intensive culture systems have been introduced, relying on the environmental stability of large rearing tanks, the use of "green water" technique and low larval density in order to produce good quality fish larvae, at an affordable cost (DIVANACH, 2002; BEN KHEMIS *et al*., 2006).

In 2002 a mesocosm hatchery was installed at Centro de Maricultura da Calheta (CMC), Madeira Island, following facilities and methods described by DIVANACH & KENTOURI (2000). Fish larvae production was initiated with gilthead sea bream *Sparus aurata* Linnaeus, 1758. Since then the rearing methodology has been adapted for production trials and the study of the biology and the culture requirements of several local species, including red porgy *Pagrus pagrus* Linnaeus, 1758 (ANDRADE *et al.*, 2010).

Red porgy is a common fish from the Atlantic Ocean and Mediterranean Sea, and a promising species for aquaculture (DIVANACH, 2002). An improved knowledge of the larval biology of this species has been crucial for developing adequate rearing methodology.

According to KOLIOS *et al.* (1997) the larvae are sensitive to stress caused by light over 80 lux intensity at about 20 days after hatching (DAH). This is possibly due to the apparition of eye rods improving the visual capacity and the photosensitivity of larvae (ROO, 1999). At this stage the switch from rotifers to *Artemia salina* (Leach, 1819), a larger prey, will result in depressed growth and mortalities (ARISTIZÁBAL & SUARÉZ, 2006). Most red porgy larvae will not be able to digest *Artemia* before 30 DAH due to a late maturation of the digestive system compared to other sparids (DARIAS *et al.*, 2007). Also, unbalances of essential fatty acids of red porgy larval diets may cause severe skeletal anomalies and affect growth performance (ROO *et al.*, 2009). ROO *et al.* (2010) overcame partly these larval culture constraints implementing a shift of light regime from continuous light to 12 hours light and also, with the supply of *Artemia* at early stage but lower densities.

Fish larvae produced using mesocosm semi-intensive methodology generally present higher growth performance and lower deformities incidence, partly due to constant live feed availability (BEN KHEMIS *et al.*, 2006; ROO *et al.*, 2010). However, large scale rearing tank systems, as used in mesocosm semi-intensive culture, have a counter part in the difficulty to control environmental, dietary and health factors (SHIELDS, 2001). Rotifers, the first larval prey, present a particular management challenge as the daily supply will have to take into account the population dynamics in the rearing tank. Due to the higher residual time in large rearing tanks there is an intrinsic rotifer population growth.

This work describes red porgy larvae production in a semi-intensive mesocosm system until 30 days after hatching (DAH). The rearing methodology was adapted concerning the light regime and live food supply to meet the specific red porgy larvae requirements. New approaches to evaluate the eggs hatching rate and rotifers population density in the culture tank were made in order to optimize production management and ultimately, the larvae performance.

MATERIAL AND METHODS

The rearing trial was performed in a fiberglass cylinder culture tank with 40 m^3 water volume. The tank was stocked with 320,000 eggs obtained from spontaneous spawning of wild *P. pagrus* broodstock kept at CMC. Filtered seawater (10 µm) was used and provided with a gentle aeration. Daily water renewal started at 3 DAH increasing from 10 to 150% at the end of trial. An initial photoperiod of 24 hours light, at 1000 lux at the water surface was provided by fluorescent lamps, switched to 12 hours light at 19 DAH.

Regarding the rearing protocol a "green water" technique was used by adding the microalgae *Nannochloropsis oculata* (Hibberd, 1981) daily and the density kept at 250 x 10^3 cell ml^{-1} (Table 1). Larval feeding was initiated 2 DAH with enriched rotifers *Brachionus plicatilis* (Mueller, 1786) (DHA Protein Selco, INVE Aquaculture, Belgium). Rotifers were

supplied twice a day and kept at a density of 2-4 rotifers ml^{-1}. *Artemia* was supplied at low densities from 19 DAH (Table 1). Larvae were fed *Artemia* nauplii and enriched metanauplii (Protein Selco, INVE Aquaculture, Belgium), at 5 to 300 nauplii l^{-1}. Co-feeding with dried diets (Lansy, INVE Aquaculture, Belgium) was initiated simultaneously with enriched *Artemia*. Water temperature and oxygen were measured daily by probe (OxyGuard, Handy Polaris), as well as salinity by refractometer (Atago, S-10E). Standard methods were followed for the determination of total ammonia nitrogen, TAN (KOROLEF, 1983) and nitrite nitrogen, $N\text{-}NO_2$ (APHA, 1998) at 2 to 4 days intervals.

Eggs hatching rate was determined using 3 cylinder mesh (100 µm) baskets of 500 ml placed floating at the surface of the mesocosm tank. Each mesh basket was inoculated with 100 eggs of same batch as the culture tank and the mean hatching rate (± standard deviation) calculated as percentage of larvae hatched at the end of the incubation period.

About twenty larvae were collected every 3 days and photographed from left side with a SoundVision SV Micro camera mounted on a stereoscopic microscope. Larvae total length was measured to the nearest 0.01 mm from the photographs using Zeiss Ks 300 software package. Deformed or curled larvae were excluded from sampling. Larvae mortality was estimated from cumulative number of larvae siphoned daily from bottom of the tank. Differences of size among larvae groups from the centre and from the margin of the tank were tested by ANOVA, and considered statistically significant at $p < 0.05$. Statistical testing was carried out using SPSS for Windows, v12.0.1 (SPSS, Chicago, IL, USA).

Rotifers availability (RA) in the culture tank *per* ml was evaluated as:

$RA = RR + RS + RG - RL$

with RR as number of rotifers remaining in tank, *per* ml; RS as the number of rotifers supplied per day, *per* ml; RG the culture tank intrinsic population growth, *per* ml; RL the rotifer losses by the water outlet (daily number of rotifers trapped by a 55 µm mesh bag). Mean rotifers density was estimated from 3 samples from the entire water column following KENTOURI *et al*. (1994).

RG was estimated as the product of the rotifers density by the specific growth rate. Rotifers growth rate varies with population density. Thus, the specific growth rate was determined for cultures at different starting densities (3, 4 and 5 rotifers *per* ml) and similar culture conditions to the larval trial, including fertility rate (20%), although in the absence of fish larvae. For RG calculation we used the specific growth rate corresponding to the nearest rotifer population density. The specific growth rate (µ) was calculated following SUANTIKA *et al*. (2003):

$\mu = (LnN_t - LnN_0) / t$

with N_t as rotifer density after culture period t (day); N_0 as initial rotifer density.

Rotifers daily consumption by red porgy larvae was calculated as the difference between estimated RA and the rotifers count in tank, *per* ml.

TABLE 1 - Feeding regime used in the culture of red porgy larvae under semi-intensive mesocosm protocol.

RESULTS

Larvae hatching occurred between 60 to 72 hours from spawning, at 18.5º C. Mean hatching rate was 72.00 ± 15.52%.

Larval rearing conditions occurred at temperatures and dissolved oxygen of 18.5-19.7º C and 6.9 ± 0.5 mg l^{-1}, respectively. Salinity was stable at 36 ± 0.5 ‰. Total ammonia nitrogen and nitrites nitrogen concentration in the culture tank were low, presenting a similar increasing pattern with maximum values of 0.2 mg l^{-1} at 16 DAH and approximately 0.02 mg l^{-1} at 12 DAH, respectively (Fig. 1).

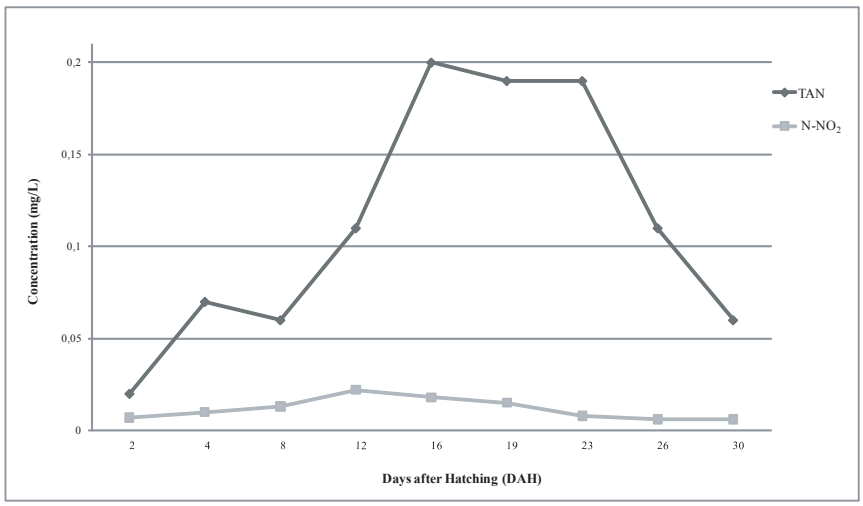

Fig. 1 - Total ammonia nitrogen (TAN) and nitrites nitrogen ($N\text{-}NO_2$) in mg *per* l in the rearing water during the mesocosm larval culture trial.

Rotifer population specific growth in the absence of larvae was 0.27 at 2 rotifer ml^{-1}, 0.31 at 3 rotifers ml^{-1} and 0.32 at 4 rotifers ml^{-1}.

Larvae exogenous feeding started at 3 DAH (Fig. 2). A trend of increasing rotifer consumption to a maximum 3 rotifers ml^{-1} was noticed a 7 and 11 DAH. From then, larvae decreased significantly rotifer consumption to about 0.5 rotifers ml^{-1} at 17 DAH. Naturally occurring copepods (CALANOIDA: ACARTIIDAE) were frequently detected on the tank walls from 9 DAH until 24 DAH. Swimbladder inflation was initiated at 5 DAH and a second period inflation occurred from 12 to 17 DAH. Approximately 90% of larvae had functional swimbladder by the end of trial.

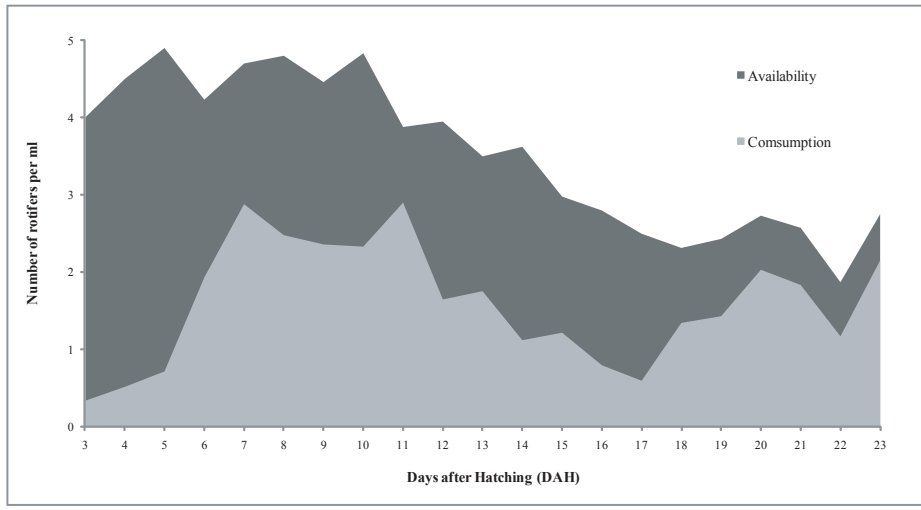

Fig. 2 - Estimated rotifers availability and red porgy larvae consumption *per* day in mesocosm tank until 23 days after hatching.

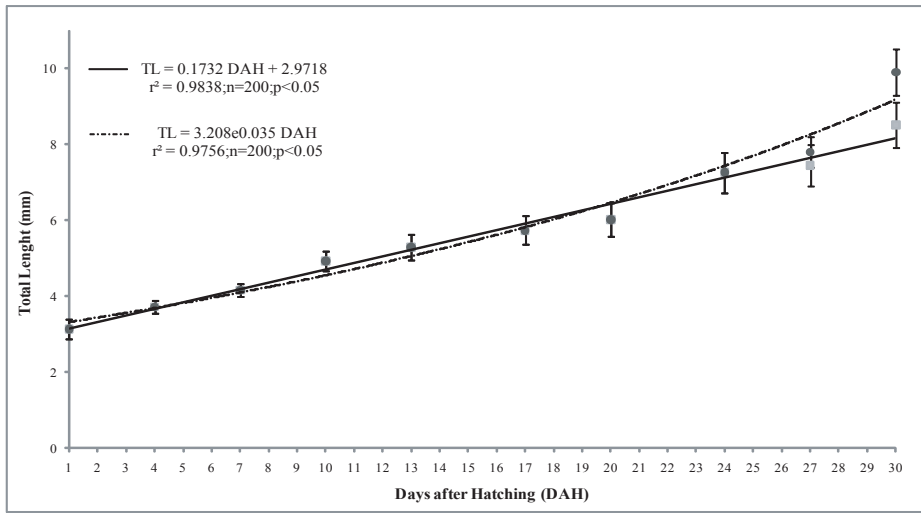

Fig. 3 - Total length (TL) of red porgy larvae during mesocosm rearing trial. Growth curves for populations in the tank margin ($TL = 0.1732\ DAH + 2.9718$; $n = 200$; $r^2 = 0.983$; $p < 0.05$) and centre of the tank ($TL = 3.208e^{0.035\ DAH}$; $n = 200$; $r^2 = 0.9756$; $p < 0.05$).

Larvae presented initially a linear growth (Fig. 3). A period of virtually no growth was detected at 27 DAH, concurrent with cannibalistic behaviour by larger larvae situated at the centre of tank. At this stage significant differences on larvae growth were found between larvae concentrated in the centre of the culture tank and from the tank margin ($p < 0.05$). Mortality was noticeable between 20 and 22 DAH and a second period of mortality was registered from 28 DAH to the end of trial. Total larvae survival at the end of the trial period was 16%.

DISCUSSION

In this study the mesocosm methodology was adapted for the rearing of red porgy, according to the environmental and feeding requirements of the species.

The hatching rate of eggs determined for the mesh bags was lower than 90-95% obtained by Büke *et al*. (2005), although these authors used incubators at 13-80 times lower egg density than the present study. The high hatching rate is indicative of good egg quality and incubation conditions, potential sources of constraints for larval studies.

Timing of larvae developmental events such as opening of mouth and swimbladder inflation coincided with earlier description for intensive culture (Kolios *et al*., 1997; Büke *et al.*, 2005) and semi-intensive culture (Roo *et al.*, 2010).

Red porgy larval growth performance was similar to reported from intensive culture (Kolios *et al.*, 1997; Mihelakakis *et al.*, 2001; Büke *et al.*, 2005) and semi-intensive culture (Roo *et al.*, 2010). Red porgy larvae tend to present a high size variation from 20 DAH onwards as previously mentioned by Mihelakakis *et al.* (2001). Differences in size are the main cause of cannibalism among larvae, which on the other hand have an effect on the size variation (Kestemont *et al.*, 2003). Actually, by 27 DAH two distinct populations of larvae occurred in the tank. Smaller larvae were by the tank margin, whereas the larger larvae (TL approximately 9% higher) occupied the central part of the tank. This pattern of larvae distribution seemed to have resulted from the cannibalistic pressure by larger larvae.

It is unlikely that feeding unbalances have originated population size variation at early development stages. In fact, rotifers availability in the culture tank was always higher (over 0.5 rotifers ml^{-1}) than required for the larvae consumption. Peaks of consumption were associated with higher growth periods. However, nutritional deficiencies rather than food availability has already been suggested as the cause for *P. pagrus* larvae high incidence of column deformities (Roo *et al.*, 2009) and consequent detrimental impact on growth.

Decreasing consumption of rotifers was observed from 11 DAH in opposition to previously reported by Papandroulakis *et al.* (2004) for intensive culture conditions. This may be explained by the competition from other live preys in tank, such as the accidental presence copepods and the *Artemia* supply from 19 DAH. Fortuitous occurrence of copepods in mesocosm semi-intensive culture has been reported and is considered an advantage of this

rearing technology (PAPANDROULAKIS *et al.*, 2005). At 20 DAH the larvae lethargic behaviour and mortality might have also contributed to reduce rotifer consumption.

Larval survival of 16% at 30 DAH was higher than 5-12% reported by ROO *et al.* (2010) for intensive culture, but within the range of 15-25% survival obtained by BÜKE *et al.* (2005).

TAN and nitrite nitrogen levels followed the increasing food consumption and metabolism of larvae when switching to exogenous feeding. From 23 DAH both nitrogen compounds lowered drastically by water dilution as a result of increasing water renewal rate and bottom of tank siphoning. Since ammonium nitrogen is 2-4% of TAN (PERSON-LE RUYET, 1997) the maximum TAN value registered corresponded to 0.004-0.008 mg l^{-1} of NH_3-N. Therefore, both amonium nitrogen and nitrite nitrogen were bellow critical levels of 0.024 mg l^{-1} and 200 mg l^{-1} respectively for seabream larval culture (PARRA & YÚFERA, 1999).

Concerning the adaptation of mesocosm rearing methodologies for red porgy, the shift to 12 hours light photoperiod at 19 DAH avoided the formation of larvae swirls at the tank surface, a sign of larval stress. This has been associated to long light photoperiods in previous trials. The abundant microalgae in mesocosm culture and its shade effect seemed to have prevented high mortalities caused by high light intensity, as previously reported by KOLIOS *et al.* (1997). No evidence of indigested food was detected in the sampled larvae confirming low densities of *Artemia* may be supplied at early developmental stages.

CONCLUSIONS

Based on the present results it is concluded that the adaptation of mesocosm semi-intensive methodology has ensured high growth and survival rates of *P. pagrus* larvae. Larval size heterogeneity and occurrence of cannibalism by the end of trial emphasized the need for the early size sorting of this species. This may be facilitated by the different spatial distribution of two population size classes within the rearing tank. Advances in the evaluation of egg hatching rate and live food (rotifers) density improved control over the larvae population in the large rearing tanks. Future research work and *P. pagrus* larvae production trials may benefit from methods that prevent risks of food limitations in culture conditions.

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