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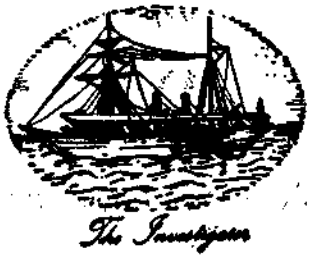

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ON CONTROLLED SPAWNING OF INDIAN PEARL OYSTER *PINCTADA FUCATA* (GOULD)

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ABSTRACT

Induced spawning of pearl oysters serves a dual purpose in pearl culture. Besides providing the gametes for the hatchery production of seed, it conditions the oyster for nucleus insertion for the production of cultured pearls. Experiments were conducted on induction of spawning in *Pinctada fucata* using hydrogen peroxide, Tris buffer, sodium hydroxide and combinations of hydrogen peroxide+Tris/NaOH. In other experiments ammonium hydroxide (N/10) was injected into the adductor muscle or foot of the oyster. Thermal stimulation was also attempted.

Spawning response to H_2O_2 treatment was not quite satisfactory. Concentrations of 3-6 mM peroxide was found to evoke some response. Hydrogen peroxide in alkaline medium using Tris gave slightly better results. Tris-buffered sea water with a pH of 9.0 by itself was found to induce 78.6% of the pearl oysters to spawn. Similarly, the alkaline sea water medium with NaOH stimulated spawning in 68.4% of the oysters at pH 9.5. Injections of 0.2 ml of N/10 NH_4OH resulted in the spawning of 48.1% of the treated oysters. Thermal stimulation by raising the sea water temperature from 28.5°C to 35.0°C, gave good results on the occasion when 87.5% of the oysters spawned. But in several other experiments the response was either nil or poor. The present study has indicated that an alkaline sea water medium (pH 9.0-9.5) would be useful for the controlled spawning of the Indian pearl oyster.

INTRODUCTION

MANAGEMENT of reproduction is one of the facets of aquaculture and it serves the primary need of providing seed for stocking the farms. Great strides have been made in controlled spawning of molluscs in shellfish hatcheries. Loosanoff and Davis (1963), Loosanoff (1971) and Ino (1972) have reviewed the methods employed for out-of-season spawning of molluscs. The commonest technique used for inducing spawning is conditioning the molluscs for accelerated development of gonad through thermal stimulation and spawning them by a quick rise in temperature to the optimum level and adding egg or sperm suspension (Loosanoff and Davis, 1963). This method has been particularly successful for species in the subtropical and temperate regions.

The Japanese workers have mostly relied on chemical stimulation for spawning the molluscs. The methods include spawning the animals in ammoniated sea water (Wada, 1942 ; Sagara, 1958 a) and injection of neutral potassium salts (Iwata, 1948 a, b) or ammonium hydroxide (Sagara, 1958 b). Stripping the gonad and treating the eggs with a weak solution of ammonium hydroxide has also given good results in many cases (Loosanoff and Davis, 1963).

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Methods such as giving a mild electric shock (Iwata, 1950) and pricking or severing the adductor muscle (Loosanoff and Davis, 1963) have proved useful for spawning the mussels. A more recent technique which has been successful in the case of a variety of molluscs is the addition of hydrogen peroxide to alkaline sea water (Morse *et al.*, 1976, 1977, 1978).

Induced spawning of pearl oyster serves a dual purpose in pearl culture. Besides providing the gametes for the hatchery production of seed, it conditions the oysters for nucleus insertion for the production of cultured pearls. The best site in the body of the pearl insertion of nucleus is the gonad and when a gonad contains eggs or sperms it is difficult to obtain good results (Mizumoto, 1979). Therefore, the pearl culturists have invented the method of forced ovulation called 'egg extraction' in order to produce large sized good quality pearls (Alagarswami, 1970, Wada, 1973). Wada (1947), Kobayashi and Yuki (1952) and Yamaguchi (1957) have worked on artificial breeding of *Pinctada martensii*, Wada (1942) on *P. maxima* and Setoguchi (1959) on *P. margaritifera*. In view of this importance, experiments were carried out on controlled spawning of the Indian pearl oyster *Pinctada fucata*, using some of the established techniques during August-September 1979 and the results are presented in the paper.

The authors are grateful to Dr. E. G. Silas, Director, Central Marine Fisheries Research Institute, Cochin for the encouragement given to this work.

Material and Methods

Pearl oysters brought from the pearl culture farm were examined for the maturity condition of the gonad. Oysters with fully ripe gonads were selected for the experiments on induction of spawning. They were kept in glass vessels containing 6 or 3 litres of the experimental solutions. Aeration was given only after the oysters were transferred from the experimental solutions to fresh sea water. All the experiments were conducted under ambient temperature conditions.

Experiments were conducted on induction of spawning using hydrogen peroxide, Tris-buffer, sodium hydroxide and combinations of hydrogen peroxide+Tris-buffer/sodium hydroxide. Ammonium hydroxide was injected into the adductor musle and foot of the oysters. The experimental procedures are given under the respective sections.

Results

Spontaneous spawning of pearl oysters

Pinctada fucata has been observed to spawn spontaneously in the laboratory. Usually it happened with a change of sea water in the vessels containing oysters for some time. In some instances, when oysters brought from the farm were cleaned and placed in troughs and sea water which had been kept standing in carboys was added, spawning occurred. On a few occasions, during November-December, pearl oysters collected from the natural beds (depth 15m), when brought up and immediately transferred to a vessel containing sea water drawn from the surface of the sea, spawned vigorously. In all such cases of spontaneous spawning, change of fresh sea water drawn from a source which is different from the one the oysters were living prior to the change, has brought about spawning. Although the factors responsible for inducing the oysters to spawn have not yet been identified, it appears that differences in density and temperature, or physiological stress might trigger the spawning act.

During the period 1973-79, spontaneous spawning was observed on 23 occasions: January-3, February-1, June-1, July-3, August-4, September-2, October-1, November-3 and December-5. Even with these limited data,

it is found that the frequency of spawning is more during November-January, which is about the Northeast monsoon period, and July-August, the period of southwest monsoon.

Experiments on induced spawning

Effect of hydrogen peroxide : Morse *et al.* (1976, 1977, 1978) have originally developed the technique of inducing spawning in abalone, mussel, scallop and the mangrove oyster by the addition of hydrogen peroxide to sea water, which is normal or alkaline. The technique was applied on the pearl oyster.

From 30% (weight) stock solution of hydrogen peroxide (guaranteed reagent grade ; stored at 0-4°C), a 6% solution was prepared. The experiments were carried out at concentrations of 1.532, 3.064 and 6.128 milliomolars (mM) prepared by adding 6.25 ml, 12.5 ml and 25.0 ml of 6% H_2O_2 solution respectively to 6 litres of fresh sea water. Selected oysters were kept in the laboratory for accilimatisation for at least 12 hours before using them in the experiments. Since it was difficult to distinguish males and females based on external appearance of the gonad, no attempt was made to sex them prior to experiments.

Sea water of identical quality was used both for acclimatisation and for the experiments so that the probability of factors such as temperature, salinity and pH influencing the animals was ruled out. The oysters were kept in the experimental medium for pre-determined durations at the end of which the solution was siphoned out and fresh sea water was added without disturbing the animals. The above procedure was common for all the experiments described in the paper except for the one on injection of NH_4OH.

Five experiments were conducted on different dates involving a total number of 111 oysters in the H_2O_2 medium and 37 oysters in the controls. The pooled results are given in Table 1.

TABLE 1. *H_2O_2 induction of spawning in the pearl oyster* Pinctada fucata

Treatment	No. of oysters		
	Tested	Spawned	
		Male	Female
H_2O, pH 8.1-8.2 (control)	37	0	0
H_2O_2, 1.532 mM ..	37	0	0
H_2O_2, 3.064 mM ..	37	3	1
H_2O_2, 6.128 mM ..	37	2	2

The temperature of sea water ranged 26.5°-29.0°C for all the five experiments and the difference within each experiment varied from 0.4° to 1.1°C. The immersion time in H_2O_2 was 2½ hr in two experiments 4 hr in one and 8 hr in two. Spawning took place from 2 hr 40 min to 3 hr 03 min after immersion in H_2O_2 in the treatments of 2½ hr and from 5 hr 52 min to 6 hr 05 min in the treatment of 5 hr duration. Spawning was observed always after the change to fresh sea water.

Effect of Tris buffer

Tris (Hydroxymethyl)-Aminomethane was used to increase the pH of sea water. The buffer was slowly added to fresh sea water reading the pH. Sea water with pH values of 8.5, 9.0, 9.5 and 10.0 was used as experimental solution. Normal sea water with a pH of 8.1-8.2 was used for control. A total of 94 oysters were used in Tris solution and 27 as controls in three sets of experiments. The pooled data are given in Table 2.

TABLE 2. *Tris induction of spawning in pearl oyster* Pinctada fucata

Treatment	No. of oysters		
	Tested	Spawned	
		Male	Female
H_2O, pH 8.10-8.25 (control)	27	0	0
Tris, pH 8.5 ..	28	0	0
Tris, pH 9.0 ..	28	4	18
Tris, pH 9.5 ..	28	9	2
Tris, pH 10.0 ..	10	2	0

The duration of immersion in Tris solution was 3 hr in one experiment and 4 hr in the other two. The temperature for all the experiments ranged 26.9°-28.9°C, but within each experiment the difference was between 0.4°-1.2°C. There was no spawning in the controls. Experiment with pH 10 was conducted only once and 2 males spawned very mildly. Satisfactory results were obtained in pH 9.0 and 9.5. In most cases (22 oysters out of 35 spawned) profuse spawning took place in the Tris medium itself and in some animals, at pH 9, spawning occurred after changing the medium to fresh sea water. The spawning response was observed within one hour after immersion in 15 oysters, between 1-2 hr in 5 oysters and after 2 hr 10 min in the remaining 2 oysters. Thirteen oysters spawned after changing to fresh sea water and the response came from 3 hr 15 min to 4 hr 44 min after the time of the immersion in Tris.

Effect of alkali (NaOH)

By dissolving pure pellets of sodium hydroxide in sea water, solutions having a pH of 8.5, 9.0, 9.5 and 10.0 were prepared. The solution turned rather opaque at the two higher concentrations. Controls, using normal sea water, had a pH of 8.0 and 8.1. A total of 67 oysters were used in the alkaline medium and 20 as controls in two experiments. The combined data of the two experiments are given in Table 3.

TABLE 3. *NaOH induction of spawning in pearl oyster* Pinctada fucata

Treatment	No. of oysters		
	Tested	Spawned	
		Male	Female
H_2O, pH 8.0-8.1 (Control)	20	0	0
NaOH, pH 8.5 ..	19	0	0
NaOH, pH 9.0 ..	19	0	0
NaOH, pH 9.5 ..	19	6	7
NaOH, pH 10.0 ..	10	0	0

The immersion time was 3 hr in one experiment and 4 hr in the other. The temperature range was from 26.9° to 27.8°C. In both the experiments spawning occurred only at pH 9.5. Of the 13 oysters, 9 spawned in the NaOH medium itself, 8 of them between 1-2 hr and one 2 hr 58 min after immersion. The 4 oysters which spawned in fresh sea water after the change responded from 3 hr 27 min to 5 hr 03 min after introduction to the alkaline medium.

Effect of H_2O_2 in alkaline medium (H_2O_2 + Tris)

Morse *et al.* (1978) reported that addition of Tris buffer, though not essential for induction of spawning, acts to increase the proportion of animals that will spawn in response to a given concentration of peroxide. They found that Tris at pH 9.1 was effective in the case of abalones. Two experiments were conducted on the pearl oyster to ascertain the spawning response in H_2O_2 buffered by Tris to pH 9.1. The experiments were carried out concurrent to those on H_2O_2 induction. Animals in Tris solution at pH 9.1 formed controls. The results are presented in Table 4.

TABLE 4. *Induction of spawning in pearl oyster* Pinctada fucata *by H_2O_2 in alkaline medium of Tris*

Treatment	No. of oysters		
	Tested	Spawned	
		Male	Female
Tris, pH 9.1 (control)	16	0	1
Tris, pH 9.1 + H_2O_2, 1.532 mM	16	0	0
Tris, pH 9.1 + H_2O_2, 3.064 mM	16	3	7
Tris, pH 9.1 + H_2O_2, 6.128 mM	16	0	0

Spawning occurred only at 3.064 mM concentration of peroxide in both the experiments, after the oysters were changed to fresh sea water at the end of 4 hr of treatment. Spawning response came actually 6½ to 8 hr after the

immersion in the experimental medium. One of the oysters in the control spawned mildly.

Effect of H_2O_2 in alkaline medium ($H_2O_2 + NaOH$)

Morse *et al.* (1978) found that use of sodium hydroxide to adjust the sea water to pH 9.1 was apparently as effective as Tris in facilitating induction of spawning in the abalones by hydrogen peroxide. This was tested on the pearl oyster in three sets of experiments carried out concurrently with those on H_2O_2 induction. The pH was adjusted to 9.0 by the addition of NaOH pellets. The results are presented in Table 5.

TABLE 5. *Induction of spawning in pearl oyster* Pinctada fucata *by H_2O_2 in alkaline medium of NaOH*

Treatment	No. of oysters		
	Tested	Spawned	
		Male	Female
NaOH, pH 9.0 (control) ..	21	5	5
NaOH, pH 9.0+H_2O_2, 1.532 mM	21	0	0
NaOH, pH 9.0+H_2O_2, 3.064 mM	21	0	0
NaOH, pH 9.0+H_2O_2, 6.128 mM	21	2	0

The duration of immersion was 2 hr 30 min in two experiments and 5 hr in the third. At 6.128 mM concentration mild spawning occurred in two males on changing to fresh sea water, 2 hr 35 min after immersion in the solution. The oysters did not spawn in other concentrations. On the other hand, spawning was observed in the controls in all the three experiments.

Effect of injection of ammonium hydroxide

A dilute solution of 0.1 normal NH_4OH was prepared from a stock solution and a total of 47 pearl oysters were treated with injections of 0.1, 0.2 or 0.3 ml of the dilute solution. Controls were kept without injection. Ten oysters each which were given 0.1 ml and 0.3 ml of ammoniated sea water did not show any spawning response. Among 27 oysters which were injected with 0.2 ml solution, 13 spawned profusely. In one experiment, when the injection was given at the base at the foot, all the seven oysters spawned profusely. In another, injection administered in the adductor muscle stimulated all the five oysters to spawn. Oysters of the control did not spawn on either occasion.

DISCUSSION

Morse *et al.* (1978) indicated that spawning in molluscs may result from a peroxide-induced stimulation of the endogenous enzymatic synthesis of potent hormone-like prostaglandin molecules. They found that the alkaline medium, though not essential for the induction of spawning, increases the proportion of animals that will spawn in response to a given concentration of hydrogen peroxide. Whereas they succeeded in spawning molluscs with hydrogen peroxide, they did not with several other oxidising agents (Morse *et al.*, 1976). The results on H_2O_2 induction presented in Table 1 indicate poor response of *Pinctada fucata* to this treatment. While Morse *et al.* (1976) have obtained spawning in 30 abalones out of 31 and in all the 6 groups of mussels in the concentration of 5 mM of H_2O_2, in *P. fucata* spawning was observed only in 4 specimens each, out of 37, in the concentrations of 3.064 mM and 6.128 mM. Spawning took place, as observed by Morse *et al.*, after replacing the medium with fresh sea water and within 3 hr ± 30 min after the first addition of H_2O_2 in the experiments where the oysters were treated for 2½ hr.

Testing in the alkaline medium (pH 9.1) of H_2O_2, using Tris (Hydroxymethyl)—Aminomethane, the result was better at 3.064 mM

concentration with 10 out of 16 oysters responding (Table 4). In other two concentrations (1.532 mM, 6.128 mM) there was no spawning. Spawning response in the alkaline medium (pH 9.0) of H_2O_2, using NaOH, was not satisfactory as only 2 oysters out of 21 spawned in 6.128 mM solutions, with no induction in the other two concentrations (Table 5). On the other hand, 10 oysters from among 21 spawned in the controls. Although Morse *et al.* (1978) have not given the percentage of animals spawning in the alkaline medium (using Tris as well as NaOH), it has been stated that alkalinity promotes both the peroxide activation and the induction of spawning.

Morse *et al.* (1978) found 2-4 mM peroxide optimal for spawning abalones, mussels, scallop and the mangrove oyster. The present study would show that concentrations between 3-6 mM can induce spawning in *Pinctada fucata*. Although spawning induction has been observed the percentage of response is relatively low. Perhaps some more experiments could decide the usefulness of this technique for large-scale spawning of pearl oysters. Peroxide induction has been found to be better in the alkaline medium of Tris. However, Tris by itself has been found to give superior results.

Alkaline sea water has been widely used by the Japanese workers for inducing spawning and artificial fertilisation in pearl oysters. Kobayashi (1948) found a pH of 8.6 optimum for the artificial fertilisation of the eggs of the Japanese pearl oyster *Pinctada martensii* (= *P. fucata*) and he was unable to obtain fertilisation under lower pH conditions. Kobayashi and Yuki (1960) found that the rate of fertilisation reached almost 100 per cent by increasing the pH from 8.3 to 8.6 by adding ammoniated sea water. Wada (1942) induced spawning in *P. maxima* by half per cent 0.1 normal NH_4OH sea water. Wada (1947) obtained artificial fertilisation of *P. martensii* by application of 1/10,000 to 1/1000 normal ammoniated sea water. Setoguchi (1959) got good results in *P. margaritifera* using 1.2-1.5% 0.1 normal NH_4OH sea water. Setoguchi (1957, 1958) reported that a pH of 8.7-8.9 was effective in inducing spawning of *Pteria macroptera* which is another species used in pearl culture. In the present study, alkaline sea water was prepared with Tris-buffer and sodium hydroxide and the spawning trials were carried out in pH 8.5, 9.0, 9.5 and 10.0. With Tris, 78.6% of the oysters spawned at pH 9.0, 39.3% at pH 9.5 and 20.0% at pH 10.0 and none in controls with pH 8.10-8.25 (Table 2). With NaOH, 68.4% of the oysters spawned at pH 9.5 (Table 3) and 47.6% at pH 9.0 (Table 5). Unlike with H_2O_2, profuse spawning takes place in the alkaline sea water itself in the case of Tris as well as NaOH. The spawning response in Tris is relatively higher than in NaOH. In both cases, pH 9.0-9.5 appears optimal for induced spawning. While pH 8.5 evokes no response, pH 10.0 with Tris has triggered spawning in 20% of the oysters. The results obtained in the present experiments would show that higher alkaline condition (pH 9.0-9.5) is even a better medium than hydrogen peroxide for inducing the pearl oysters to spawn.

Sagara (1958 b) has induced spawning in the clam *Meretrix* by injection of NH_4OH 1/20 N in the gonad. Iwata (1948 a, b) has succeeded in spawning several species of clams by injection of 2 ml of neutral potassium salt solutions into the visceral cavity. In the present study injection of 0.2 ml of N/10 NH_4OH administered in the foot or the adductor muscle has resulted in the spawning of 48.1% of the oysters treated. The spawning response of pearl oysters for NH_4OH injection has been proved. Further experiments are needed to determine the optimum concentration of the ammoniated sea water required for achieving higher spawning rates.

As in the case of edible oysters and clams, some success has been achieved in thermal

stimulation of spawning in the pearl oyster. Kuwatani *et al.* (1974) observed mature and spawned individuals in tanks with circulating sea water of higher temperature (26° and 30°C) and suggested that spawning might be induced by this method in winter. Wada (1976) induced spawning in the pearl oyster by raising the temperature from 25°C to 30°C and found that more than 700-800 degree-days would be required for the gonads to mature in the Ago Bay. During the course of the experiments reported here, six trials were carried out to spawn the pearl oyster under thermal stimulation. While the spawning response was nil in three and poor in two experiments, good results were obtained in 14 among 16 pearl oysters by raising the temperature from 28.5° to 35.0°C in the sixth experiment. Rao *et al.* (1976) obtained spawning in the mussel *Mytilus viridis* by increasing the temperature from 26.5°-28.0°C to 32.0°-35.0°C. These two results coming from the Indian waters might suggest, although the data are admittedly inadequate for attempting any generalisation, that in the tropical species of molluscs thermal stimulation for artificial spawning may require raising the temperature to a higher level such as 35°C or about 7°C above the ambient temperature.

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