The Prototype Design and Function of a low Maintenance Brachyuran Early Larval Rearing System

Ali Al-Aidaroos

Faculty of Marine Science,
King Abdulaziz University,
P O Box 80207, Jeddah, Kingdom of Saudi Arabia

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Corresponding Author:
Ali Al-Aidaroos
E-mail: aaidroos@hotmail.com

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ABSTRACT

The prototype design and function of a low maintenance larval rearing system for Brachyuran larvae are described in this paper. The rearing system consisted of an array of 1000 ml beaker-shaped polypropylene unit series. The model conceived and constructed in the present study finally concluded upon 1000 ml rearing vessels than 50 ml or 500 litre modules. Water change in this new system is not only far less stressful to the larvae but also less time consuming. The novel brachyuran early larval rearing system designed in this study may be useful to overcome the mortality of larvae due to stress and as it is simple and low maintenance, it could be easily applied in any aquaculture/fisheries units.

Introduction

The majority of brachyuran have typically benthic adults and metamorphic life cycle with planktonic larval stages. Larvae taken from the planktonic have traditionally been difficult to identify (Clark and Paula, 2003), while hatching and rearing of crabs in the laboratory have allowed larvae to be identified unequivocally since the adult are of known species (Clark et al., 2005). Rearing of decapod larvae was once considered difficult, but the use of *Artemia* nauplii as a food source has opened up the field. Obtaining all developmental stages from an ovigerous female is now common in the laboratory. This is a significant advance for descriptive studies (alpha taxonomy), systematics, phylogenetic and evolutionary theory (Clark, 2009). However, larval rearing is not without its disappointments and failures. For no apparent reason, larval cultures occasionally crash (Clark, 2009). These, frustrations aside, there are distinct advantages to rearing larvae in the laboratory as opposed to studying plankton-collected material, such as collecting all life stages with verification from exuviate, providing sufficient specimens for morphological studies, and confirming the identification of the larvae by examining the spent female. The ability to positively identify the species is the distinct advantage that laboratory-reared material has over describing plankton-caught larvae (Clark, 2009).

Buchanan *et al.,* (1975) devised a flowing water system for culture of brachyuran crab larvae. The design minimizes the problem of larval entrapment in water exit screens. Survival of *Cancer magister* zoeae in rearing tests lasting up to 60 days compared favorably with survivals obtained previously using static culture methods. Previous studies by Charmantier and Charmantier (1991), Davis *et al.,* (2005) and Ventura *et al.,* (2008) also reported the rearing system for crab larvae. In the present study, an attempt was made to rear selected brachyurans from the Central Red sea employing various rearing containers. On an empirical approach, after facing failures in varying designs (plastic compartmentalized boxes), altering, adapting the framework to improvise newer structures and integrate better bioengineering functions to achieve the targeted rearing process with better degree of success. Brachyuran zoea of Xanthidae and Leucosiidae, unlike Grapsidae and Portunidae are too miniature-sized and are susceptible to shear stress. Mortality was observed to be a linear function of shear stress for all species. Micro-fractionated air bubbles are hyper surface tensive and can immobilize the first zoea. Sticking to the surface film due to water surface tension also incapacitate the tiny first formed zoea. So, the need for an innovative design that overcomes the practical constraints was felt wanting. The design reported in this paper may be useful for brachyuran early larval rearing with low maintenance.

Materials and Methods

The rearing system consisted of an array of 1000 ml beaker-shaped polypropylene unit series. The inner bottom of the rearing vessel was painted white and the inner lateral walls painted black to promote colour contrast perceptibility and silhouette identification of the ss-rotifer prey by the first zoea. A lateral LED light bank was provided and covered with a double bolting screen cloth to attenuate the incident rays to attain 150 lux of illumination. This ensured a diffused radiation flux per unit culture volume of rearing media meaning to imply a photon flux density of 50 lux in the rearing mesocosm. Sand was sparingly spread on the bottom to prevent sticky mucus adhesion of the zoea to the floor. A 20 mm central plastic standpipe (removable columnar filter) with a 55 micron mesh casing was centrally planted as articulated to an appropriate socket which leads to the exterior in the form of a swivel pipe (20 mm diameter) that can be moved 180 C maximum to adjust water flow. The columnar filter can be removed and replaced with a 100 micron mesh filter (to retain only the egg bearing females that release young rotifer larvae to be an immediate prey for the brachyuran zoea) or a 150 micron filter (to expel unused rotifers) ; implant 218 micron mesh filter (to expel unused Instar II artemia out of the system). All the units were covered with an acrylic plastic sheet to prevent evapo-transpiration of seawater from the rearing media and thereby arrest salinity increases from the ambient 40 ppt.

A centri-convective circum air-frame floats on top of the rearing media and the inner side of main frame (facing the larval medium) is drilled along the perimeter with 0.3 mm size holes at an equal distance of 1.5 cm. This circular air-frame is connected to an air source and through the air- perforations placed equidistant blows a uniform gentle stream of air on the water surface to micro-hammer the surface film otherwise caused by surface tension. A 5 mm bored glass tube is inserted into the rearing container which constantly causes a continuous serial uplifting of vertically rising whole air cells (VRWAC). The 5 mm spouted orifice floats bioproteins and wall-slimes the bio-organics. A 3000 ml separating funnel is lodged above the unit to dispense clean seawater dosed with sodium nifurstyrenate in proportion to the angular swivel dripping rate of culture media. Naturally aged estuarine water was used after being shifted through a 0.45-mm pore filter and activated charcoal to remove dissolved organic matter and trace metals.

Results and Discussion

The prototype of the brachyuran early larval rearing unit designed in this study is given in figure 1. Table 1 show the comparison of results obtained in the present study with that of reported by Andres *et al.,* (2010).

Table 1: Comparison of *Grapsus albolineatus* rearing performance – improvised rearing vessel presently studied with non-improvised control rearing vessel for *Portunus pelagicus* (Andres *et al.,* 2010)

(600 mL glass beakers filled with (Improvised 1000 mL glass beaker filled)		
Factors	**500 mL UV**	**Filtered Seawater**
Rearing Volume	600 ml	1000 ml
Culture Holding Volume	500 ml	500 ml
Larval Density per vessel	25	25
Maximum stage attained	Zoea 1	Zoea 4
Survival until Zoea 1	10 %	50 %
Survival until Zoea 2	00	35 %
Survival until Zoea 3	00	23%
Survival until Zoea 4	00	10%
Days elapsed to reach Z1	08	07
Days to reach Z2 from Z1	00	11
Days to reach Z3 from Z1	00	14
Days to reach Z4 from Z3	00	20

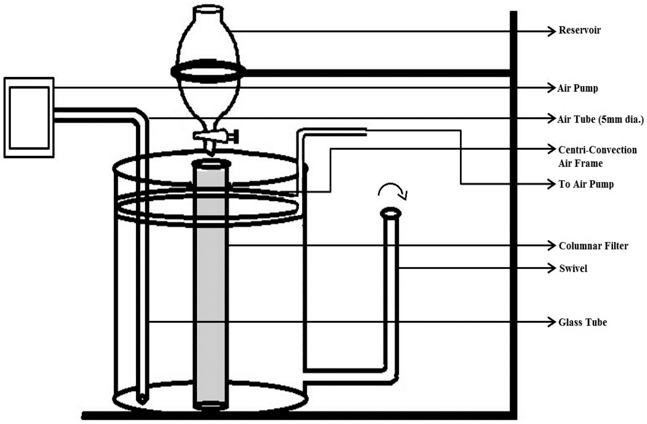

Figure 1: The prototype design of the Brachyuran Larval rearing system.

The model conceived and constructed in the present study finally concluded upon 1000 ml rearing vessels than 50 ml or 500 litre modules. Hamasaki *et al.,* (2007) also experienced larval mass mortality but found relatively smaller vessels better efficient in survival. The survival rate of snow crab larvae in 500 liter tanks rapidly decreased and few larvae molted to the second zoea (Kogane and Hamasaki, Unpubl. Data). However in one litre beakers, mean survival to the first crab stage reached circa 30 % (Kogane *et al.,* 2005). Differences between survival rates in large tanks and small vessels were also observed for other brachyuran species (Hamasaki et al., 2002; Hamasaki, 2003). In the present study also, it was confirmed that 1000 ml containers could be easily replaced every 7 days with a new vessel and thus get rid of the dead larvae, injured bottom-schooling live prey and faecal pellets.

The light bank of 150 lux was purposely planned to homogenously distribute the larvae in the rearing system. Newly hatched larvae showed positive phototaxis and to reduce the upschooling frequency, this mild dullish and diffuse areal illumination of 150 lux was instituted and a PFD of 50 lux achieved within culture media by progressive attenuation with swiss nytal thal bolting screens. As the zoea grows, positive geotaxis is revealed and the PFD is increased from 50 lux to 150 lux for achieving certain degree of negative geotaxis. Rearing salinity was increased gradually from 36 ppt by first zoea to 40 ppt by Zoea 4. This was done to reduce the larval sinking speed, a characteristic feature of metamorphosing late zoeal stages.

According to Ikhwanuddin *et al.* (2011) the highest percentage survival was observed in the dark-grey tanks when the stocking density of *Portunus pelagicus* larvae was 20 larvae L^{-1}. No larva reached the juvenile crab size in white tanks. It is well known that the nutritional value of enriched brine shrimp metanauplii decreases rapidly 24 h after being supplied to the larvae (Narciso et al., 1999; Navarro et al., 1999). Therefore, the daily cleaning and maintenance of the rearing tanks, particularly the daily washing and exchanging of the 200- and 500-μm mesh screens, not only allowed the maintenance of good water quality but also the daily replacement of 24-h-old enriched metanauplii by newly enriched ones. This procedure played a vital role during the entire rearing process since it enabled the larvae to feed on highly nutritional preys. The traditional water change of other rearing systems often requires larval manipulation and induces stress to the larvae. Water change in this new system is not only far less stressful to the larvae but also less time consuming.

The control of water velocity of the upwelling flux in each rearing tank was also very important for the successful larval culture of these species. The upwelling water flux caused by the vertical rise up of whole air cells kept in suspension the larvae of all stages. This upwelling flow prevented larval accumulation on the bottom of the rearing tank and the consequent larval "tangling". In conclusion, the novel brachyuran early larval rearing system designed in this study may be useful to overcome the difficulties reported by previous investigators. As it is simple and low maintenance, it could be easily applied in any aquaculture/fisheries units.

Conclusion

This design commands a new window of opportunity for the difficult-to-rear brachyuran first zoea. Apart from conventional rearing systems, this small research scale system where water change is less stressful sounds remarkable. Vigorous pipetting of live larvae to another unit is not needed. Exuviae collection for morphologic examination is easier in this system owing to the straining function of the central filter during water exchange. Profound morphologic changes at specific molts of various stage sequences can be precisely detected through molted exuviae examinations. Larval descent swimming speed can be outwardly regulated by the bottom charged air vent hole. The control of water velocity of the upwelling flux of air cells in each rearing tank is also very important for the successful larval culture of these species. Vertical rise up of whole air cells (without microfractionative diffusers) helps to skim dissolved organics by dissolved air floatation technique. The surfacial air ring frame thwarts the upsticking larvae that firmly clanged onto the otherwise formed surface tensive film that causes immobility of appendages. A LED illuminator for the rearing system sounds befitting for controlling the radiation flux per unit rearing volume. The overhead funnel could help to incubate and drip any unconventional microbial diets on piece-meal basis for future research trials elsewhere the globe. This design is thus contributory to the science and design engineering of difficult-to-rear brachyuran early larviculture.

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