Aquaculture Research
August 2015, Volume 46, Issue 8, Pages 1858-1865
http://dx.doi.org/10.1111/are.12341

Effects of stocking density on intermediate culture of the razor clam *Ensis arcuatus* (Pharidae: Bivalvia)

Fiz da Costa[1,a,*], Berta Barreiro[2], Justa Ojea[1], Susana Nóvoa[1], Dorotea Martínez-Patiño[1]

[1] Centro de Investigacións Mariñas, Consellería do Medio Rural e do Mar, Xunta de Galicia, Ribadeo (Lugo), Spain
[2] Cofradía de Pescadores de Cangas, Cangas (Pontevedra), Spain

[a] Present adress : Ifremer Laboratoire des sciences de l'Environnement Marin (UMR 6539 LEMAR), Plouzané, France

*: Corresponding author : Fiz da Costa, email address : fiz.da.costa.gonzalez@ifremer.fr

Abstract:

Commercial production of most bivalve species involves a phase of intermediate cultivation during which juveniles are grown under protected conditions until they can be transferred to the final grow-out location. Consequently, the aim of this study was investigating the effect of density on growth and survival in the intermediate culture of the razor clam *Ensis arcuatus* in raft. Two series of experiments were performed, using in the first experiment two holding systems: 5-L plastic bottles and PVC cylinders, both perforated, covered with a 1 mm mesh net to prevent razor clam escape and a 10-cm layer of coarse grained sand (300–1200 µm grain diameter). In this trial, two stocking densities were tested: 0.15 and 0.30 kg m^{-2}. In the second one-two densities were essayed (0.62 and 1.24 kg m^{-2}) in the PCV cylinders. In the first experiment after 27 of trial higher growth in cylinders at low density was observed. At the end of this trial all juveniles died in the 5-L plastic bottles and higher growth and survival were recorded in the cylinders at low density. In the second experiment, significant differences in growth and survival were found on day 70, with the best results at low density. Five-L plastic bottles must be rejected as holding system for the intermediate culture of *E. arcuatus* and the PVC cylinders at low densities could be a good alternative. At the end of the experiment razor clams reached the adequate size for their transfer into the grow-out system.

Keywords: *Ensis arcuatus* ; intermediate culture ; density ; raft

Introduction

The razor clam *Ensis arcuatus* (Jefreys, 1865) is the most abundant Pharidae species in Spain. *E. arcuatus* lives buried in sand in low intertidal and subtidal zones from Norway to Spain and it is found along the British coast (Hayward, Wigham & Yonow 1998). Razor clams have a slender and thin-bladed shell shape. They are extremely fast burrowers and they require a substrate to allow burrowing, which in£uences culture conditions needed in hatchery, nursery and on-growing culture (da Costa, Darriba, Martínez-Patiño & Guerra 2011b).

Commercial production of most bivalve species involves a phase of nursery cultivation during which juveniles are grown under protected conditions, using natural phytoplankton as food, until they are large enough to be less prone to predation and can be transferred to the final grow-out location. Bivalve juveniles produced within a hatchery have to be transferred to natural beds when they reach a certain length, because the cost of maintaining them in a hatchery is too high. Microalgal culture represents an essential and demanding step for spat production in bivalve hatcheries mainly because of its high cost, which may represent 30% or more of the entire hatchery management costs (Coutteau & Sorgeloos 1992). Phytoplankton demand increases as juvenile biomass does due to the exponential rise in growth rates and filtration after settlement. If juveniles are introduced into natural beds when they are too small they are subject to very high levels of mortality, mainly as a result of predation.

Clam seed can be grow out using different methods, such as net-covered bottom culture (Spencer, Edwards & Millican 1992), oyster bags (Cigarría & Fernández 1998) suspended lantern-nets (Boscolo, Cornello & Giovanardi 2003) and up-flow nursery systems in ponds (Manzi, Hadley & Maddox 1986).

A major barrier in razor clam juvenile production is the need for a substrate to allow burrowing (da Costa et al. 2011b). Literature on intermediate culture in razor clams are very scarce, as the study in *Ensis macha* (Lépez, Véjar & Arriagada 2011). In this study author tested three different culture systems: cylinders held in long lines, nursery pond culture and on-bottom culturing methods. Regarding to European razor clam species, one study tested intermediate culture in 5-L bottles in *E. arcuatus* (da Costa et al. 2011b) and in cylinders in *Ensis siliqua* and *Solen marginatus* (da Costa, Cerviño-Otero, Aranda-Burgos, Louzán & Martínez-Patiño 2011a; da Costa, Fernández-Pardo, Louzán, Nóvoa, Ojea & Martínez-

Patiño 2011c). Therefore, the objective of the present study is investigating the effect of density on growth and survival using two holding systems for intermediate culture of *E. arcuatus* juveniles in raft.

Material and methods

Intermediate culture experiments

E. arcuatus juveniles were produced at the Centro de Cultivos Marinos de Ribadeo hatchery facilities from wild broodstocks as described in da Costa et al. (2011b). Two sets of experiments were carried out during spring-summer 2011 in a raft belonging to Cangas fishermen assotiation located in Moaña (42º 17' N, 8º 13' O) in Ría de Vigo (NW, Spain). In the first experiment two different containers for intermediate culture were tested: 1: 5-L plastic bottles as the structure used in da Costa et al. (2011b) and 2: PVC cylinders with a diameter of 30 cm, consisting on 2 pieces of 50 cm high each one, that were perforated with 6 cm holes in the sides (Fig. 1). Each structure had a 150 μm mesh net in the bottom to retain the sand, yet allowing its aeration. Both intermediate culture systems (1 and 2) were perforated and they had an 1 mm mesh net to cover the structure to prevent razor clam escape. The structures contained a 10 cm layer of coarse grained sand (300-1200 μm grain diameter) for razor clam burrowing. All containers were held at 4-5 m depth. The experiment started 26th May and it lasted for 85 days. Initial length the juveniles was 8.04 ± 1.95 mm (14.65 ± 3.78 mg). Razor clams were samplet at day 27 and at the end of the experiment. Every sampling, containers were emptied and individuals were sieved. A sub-sample of 30 individuals was measured. Three sub-samples of approximately 30 individuals were weighted and counted to calculate survival using total weight of the sample. Two holding desities were tested: 0.15 and 0.30 kg m^{-2}. In the second experiment two holding densities were investigated (0.62 and 1.24 kg m^{-2}) in only one holding system, the PVC cylinders. The lowest density used in the present study was chosen as a standard density that previously exhibited good growth and survival in *E. arcuatus* intermediate culture (da Costa et al. 2011b). Densities in the second experiment were doubled to determine maximum density that the system can afford. The experiment started in 28th July 2011 and it lasted for 70 days, with an intermediate sampling at day 49. Initial length of the juveniles was 12.65 ± 2.38 mm (58.49 ± 3.48 mg). All treatments in both experiments were performed in duplicate.

Environmental conditions

Temperature, salinity and chlorophyll *a* concentration in the water column was recorded weekly by INTECMAR (Instituto Tecnolóxico para o Control do Medio Mariño-Xunta de Galicia) at a location close to the raft in Moaña (Ría de Vigo) (our experimental raft and environmental sampling station were separated less than 500 m). Chlorophyll *a* concentration was determined by spectrofluorometric methods.

Data analysis

Length and weight data of the juveniles were used to calculate instantaneous growth rates in length and weight (Winberg 1971), using the following equations (ln: Napierian logarithm):

G_{L30}= (ln final length - ln initial length) x 30 x 100 / number of days

G_{W30}= (ln final weight - ln initial weight) x 30 x 100 / number of days

Group normality was initially evaluated using Shapiro-Wilk test. All results were tested by a Levene's test for homogeneity of variances. Length, survival and instantaneous growth rates were compared using a two-way analysis of variance (ANOVA) in the first experiment and with an one-way ANOVA in the second experiment using SPSS software (version 15.0). Percentage data were arcsine-transformed to normalize variance (Sokal & Rohlf 1995).

Results

Experiment 1

A significant effect on growth of system and density ($p<0.0001$) was observed after 27 days of intermediate culture, without any significant interaction between factors (Table 1). Juveniles reared in cylinders at low density achieved a greater length (16.13 mm) than those held at high density (13.95 mm) ($p<0.0001$) (Fig. 2A). No significant effect of holding system on survival on day 27 was found ($p>0.05$),

whereas the increase in stocking density brought about a decrease in survival ($p<0.05$) (Table 1). At the end of the trial (day 85) total mortality of the juveniles held in plastic bottles irrespective of stocking density was recorded (Fig. 2B). Moreover, a combined effect of holding system and stocking density was found. Juvenile survival at the end of the experiment in cylinders at low density was 97%, whereas at high density only 30% survival was recorded, with total mortality in one of the replicates (Fig. 2B). At day 85, significantly higher growth was observed in cylinders held at low density ($p<0.0001$) (Fig. 2B).

Instantaneous growth rates in length (G_{L30}) and weight (G_{W30}) were higher in cylinders at low densities than in other condition between start of the experiment and day 27(Fig. 3A). A significant effect of holding system and density was observed for both growth rates ($p<0.05$, table 1). Similar values of G_{W30} were recorded at both densities for cylinder system at the end of the experiment (Fig. 3B).

Experiment 2

Higher growth was recorded at low density on both samplings ($p<0.05$, Fig. 4A). No differences were exhibited in survival on the first sampling (49 days, $p>0.05$), whereas lower survival was found at high density (79%, $p<0.05$) compared to 99% of survival at low density (Fig. 4B).

G_{L30} and G_{W30} were higher at low density (Fig. 5) between the beginning of the trial and the first sampling ($p<0.05$). On the contrary, no differences were observed on both rates (G_{L30} and G_{W30}) between the first and second sampling (49-70 days, $p>0.05$).

Environmental conditions

Mean seawater temperature was 16.6 ºC during the experimental trials (Fig. 6A). Mean recorded salinity was 35.5 ‰. Fig. 6B shows the variation pattern of the concentration of chlorophyll *a* in the water column during the study. The highest values were found in July (2 peaks) and October (1 peak).

Discussion

The development of the intermediate culture technology for the razor clam *E. arcuatus* is of utmost importance since once this razor clam surpass 1 mm in length holding them in nursery facilities is unfeasible due to the low survival that they exhibited (da Costa et al. 2011b). In the first reported trial in the literature dealing with intermediate culture of *E. arcuatus* in 5-L plastic bottles no differences in growth and survival were recorded due to the short period of study (30days) (da Costa et al. 2011b). Nevertheless, in the present study during the first experiment significant differences in growth and survival, related to holding system and stocking density, were observed. In addition to that, instantaneous growth rates in length (G_{L30}) and weight (G_{W30}) were higher in cylinders than in bottles at both densities. Moreover, total mortality of the juveniles held in 5-L plastic bottles was recorded at the end of the first trial. This mortality may be explained by a lower circulation of water currents inside the structure, which may hinders suitable juvenile development. The problem with this type of container is that it is time-consuming to fill it with juveniles and to sample them subsequently; this would preclude their large-scale use in a commercial situation. Contrary to that, the design of the PVC cylinders allowed improving the intermediate cultivation of razor clams using readily-available cheap equipment and protocols that could be rapidly adopted by commercial producers. This system is similar to the one used for intermediate culture of *E. macha* in long-lines which allows reaching commercial size (López et al. 2011). The use of PVC cylinders as culture device allows a better use of the space and food than in plastic bottles because they could be held at different depths. However, this culture system needs large amounts of sand, affecting raft weight and flotation costs. It is easy to manage and maintain, and allows working with high seed densities when the culture is starting.

Similar G_{W30} were recorded at both densities between 0-27 days and 27-85 days in the first experiment, whereas in the second one G_{W30} was lower at the end of the experiment. This fact may be explained by the lower food availability (expressed as chlorophyll *a*) and seawater temperature recorded at the end of the study. We hypothesize that the chlorophyll *a* peak observed in October may not be enough to promote growth in weight of the juveniles. The low values of G_{L30} at the end of the both experiments may be explained by the limited substrate depth inside containers, which may hinders growth in length.

Significant growth was recorded under all stocking densities in the PVC cylinders. In both experiments, final length of *E. arcuatus* juveniles (2.1-2.5 cm) was adequate to be safely transferred to the final grow-out location under protection in burrowing cages. These holding containers were used for on-botton culture of juveniles of the same species with an initial length of 6 cm (da Costa et al. 2011b). However, da Costa & Martinez-Patiño (2009) reported on-growing of juveniles of the razor clam *Solen marginatus* with an initial length of 2 cm, with good survival during a 3-year study. Consequently, this transfer of juveniles to bottom culture may be benefitial for reducing the substrate needs of intermediate culture in rafts.

Despite our data of both trials with the cylinders showed that growth and survival were highly dependent on stocking densities, the higher initial stocking density used in our study (1.24 Kg m^{-2}, which corresponded to 21,220 individuals m^{-2}) exhibited fairly good survival results (79%) and good growth. This density (21,220 individuals m^{-2}) is slighly lower than those used for suspended culture in long-lines of *E. macha* (24,000 individuals m^{-2}) for a similar initial size (1 cm) (López et al. 2011). Therefore, future research must investigate even higher stocking densities and determine which density is the better one in terms of maximising growth and survival. Nonetheless, the great variation in survival observed during the first experiment in the cylinders at high density (with the total mortality of one replicate) may be explained by the deleterious effects of fouling during summer (i.e. barnacles, fixed mussel seed, macroalgae, bryozoans, tunicates, etc.), as cleaning operation could not be performed due to the inability of accessing to the raft. Consequently, protocols for cleaning operations during the seasons when higher growth rates are recorded (spring and summer) due to high temperature and food availability must be developed in order to avoid deleterious effects of fouling in growth and survival. Moreover, as the longer experiment carried out in this study lasted for less than 3 months grading was not considered. However, we can not dismiss the effect of shelf-thinning (natural density reduction). Future research may investigate the effect of grading on growth and survival.

The present study indicates that it is feasible to conduct intermediate cultivation in suspended culture systems using cheap and readily-available materials. However, more research will be needed for the development of suspended intermediate culture of the razor clam *E. arcuatus* to a semi-industrial scale. A cost-benefits analysis of the production , using growth and survival data vailable, may be carried out in order to maximise yields and minimise production costs in terms of man-hours used.

Conclusions

It can be concluded that plastic bottles are not an appropriate culture device for suspended intermediate culture of *E. arcuatus* during long periods of time, likely due to the low water circulation inside the structure. However, PVC cylinders may be a viable culture system for *E. arcuatus* due to their easiness of filling and sampling. Juveniles growing at higher densities were more variable in growth and survival than razor clams growing at lower densities. Our study showed than even the higher initial stocking density used herein (1.24 Kg m^{-2} for juveniles of 13 mm) showed adequate results, and it can be defined as reference density.

Acknowledgements

We are grateful to the staff of the Centro de Cultivos Marinos de Ribadeo-CIMA (Xunta de Galicia) for their assistance. We also like to acknowledge Cofradía de Cangas for broodstock provision and the help of their staff in sampling. F. da Costa was funded by a Fundación Juana de Vega (Spain) postdoctoral fellowship at IFREMER during data analysis and manuscript preparation.

References

Boscolo R., Cornello M. & Giovanardi O. (2003) Condition index and air survival time to compare three kinds of Manila clam *Tapes philippinarum* (Adams & Reeve) farming systems. *Aquaculture International* **11**, 243-254.

Cigarría J. & Fernández J. (1998) Manila Clam (*Ruditapes Philippinarum*) culture in oyster bags: Influence of density on survival, growth and biometric relationships. *Journal of the Marine Biological Association of the United Kingdom* **78**, 551-560.

Coutteau P. & Sorgeloos P. (1992) The use of algal substitutes and the requirement, for live algae in the hatchery and nursery rearing of bivalve molluscs: an international survey. *Journal of Shellfish Research* **11**, 467-476.

da Costa F., Cerviño-Otero A., Aranda-Burgos J.A., Louzán A. & Martínez-Patiño D. (2011a) Effects of substratum and stocking density on the performance of juveniles of the razor clam *Ensis siliqua* on an intermediate cultivation system. In: *Aquaculture Europe 2011*, Rhodes, Greece.

da Costa F., Darriba S., Martínez-Patiño D. & Guerra A. (2011b) Culture possibilities of the razor clam *Ensis arcuatus* (Pharidae: Bivalvia). *Aquaculture Research* **42**, 1549-1557.

da Costa F., Fernández-Pardo A., Louzán A., Nóvoa S., Ojea J. & Martínez-Patiño D. (2011c) Efecto del sustrato y de la densidad en un sistema de preengorde de *Solen marginatus*. In: *XII Congreso Nacional de Acuicultura*, Castelldefels, Spain. (In Spanish).

da Costa F. & Martinez-Patiño D. (2009) Culture potential of the razor clam *Solen marginatus* (Pennant, 1777). *Aquaculture* **288**, 57-64.

Hayward P.J., Wigham G.D. & Yonow N. (1998) Mollusca (Phylum Mollusca). In: *Handbook of the Marine Fauna of North-West Europe* (ed. by Hayward PJ, Ryland JS). Oxford University Press, Oxford, U.K., pp. 484-628.

Lépez I., Véjar F.E. & Arriagada D. (2011) Razor clam (*Ensis macha*) culture in Chile. In: *Razor clams: Biology, aquaculture and fisheries* (ed. by Guerra D, Lodeiros C, Gaspar MB, da Costa F). Consellería do Mar, Xunta de Galicia, Santiago de Compostela, pp. 219-226.

Manzi J.J., Hadley N.H. & Maddox M.B. (1986) Seed clam, *Mercenaria mercenaria*, culture in an experimental-scale upflow nursery system. *Aquaculture* **54**, 301-311.

Spencer B.E., Edwards D.B. & Millican P.F. (1992) Protecting Manila clam (*Tapes philippinarum*) beds with plastic netting. *Aquaculture* **105**, 251-268.

Sokal R.R. & Rohlf F.J. (1995) *Biometry. The principles and practice of statistics in biological research,* Freeman, New York.

Winberg G.G. (1971) *Methods for the estimation of production of aquatic animals,* Academic Press, London.

Tables

Table 1

Two-way ANOVA of growth, survival, instantaneous growth rates in length (G_{L30}) and in weight (G_{W30}) of intermediate *E. arcuatus* culture during the first experiment. * $p<0.05$.

Source of variation	**DF**	**MS**	**F**	**P**
Length (mm) on day 27				
Holding system	1	781.565	5104.606	0.000*
Density	1	207.539	27.777	0.000*
Interaction	1	6.150	0.823	0.365
Residual	236	7.472		
Survival (%) on day 27				
Holding system	1	0.002	0.041	0.850
Density	1	0.322	7.800	0.049*
Interaction	1	0.034	0.830	0.414
Residual	4	0.041		
Survival (%) on day 85				
Holding system	1	1.347	24.462	0.008*
Density	1	0.501	9.090	0.039*
Interaction	1	0.501	9.090	0.039*
Residual	4	0.055		
G_{L30} 0 - 27 days				
Holding system	1	1851.534	91.955	0.001*
Density	1	488.318	24.252	0.008*
Interaction	1	0.656	0.033	0.865
Residual	4	20.135		
G_{W30} 0 - 27 days				
Holding system	1	11024.146	86.960	0.001*
Density	1	2326.792	18.354	0.013*
Interaction	1	11.009	0.087	0.783
Residual	4	126.773		

Fig. 1. A. Raft for suspended culture. B. Plastic bottle for razor clam intermediate culture. C. System of PVC cylinder.

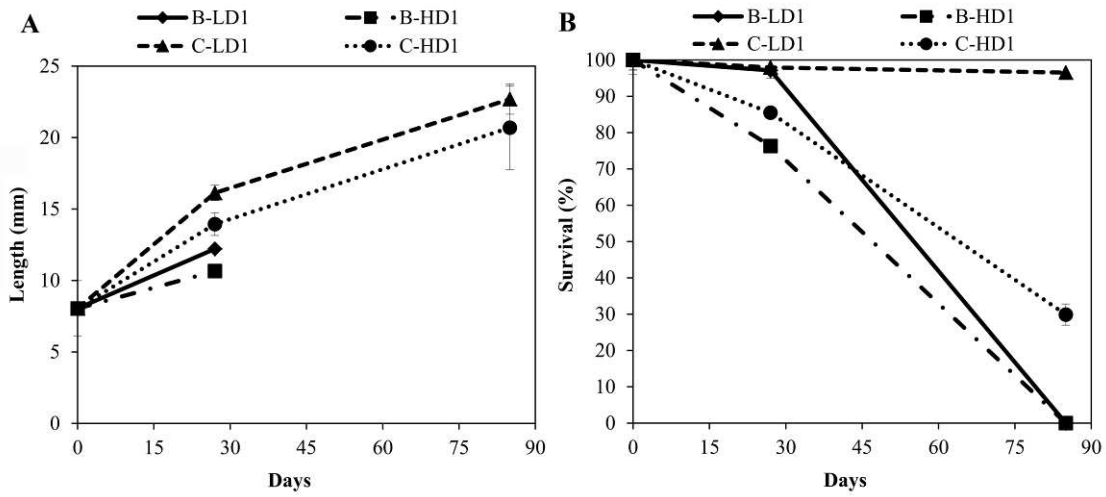

Fig. 2. Results of the first trial. A. Growth in length (mm). B. Survival. B: 5-L plastic bottle; C: cylinder; LD1: low density; HD1: high density.

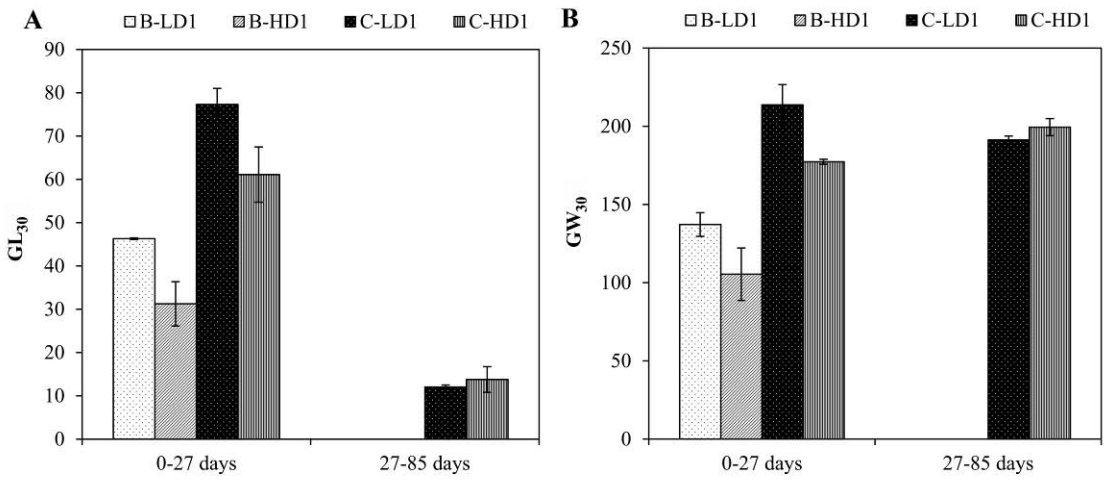

Fig. 3. Instantaneous growth rates in length (A, G_{L30}) and in weight (B, G_{W30}) during the first experiment. B: 5-L plastic bottle; C: cylinder; LD1: low density; HD1: high density.

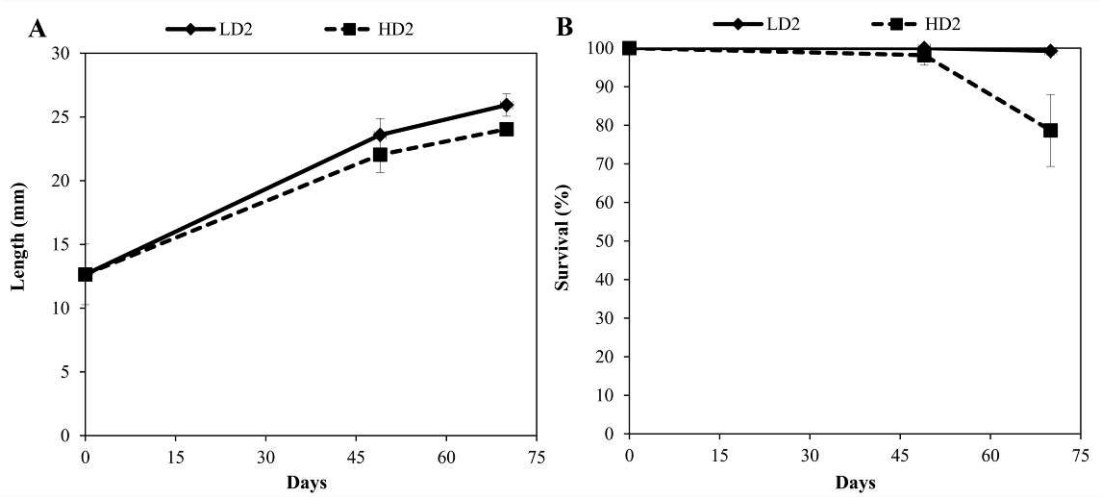

Fig. 4. Results of the second trial. A. Growth in length (mm). B. Survival. LD2: low density; HD2: high density.

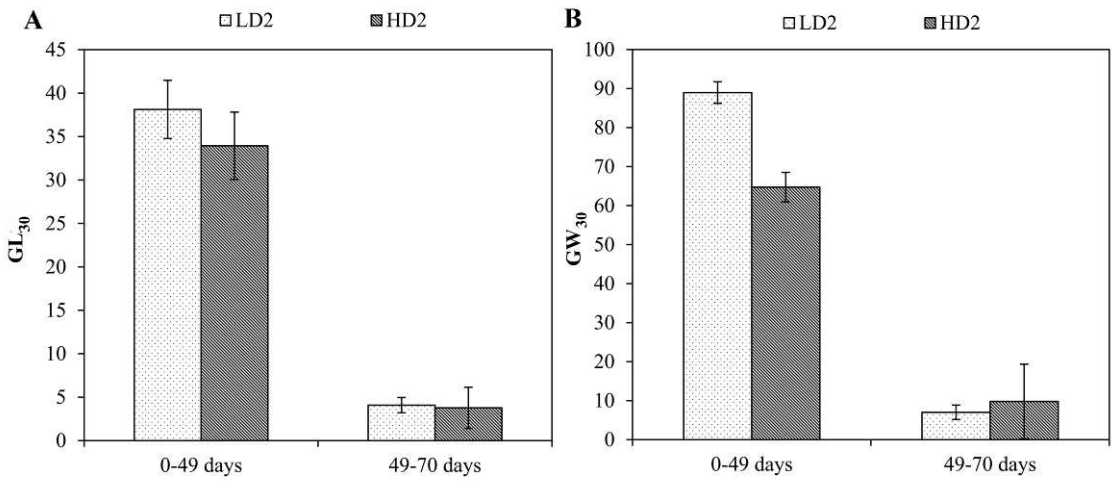

Fig. 5. Instantaneous growth rates in length (A, G_{L30}) and in weight (B, G_{W30}) during the second experiment. LD2: low density; HD2: high density.

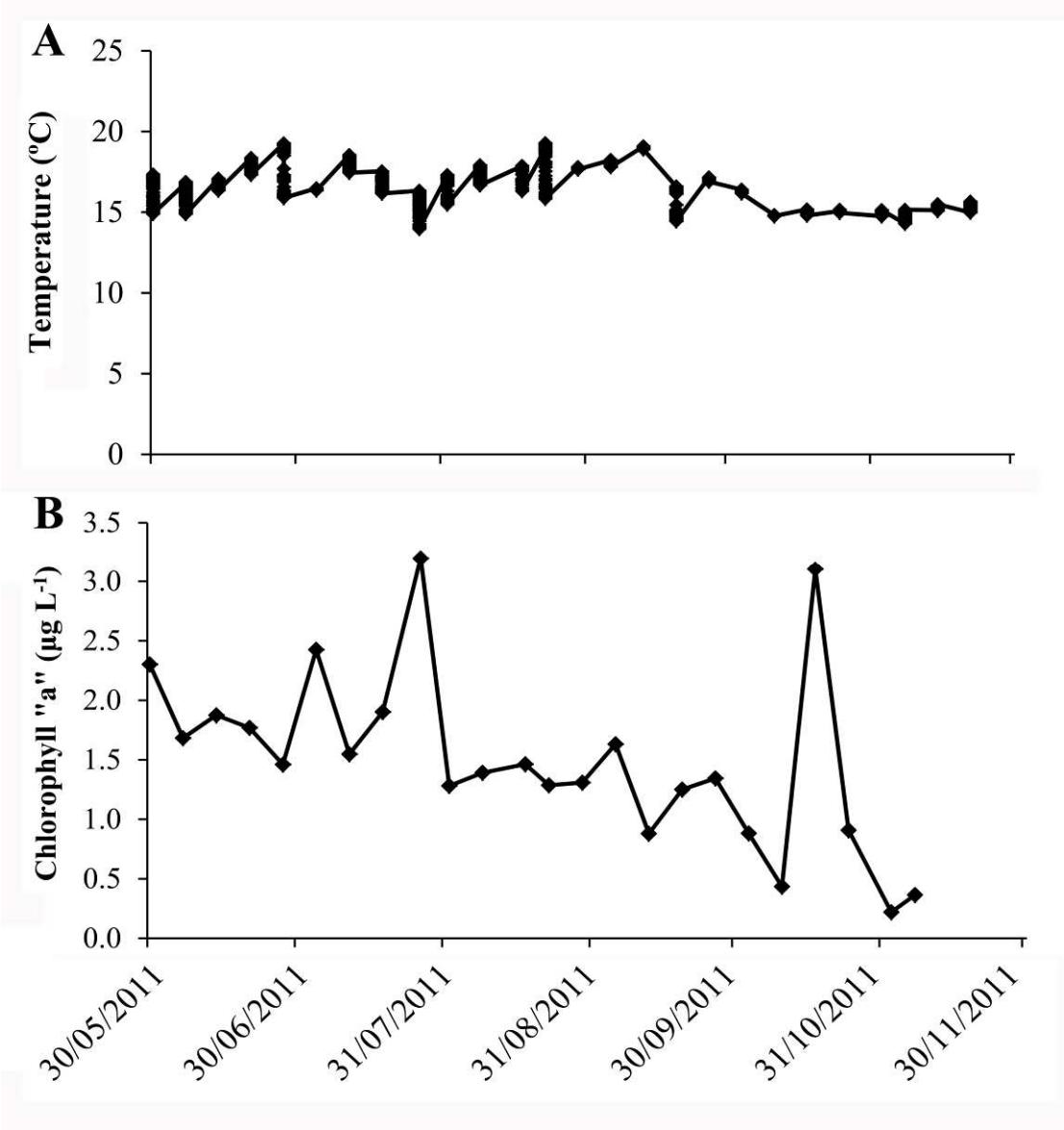

Fig. 6. A. Temperature variation pattern in the water column. B. Chlorophyll *a* concentration in the water column.