pH AS A FACTOR INFLUENCING MATURATION OF *PENAEUS INDICUS* IN CAPTIVITY

M. S. MUTHU, A. LAXMINARAYANA AND K. H. MOHAMED
Central Marine Fisheries Research Institute, Cochin-18.

ABSTRACT

Unilaterally eye-ablated females of *Penaeus indicus* have matured and spawned in 3-4 days time when the pH of the maturation pools was maintained at a level similar to that of the sea, between 8.0 and 8.2, by daily addition of sodium carbonate. But eye-ablated females kept in pools where the pH was allowed to fall below 7.9 did not attain full ovarian development and no spawning was obtained.

INTRODUCTION

Penaeus indicus has been induced to mature and spawn in captivity by the unilateral-eyestalk-ablation technique at the Narakkal Prawn Hatchery (NPH) of the Central Marine Fisheries Research Institute (Muthu and Laxminarayana 1980). In the course of further work at the hatchery, it was found that, when the pH of the maturation pool declined below 7.9, the developing ovaries of eye-ablated prawns regressed, indicating that pH of the medium could be an important factor influencing the maturation process. This made us think that, for successful maturation and spawning, it may be necessary to have the pH of the medium around 8.2, the normal pH of water in the sea where the prawns naturally mature and spawn. To test this hypothesis, controlled experiments were conducted and the results are presented in this communication.

MATERIAL AND METHODS

Source of prawns: Specimens of *Penaeus indicus* larger than 30 mm in carapace length were collected from the grow-out ponds of the NPH. The females were immature but impregnated, and the males were mature with well-developed spermatophores at the base of the 5th walking legs. The females were subjected to unilateral eyestalk ablation using an electrocautery apparatus. The males were not eye-ablated. The prawns were introduced into the maturation pools soon after eyestalk ablation.

Maturation pools: The experiments were conducted in 10-tonne-capacity, circular, plastic-lined pools, kept in a tile-covered shed open on the sides. Each pool was fitted with a 2 m x 2 m sub-gravel biological filter through which the

seawater was continuously recirculated with the help of 9 airlifts, at the rate of 150 litres minute. The airlifts, operated by oil-free air from an air-blower, apart from recirculating the water through the biological filter, served to keep the water well aerated. The depth of water in the pools was 90 cm. The natural light intensity at the surface of the water was 500-3600 lux during the day. No artificial illumination was provided.

Experimental setup: A series of three experiments were conducted. For each experiment, equal number of similar-sized prawns were kept in two identical maturation pools. In one pool the pH was not controlled; whereas, in the other pool, the pH was so regulated by adding adequate quantity of anhydrous Na_2CO_3 (dissolved in 2 litres of tap water), everyday at 0900 hrs, that the pH of the water one hour after addition of Na_2CO_3 stood at ca. 8.2. All the physical and other hydrological parameters were identical in both the pools. In the first experiment, 8 eye-ablated females and an equal number of males were kept in each pool. In the second set, in each pool 6 females and 6 males were kept and, in the third set, 5 females and 5 males. The prawns were fed *adlibitum* with fresh clam meat. The uneaten food and faecal matter were siphoned out everyday. The depth of water in the pools was maintained at 90 cm by addition of seawater.

The temperature, salinity, pH, dissolved O_2, total ammonia and NO_2 of the seawater in the pools were monitored at 0900 hrs everyday. In the pH-regulated pool, the pH was measured before and one hour after the addition of Na_2CO_3. Total ammonia and NO_2 were estimated using the methods of Solorzano (1966) and Strickland and Parsons (1968), respectively, making use of a spectrophotometer. The pH was measured with a digital pH meter having a sensitivity of 0.01.

At the end of each experiment the seawater in the pools was completely changed and a fresh set of specimens introduced for the next experiment. The pool in which pH was regulated during the first experiment was used as the control pool without pH regulation in the second experiment and *vice versa*. In the third experiment, pH treatment in the pools was again reversed. This was done to eliminate any intrinsic advantage that one pool might have had by virtue of its position in the shed.

The developing ovary in the females was visible through the transparent dorsal cuticle. So the stage of ovarian development was noted every day without disturbing the prawns. Females with fully developed ovary were caught with a dipnet and placed individually in 200-litre-capacity, cylindroconical, fibreglass tanks containing clear, filtered seawater and provided with good aeration. Spawning took place in the night and the spent spawner was returned to the maturation pool soon after spawning. The total number of eggs spawned and the number of nauplii that hatched out were computed from counts made from 5 aliquot samples.

RESULTS

In each experiment the environmental conditions in the two maturation pools were identical except for the difference in pH. In the pools where pH was not regulated the pH steadily declined from the initial value of ca. 8.2 to ca. 7.2 in the course of 10-12 days, due to the metabolic processes taking place in the pool. In the pH-regulated pools the pH declined slightly to ca. 7.95 in the mornings but was increased to ca. 8.2 by daily addition of Na_2CO_3. The total ammonia and NO_2 levels in the pools ranged from 0.015 to 0.035 ppm and 0.003 to 0.012 ppm, respectively, and were far below the sub-lethal values reported by Wickins (1976) for penaeid prawns. The temperature in the pools ranged from 26.5°C to 28.3°C and the salinity from 32.1 to 34.1 ppt. The dissolved-O_2 levels remained high, around 6.4 mg|1.

In the pH-regulated pools all the eye-ablated females, in all the three experiments, matured and spawned viable eggs 3-4 days after eyestalk ablation (Tables 1 and 2). The 19 females produced a total of 1,455,300 eggs, i.e., 76,595 eggs per female. The average number of nauplii produced per female was 70,674 giving a hatching rate of 92.3%. The nauplii were healthy and were reared up to the postlarval stage in the NPH.

TABLE 1. *Summary of experiments to show the influence of pH regulation on maturation and spawning of eye ablated* Penaeus indicus.

Exp. No.	Duration of experiment	with pH regulation			without pH regulation		
		Size range of females used. C.L. in mm	No. of ablated females kept	No. of females that spawned viable eggs	Size range of females used. C.L. in mm	No. of ablated females kept	No. of females that spawned viable eggs
1	8-2-82 to 20-2-82	30.0 to 34.5	8	8	30.5 to 34.5	8	nil
2	1-3-82 to 9-3-82	31.0 to 37.0	6	6	31.5 to 37.0	6	nil
3	10-3-82 to 18-3-82	29.5 to 32.0	5	5	30.0 to 32.0	5	nil

But, in the pools where pH was not regulated, in all the experiments, the females reached only the II-III stage of ovarian maturity and then re-absorbed the ovary. None of them attained full ovarian development although they were kept in the pools for 9-14 days.

TABLE 2. *Details of maturation and spawning of unilaterally eye ablated* Penaeus indicus *kept in maturation pools with pH regulation.*

Sl. No.	Carapace length mm	Date of ablation	Date of spawning	Nature of spawning	No of eggs spawned $\times 10^3$	No. of nauplii hatched $\times 10^3$	Hatching rate
1	34.5	8-2-1982	11-2-1982	Full	106.5	96.4	90.5
2	30.9	—do—	—do—	—do—	86.4	82.2	95.1
3	31.5	—do—	—do—	—do—	98.5	71.7	72.8
4	30.5	—do—	—do—	—do—	71.5	69.2	96.8
5	30.2	—do—	—do—	—do—	68.4	60.5	88.5
6	31.5	—do—	—do—	Partial	66.1	59.1	89.4
7	32.0	—do—	12-2-1982	Full	88.1	86.4	98.0
8	31.8	—do—	—do—	—do—	96.1	92.5	96.3
9	31.0	1-3-1982	4-3-1982	Full	64.0	48.0	75.0
10	37.0	—do—	—do—	—do—	116.2	114.6	98.6
11	31.0	—do—	—do—	—do—	65.3	63.8	97.7
12	31.0	—do—	—do—	—do—	93.1	90.0	96.8
13	31.0	—do—	5-3-1982	—do—	82.5	80.9	98.0
14	31.0	—do—	—do—	—do—	45.7	43.0	94.1
15	30.0	10-3-1982	13-3-1982	Full	51.8	44.9	86.7
16	32.0	—do—	—do—	—do—	52.7	44.8	85.0
17	30.0	—do—	—do—	—do—	70.1	66.5	94.8
18	30.0	—do—	—do—	—do—	71.3	69.1	96.9
19	30.5	—do—	—do—	Partial	61.0	59.2	97.0

These results clearly established the importance of pH as a factor influencing the maturation of *P. indicus*. Maturation was rapid in the pools where the pH was not allowed to fall below 7.95. It appears that a pH range of 8.0-8.2 is conducive to the development of the ovary.

It may be noted that the males were superfluous, as the eye-ablated females treated in the experiments were already impregnated. They too were included only to ensure impregnation of any female that might moult during the course of the experiments, which, however, did not happen.

Discussion

The results obtained in these experiments point to the importance of environmental pH in ovarian development of female *P. indicus*. In nature *P. indicus* matures and spawns only in the sea, where the pH is more or less steady at 8.2. Although the juveniles reach adult size in brackishwater grow-out ponds, here the females do not attain maturity. This may be due, among other things, to the highly fluctuating pH of the grow-out ponds, where the pH has been found to vary from 6.5 in the early mornings to 9.5 in the afternoons, especially when phytoplankton blooms occur (personal observation). But the males do not appear to be affected by these pond conditions as they attain maturity and even mate with the immature females in the pond.

In closed recirculating seawater systems the decline in pH is one of the consequences of the oxidation of ammonia (excreted by the cultured animals) to nitrite and nitrate by the nitrifying bacteria growing in the biological filter (Wickins 1976). Oxidation of 1 mg of NH_4-N to nitrate produces 0.14 mg of H^+. Therefore, the greater the amount of ammonia oxidized the greater the increase in hydrogen-ion concentration and the lower the pH of the medium. Wickins (1976) also pointed out that nitrification led to loss of inorganic carbon from recirculated water and that this could affect the moulting process in prawns. The addition of Na_2CO_3 daily to the water during the present experiments, apart from compensating the decline in pH, might also have maintained the inorganic carbon level in the maturation pools. But the inorganic carbon was not measured during this study.

In the flow-through systems like the ones used by Aquacop (1975) and Primavera et al (1982) the pH would not decline provided the flow-through rate is high enough. In maturation pools, with closed recirculation of seawater, an automatic pH controlling device will be very useful in maintaing a steady pH.

It is clear from our experiments that the pH level of 8.0-8.2 is helpful for the development of the ovary in female *P. indicus*. But how exactly the pH of the environment influences the maturation process is not known. Whether the

pH of the external medium affects the hydrogen-ion concentration of the haemolymph which in turn influences the physiological processes leading to vitellogenesis needs to be investigated.

ACKNOWLEDGEMENTS

We are grateful to Dr. E. G. Silas, Director, and Dr. M. J. George, Head of the Division of Crustacea, Central Marine Fisheries Research Institute, for their encouragement and guidance in the preparation of this paper.

REFERENCES

AQUACOP. 1975 Maturation and spawning in captivity of penaeid prawns *Penaeus merguiensis* de Man, *Penaeus japonicus* Bate, *Penaeus aztecus* Ives, *Metapenaeus ensis* de Haan and *Penaeus semisulcatus* de Haan. *Proc. 6th Annual Workshop, World Mariculture Society*, 6: 126-132.

MUTHU, M. S. AND A. LAXMINARAYANA. 1980. Induced maturation and spawning of Indian penaeid prawns. *Indian J. Fish.*, 24 (1 & 2): 172-180.

PRIMAVERA, J A., T. YOUNG AND C. D. DE LOSREYES. 1982. Survival, maturation, fecundity and hatching rate of unablated and ablated *Penaeus indicus* H. M. Edwards from brackishwater ponds. *Proc. Symp Coastal Aquaculture*, 1: 48-54.

SOLORZANO, L. 1969. Determination of ammonia in natural waters by the phenol hypochloric method. *Limnology and Oceanography*, 14(5): 799-801.

STRICKLAND, J. D. H. AND T. R. PARSONS. 1968. A practical handbook of seawater analysis. *Bull. Fish. Res. Bd. Canada*, 167: 311 pp.

WICKINS, J. F. 1976. The tolerance of warm water prawns to recirculated water. *Aquaculture*, 9: 19-37.