The comparative growth and survival of juvnile tropical oyster (*Magallana bilineata,* Roding, 1798) using different intensive nursery systems

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Received: November 2021 Accepted: May 2022

Abstract

The bottleneck of the oyster industry worldwide is imposed by the limitation in oyster seed supply and long culture cycles. In developing technology seed production for hatchery seed production to the commercial plant scale, the main challenge lies not only in getting the D- hinged larvae to the settling stage (eyed larvae) but also in growth and survival rates of the juvenile oysters to economically viable levels. Subsequently, efforts are concentrated on research designed at defining optimal conditions for growth and survival of the juvenile oysters cultured using different intensive rearing systems. The nursery systems used in this project are modified and refined technologies used by most hatcheries in the world. Attention is focused on developing oyster seeds or juvenile oysters with good growth and survival rate. Different systems of intensive nursery systems to obtain juveniles with regular shape and size were used. The systems applied in this project were the down-welling system, up-welling system, the rain-down system and the "coke-bottle" system. All these four systems produced cultchless spat, which grew into single oysters. The results of the survival of juveniles (from 1 week to 4 weeks) using different nursery systems showed that the best survival and highest growth rate was observed in the up-welling system (89.3±7.3%), followed by the rain-down system (76.5±4.1%) and coke-bottle system (72.2±12.0%). The down-welling system showed the lowest survival rate. This may be due to the down-flowing water of the system that made it difficult for the water to flow efficiently in the system, with the juveniles blocking the nitex screen. In general, there are no significant differences in the up-welling system, rain-down system and coke-bottle system for culturing the smaller juveniles. The survival of juveniles (from 4 weeks to 2 months) using different nursery systems showed the best survival, and the highest growth was observed in the Coke-bottle system (93.2 ± 9.1%), followed by the up- welling system (85.6± 7.4%). The down-welling system showed the lowest survival rate. The coke-bottle system showed the highest survival rate because of its flow of water moving upwards in a narrower diameter (compared to the up-welling system), which enables the juveniles to be rotated regularly in the system and the food to be distributed evenly in the system.

Keywords: Tropical oyster, Seed production, Intensive rearing systems, Straits of Malacca

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Introduction

The current oyster trade in Malaysia is at 1,613.78 metric tonnes (Malaysia's Trade Statistics) in 2018. Only 28% of this demand is from local production, and 72% is imported (ASFSR, 2000-2018). This demand is imposed by the limitation in oyster seed supply and long culture cycles. The problem is insufficient good quality oyster seeds to support the industry. Seeds are collected from the wild and dependence on wild collected oyster seed is of course a problem. Supply is inconsistent and often inadequate. Producers are exposed to the vagaries of the seasons, seed quality is poor and it is definitely of variable size and age. The solution to overcome this lack of seed supply is laboratory-produced high quality seed on demand.

The oyster industry in Malaysia has progressed from using mangrove sticks, as substrates for both the collection and growth of oysters, to the use of asbestos collectors (Mohamad Yatim, 1993). The tropical oysters (*Magallana bilineata*) are generally caught and either grown to market size on the stick or knocked off the sticks and grown on plastic trays; however, many oyster farmers now use "single-seed" culture techniques, taking advantage of either natural spat (removed from synthetic collectors) or hatchery-produced spat. A nursery phase follows where the unattached single oysters are grown in sectionalized trays or PVC cylinders. Single-seed culture eliminates laborious culling (separation of oysters), allows machine grading and ensures oysters are of more uniform shape with a larger shell cavity volume than oysters grown on sticks. As single-seed oysters are grown, they are moved into nursery units with larger mesh sizes to allow greater water flow and to reduce the problem of fouling (Helm *et al*., 2004; Tan *et al*., 2014).

The challenges faced by many commercial hatcheries in South East Asia are getting sufficient mature broodstock, obtaining high survival rate from D-hinged larvae to settlement stage, improving nursery and grow out systems which are economically viable (Tan *et al*., 2014). It is of ultimate importance to be able to produce sufficient seeds to sustain the oyster industry in Malaysia. Due to lack of hatchery space and the needs for high quality and quantity oyster seeds (Mohamad Yatim, 1993; Tan *et al*., 2014), research needs to be focus on intensive nursery systems. This project will focus on the viability of different methods of intensive nursery systems with the aim of obtaining juveniles with regular shape and size, besides aiming at good growth and survival rate.

Materials and methods

The methods applied in this project were the down-welling system, up-welling system, the rain-down system and the "coke-bottle" system (Mohamad Yatim, 1993; Roels *et al*., 2009). The experiment was conducted using the facilities of a commercial hatchery, SeaHarvest Aquamarine (M) Sdn Bhd. All the four methods were tested during the nursery stage. All these four methods would produce cultchless spat which will grow into single oysters (Mohamad

Yatim, 1993). The design of these four systems is shown in Table 1.

The down-welling, up-welling and rain-down systems were held in shallow troughs (length 245 cm x width 65 cm x height 35 cm). Each of systems were supplied with seawater from the reservoir tanks (length 160 cm x width 95 cm x height 95 cm) , where water was returned to the reservoir tanks via a stand-pipe fitted in each trough which maintain the water depth in the troughs. Water is pumped from the reservoir back to the troughs. The water for the "coke bottle" system went directly to the reservoir and pumped back to the "coke bottles" directly. The seawater flow rate of all the four intensive nursery systems were kept constant at 1 L per minute. Each nursery systems were conducted in five (5) replicates.

The initial density of juveniles at settlement stage with size around 0.3 mm shell length placed at each container in every nursery systems were 500 individuals for the early stage experiment from 1 week to 4 weeks, while 300 individuals of 10 mm shell length juveniles were used for the 4 weeks to 2 months experiment. The temperature monitored at each system were 28±2°C throughout the whole experiment. A total of 3 L of phytoplankton (1×10^6 cells/mL) was added into each reservoir every day. Phytoplankton fed to all the juveniles comprised of *Chaetoceros calcitrans* and *Tetraselmis* spp. .

As in closed intensive nursery systems, water quality was maintained by changing the water in the reservoir tanks completely on alternate days. The cylinders or "coke bottles" containing the juveniles were removed and each was sprayed gently with a seawater jet to dislodge and remove detritus adhering to the juveniles and to the mesh of the containers. The reservoir and holding troughs were cleaned and refilled before returning the juveniles to the cylinders or containers.

The growth and survival of the juveniles were measured on week 4 for short term culture and also measured after 2 months in the nursery systems for intermediate term culture. The growth of the juveniles from each intensive nursery system were measured based on the shell length (from the hinge till the farthest end of the shell), to the nearest 0.1 mm using vernier caliper. One-way ANOVA was conducted to compare the growth and survival differences of the intensive nursery systems for the experiment duration of 4 weeks and 2 months The end results are displayed as average percentage for survival and growth of juveniles cultured using different intensive nursery systems.

Results

Experiments on culturing juveniles using different intensive nursery systems from 1 week to 4 weeks and 4 weeks to 2 months have been conducted. The survival and growth of the juveniles were recorded. Figure 1 shows the survival of juveniles (from 1 weeks to 4 weeks) using different intensive nursery systems.

Table 1: The description of the nursery systems used in this project.

Settlement system	Set-up	Description
Down-welling		The flow of the water in this system is from the top of the inlet pipe onto the settling ring. The water is pushed out of the ring downwards through the nitex screen and into the holding tank. Diameter of the ring: 40 cm Height: 25 cm
Up-welling		The flow of the water in this system is from the bottom of the nitex screen and pushed upwards into the settling ring. The water from the settling ring is pushed out through the outlet screen into the holding tank. Diameter of the ring: 40 cm Height: 25 cm
Rain-down		The flow of the water in this system is sprinkled like raindrops from a tube above the settling ring. The water is then pushed out from the settling ring through the nitex screen into the holding tank. Diameter of the ring: 40 cm Height: 25 cm
"Coke Bottle"		The flow of the water in this system is similar to the up-welling system, where the water will be pushed upwards. No nitex screen is used in this system. Diameter: 8 cm Height: 40 cm

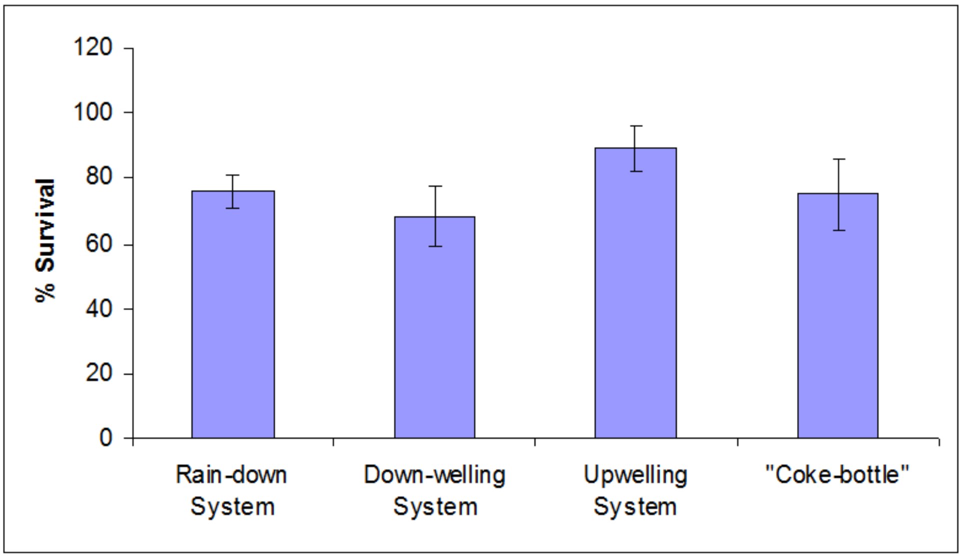

Figure 1: Percentage survival of 4 weeks old juveniles using different nursery systems (monitoring from 1 weeks to 4 weeks).

The results showed that the best survival and highest growth rate was observed in the up-welling system (89.3±7.3%), followed by the rain-down system (76.5±4.1%) and “coke-bottle” system (72.2±12.0%). The down-welling system showed the lowest survival rate. The One-Way ANOVA analysis showed no significant differences between the up-welling system, rain-down system and “coke-bottle” system for culturing the smaller juveniles from 1 week to 4 weeks. However, the survival recorded in the up-welling system was significantly higher than the percentage survival of juveniles cultured in the down-welling intensive nursery system.

Figure 2 shows the growth of the early settled juveniles (1 week) to 4 weeks. The growth of juveniles cultured in down-welling nursery system showed the smallest growth (6.2±3.1 mm shell length) by week 4 compared to the other nursery systems. The juveniles cultured in the up-welling nursery systems showed the fastest growth with mean shell length of 15.6±0.7 mm at week 4. However, the shell length of the juveniles cultured in up-welling nursery system showed no significant differences with the juveniles cultured in rain-down and “coke bottle” intensive nursery systems.

Figure 3 shows the survival of juveniles (from 4 weeks to 2 months) using different intensive nursery systems. The results showed that the best survival and highest growth was observed in the “Coke-bottle” system (93.2±9.1%), followed by the up-welling system (85.6±7.4%). The lowest survival rate was recorded from the down-welling nursery system. The One-Way ANOVA analysis showed no significant differences between the up-welling and “coke-bottle” systems for culturing juveniles from 4 weeks to 2 months. However, both u-welling and “coke-bottle” nursery systems are significantly different in percentage survival compared to rain-down and down-welling nursery systems.

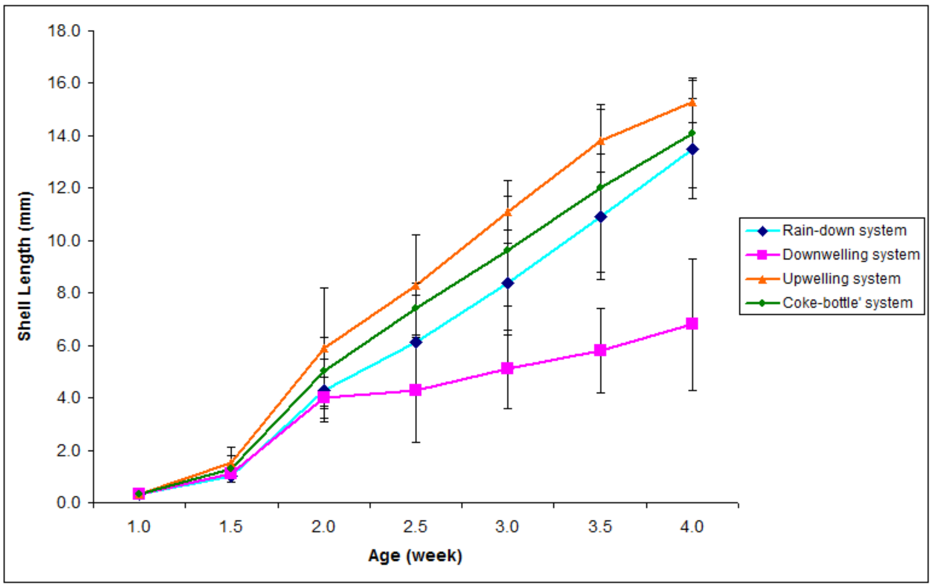

Figure 2: The growth of juveniles using different intensive nursery systems (monitoring from 1 week to 4 weeks).

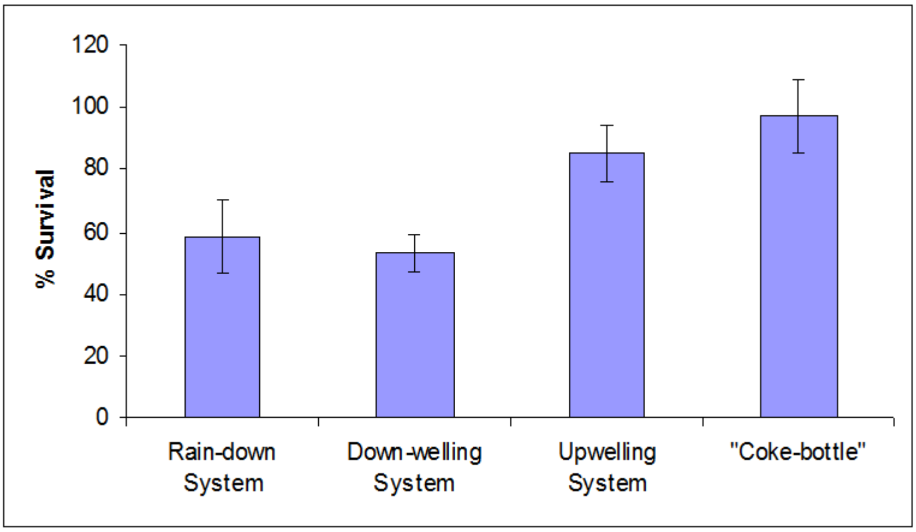

Figure 3: Percentage survival of 2 months old juveniles using different nursery systems (monitoring from 4 weeks to 2 months).

Figure 4 shows the growth of juveniles of 10 mm shell length (4 weeks old) to 2 months. The growth of juveniles cultured in "coke-bottle" nursery system showed the fastest growth (26.4±0.6 mm shell length) by month 2 compared to the other nursery systems. The growth of juveniles cultured in "coke-bottle" system was significantly different compared to the other intensive nursery systems. The juveniles cultured in the up-welling nursery system showed the second fastest growth with mean shell length of 20.8±4.7 mm at month 2. However, the shell length of the juveniles cultured in up-welling nursery system showed no significant differences with the juveniles cultured in rain-down and down-welling intensive nursery systems.

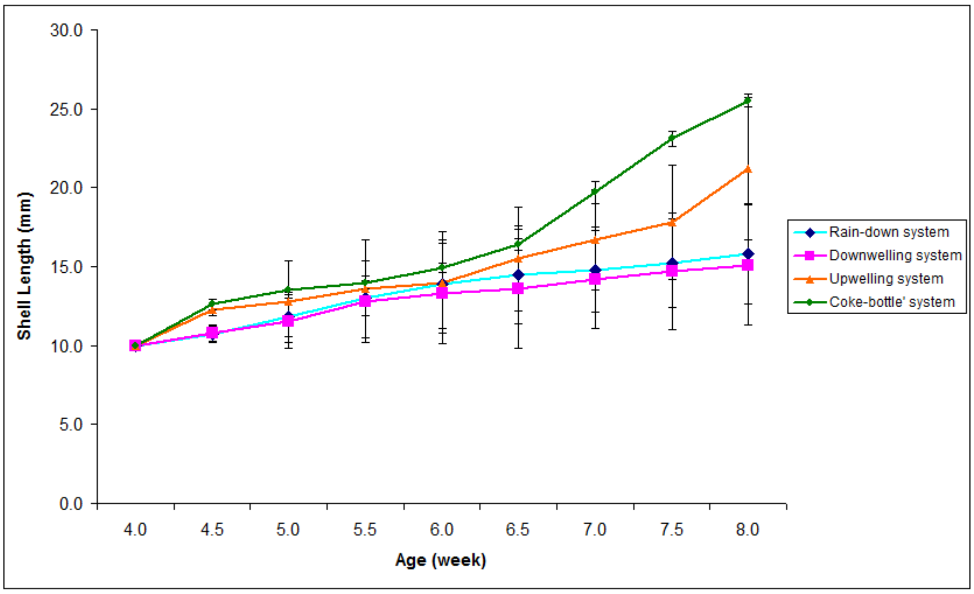

Figure 4: The growth of juveniles using different intensive nursery systems (monitoring from 4 weeks to 2 months).

The juveniles cultured using the "coke-bottle" intensive nursery systems displayed very consistent shape as single seed, from 1 week until 2 months, while the juveniles cultured using the up-welling nursery system showed fairly consistent shape until 2 months. Juveniles cultured using the down-welling and rain-down intensive nursery systems showed fairly consistent shape from 1 week to 4 weeks but the shape slowly became inconsistent from 4 weeks to 2 months.

Discussion

There is a high demand for oyster seeds in Malaysia especially when the quantity of seeds from the wild is limited and inconsistent to support the oyster industry (Tan *et al.*, 2014). Besides its inconsistency in supply, the seeds from the wild are also of irregular shapes and poor quality. Oyster seeds of required quality and quantity can be made available using the present technology applied during the nursery stage (Helm, 1994).

Intensive nursery systems for oyster culture using upwelling system are relatively common and had been applied to many commercial hatcheries in USA and Canada. However, the comparison of growth and survival of juveniles cultured using different intensive nursery systems such as down-welling, rain-down and "coke-bottle" systems have not been conducted. This project has looked into the feasibility as well as the performance of seeds been grown using different intensive nursery systems. Survival and growth of the oyster juveniles of *Magallana bilineata* were measured from 1 week to 4 weeks and from 4 weeks to 2 months.

The results of juveniles *M. bilineata* cultured from 1 week to 4 weeks showed that the best survival and highest growth rate were observed in the up-welling system, followed by the rain-down system and "coke-bottle" system. The down-welling system showed the lowest survival rate and significantly lower than the up-welling intensive nursery system. This may be due to the down-flowing water of the system that made it difficult for the water to flow efficiently in the system with the juveniles blocking the nitex screen (Roels *et al*., 2009; Baldwin *et al.*, 1995; Warfel, 2002; Williams, 1981).

The survival of *M. bilineata* juveniles (from 4 weeks to 2 months) using different intensive nursery systems showed that the best survival and highest growth was observed in the "Coke-bottle" system, followed by the up-welling system. The lowest survival rate was recorded from the down-welling nursery system. This may be due to the down-flowing water of the system that made it difficult for the water to flow efficiently in the system with the juveniles blocking the nitex screen (Roels *et al*., 2009; Warfel, 2002). The "coke-bottle" system showed the highest survival rate because of its flow of water moving upwards in a narrower diameter (compared to up-welling system), which enable the juveniles to be rotated regularly in the system and the food to be distributed evenly in the system.

Table 2 below summarises the overall results of survival, growth and consistency in shapes and sizes of *M. bilineata* juveniles cultured using different intensive nursery systems. From the experiments above, we have found that it is best to use either the upwelling system or rain-down system for the nursery stage when the *M. bilineata* juveniles are 1 week to 4 weeks old but the nursery system has to be changed to "coke-bottle" system when the juveniles grow bigger (4 weeks to 2 months).

Table 2: Overall results of survival, growth and consistency in shapes and sizes of *Magallana bilineata* juveniles cultured using different intensive nursery systems.

	Rain-down system	**Down-welling system**	**Upwelling system**	**"Coke bottle" system**
Survival				
1-4 weeks	High	Lowest	Highest	High
1-2 months	Average	Lowest	High	Highest
Growth				
1-4 weeks	High	Lowest	Highest	High
1-2 months	Average	Lowest	High	Highest
Shape and Size				
1-4 weeks	Fairly consistent	Fairly consistent	Fairly consistent	Very consistent
1-2 months	Not consistent	Not consistent	Fairly consistent	Very consistent

Upwelling is particularly useful in the culture of post-settlement oysters (Helm *et al*., 2004; Warfel, 2002; Williams, 1981). Small juveniles around the size of 0.3 mm to 1.0 mm are amenable to stocking in depth at high density, i.e. layered one above the other. Holding the juvenile oysters in this way with a sufficient flow of water will prevent the juveniles from clustering and cement to each other, leading to the production of consistent shape single oysters (Roels *et al*., 2009). Cluster formation can be a problem in oyster species if the spat are not kept moving - for example, if they are grown in the downwelling and rain-down intensive nursery systems. Up-welling and "coke-bottle" intensive nursery systems are also more efficient in keeping the juveniles free of faecal deposits compared to down-welling system, where faeces tends to accumulate on and around the juveniles. The mesh in the container can be clogged by the faeces and hindered the water flow. This clogging incident is less prominent in the up-welling containers or the "coke-bottle" nursery systems.

Down-welling and rain-down intensive nursery systems are at their optimal production if the juveniles are placed in a single layer and spread over a much greater surface area to avoid overcrowding and to allow continuous water exchange. This will also ensure that the juveniles do not cluttered together (Helm *et al*., 2004; Roels *et al*., 2009; Helm, 1994).

The survival and growth are best in "coke-bottle" intensive nursery systems due to the narrower diameter of the cylinder which allowed faster flow of water and the aeration and flow of seawater in the system are strong enough to uplift the juveniles continuous, creating juveniles with very consistent shape and sizes in the system.

In order to increase production of oysters in hatcheries and nurseries, the development of an intensive nursery system is important (Helm *et al*., 2004; Roels *et al*., 2009; Helm, 1991). Up-welling intensive nursery and "coke-bottle" systems have several advantages: high densities of the species can be cultured resulting in a cost-effective production system; optimal flow rate, stable water quality and sufficient good quality phytoplankton maximize production. Similar practices have been implemented with other oyster species such as *Crassostrea rhizophorae* and European oysters (Helm, 1991; Helm, 1992), and also other bivalve species (Sami *et al*., 2019; Gribben *et al.*, 2002; Chessa *et al.*, 2013; Serdar *et al.*, 2007). However, the optimum production is also influence by the density of juveniles been placed in the intensive nursery systems, flow rate, diameter of the containers, quality and quantity of the feed provided, water quality, temperature and frequency of water change (Liu *et al.*, 2011; Kamermans *et al.*, 2016).

Acknowledgements

We thank members of Marine Science Laboratory and Centre For Marine & Coastal Studies, Universiti Sains Malaysia for assistance and SeaHarvest Aquamarine (M) Sdn Bhd. for providing the facilities for this project. The work was aimed to ensure a sustainable food production system, which is in line with the United Nation's Sustainable Development Goal (SDG), Goal 14: Life below water and Goal 2: Zero hunger. This project was financially supported by Ministry of Higher Education's Translational Research Malaysia Grant (TR@M) 203/PPANTAI/6790002.

References

ASFSR (Annual States Fisheries Statistics Report), 2000-2018. Vol. 2 Department of Fisheries, Ministry of Agriculture (Kuala Lumpur: Malaysia) Retrieved from https://www.dof.gov.my/en/resources/fisheries-statistics-i/

Baldwin, R.B., Mook, W., Hadley, N.H., Rhoses, F.J. and DeVoe, M.R., 1995. Construction and operations manual for a tidal-powered upwelling nursery system. National Coastal Research Development Institute, NOAA contract AQ66.90-5228-03.

Chessa, G., Serra, S., Saba, S., Manca, S., Chessa, F., Trentadue, M. and Fois, N., 2013. The floating upwelling system (FLUPSY) for breeding of *Venerupis decussata* (Linnaeus, 1758) juveniles in a coastal lagoon in Sardinia (Italy). *Transitional Waters Bulletin*, 7(**2**), 53-61.

Gribben, P.E., Creese, R.G. and Hooker, S.H., 2002. Growth rates of the venus clam *Ruditapes largillierti* grown under experimental culture conditions in New Zealand. *Aquaculture*, 213, 187-197.

Helm, M.M., Bourne, N. and Lovatelli, A. (comp./ed.), 2004. Hatchery culture of bivalves. A practical manual. FAO Fisheries Technical Paper. No. 471. Rome, FAO, 177 P.

Helm, M.M., 1991. Development of industrial scale hatchery production of seed of the mangrove oyster, *Crassostrea rhizophorae*, in Cuba. Food and Agriculture Organization of the United Nations. FAO: TCP/CUB/8958: 46 P.

Helm, M.M., 1992. Operation of a managed, shore-based nursery for the early growth of hatchery produced European oyster seed. Department of Aquaculture and Fisheries, Halifax, Nova Scotia, March 1992, 35 P.

Helm, M.M., 1994. Towards reliable bivalve seed supply in Nova Scotia. *Bulletin Aquaculture Association of Canada*, 94(**4**), 9–14.

Kamermans, P., Garcia, A, Joaquim, S., Matias, D., Magnesen, T., Nicolas, J., Petton, B. and Robert, R., 2016. Recirculation nursery systems for bivalves. *Aquaculture International*, 24(**3**), 827-842.

Liu, W., Pearce, C.M., Alabi, A.O., Beerens, A. and Gurney-Smith, H., 2011. Effects of stocking density, ration, and temperature on growth of early post-settled juveniles of the basket cockle, *Clinocardium nuttallii*. *Aquaculture*, 320, 129-136

Mohamad Yatim, N., 1993. A guide to oyster culture in Malaysia. Manuals and Guides _ BOBP/MAG/18.

Roels, O., Sunderlin, J. and Laurence, S., 2009. Bivalve molluscan culture in an artificial upwelling system. *Proceedings of the World Mariculture Society*, 10, 122-138.

Sami, M., Mohammad, D.A., Sallam, A.E. and Mohammed, S.Z., 2019. Seed rearing of *Ruditapes decussatus* (Bivalvia: Molluscs) using two culture techniques. *Al Azhar Bulletin of Science*, 27(**2**), 51-66.

Serdar, S., Lök, A., Köse, A., Yildiz, H., Acarli, S. and Goulletquer, P.,

2007. Growth and survival rates of carpet shell clam (*Tapes decussatus* Linnaeus, 1758) using various culture methods in Sufa (Homa) Lagoon, Izmir, Turkey. *Aquacultural Engineering*, 37(**2**), 89-99.

Tan, S.H.A., Chang, G.O, Yen, P.K. and Peng, T.C., 2014. Oyster culture in Malaysia: Opportunities and challenges. *Journal of Science and Technology in the Tropics*, 10, 99-108.

Warfel, P.E., 2002. Growth and economic advantages of distributed powered upwellers: creating a new aquaculture niche. Presented, National Shellfisheries Association Meeting, Mystic, Conn. 2002.

Williams, P., 1981. Offshore nursery culture using the upwelling principle. In: Claus, C., De Pauw, N. & Jaspers, E. (ed.) Nursery culturing of bivalve molluscs. EMS Spec. Pub. No. 7. European Mariculture Society, Bredene, Belgium.