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Short Communication

Larval Culture of *Scylla serrata:* Maintenance of Hygiene and Concepts of Experimental Design

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Abstract

Significant progress has been achieved towards sustainable commercial production of mud crabs from hatcheries. However, the larval rearing protocol used has not been the most efficient. In the culture of mud crab larvae, the maintenance of hygiene and the control of pathogenic organisms are critical to survival and metamorphosis. The control of pathogens in larval culture should not be dependent on antibiotics, as resistant organisms will develop. Biological control of pathogens using microbially mature seawater or probiotics has potential for the development of successful larval culture.

Worthwhile experimentation with larval cultures must embody good statistical design and execution. Replications from at least two hatchings are necessary to establish firm conclusions on treatment effects. Factorial experiments can be used to reduce experimental error and wastage of materials as well as to investigate treatment interactions.

Introduction

For decades the mariculture of *Scylla* spp. has been hindered by the failure of investigators to reliably produce larvae from zoea1 to megalopa and then to first crab stage (C1) and beyond in commercial numbers. During the last three years, progress in Southeast Asia and Australia has shown that barriers to larval survival and metamorphosis can be broken, paving the way for the development of a complete and reliable mariculture of the mud crab.

However, the high variability in culture success and the low repeatability experienced by investigators are signs that experimentation has not been efficient. The basic essential methods for the maintenance of hygiene have been neglected, and the principles of experimental design and analysis were not consistently followed.

Hygiene

Hygiene begins with the preparation of the broodstock and continues up to the metamorphosis of megalopa to crablets. The aim is to restrict the growth of potential pathogens, including bacteria, fungi, viruses and protozoa in the culture system. It can be safely assumed that all inputs into a culture tank are potential sources of infection, that may reduce rates of larval survival and metamorphosis. All tanks and equipment used in the culture must first be effectively sterilized following standard methods before use as a simple precautionary measure.

Seawater appears to be an excellent medium for bacterial survival and the microbiological safety of all sea- and freshwater used must be assured to make it the first line of defense against harmful bacterial contamination from other sources.

Broodstock are potential sources of fungal and bacterial infection. Egg-carrying crabs need continued exposure to clean, high quality seawater. This can be achieved by utilizing flow through, UV-treated water. Direct immersion in antibacterial agents will also reduce bacterial loads. It has been shown that newly hatched larvae should be removed within an hour from the hatch tank otherwise, microbial proliferation will occur and larval survival may be severely compromised (Mann, D. personal communication).

Cultures of algae and rotifers should be maintained in hygienic conditions. Regular inspection of culture quality is essential and may include testing for pathogenic bacterial agents. Rotifers have been recognized as a source of a debilitating, but so far unidentified infection of mud crab larvae in large tanks. (Mann, D. personal communication). *Artemia*, another potential carrier of infection, have been linked to massive larval destruction caused by *Vibrio* spp. As a result, careful preparation of A*rtemia* nauplii is essential. If facilities are available microbial checks should be run to determine types and densities of potentially pathogenic organisms.

When several tanks are in use for larval culture, cross-contamination between staff and their equipment must be avoided. As much as possible, the hatchery should be physically isolated from the broodstock and other crustacea. Those working in the broodstock area should routinely undergo a clean up before entering the hatchery area.

Cultures may repeatedly crash because of infection and the knee-jerk reaction in aquaculture and in many land-based animal industries, has been to use antibiotics immediately. Intensive animal production exposes stock to greater risks of disease. Throughout the world, antibiotics have been used to both prevent and treat diseases in man and animals as well as to enhance livestock production. A major consequence has been the proliferation of resistant bacteria and the transmission of resistance to other bacterial species (Benson 1998). Seawater, as the medium for mariculture, is a perfect vehicle for bacterial spread, particularly by contamination of input water caused by inappropriate siting of effluent disposal from culture sites. Shrimp hatcheries worldwide are now plagued by multi-antibiotic resistant strains of virulent bacteria. The fast transmission of pathogenic

bacteria and the occurrence of antibiotic resistance means that the public health methods developed more than 100 years ago for the control of waterborne diseases in people must be reapplied to aquaculture using the principles of hazard analysis and quality control (Blackshaw et al. 1999). The aim is to restrict the growth of potential pathogens including bacteria, fungi, viruses, and protozoa in the culture system. Whenever possible, antibiotics should be avoided and the problems of pathogens should be solved through hygienic measures and biological control.

There is increasing evidence that a process of microbial maturation (Skjermo and Vadstein 1999) can control the composition of the bacterial flora of seawater. Settling of the water for a number of days, results to a diverse bacterial flora, dominated by non-opportunists, that act as a stable buffering system restricting the growth of opportunistic and potentially pathogenic bacteria. An alternative procedure is the use of probiotics wherein specific, desirable bacteria are added to the seawater to create a similar environment unfavorable to pathogens. Microbial maturation of seawater has been used in the culture of Atlantic halibut and turbot larvae. Survival, growth rate, and feeding were all improved over control treatments (Skjermo et al. 1997).

Although a commercial probiotic was tested at the Bribie Island Aquaculture Research Centre (BIARC) without success in the culture of *S. serrata* larvae (Mann, D. personal communication) a number of reports have shown that pathogens can be controlled by some probiotics. Significant control of V*ibrio tubiashii* was achieved in the culture the Pacific oyster (*Crassostrea gigas*) larvae using *Aeromonas media* (strain A199) by Gibson et al. (1998). The black tiger shrimp *P. monodon* postlarvae was completely protected from damage by *Vibrio harveyi* using cultures of *Bacillus S11,* isolated from shrimp habitats (Rengpipat et al. 1998). Addition of the bacterium (*Thalassobacter utilis* (strain PM4)) to larval cultures of the swimming crab (*Portunus trituberculatus)* repressed the growth of *Vibrio harveyi* and the fungus *Haliphthoros* spp and significantly improved survival rates (Nogami et al. 1997).

Recent trials at BIARC have shown that if mud crab larval cultures are maintained in seawater that has stood for several days in a sealed tank before use (settled water), the growth of pathogenic *Vibrio* species is reduced. The process is similar to that developed as microbial maturation by Skjermo and Vadstein (1999). The conditioning of water has been recognized among marine tropical fish culturists. Emmens (1985) recommended that aquarium water stand for 2 to 3 weeks before being used for tropical marine fish culture.

Experimental design

There are many factors affecting larval survival and their effective statistical analysis requires adequate design and execution of laboratory and tank trials. All experiments must be systematically planned for appropriate statistical analysis. The actual conduct or misconduct of experiments

using *ad hoc* changes may negate careful design and analysis, and may make conclusions erroneous. Training and supervision of hatchery staff performing an experiment is essential. Principals may not be able to personally supervise the conduct of each experiment, but they must delegate the task of close supervision to colleagues who are experienced in hatchery work. They may just conduct regular spot checks on the performance of the staff.

The design of the laboratory (up to several liters) and hatchery (250 to 10,000 l tanks) may be different. Multifactor trials can be easily performed in the laboratory, but in the hatchery the size and availability of large tanks will dictate the use of simpler designs. However, a 5000 l tank is a single unit and statistically it gives no more information than a 3 l pot. In some cases tank subdivision will allow several factors to be examined under similar conditions. In a number of reported results a common design and implementation fault is the use of a larval hatching from a single crab (no external replication). Although often believed to be indicative, single batch experiments have no formal validity for drawing firm conclusions on treatment effects. In some cases, different hatchings using the same treatments have been analyzed separately, losing valuable information on batch effects and possible interactions. On the other hand, multiple replication of identical treatments oftentimes result to waste of time and materials. The use of simple factorial experiments will provide this internal replication, as well as allow more treatments and provide information on interactions. It is incumbent on the experiment designers to seek advice and confirmation of design principles and methods of analysis from institute statistical advisers.

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