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PH AS A FACTOR INFLUENCING MATURATION AND SPAWNING OF THE PRAWN PENAEUS JAPONICUS (Bate)

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ABSTRACT

Unilateral eyestalk extirpation in females of ***Penaeus japonicus*** *(Bate) is sufficient to induce maturation and spawning in 3-5 days time when the pH of the maturation tanks was maintained at a level similar to that of the sea water (between 8.2 and 8.4) by daily addition of 1N sodium carbonate. But eye-ablated females kept in tanks where the pH was allowed to fall below 7.9 did not attain full ovarian development and no spawning was obtained.*

INTRODUCTION

Maturation through eyestalk ablation has been induced successfully in many penaeid prawn species by keeping the individuals, after ablation, either in the sea or under condition similar to those in nature. Halder (1978) induced maturity in ***Penaeus monodon*** by eyestalk ablation, but failed to obtain viable eggs. In the same species, Santiago, (1977) induced maturation and breeding of pond-reared specimens by using the eyestalk ablation technique in marine ponds, and obtained viable eggs, while Primavera (1977) went further and successfully induced maturity and spawning in a land-based tank containing filtered sea water.

The observations of Adiyodi and Adiyodi (1970) and Abd El- Hamid (1990), on precocious maturation and moulting in destalked decapoda led to the development of the eyestalk ablation technique for the induction of gonadal maturation and spawning in penaeid prawns under controlled conditions.

It has also been established that sites in the eyestalk glands of prawns, carbs and other decapod crustaceans (the X-organ and the sinus gland) store two hormones (the gonad inhibiting hormone and the moult inhibiting hormone). Removal or destruction of the eyestalk eliminates these inhibitors and leads to moulting and maturation, according to the stage of the moult cycle at which ablation was performed. Under natural conditions, a decrease in the gonad-inhibiting hormone is triggered by certain environmental factors, which need to be thoroughly understood.

Muthu and Laxminarayana (1980) pointed out that, when the pH of the maturation tanks declined below 7.9, the developing ovaries of eye-ablated prawns of ***Penaeus indicus*** regressed, indicating that pH of the medium could be an important factor influencing the maturation process.

Parado-Estepa ***et al.*** (1990) found that pH range of 7.3-8.5 is suitable for shrimp hatchery, while Sultan Noor Hamid ***et al.*** (1994) suggested that pH must be narrowed to 7.0-8.0 to induce maturation and spawning in the prawn ***Penaeus monodon.***

The aim of the present study is to investigate the effect of pH on maturation and spawning in ***Penaeus japonicus*** (Bate) after unilateral eyestalk ablation.

MATERIALS AND METHODS

Healthy adult of both sexes of ***Penaeus japonicus*** (Bate) larger than 130 mm in total length were collected from Abu-Kir Bay (east of Alexandria). After two days of proper acclimation, the females were subjected to unilateral eyestalk ablation by cutting through the flexible membrance at the base of the eyestalk, using sterilized pair of scissors and without cautery. The males were not eye-ablated. The prawns were introduced into glass tanks soon after eyestalk ablation with a small amount of circulating filter sea water created by pumps.

A series of three experiments were conducted. For each experiment, equal number of similar sized prawns were kept in two identical glass tanks, each measuring 30x 50x 70cm. In one tank the pH was not controlled; whereas, in the other tank, the pH was so regulated by adding adequate quantity of 1N

anhydrous Na_2CO_3 (dissolved in tap water), everyday, that the pH of the water one hour after addition of Na_2CO_3 stood at pH 8.4 All the physical and other hydrological parameters were identical in both tanks.

In the first experiment, 10 eye ablated females and an equal number of males were kept in each tank. In the second set, in each tank 8 females and 8 males were kept and in the third set, 6 females and 6 males. The prawns were fed on minced fresh prawn of the rate about 10 % of their body weight. The uneaten food and faecal matter were siphones out every day. Three groups of prawns were prepared under the same conditions [temperature (27-32°C), salinity (32.5-37.5 per mille)], and no artificial illumination was provided.

For specimen, length, weight, stage of maturity and number of moulting were determined. In addition temperature, salinity, pH, dissolved 0_2, total ammonia, NO_2 of sea water in the glass tanks were monitored every day. The pH was measured in the regulated tank befor and one hour after the addition of Na_2CO_3. Total ammonia and NO_2 were estimated using the methods of Solor zano (1969) 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 sea water in the tank was completely changed and a fresh set of specimens introduced for the next experiment. The tank in which pH was regulated during the first experiment was used as the control tank without pH regulation in the second experiment and vice versa. In the third experiment, pH treatment in the tanks was again reversed. This was done to eliminate any intrinsic dvantage that one tank 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 and placed individually in glass tanks containing clear, filtered sea water and provided with good aeration. Spawning took place during night and 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 glass tanks were identical except for the difference in pH. In the tank where pH was not regulated the value of pH steadily declined from the initial value of pH 8.4 to 7.4 in the course of 10-12 days, due to the metabolic processes taking place in the tank. In the pH -regulated tanks the value of pH declined slightly to 8.0 in the mornings but was increased to pH 8.4 by daily addition of Na_2CO_3. The total ammonia and NO_2 levels in the tanks 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 tanks ranged from 27-32°C and the salinity from 32.0 to 37.5 ppt. The dissolved O_2 levels remained high, around 6.5 mg/L.

Penaeus japonicus in the unilateral ablated groups started to moult earlier than intact animals. Nearly all eyestalkless prawn had moulted before the control animals began to moult. Duration between 1st and 2nd ecdysis post treatment was shorter in treated groups than in the intact group. Further, this parameter is significantly different between all treated groups specially with pH regulated once. The efficacy in accelerating moulting and maturation of the ovaries were in the order of eyestalk ablation and regulated the pH at 8.4. The duration between the first and second ecdysis after treatment was shown in Table (1). The moult cycle duration of the intact shrimp was longer than that of treated animals.

In the pH-regulated tanks all eye-ablated mature females, in all the three experiments, ripeness and spwned viable eggs 3-5 days after eyestalk extirpation (Table 2 and 3). The 24 females produced a total of 2,152,200 eggs, i.e., 89,675 eggs per female. The average number of nauplii produced per female was 76.928 giving hatching rate of 84.3 %. The nauplii healthy and were reared up to the postlarval stage.

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

Table (1): Number of days to reach the first ecdysis after treatment and the duration between the first and second ecdysis post treatment in three groups of ***Penaeus japonicus*** (Bate).

	Control	***Eyestalk ablated***	***pH regulated without ablation***	***with ablation***
Days moulted after treatment	10.0±2.1 (20)	6.5±1.0*** (20)	8.0±1.6** (20)	5.3±1.1**** (20)
Duration between 1st & 2nd ecdysis post treatment	13.2±2.3+ (15)	9.0±1.4++ (15)	10.0±1.6++ (15)	7.0±1.2++ (15)

Values are presented as mean ± S.D. and between brackets the no. of individuals. Within the same row, different no. of * or + are significantly different ($P < 0.05$; student's t-test).

These results clearly established the importance of pH as a factor influencing the maturation of ***Penaeus japonicus***. Maturation was rapid in the glass tanks where the pH was not allowed to fall below 8.0. It appears that a pH range of 8.2-8.4 is conductive to the development of the ovary.

DISCUSSION

The results obtained in these experiments point to the importance of environmental pH in ovarian development of eyestalkless female ***Penaeus japonicus.***

Sreenivasula (1990), reported that, the decline in pH is one of the consequences of the oxidation of ammonia (excreted by the cultured animals) to nitrate and nitrite by the nitrifying bacteria growing in the biological filter.

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. Michele (1987) 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, a part from compensating the decline in pH, might also have maintained the inorganic carbon level in the tanks. But the inorganic carbon was not measured during this study.

In the flow-through systems like the ones used by Aquacop (1975); Primavera *et al.* (1982) and Muthu *et al.* (1984) the pH would not decline provided the flow-through rate is high enough. In tanks, an automatic pH controlling device will be very usefull in maintaining a steady pH.

Table (2): Summary of experiments to show the influence of pH regulation on maturation and spawning of sys-ablated *Penaeus japonicus*.

Exp.	*Duration of Exp.*	*With pH regulation*			*Without pH regulation*		
		Size range of o used total length in mm	No. of ablated females	No. of females that spawned viable eggs	Size range of o used total length in mm	No. of ablated females	No. of females that spawned viable eggs
1	10-1-1993 to 20-1-1993	130.0 to 135.0	10	10	102.0 to 106.0	10	nil
2	10-2-1993 to 20-2-1993	140.0 to 145.0	8	8	110.0 to 117.0	8	nil
3	10-3-1993 to 20-3-1933	150.0 to 155.0	6	6	122.0 to 126.0	6	nil

Table (3): Details of maturation and spawning of unilaterally eye-ablated *Penaeus japonicus* kept in glass tanks with pH regulation.

Exp No.	Total length mm	Date of ablation	Date of spawning	Nature of spawning	No. of eggs spawned X 10^3	No. of nauplii hatched X 10^3	Hatching rate
I	130.0	10-1-1993	13-1-1993	full	110.7	101.1	91.3
	131.5	do	do	do	96.2	83.6	86.9
	133.0	do	14-1-1993	do	90.3	77.4	85.7
	135.1	do	do	partial	65.6	47.4	72.3
	134.7	13-1-1993	18-1-1993	do	70.2	52.9	75.3
	130.0	do	do	full	72.3	57.8	79.9
	132.6	do	do	do	100.1	82.6	82.5
	130.1	5-1-1993	20-1-1993	do	106.5	94.1	88.3
	134.9	do	do	partial	62.3	37.9	60.9
	135.2	do	do	full	96.3	89.1	92.5
II	145.0	10-2-1993	14-2-1993	full	110.6	102.9	93.1
	140.2	do	do	do	91.2	82.5	90.5
	143.0	do	do	do	96.3	88.2	91.6
	144.8	do	15-2-1993	do	103.2	96.4	93.6
	140.8	do	do	partial	60.5	47.9	79.2
	141.2	16-2-1993	20-2-1993	do	83.6	63.5	75.9
	144.0	do	do	full	93.1	82.6	88.7
	143.2	do	do	full	90.0	79.2	87.1
III	155.0	do	do	full	90.9	79.2	87.1
	153.2	10-3-1993	14-3-1993	full	114.6	111.5	97.3
	151.0	do	15-3-1993	partial	61.2	49.8	81.5
	155.0	16-3-1993	19-3-1993	do	67.6	49.41	73.1
	153.1	do	do	full	102.5	88.4	86.2
	154.0	do	20-3-1993	do	106.1	94.7	89.3
		do	do	do	100.3	81.4	81.2

Different periods have been reported for the attainment of full maturity and spawning after eyestalk ablation and regulated pH at 8.4. Some females in this study completed maturation and spawning within 3-5 days after treatment. According to Primavera (1977) observed that complete maturation and spawning were attained in some female of ***Penaeus monodon*** require 12 days after ablation.

Unilateral eyestalk ablation is sufficient to induce maturation and spawning whereas pH level of 8.2-8.4 and ablation of eyestalk is helpful for the acceleration of development the ovary in female ***Penaeus japonicus***.

A higher salinity and temperature are usually more favourable for inducing maturity. Alikunhi et al. (1975) achieved maturation of ***Penaeus monodon*** at a salinity of 24-31 per mille and temperature of 27.7-30.5°C, while Primavera (1977) found salinity of 30-34 % to be more conductive to maturation and spawning of the same species. In the present investigation, it is observed that the salinity of 32.5-37.5 per mille a temperature of 27-32°C and pH at 8.2-8.4 were more suitable for maturation and spawning of the prawn ***Penaeus japonicus*** (Bate).

It is clear from the present experiment that the pH level of 8.2-8.4 is helpful for the development of the ovary in female ***Penaeus japonicus***. 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.

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