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Problems Associated with Tank-held Mud Crab (*Scylla* spp.) Broodstock

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Abstract

To support studies on the development of broodstock and hatchery technology for mud crabs under the genus *Scylla*, the SEAFDEC Aquaculture Department maintains captive broodstock in land-based tanks. Disease problems seen in broodstock after being held for three months in these tanks include shell disease due to a combination of fouling organisms and chitinoclastic bacteria, bacterial contamination of the hemolymph, parasitic infestation on the gills and shell, and loss of appendages. Shell disease was manifested as off-white and black patches on the shell, that progressed and became perforations exposing underlying tissues. The hemolymph of a significant number of newly recruited crabs harbored mixed populations of sucrose-fermenting vibrios. Pedunculate cirripedes were found in large numbers both in the gill region and on the shell, boring through and creating perforations in the latter. Nematodes and other saprophytic organisms enter the crab through these perforations. The fouling problems that affect the integrity of the shell are considered to reduce the life span and reproductive potential of captive broodstock under tank conditions; therefore, regular cleaning of the shell is recommended to minimize shell fouling.

Introduction

Mud crabs belonging to the genus *Scylla* are large portunids with high commercial value. Since the 1900s, the biology, ecology, and culture of these crabs have been intensively studied in many parts of the world (Brackish Aquaculture Information System 1989). Its culture in the Philippines is a significant source of income for small-scale farmers. However, a major constraint to grow-out culture activities is the insufficient supply of crab juveniles, gathered from the wild. To answer this problem, research on developing techniques for broodstock management and larval rearing of the mud crab has been intensified since 1995. However, the quality of spawners declines in captivity, and survival rates of the larvae and megalops in the hatchery remain low and inconsistent. This study documents the disease

problems affecting captive broodstock aimed at identifying measures for their prevention and control.

Materials and Methods

Pond-grown mature crabs (240 to 790 g body weight; 8.04 to 12.53 cm carapace length) were purchased from commercial crab dealers and allowed to spawn at the SEAFDEC Crustacean Hatchery. They were held in 10 t concrete tanks provided with sand as substrate and wooden blocks for shelter. The tanks were supplied with either a partial flow-through ($3h \cdot day^{-1}$) of chlorinated and neutralized seawater or a continuous flow-through of unchlorinated seawater. Sand substrates were cleaned twice a week. Crabs were fed mussel meat and fish-by-catch or a formulated diet (modified from Millamena et al. 1986), or a combination of both diets given twice a day at 3% body weight (Millamena and Quinitio 2000). Excess feeds were siphoned out prior to feeding.

External examination

Crab broodstock were regularly examined macroscopically for shell discoloration, presence of fouling organisms and lesions. Parasites and protozoans were examined using fresh squashes of affected organs. Parasites and symbionts recovered were preserved in 10% buffered formalin for further identification.

Bacterial examination

The first batch of samples was composed of 15 crabs with severe shell disease. The discolored areas on the shell were aseptically examined for bacteria by scraping with a sterile scalpel. The scrapings were placed in a homogenizing tube, weighed, homogenized, and suspended in sterile seawater. Dilutions were made using standard procedures (Austin 1988). Aliquots (0.1 ml) were plated on duplicate plates of nutrient agar (NA) with 1.5% NaCl and thiosulfate citrate bile sucrose (TCBS) agar and incubated at room temperature (28 to 30ºC). Bacterial colonies were counted after 24 hours. Chitinoclastic bacteria were enumerated on chitin agar (West and Colwell 1984) and counted after two weeks of incubation. The biochemical characteristics of the dominant bacteria were determined using tests prescribed by West and Colwell (1984). Bacterial identification was based on the scheme of Alsina and Blanch (1994).

The second batch of samples was composed of 45 newly-recruited crab spawners. They were monitored for presence of bacteria in the hemolymph. The spawners belong to the following species: *Scylla tranquebarica* (13 individuals), *S. olivacea* (19 individuals), and *S. serrata* (13 individuals). At least 0.5 ml hemolymph was withdrawn aseptically. Hemolymph samples (0.1 ml) were plated in duplicate on NA and TCBS plates and incubated at

28 to 30°C for 24 hours. For comparison, the hemolymph of some broodstock from the same batch was re-examined after being held in tanks for three months using the same media and procedures.

Results and Discussion

Ovigerous crabs reared in land-based 10-ton concrete tanks successfully spawned two to three times within a three-month period. However, captivity beyond this period resulted in disease problems. Although no significant mortality was observed in the captive broodstock, the diseases significantly affected their appearance, health and reproductive performance. The following diseases are considered significant:

Shell disease

This condition affected 100% of the spent broodstock after being held in tanks for three months, but was rarely found in newly-recruited crabs. Shell disease appeared initially as discolored patches on the carapace (Fig. 1), that later spread over the chelipeds (Fig. 2). The fuzzy mats that developed on the shell were composed of a community of filamentous blue-green algae; bacteria; sessile, ciliated protozoans; saprophytic ciliated protozoans and some flagellates. The shell disease in the first batch of crabs

Table 1. Number of bacteria isolated from the severely-diseased exoskeleton of three species of crab broodstock held captive in land-based tanks for three months

Species/Number of samples	Total Bacterial Count (cfu/0.1g)	Presumptive *Vibrio* count (cfu/0.1g)	Chitinoclastic bacteria*
S. tranquebarica (5)	1.7×10^4 - 5.8×10^6	7.5×10^3 - 7.0×10^5	50 %
S. olivacea (4)	2.2×10^4 - 8.0×10^6	5.0×10^3 - 7.0×10^5	75 %
S. serrata (6)	3.5×10^4 - 8.0×10^7	4.5×10^2 - 6.0×10^6	75 %

*Percentage of total bacterial count.

Fig. 1. Crab broodstock showing extensive off-white patches on the carapace, which are the initial signs observed in shell disease development. The blackened patches in the center are depressions created mainly by chitinoclastic bacteria.

Fig. 2. The chelipeds of crab broodstock showing black and white areas affected by shell disease

was considered severe because more than 75% of the dorsal region of the shells was affected. Many parts of the exoskeleton were soft and black lacking calcified tissue underneath. These areas easily became perforated, exposing underlying tissues. New recruits possessed a shiny exoskeleton. The bacterial loads of the discolored and diseased patches of the shell are presented in table 1. The bacterial population was very high with up to 10^7 total bacteria obtained per 0.1 g of sample, 50 to 75% of these were chitinoclastic. Several species of sucrose-fermenting and non-sucrose-fermenting vibrios were identified such as *Vibrio vulnificus, V. splendidus,* and *V. orientalis* (Table 2). Aside from chitinase, these vibrios also possess the enzymes gelatinase and lipase considered as compounding factors that enhance shell degradation. It is probable that the microbiological aggregate formed on the shell provided a good environment for chitinoclastic vibrios to settle, caused gradual damage and resulted to perforation. Shell disease in crabs and other crustaceans with a chitinous exoskeleton is rather common (Rosen 1970; Fisher et al. 1976; Baross et al. 1978; Getchell 1989; Sindermann 1989; Lio-Po and Lavilla-Pitogo 1990), but the prevalence in

Table 2. Biochemical characteristics of bacteria associated with the shell lesions of captive mud crab broodstock

Test	Reaction		
Colony on TCBS agar	yellow	green	yellow
Enzyme production			
Lipase	+	+	+
Gelatinase	+	+	+
Chitinase	+	+	+
Arginine decarboxylase	-	+	-
Lysine decarboxylase	+	-	+
Ornithine decarboxylase	+	-	-
Indole	-	-	+
Citrate	+	-	+
Hydrogen sulfide	-	-	+
Acid from			
Arabinose	-	-	-
Cellobiose	+	+	+
Fructose	+	+	+
Galactose	+	+	+
Glucose	+	+	+
Inositol	-	-	-
Lactose	-	-	-
Maltose	+	+	+
Mannitol	+	-	+
Mannose	+	+	+
Sucrose	+	-	+
Xylose	-	+	+
Triple sugar iron test			
Slant/butt	A/A	K/K	A/A
H_2S	-	-	-
Gas production	-	-	-
Growth in			
0% NaCl	-	-	-
3% NaCl	+	+	+
6% NaCl	+	-	+
8% NaCl	-	-	-
Identification	*V. vulnificus*	*V. splendidus*	*V. orientalis*

older animals is more common due to longer intermolt periods. Injury inflicted during handling and crowding, and exposure to pollutants are some of the predisposing factors implicated. The condition seldom leads to mortality, but extensive shell erosion and perforation may create portals of entry for secondary bacterial or parasitic infections. In the immediate vicinity of the perforated regions of the shells, there were small populations of saprophytic ciliated protozoans and nematodes, although no such organisms were seen circulating in the hemolymph.

It is interesting to note that shell disease was confined to the dorsal areas of the shell. The frequent brushing of the ventral region with the sand substrate during burrowing may have a cleansing effect on the shell. In this connection, incorporation of cleaning procedures such as light brushing and swabbing with iodine solution during captivity of broodstock may minimize the buildup of fouling organisms. These provide a favorable substrate for the establishment of chitinoclastic bacteria on the dorsal regions of the body.

Microbial contamination of the hemolymph

A significant number of newly-recruited crabs (37 to 67%) harbored mixed populations of bacteria in the hemolymph, mainly dominated by sucrose-fermenting vibrios. Table 3 summarizes the health status of newly-recruited broodstock and those held for three months. The presence of bacteria in the hemolymph of new recruits, where shell disease was not seen, shows that shell perforation is not necessarily a precursor to internal contamination. It is interesting to note that contamination was highest in *S. serrata* both in the new recruits and those captive for three months. The presence of bacteria in the hemolymph was reported in crawfish by Scott and Thune (1986), who also cited several reports about bacteria in the hemolymph of crustaceans. Compared with the study of Davis and Sizemore (1982) that reported bacterial infection of up to 3×10^7 bacteria·ml of

Table 3. Comparison of the health status of 3 species of newly-recruited and captive crab broodstock based on shell quality and presence of bacteria in the hemolymph. Captive crabs were held in land-based tanks for at least three months.

	Scylla serrata		*Scylla tranquebarica*		*Scylla olivacea*	
	New recruit	Captive	New recruit	Captive	New recruit	Captive
Number of samples	13	6	13	6	19	10
With shell disease (%)	0	100*	0	100*	0	100*
With bacteria in hemolymph (%)	67	67	46	0	37	10
Range of bacteria in hemolymph (cfu/ml)	$0\text{-}5\times10^3$	$0\text{-}4\times10^1$	$0\text{-}8\times10^1$	0	$0\text{-}5\times10^1$	$0\text{-}3\times10^1$
Dominant strain	sucrose-fermenting vibrios		sucrose-fermenting vibrios		sucrose-fermenting vibrios	

*With light to moderate shell disease = affected 25 to 50% of dorsal shell surface, no perforation

hemolymph, bacterial populations in the present samples are comparatively light. Nevertheless, since about half of the newly-recruited crabs without shell disease harbored bacteria in their hemolymph, their presence should be noted as abnormal.

Although shell disease occurred in 100% of crabs after three months of captivity, there was no increase in the number of individuals that harbored bacteria in the hemolymph. The shell disease in this batch of crabs was graded as light to moderate affecting 25 to 50% of the dorsal shell surface. No perforation of the shell was observed.

Parasitic diseases

The observations were limited to macroparasites observed on the shell and on the gills. Pedunculate (Lepadomorph) cirripeds, that are morphologically similar to *Octolasmis cor* as described by Arudpragasam (1967) and Hudson and Lester (1994), were observed around the carapace at the edge of the inhalent aperture, at the base of the cheliped, and on the second and third maxillipeds (Fig. 3). Although only one live specimen was observed to be infested with the cirripeds, five dead broodstock were observed to harbor them on the gills. Previous reports about the association of this organism with *S. serrata* (Jeffries et al. 1985) showed a commensal or symbiotic relationship. However, we have observed that organisms on the shell actually bored through it and were anchored to the crabs through the peduncle. Some of the negative effects of *O. cor* infestation are competition for oxygen and blockage of the gills due to accumulation of debris on colonized respiratory surfaces (Overstreet 1978, 1988; Hudson and Lester 1994). A balanid cirriped *Chelonibia patula* and other barnacles were also found attached to the carapace and chelipeds of crabs. These organisms, though not causing mortality, may affect the mobility of the crabs due to the extra weight of the barnacles (Overstreet 1988).

Loss of appendages

Due to severe muscular emaciation in some captive broodstock, handling may result in the loss of appendages such as periopods and chelipeds (Fig. 3). Regeneration of the lost part does not immediately restore its original function because of the relatively small size of the new appendage. The loss of a major appendage like the cheliped may lead to impaired feeding, mating, and capability for defense.

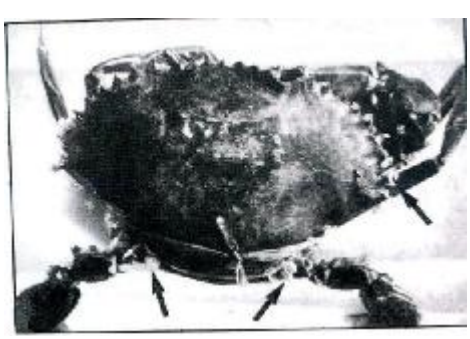

Fig. 3. Crab broodstock colonized by pedunculate cirripeds around the edge of the carapace and at the base of appendages (arrows). Note the missing cheliped on the left.

Conclusions and Recommendations

Conditions during maintenance of broodstock in tanks resulted in severe fouling that led to either shell perforation or parasite settlement. These were brought about by inappropriate holding conditions, overcrowding, and unsuitable environmental factors that enhanced the dominance of fouling organisms. Once the integrity of the shell was damaged, portals of entry for secondary and opportunistic pathogens were created. There is a need to improve the conditions within the rearing tank to maintain the health of the broodstock beyond this period and prevent emaciation.

It is equally important to provide a sandy substratum of appropriate thickness under which the crabs could burrow. Provision of an optimum amount of substrate may not only reduce stress, but also reduce the build-up of fouling organisms on the crabs. It is also recommended to regularly lightly brush and wipe the dorsal region of the exoskeleton using cotton dipped in iodine solution to prevent fouling during captivity.

In view of the presence of bacteria in the hemolymph of both newly-recruited and captive crab broodstocks, it is important to study if such a condition leads to problems like delayed clotting, impaired defense mechanisms, and increased susceptibility to other infectious microorganisms.

References

Alsina, M. and A.R. Blanch. 1994. A set of keys for biochemical identification of environmental *Vibrio* species. Journal of Applied Bacteriology 76: 79-85.

Arudpragasam, K.D. 1967. Distribution and variation in form of the Cirripede *Octoclasmis cor* (Aurivillius, 1893) in relation to the respiratory current of its host *Scylla serrata*. Ceylon Journal of Science 7:105-115.

Austin, B. 1988. Methods in Aquatic Bacteriology. John Wiley and Sons, New York, USA. 425 pp.

Baross, J.A., P.A. Tester and R.Y. Morita. 1978. Incidence, microscopy and etiology of exoskeleton lesions in the tanner crab, *Chionoecetes tanneri*. Journal of Fisheries Research Board of Canada 35:1141-1149.

Brackish Aquaculture Information System (BRAIS). 1989. Mud Crab Abstracts. Aquaculture Department, Southeast Asian Fisheries Development Centre, Tigbauan, Iloilo, Philippines. 136 pp.

Davis, J.W. and R.K. Sizemore. 1982. Incidence of *Vibrio* species associated with blue crabs (*Callinectes sapidus*) collected from Galveston Bay, Texas. Applied Environmental Microbiology 43: 1092-1097.

Fisher, WS, T.R. Rosemark and E.H. Nilson. 1976. The susceptibility of cultured lobsters to a chitinoclastic bacterium. In: Proceedings of the 7th Annual Meeting of the World Mariculture Society (ed. J. Avault), Vol. 7:511-520. World Mariculture Society, USA.

Getchell, R.G. 1989. Bacterial shell disease in crustaceans: a review. Journal of Shellfish Research 8:1-6.

Jeffries, W.B., H.K. Voris and C.M. Yang. 1985. Growth of *Octolasmis cor* (Aurivillius, 1892) on the gills of *Scylla serrata* (Forskal, 1755). Biological Bulletin 169: 291-296.

Hudson, D.A. and R.J.G. Lester. 1994. Parasites and symbionts of wild mud crabs *Scylla serrata* (Forskal) of potential significance in aquaculture. Aquaculture 120: 183-199.

Lio-Po, G.D. and C.R. Lavilla-Pitogo. 1990. Bacterial exoskeletal lesions of the tiger prawn *Penaeus monodon*. In: The Second Asian Fisheries Forum (eds. R. Hirano and I. Hanyu), pp. 701-704. Asian Fisheries Society, Manila, Philippines.

Millamena, O.M., J.H. Primavera, R.A. Pudadera, R.V. Caballero. 1986. The effect of diet on reproductive performance of pond-reared *Penaeus monodon* Fabricius broodstock. In: The First Asian Fisheries Forum (ed. J.L. Maclean, L.B. Dizon and L.V. Hosillos), pp.593-596. Asian Fisheries Society, Manila, Philippines.

Millamena, O.M. and E. Quinitio. 2000. The effects of diets on reproductive performance of eyestalk-ablated and intact mud crab *Scylla serrata*. Aquaculture 181:81-90.

Overstreet, R.M. 1978. Marine maladies? Worms, germs, and other symbionts from the northern Gulf of Mexico. Mississippi-Alabama Sea Grant Consortium, Sea Grant Publ. No. MASGP-78-021, 140 pp.

Overstreet, R.M. 1988. Aquatic pollution problems, Southeastern U.S. coast: histopathological indicators. Aquatic Toxicology 11: 213-239.

Rosen, B. 1970. Shell disease of aquatic crustaceans. In: A Symposium on Diseases of Fishes and Shellfishes (ed. S.F. Snieszko). American Fisheries Society Special Publication No. 5, Washington DC.

Scott, J.R. and R.L. Thune. 1986. Bacterial flora of hemolymph from red swamp crawfish, *Procambarus clarkii* (Girard), from commercial ponds. Aquaculture 58:161-165.

Sindermann, C.J. 1989. Shell disease in marine crustaceans – a conceptual approach. Journal of Shellfish Research 8(2):461-462

West, P.A. and R.R. Colwell. 1984. Identification and classification of Vibrionaceae - an overview. In: Vibrios in the Environment (ed. RR Colwell), pp. 283-363. John Wiley, New York, NY.