OBSERVATIONS ON THE PROBABLE EFFECTS OF SALINITY ON THE SPAWNING, DEVELOPMENT AND SETTING OF THE INDIAN BACKWATER OYSTER, *OSTREA MADRASENSIS* PRESTON*

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1. INTRODUCTION

THE salinity of sea-water, like its temperature, is one of the abiotic factors having a remarkable influence on the development of oysters as is well known from the work of Amemiya (1921, 1926 and 1928), Nelson (1921), Seno, Hori and Kusakabe (1926), Hopkins (1931), Gaarder (1932, 1933), and Gaarder and Bjerkan (1934) on different species of European, Japanese, Portuguese and American origin. Very little scientific information is available on the behaviour of the edible Indian backwater oyster in varying salinities. Hornell (1910 *a*) observed maximum sexual activity in this

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oyster on the east coast rivers and backwaters synchronizing with the heavy rains of October and November, and held that the chief stimulating factor in its spawning was not rise in temperature but a fall in salinity. Hornell (1922) observed also that on the Coromandal Coast there was a spawning maximum in March-April with cases of stray spawning individuals in between; in his view the spawning was induced not only by rise in temperature but also by fall in salinity. Sundar Raj (1930) remarked that "the salinity favoured by the breeding oysters, their eggs, and larvæ was found to range from $8 \cdot 42°/_{oo}$ to $29 \cdot 99°/_{oo}$ with a specific-gravity of $1 \cdot 007$ to $1 \cdot 020$." Panikkar and Aiyar (1939) observed motile sperms in the male oysters of Adyar River in two successive years, *e.g.* (November) 1934 and (October and November) 1935. Paul (1942) found them breeding in the Madras Harbour from April to October which constitute their reproductive period, and, on a minimum scale, during the non-reproductive period also. It will be seen that while Hornell's observations were based on backwater oysters, Paul's were confined to the same species found in the Madras Harbour. No salinity readings appear to have been recorded by either of them. Sunder Raj's statement quoted above lacks details, and the range of salinity stated to have been observed is too wide to be of any significance. The present studies are based on laboratory work and field observations both under marine and estuarine conditions over a continuous period of 18 months from March, 1948 to August, 1949. The diversity of breeding habits of the same species of oyster in two different localities due to direct or indirect effect of variations in salinity is of considerable scientific interest.

2. Material and Methods

Regular supplies of living *Ostrea madrasensis* Preston* were obtained for study at intervals of fifteen days both from the Madras harbour, which presents marine conditions, and the Adyar river-mouth with its adjoining backwaters, which has estuarine and brackish-water conditions. The gonads were examined macroscopically and also microscopically either by the direct observations of smears or by serial sections. Artificial fertilisation was tried to ascertain the fitness of the reproductive elements to undergo development. Records of the salinity and temperature of the waters of

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* The Indian backwater oyster is very variable in form and different authors ascribed it to different species as follows: Hornell (1910 *a*)—*Ostrea cucullata* Born, Preston (1916)—*O. madrasensis* n. sp.; Annandale and Kemp (1916)—*O. virginica* Gmelin; Hornell (1922)—*O. virginiana* Gmelin; Moses (1928)—*O. virginiana* var. *madrasensis* Preston; Winckworth (1931)—*O. arakanensis* Sowerby; Awati and Rai (1931) and Paul (1942)—*O. madrasensis* Preston. In the present state of our knowledge, it would be best to retain the specific name *O. madrasensis* Preston, for the backwater oyster of Madras.

the sea and the estuary were maintained for the entire period of observations. During the spawning season which was ascertained by actual observations on spawning oysters both in the natural habitat and in the laboratory, the gonads of the female oysters were carefully cut open with a sterilised scalpel and the contents pipetted out into a petri-dish were mixed with similarly extracted male gonadic contents to bring about fertilisation by the dry method. For determining the favourable salinity medium for development, this mixture of eggs and sperms was placed in samples of water of varying grades of salinity in separate finger-bowls. The salinity of sea-water filtered several times through a plug of cotton-wool was estimated by titrating it with silver nitrate solution and checked up with standard solution of sodium chloride containing 32·34 gm. per litre. For all lower grades of salinity the sea-water was diluted with measured quantities of distilled water. For preparing grades of salinity higher than that of sea-water two methods were followed: (1) the addition of sodium chloride to fresh sea-water and (2) evaporation of sea-water in sunlight in enamel trays for raising its concentration. In both cases the exact salinity after the treatment was determined by titration with silver nitrate, and suitable grades were prepared by dilution of the medium with distilled water. The results obtained did not materially differ under experimental conditions. The first method was found suitable when a salinity of a desired grade had to be prepared fresh at the time of the experiment, whilst the second had the disadvantage of producing an adverse effect over development by the spoilage of the medium when kept for over a fortnight. The possibilities of the deleterious effect on developing eggs due to the proportions of the chemical constituents of sea-water being disturbed by the addition of sodium chloride were foreseen and the higher grades of salinity prepared in this way were, therefore, checked up with results obtained by the second method. Six drops of the mixture of the eggs and sperms were transferred simultaneously into separate finger-bowls holding mixtures of water of varying grades of salinity. The mixtures were kept cool by the bowls being placed in trays holding water one inch deep and being kept covered by thin moist cotton gauze to minimise effects of evaporation. The embryos and the larvæ developed in the various bowls were, from time to time, examined microscopically. Water in the finger-bowls was changed once in twenty-four hours with a siphon plugged with cotton-wool to prevent escape of the larvæ. Laboratory observations thus made on the developing eggs and larvæ in response to variations in salinity formed a convenient basis for the interpretation of the field observations. Regular plankton collections were obtained throughout the period of observations, from the sea in the vicinity of the harbour and also from the

Adyar river and backwaters to study the frequency of occurrence of the larvæ. Grown-up larvæ of oysters of recognisable shape were picked up under the binocular microscope with a finely pointed pipette and transferred to finger-bowls containing varying grades of salinity of water with a view to determine a medium suitable for the setting of spat. For want of suitable equipment and other facilities no elaborate field observations could be made; it was not possible, for instance, to collect plankton and water samples at different depths to ascertain the vertical distribution of the larvæ and correlate the same with variations in salinity in different strata, if any. All collections of plankton were made by the surface hauls and of the water samples in the closest proximity to the oyster beds. Cultch laid in the backwaters was invariably disturbed by fishermen who frequented the place, and the observations on setting of spat were therefore confined to frequent examination of all submerged objects and heaps of shells placed in the backwaters at suitable places for the larvæ to settle.

3. Salinity and Temperature of Waters of Adyar River and of Madras Harbour

The estuary of the Adyar river[1] with its adjoining backwaters and the Madras Harbour[2] to which the present investigations were confined present typical conditions of varied environments under which the oysters normally thrive. In Adyar the oysters occur on stones close to the mouth of the river, at the base of the southern embankment of the estuary extending upto a distance of about a mile by the side of the Theosophical Society, and also on the boulders of the Elphinstone Bridge and the smaller Bridge near the Adyar cemetery. This estuary, as in the case of nearly all coastal backwaters of the east coast, has no permanent communication with the sea. During the floods of the north-east monsoon the level in the waters rises and communication is established with the sea; and when there is a fall in the level of the waters thereafter, the breakers at the mouth of the river silt up sand to form a 'bar' cutting off the opening into the sea. Thus the opening and the closing of the bar is intermittent; hence fluctuations in salinity are very marked (*vide* Table IV and Fig. 1). In the harbour, the oysters thrive under marine conditions over the boulders, piers, rocks and derelict vessels. The waters of the harbour are always in open communication with the sea.

Salinity Variations in Adyar.—From 13th March 1948 to 6th July 1948 the salinity was steadily rising from 20·32 to 38·47‰ (*vide* Table IV and

[1, 2] Detailed descriptions of the Adyar estuary and the Madras Harbour are given by Panikkar and Aiyar (1937) and Paul (1942).

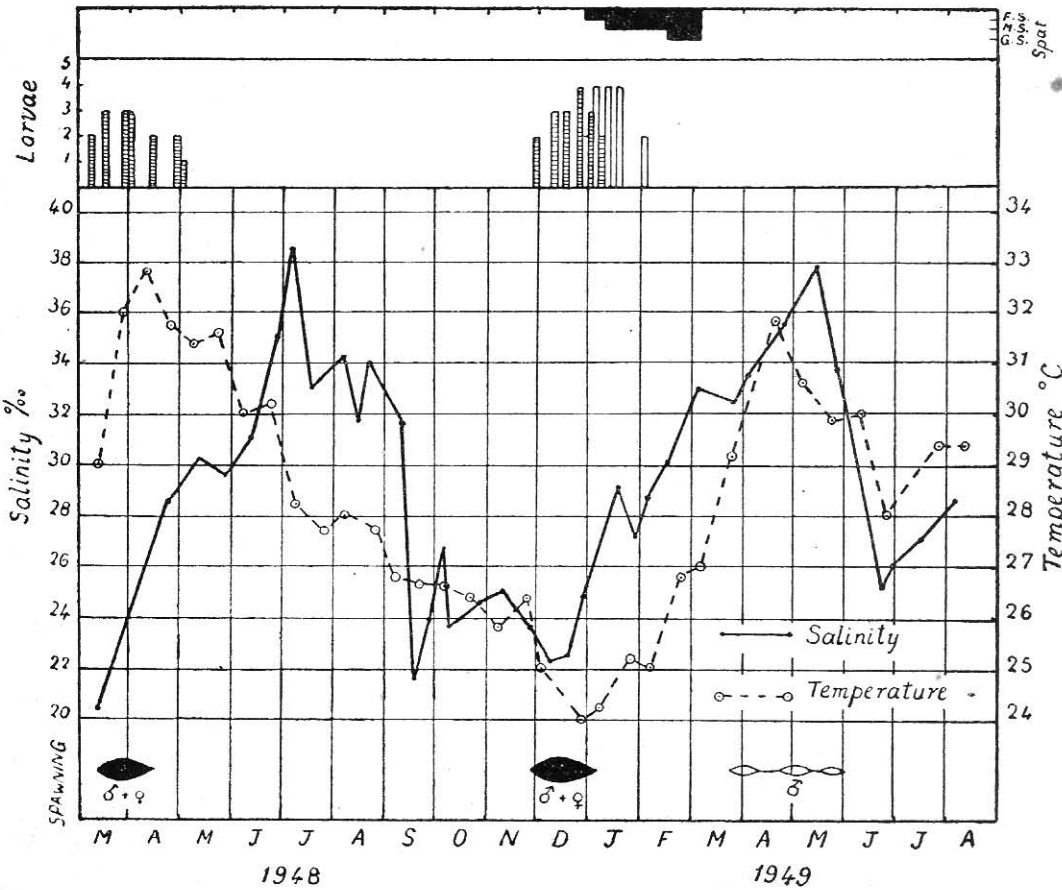

FIG. 1. Periods of spawning and frequency of occurrence of larvæ and spatfall of *Ostrea madrasensis* in relation to variations in salinity and temperature in Adyar estuary. Data presented in Table IV. (Explanation of lettering is as for Table IV: histograms with cross stripes indicate larvæ of young size with straight hinge stage, and those without cross stripes represent larvæ of setting stage.)

Fig. 1). There was a rapid rise in the temperature of the waters of the estuary owing to its shallowness and the consequent rapid evaporation. The loss of water due to evaporation could hardly be made good by the very meagre inflow of river water which also contributed to the bar being closed during this period. The local rainfall was poor.

On the 20th July 1948 the salinity dropped to 33·01‰ and varied thereafter upto 12th September 1948 between 34·43 and 31·74‰. During this period the temperature was falling resulting in lesser evaporation than before, and there was also more river water entering the estuary to reduce the salinity. The bar was still closed.

On the 26th September 1948 there was a sudden drop in salinity of the estuary due to heavy floods in the river. This happens almost every year before communication is established with the sea.

During October, November and December of 1948 the salinity continued to be low, except for a slight rise up to $26 \cdot 8°/_{oo}$ on 11th October 1948 due probably to the establishment of a communication with the sea for a short time which escaped attention.

From 29th December 1948 to the last week of January 1949 the salinity ranged between $24 \cdot 89$ and $29 \cdot 01°/_{oo}$ when the bar was open and the estuary communicated with the sea.

During February 1949 when the communication of the estuary with the sea was intermittent the salinity was $28 \cdot 87°/_{oo}$.

From 5th March 1949 to 14th May 1949 there was a rise in salinity from $33 \cdot 00$ to $37 \cdot 94°/_{oo}$ due to the closure of the bar and the prevailing high temperatures and the consequent rapid evaporation of the water as in the corresponding period of the year 1948.

The fall in salinity recorded in June 1949 was due to heavy local rainfall in the previous month.

The Salinity Variations of the Sea-water in the Vicinity of the Harbour.—For the most part of the year the salinity ranged between $32 \cdot 00$ and $35 \cdot 00°/_{oo}$, except for sharp variations due to rise in temperature in March and April 1948 and April 1949 up to $36 \cdot 97°/_{oo}$ and $37 \cdot 25°/_{oo}$ and $36 \cdot 65°/_{oo}$ respectively, and rapid fall ranging between $30 \cdot 55$ and $24 \cdot 6°/_{oo}$ from the second week of October 1948 to January 1949 due to influx of flood water from land after the monsoon rains.

Water Temperatures in Adyar.—The highest maximum temperature of the waters recorded from the oyster beds in Adyar was 32·8° C. in April 1948 and the lowest 24·2° C. in December 1948. In general high temperatures about 30 to 33° C. prevailed in April, May and June, and somewhat lower temperatures of 28° to 29° C. in July and August. During December and January the temperature readings were the lowest, ranging between 25 to 24° C., and by the end of February there was a tendency to rise again.

Water Temperatures of the Harbour.—The maximum temperature readings of 29° to 31° C. for the coastal waters of the sea in the vicinity of the Harbour were recorded during the periods April to June 1948 and May to August 1949. During the periods September-November and December-January the temperature came down to about 26° to 27° C. and 25° to 24° C. respectively. By the end of February it was shooting upto about 27° C.

In general, the maximum temperature during March to June in Adyar was a little higher than in the vicinity of the harbour which was to be expected in a relatively smaller and shallower area of the estuary which takes up heat more readily than the waters of the open sea.

4. Gonadic Condition and Spawning of the Backwater Oyster

The gonads are paired and very much alike in external appearance in both the sexes. They are creamy white, much branched with the ramifications of the tubular follicles spreading beneath the general epithelium and filling up the bulk of the visceral mass when the oyster is ripe. The follicles of the gonad of the two sides almost coalesce at the hinge, where they form a very thick mass, but are absent beneath the œsophagus and also in the region of the pericardium. The gonoduct of each side opens into a urinogenital cleft close to the adductor muscle. The ramifications of the gonadic tubules in a fully ripe female oyster are usually clearly seen through the epithelium whereas those of the ripe male are indistinct. In a partially spent female or male the part of the gonad close to the hinge is flabby with the accumulation of a certain amount of a watery fluid within, but farther down this region it is full of the whitish reproductive elements in the follicles. The amount of watery fluid varies depending upon the extent of spawning. The fully spent female oyster presents usually a more flabby appearance than its male counterpart, as the amount of the residual ova is always much less than the residual sperm in the spent gonads.

Gonadic Condition of Oysters from Adyar.—The condition of gonads of Adyar oysters exceeding two inches in length during the period of observations is given in Table I. From June to August both in 1948 and 1949 the proliferating activity of the gonads was observed as a preparation for the next spawning season. However, the differences in the condition of the gonads of March-April 1948 and of 1949 seem to be due to the salinity variations in the respective years. After heavy spawning and rapid resorption of the few residual reproductive elements, as it occurred in May 1948, it is difficult to distinguish the sex in a small number of oysters, and this phase may correspond to Loosanoff's (1942) 'indifferent stage' of *Ostrea virginica*, from which reversal of sex is possible. A few of the hermaphrodite phases noted in this species will be dealt with elsewhere. The occurrence of stray individuals with full gonads after the spawning period was not uncommon.

Gonadic Condition of Oysters of the Harbour.—The oysters of the Madras Harbour living under purely marine conditions do not seem to attain the

TABLE I

Changes in the gonadic condition and occurrence of spawning in relation to salinity and temperature variations in Adyar

Dates and months	Gonadic condition	Spawning	Salinity parts per thousand	Maximum temperature °C.
March to April, 1948	Full or partially spent or spent	Excellent spawning of both sexes	20—28	29—33
May, 1948	Spent	No spawning	30	31
June to 14th September, 1948	Recovering stage	No spawning	31—38	27—30
21st September to October, 1948	Full	Pre-spawning stage	21·7 to 26·8	26·4 to 26·8
November, December 1948 and 1st week of January, 1949	Full or partially spent	Excellent spawing of both sexes	22·39 to 25·08	24—25
Late January and February, 1949	Signs of recovery but no rapid proliferation	No spawning	29—30	24·8 to 26·8
March, April and May, 1949	Cytolysis and dissolution of eggs	Stray spawning of males only	32 to 37·9	27 to 31·8
June to August 1949	Stage of recovery	No spawning	27–30	28—30

proportions of those of Adyar living under the estuarine and backwater conditions either in the size of the animal or thickness of the gonad. The grown-up oysters in the Harbour were in a partially spawned condition throughout the year with relatively large number of developing oocytes in August and September.

Spawning in Oysters from Adyar.—Oysters from Adyar estuary placed in glass troughs in the laboratory spawned profusely on the 11th March 1948. At first a small male commenced emitting a steady stream of milt, which was followed by quick emission of eggs by two females of fair size; then more males and females spawned rendering the water in the trough cloudy. The salinity of the waters in Adyar was $20 \cdot 32°/_{oo}$ and of the sea water $34 \cdot 42°/_{oo}$. Spawning took place in sea-water diluted with tap-water so as to bring down its salinity to the level of the Adyar water. The temperature of the water in which spawning took place was $27° \cdot 3$ C.

Natural spawning was observed on the oyster-beds when the salinity was $20 \cdot 3°/_{oo}$ and the temperature $29° \cdot 0$ C. at 12 Noon on the 13th March 1948.

It would appear that a large number of individuals in shallow waters near the southern embankment of the estuary had spawned simultaneously. Spawning in the laboratory continued at short intervals till the third week of April 1948. No further spawning was observed in the laboratory or in the natural habitat from the last week of April 1948 to third week of November 1948. In the last week of April 1948 when the spawning had just stopped the salinity of the estuarine water had risen to $28 \cdot 59°/_{oo}$. Again on the 27th and 30th November, 1st, 6th, 24th and 26th December 1948, and 4th and 6th January 1949 there was spawning of the oysters of both the sexes from this locality. In the spawning period of November to January spawning was observed in the natural habitat only on the 6th and 26th December, but on other days it took place under laboratory conditions. From the third week of November 1948 to the first week of January 1949 the salinity and temperature ranges were respectively $22 \cdot 52$–$25 \cdot 08°/_{oo}$ and $24 \cdot 0$–$26° \cdot 4$ C.

Between the 6th January and the third week of March 1949 there was no spawning. On the 25th March, 1st April and 20th May 1949, when the water temperatures were high ranging from $29° \cdot 2$ to $31° \cdot 8$ C. only the males spawned. From the last week of March to the last week of May 1949, the salinity range was between $32 \cdot 45$ and $37 \cdot 94°/_{oo}$ which was considerably higher than it was during the previous period of effective spawning of both the sexes. Though the observations were discontinued by August 1949, grown-up larvæ were again seen in the Adyar estuary and backwaters in December 1949, which indicates spawning also in the November-December period of 1949.

From the field and laboratory observations on *O. madrasensis* it would appear that intense sexual activity occurs in the Adyar estuary during November-December, usually followed by a feeble one in March-April. All the salinity readings taken during spawning of both sexes fall within the optimum range, $22 \cdot 00$ to $26 \cdot 00°/_{oo}$ required for the development of the early veligers from fertilised eggs as corroborated by laboratory experiments. It is therefore probable that spawning in the backwaters takes place only when optimum salinity conditions prevail. The successful spawning of oysters in March and April 1948 and the failure of the same in the corresponding period of 1949 may be attributed to the favourable and unfavourable salinities prevailing respectively in the two years. It seems likely that the females and males behave differently under high salinities and temperatures, the former failing to spawn in the backwaters in increased salinities and the latter reacting positively to increased temperatures as they did in March to May 1949. It may also be observed that in March 1948 spawning oysters continued to liberate spawn for a short time under laboratory conditions in pure sea-water of high salinity of $34 \cdot 4°/_{oo}$ as well as in lower salinities. This seems to indicate that what constitutes the favourable condition for spawning is not the sudden change in salinity but the length of the period of stable salinity preceding spawning. Attempts at inducing the Adyar oysters to spawn in the non-reproductive period in samples of water of different grades of salinity met with no success.

Spawning of the Oysters of the Harbour.—Spontaneous spawning of the harbour oysters was not observed either in the natural habitat or under laboratory conditions, but induced spawning was, however, possible in April-May and October-November of 1948 and in March to May of 1949. Both sexes were induced to discharge their reproductive elements by the stimulus of the mere presence of ripe sperms and eggs in the water containing the oysters. The occurrence of oyster larvæ in the coastal plankton in all the months of the year is a positive evidence of more or less continuous spawning throughout the year.

5. Early Development of Oyster Eggs in Different Grades of Salinity

The experiments and observations of Amemiya (1921, 1926 and 1928) with fertilised eggs of oysters in various grades of salinity have shown that the optimum range of salinity for the development of shelled veligers varies with the species—$25–29°/_{oo}$ in *Ostrea virginica*, $28–35°/_{oo}$ in *Gryphæa angulata*, $15–25°/_{oo}$ in *Ostrea gigas* and *Ostrea rivularis*, $28–38°/_{oo}$ in *Ostrea circumpicta* and $30–38°/_{oo}$ in *Ostrea spinosa*. Seno, Hori and Kusakabe (1926) and Clark (1935) made similar observations on *Ostrea gigas* and *Ostrea virginica* respectively. Awati and Rai (1931) found a density of sea-water of $1 \cdot 020$ or $1 \cdot 021$ to be most suitable in the early development of *O. cucullata*. Roughley (1933) observed free-swimming stages formed in the Australian, *O. commercialis* in 8, 9 and $10\frac{3}{4}$ hours in densities of $1 \cdot 021$, $1 \cdot 015$ and $1 \cdot 011$ respectively, the rate of development decreasing as the density was lowered. In a density of $0 \cdot 005$, most of the eggs remained unfertilised, but the few that passed through the segmentation stages did not develop beyond the morula stage. Cole (1939) found a salinity range of $30 \cdot 5$ and $32 \cdot 5°/_{oo}$ suitable for the culture of *O. edulis* at Conway. The oviparous oysters are better than the larviparous ones for studying the effect of salinity as the eggs of the former can be more easily fertilised and developed normally in suitable media. As in larviparous *O. edulis* and *O. denselamellosa* artificial fertilisation was not successful, the embryos taken from the parent oysters were subjected to grades of salinity by Amemiya (1926 and 1928). The effects of very high or very low salinities appear to be either cessation of development or retarded or defective segmentation. In the latter case the resulting blastomeres tend to clump together, like small colonies of yeast cells, developing into abnormal embryos and larvæ, or get detached to grow into incomplete larvæ.

In the experimental data detailed in Table II, altogether thirty grades of salinity media were used with laboratory temperatures varying between 26°·0 C. and 28°·5 C. Development was at its best in grade 17 of salinity 26·00°/$_{oo}$. The average time taken by the eggs to reach the respective stages after fertilisation in different grades of salinities is shown in the table.

It may be seen from Table II that in grades 1 to 3 of salinities ranging from 50·10 to 48·00°/$_{oo}$ and in grade 30 of salinity 4·33°/$_{oo}$ no development was observed. In very high salinities the eggs were very much shrunk and in very low salinities they were a little swollen. In grades 4 to 29 of salinities ranging from 46·00 to 6·5°/$_{oo}$ development was initiated by the extrusion of polar bodies and segmentation, and they are grouped under the following five ranges for convenience of description.

Range I of Too High Salinity.—In grades 4 to 9 of salinity ranges between 46·00 to 37·01°/$_{oo}$ development was defective, abnormal and incomplete. In the higher grades of this range the majority of eggs remained unchanged, and in the lower grades segmentation improved. In salinities from 41·00 to 37·00°/$_{oo}$ shell-formation was commenced after the trochophore stage but was not completed. There were only varying numbers of abnormal larvæ but no shelled veligers.

Range II of High Salinity.—In grades 10 to 16, of salinity ranges between 36·00 to 28·04°/$_{oo}$ development progressed satisfactorily in varying numbers of the eggs upto the shelled veliger stage. Segmentation was good and quite normal in most cases. With the fall in salinity the number of abnormal larvæ gradually decreased while the shelled veligers increased correspondingly. In the last two grades of this range, development was good except that some did not develop into shelled veligers even after 30 hours.

It may be observed here that, judging from the occurrence of the planktonic larvæ, the oyster seems to breed under marine conditions of the harbour for the most part of the year within the salinity limits of Range II. Fall in salinity of the coastal waters in October to about the same as in Range III, was followed by an increase in the numbers of larvæ.

Range III of Optimum Salinity.—In grades 17 to 20 of salinities from 26·00 to 21·83°/$_{oo}$ development was excellent and almost all the eggs divided

TABLE II

Showing Effect of Different Grades of Salinity on the Developing Eggs of the Oyster

Range	Grade No.	Salinity °/₀₀	Extrusion of polar bodies	Nature of segmentation	Duration of formation of Blastula	Duration of early free swimming stage	Duration of formation of Trochophore	Duration of Formation of shell	Duration of Formation of early Veligers	Abnormal free swimming larvæ
No development	1	50·10	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	2	49·72	do	do	do	do	do	do	do	do
	3	48·00	do	do	do	do	do	do	do	do
Range I Salinity— too high	4	46·00	30 min.	Defective	do	do	do	do	do	do
	5	45·00	do	do	do	do	do	do	do	do
	6	43·10	do	do	do	do	do	do	do	Large Nos.
	7	41·40	15 min.	do	6 hrs.	8–9 hrs.	20 hrs. (fair)	Not completed	do	Numerous
	8	39·00	do	do	do	do	do	do	do	do
	9	37·01	do	do	do	do	do (good)	do	do	do
Range II Salinity— high	10	36·00	do	Good	3 hrs.	6 hrs.	Large Nos.	24 hrs.	30 hrs. (very few)	Large Nos.
	11	35·00	do	do	do	do	do	do	do	do
	12	33·14	do	do	2½ hrs.	do	do	do	30 hrs. (a few)	do
	13	33·09	do	do	do	do	do	do	do	do
	14	31·34	do	do	do	do	do	do	do (fair)	Good Nos.
	15	29·69	do	do	2 hrs.	4–6 hrs.	18–20 hrs.	20–24 hrs.	28 hrs. (large)	Few
	16	28·04	do	do	do	do	do	do	do	Nil
Range III Salinity— optimum	17	26·00	1st 5–10 min. 2nd 15 min.	do	1¾–2½ hrs.	3½ hrs.	12–14 hrs.	17–18 hrs.	20–22 hrs. (numerous)	Nil
	18	24·86	do	do	do	do	do	24 hrs.	26–28 hrs. (numerous)	do
	19	23·83	do	do	do	do	do	do	do	do
	20	21·83	do	do	do	do	do	do	30 hrs.	do
Range IV Salinity— low	21	19·5	do	Defective	3 hrs.	5–6 hrs.	14–18 hrs.	24–30 hrs.	34 hrs. (few)	Very few
	22	17·33	do	do	do	do	do	do	do	do
	23	16·00	do	do	do	do	do	do	do	do
Range V Salinity— too low	24	15·10	do	do	do	do	do	Not completed	Nil	Large Nos.
	25	13·00	30 min.	do	3–3½ hrs.	do	20 hrs.	Nil	do	do
	26	12·43	do	do	do	do	do	do	do	do
	27	10·83	do	do	do	do	do	do	do	Numerous
	28	8·66	do	do	do	24 hrs.	60 hrs. (a few)	do	do	do
	29	6·5	do	do	Nil	Nil	Nil	do	do	Nil
No development	30	4·33	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

normally. There were no abnormal or partial larvæ. Almost all the eggs developed to the shelled veliger stage.

The salinity limits under which successful spawning of both sexes of the oysters took place in Adyar backwaters fall approximately within this range.

Range IV of Low Salinity.—In grades 21 to 23 of salinities from 19·5 to 16°/₀₀ development was retarded, defective and abnormal in varying number of eggs. Mortality was great after the free-swimming stage was reached. Some developed into the shelled veliger stage, thus completing the early development.

Range V of Too Low Salinity.—In grades 24 to 29 of salinities from 15·0 to 6·5°/₀₀ development was very much retarded, defective, abnormal and incomplete. After the trochophore stage shell formation commenced in the higher salinity grades within this range but was not completed. In most cases development did not proceed beyond the trochophore stage. There were numerous abnormal larvæ, but no veligers.

6. Frequency of Occurrence of Oyster Larvæ in Plankton Hauls

The relative abundance of the oyster larvæ in the plankton was measured by a rough method, which is a slight modification of procedure described by Hopkins (1931). An arbitrary standard was adapted in which numbers, *viz.*, 0, 1, 2, 3, 4 and 5 indicate none, very few, few, fair large and abundant numbers of larvæ respectively in each collection. As younger larvæ in the straight-hinge stage are difficult to be distinguished from those of other bivalves, the relative abundance of only larvæ of setting size was estimated. Where appearance of younger larvæ coincided with the spent condition of the gonad their occurrence was recorded with 1* in Table IV. Every plankton collection consisted of four horizontal hauls, each of fifteen minutes duration, the net being towed from a catammaran.

Oyster Larvæ in the Adyar Backwaters.—Following spawning early veliger larvæ occurred in gradually diminishing numbers in the backwaters and the estuary from the middle of March to the first week of May 1948. The average water temperature was 32°·8 C. rising upto a maximum of 33°·2 C. on 9th April 1948. This high temperature resulted in heavy mortality of the larvæ and the absence of spat. The salinity for most part of this period ranged between 20·32 and 28·59°/₀₀ which is almost the same as the optimum requirement for early development under controlled experiments. No larvæ were observed from the second week of May to the third week of November 1948. From 26th November 1948 to 6th January 1949 there was spawning followed by the appearance of larvæ in gradually increasing numbers, the peak of occurrence of setting larvæ being in the first three weeks of January 1949 during which the salinity and temperature were $29 \cdot 01 ‰$ and $24° \cdot 2$ to $25° \cdot 2$ C. respectively. This was followed by a good spatfall. Between February 1949 and August 1949, when the observations were discontinued, no larvæ were present in the backwaters. In December 1949, however, grown up larvæ were observed in the same locality when the salinity was $21 \cdot 90$ and $22 \cdot 50 ‰$. Though spawning takes place in the backwaters under the optimum salinity of $22 \cdot 0$ to $26 \cdot 0 ‰$, the larvæ thrive well in slightly higher ranges as well, the bar opening usually at about this time letting in sea-water into the backwaters which raises the salinity.

Oyster Larvæ from the Sea in the Vicinity of the Harbour.—Varying numbers of larvæ of setting size were obtained from the plankton collections over a period of 18 months with peak occurrences in June-July 1948, November 1948 to January 1949, and May to July 1949. It may be seen from Table IV that the May–July period both in 1948 and 1949 was itself a period of very high temperatures or was preceded by a period of very high temperatures, whereas the November–January period was preceded by one of moderate temperatures and low salinity. Thus it follows that under marine conditions the backwater oyster remains sexually active throughout with intensive spawning periods which are followed by high temperatures, or low salinities suitable for early development.

7. Field and Laboratory Observations on Relation Between Salinity and Setting of Oyster Spat

Spat-Setting in the Backwaters.—The field experiments on the setting of spat did not prove a success, owing to the frequent disturbance caused in the locality by fishermen and clam gatherers. These experiments were made with (i) suspended dried shell-cultch, (ii) a small wooden rack with removable glass slides and (iii) heaped dead shells in shallow water, which could be examined, cleaned or replaced from time to time. Besides these experiments with cultch careful examination was made from time to time of all likely cultch in the locality such as the cement river embankment, the boulders of the Elphinstone Bridge across the Adyar river, and submerged stones or other hard objects on which spat may be expected to settle.

In March–April 1948 the absence of spat may have been a result of larval mortality due to high temperatures, unless a few that had settled in the cooler and deeper waters covering the boulders in the middle of the river had escaped observation. Nor did the shells of oysters and other molluscs obtained from the river bottom by diving reveal any trace of spatfall.

The November–December spawning of 1948 was followed by the occurrence of larvæ till about the end of January 1949. On 29th December 1948, when the salinity had gone up to $25 \cdot 49°/_{oo}$ (*vide* Table IV) in the backwaters of Adyar owing to the bar having opened two days previously, grown-up larvæ were obtained in the plankton and it was possible to rear them to the spat stage in the laboratory in sea-water of salinity $25 \cdot 98°/_{oo}$. Spatfall continued in the estuary and the backwaters throughout January and the first week of February 1949 when the salinity varied between $27 \cdot 32°/_{oo}$ and $29 \cdot 01°/_{oo}$. In the extensive backwaters of Ennore, eleven miles north of Madras, widespread spatfall was observed on 21st December 1949, where the salinities were $31 \cdot 09°/_{oo}$ and $30 \cdot 21°/_{oo}$ in the shallow and deeper waters respectively. The bar was open and the backwater was in communication with the sea. In the same week in the Adyar backwaters where the salinity range was $21 \cdot 92$–$22 \cdot 52°/_{oo}$ a fair number of larvæ was present but without spatfall. The observations made above seem to show that the optimum salinity for the spatfall is higher than that for spawning and early development, which under controlled experiments was found to be $22 \cdot 00$ to $26 \cdot 00°/_{oo}$. Spatfall was the best in both the backwaters when the salinities were as high as $29 \cdot 00$ to $31 \cdot 09°/_{oo}$. The absence of spat in Adyar in December 1949 may have been a result of the then prevailing low salinity owing to the bar remaining closed. To ascertain a suitable grade of salinity for setting, planktonic larvæ of setting size were placed in bowls of water of different grades of salinity.

Spat Setting in and around the Madras Harbour.—Frequent searches of the boulders, piers and other submerged objects of the locality for spat revealed that setting was at its best from (i) May to July 1948, (ii) December 1948 to January 1949 and (iii) May to August 1949, though larvæ continued to occur all the year round during the period of observations. Intensive spatfall was observed only when there was an abundance of larvæ in the sea.

Setting of Oyster Larvæ under Experimental Conditions.—Larvæ of setting size from the plankton of the inshore waters were carefully picked up under the binocular microscope with finely drawn glass pipettes and removed into finger-bowls containing different grades of salinity of sea-water, dilutions being made, with distilled water. Twenty larvæ were taken into each finger-bowl. Usually over $50°/_{o}$ of them settled on the sides of the glass bowl in about 24 hours if the medium was of a suitable grade. Most of the setting took place during the night. If the setting of larvæ was delayed, fresh sea-water of the same dilution was added after the old water had been carefully siphoned out. The experiment was repeated with different grades of salinity prepared out of estuarine water obtained from Adyar when the bar was closed.

During the periods when good spat were obtained in the vicinity of the harbour (*vide* Table IV), laboratory experiments on setting also met with success. The months and salinities of sea-water in which excellent setting took place are shown below:—

Salinity of sea water	$33 \cdot 01‰$	$28 \cdot 39‰$ & $29 \cdot 07‰$	$33 \cdot 21‰$ $33 \cdot 39‰$	$34 \cdot 33‰$	$34 \cdot 3‰$	$34 \cdot 4‰$
Months	July 1948	January 1949	May 1949	June 1949	July 1949	August 1949

The results of experiments on the effect of different grades of salinity on the setting of spat under laboratory conditions are given in Table III.

TABLE III

Showing Relative Effects of Salinity Grades of Sea-water and Estuarine Water on Setting

Salinity grades with sea-water	*$34 \cdot 33‰$ excellent	$30 \cdot 01‰$ excellent	$27 \cdot 88‰$ good	$25 \cdot 74‰$ fair	$21 \cdot 45‰$ poor	$17 \cdot 23‰$ nil	
Salinity grades with estuarine water	..	..	*$27 \cdot 02‰$ very poor	$23 \cdot 69‰$ nil	$20 \cdot 32‰$ nil	..	$13 \cdot 56‰$ nil

* Used without dilution.

It may be concluded from the field and laboratory observations that (i) the salinity of the waters of the sea are always favourable for setting, the intensity of setting being dependent on the intensity of occurrence of larvæ of setting size, and (ii) conditions in the backwaters are favourable for setting only when the bar is open and the sea-water mixes with the back-water, so as to raise the salinity of the latter to about $28–30 \cdot 0‰$. It may be seen from Table III that there is a marked difference in setting in samples of water of approximately the same grades of salinity (i) when prepared with sea-water and (ii) with estuarine water when the bar is closed, preference being shown by the larvæ to settle in grades prepared with the former. Both in the backwaters and the coastal waters of Madras the pH varied only very slightly between $8 \cdot 3$ and $8 \cdot 6$ and it cannot therefore be regarded as the deciding factor in the success or failure in the setting of

TABLE IV—(*Contd.*)

Dates	Adyar River Mouth and Backwaters					Madras Harbour and the adjoining sea			
	Salinity °/₀₀	Average Max. temp. °C.	Spawning	Freq. of larvæ	Setting of spats	Salinity °/₀₀	Average Max. temp. °C.	Freq. of Larvæ	Setting of spats
December 1948									
1— 7 ..	22·39	25·0	f. & m.	0·1*	N.S.	24·49	25·1	3	N.S.
8—14 ..	..	do	f. & m.	0·1*	N.S.	26·79	25·0	..	F.S.
15—21 ..	22·52	24·0	f. & m.	·1*	N.S.	..	24·4	0, 1*	F.S.
22—31 ..	24·89	do	f. & m.	2·1*	F.S.	25·98	24·0	4	F.S.
January 1949									
1— 7 ..	..	24·2	f. & m.	4·1*	M.S.	28·39	24·5	4, 5, 3	G.S.
8—14 ..	..	do	nil	4·1*	M.S.	28·77	24·4	5	G.S.
15—21 ..	29·01	25·2	nil	4·	G.S.	29·07	25·1	4, 5	G.S.
22—31 ..	27·32	do	nil	..	G.S.	32·09	25·1	1, 1, 1	N.F.S.
February 1949									
1— 7 ..	28·87	25·0	nil	2	G.S.	32·80	25·6	0, 0	..
8—14 ..	..	do	nil	0	N.F.S.	32·82	26·6	1, 3	N.F.S.
15—21 ..	30·01	26·8	nil	..	N.F.S.	33·15	26·6	0	N.F.S.
22—28 ..	..	do	nil	0	N.F.S.	33·47	27·0	4, 1	..
March 1949									
1— 7 ..	33·0	27·0	nil	0	N.F.S.	33·91	27·2	1, 1*	N.S.
8—14 ..	..	do	nil	0	do	33·91	27·6	1, 1	..
15—21 ..	..	29·2	nil	0	do	34·01	27·9	0	..
22—31 ..	32·45	29·2	m	0	do	34·31	28·1	1	..
April 1949									
1— 7 ..	33·53	30·5	m.	0	N.S.	34·87	28·2	1, 1	N.S.
8—14 ..	..	do	nil	0	do	36·65	28·2	..	..
15—21 ..	..	31·8	do	0	do	34·60	28·3	..	N.S.
22—30 ..	35·55	do	do	0	N.S.	35·01	28·7	2	do
May 1949									
1— 7 ..	..	30·6	m.	..	N.S.	35·27	28·4	3, 3	..
8—14 ..	37·94	do	nil	0	N.S.	34·24	28·8	3	..
15—21 ..	..	29·9	m.	0	do	33·21	29·1	3, 1	G.S.
22—31	33·71	do	m.	0	N.S.	33·39	29·4	4, 5	G.S.
June 1949									
1— 7 ..	..	30·0	..	0	N.S.	31·16	28·9	1, 3	..
8—14 ..	..	do	nil	..	do	34·33	29·0	..	G.S.
15—21 ..	..	28·0	nil	..	do	34·31	29·1	2, 2	..
22—30 ..	25·3 26·01	do	nil	..	do	34·26	29·5	1, 3, 2	F.S.
July 1949									
1— 7 ..	..	28·6	..	..	N.S.	34·46	28·9	..	M.S.
8—14 ..	27·02	..	nil	0	do	34·36	28·7	2, 1, 4	G.S.
15—21 ..	..	29·4	..	..	do	34·25	30·1	4	G.S.
22—31 ..	..	do	nil	0	N.S.	34·3	30·0	4, 4	G.S.
August 1949									
1— 7 ..	28·6	29·4	nil	0	N.S.	34·40	29·5	3	G.S.
8—14 ..	..	do	nil	0	do	..	..	4	G.S.
15—21 ..	..	..	..	..	..	..	..	..	..
22—31 ..	..	..	..	..	..	..	..	..	..

Spawning.—m. male spawning; f. & m., male and female spawning.

Frequency of Larvæ.—0, absent; 1. very few; 2. few; 3. fair; 4. large numbers; 5. abundant. Nos. 0–5 indicate larvæ of setting size; 1*. larvae of straight hinge stage (small larvæ).

Spats.—*N.S.*, No spat; *F.S.*, Few spat, *N.F.S.* No fresh spat; *M.S.*, Moderate spat; *G.S.*, Good spat.

TABLE IV

Larvæ and Spatfalls with Correlated Oservations in Adyar Backwaters and Madras Harbour

Dates	Adyar River Mouth and Backwaters					Madras Harbour and the adjoining sea			
	Salinity ‰	Average Max. temp. °C.	Spawning	Freq. of larvæ	Setting of spats	Salinity ‰	Average Max. temp. °C.	Freq. of Larvæ	Setting of spats
March 1948									
1—7	..	..	..	..	..	34·56	..	..	..
8—14	20·32	29·0	f. & m.	..	N.S.	34·42	27·8	..	..
15—21	..	29·8	f. & m.	0·1*	N.S.	36·97	28·0	1, 1	N.S.
22—31	..	32·0	f. & m.	0·1*	N.S.	34·36	28·0	1	N.S.
April 1948									
1—7	..	..	f. & m.	0·1*	N.S.	34·58	28·2	3	N.S.
8—14	..	32·8	f. & m.	0·1*	N.S.	34·74	29·0	3	N.S.
15—21	..	..	..	..	..	37·25	29·0	2	N.S.
22—30	28·59	31·7	nil	0·1*	N.S.	35·61	28·0	3	N.S.
May 1948									
1—7	..	..	nil	0·1*	N.S.	35·06	29·6	3	..
8—14	30·23	31·4	nil	..	..	35·06	29·0	2	..
15—21	..	..	nil	0	N.S.	35·33	28·8	..	..
22—31	29·67	31·6	nil	..	N.S.	..	29·1	..	F.S.
June 1948									
1—7	..	..	nil	0	N.S.	34·60	29·1	3, 4	F.S.
8—14	31·15	30·0	nil	0	N.S.	34·88	29·6	5, 3	..
15—21	..	..	nil	0	N.S.	35·71	29·4	3, 3	M.S
22—30	35·14	30·2	nil	0	N.S.	34·92	29·0	4	M S.
July 1948									
1—7	38·47	..	nil	0	N.8.	34·93	28·6	4 5	M.S.
8—14	..	28·2	nil	..	N.S.	34·79	27·8	3	..
15—21	33·01	..	nil	0	N.S.	34·89	27·3	5, 5	G.S.
22—31	..	27·7	nil	0	N.S.	34·60	26·7	4	G.S.
August 1948									..
1—7	34·07	28·0	nil	0	N.S.	34·74	28·0	..	N.F.S.
8—14	34·43	..	nil	..	N.S.	34·65	28·2	1	..
15—21	31·91	..	nil	0	N.S.	34·76	28·2	..	N.S.
22—31	34·14	27·7	nil	0	N.S.	34·86	27·4	1	
September 1948									
1—7	..	26·8	nil	0	N.S.	34·76	26·3	..	..
8—14	31·74	..	nil	0	N.S.	34·43	26·9	1	N.S.
15—21	..	..	..	..	N.S.	34·72	26·7	0	..
22- 30	21·69	26·7	..	..	..	34·73	26·8	0, 0	N.S.
	23·95								
October 1948									
1—7	..	26·6	..	..	..	34·21	26·4	0, 0	..
8—14	26·80	..	..	0	N.S.	29·26	26·6	0	..
	23·71								
15—21	..	26·4	nil	0	N.S.	28·71	26·4	..	..
22—31	24·56	..	nil	0	N.S.	27·64	26·9	2, 0, 3	N.S.
November 1948									
1—7	..	25·8	nil	0	N.S.	28·27	26·2	3	N.S.
8—14	25·08	..	nil	0	N.S.	26·01	26·0	3	N.S.
15—21	..	..	nil	0	N.S.	24·79	26·8	..	N.S.
22—30	23·84	26·4	f. & m.	0·1*	N.S.	24·60	26·6	3, 5, 0, 3	N.S.

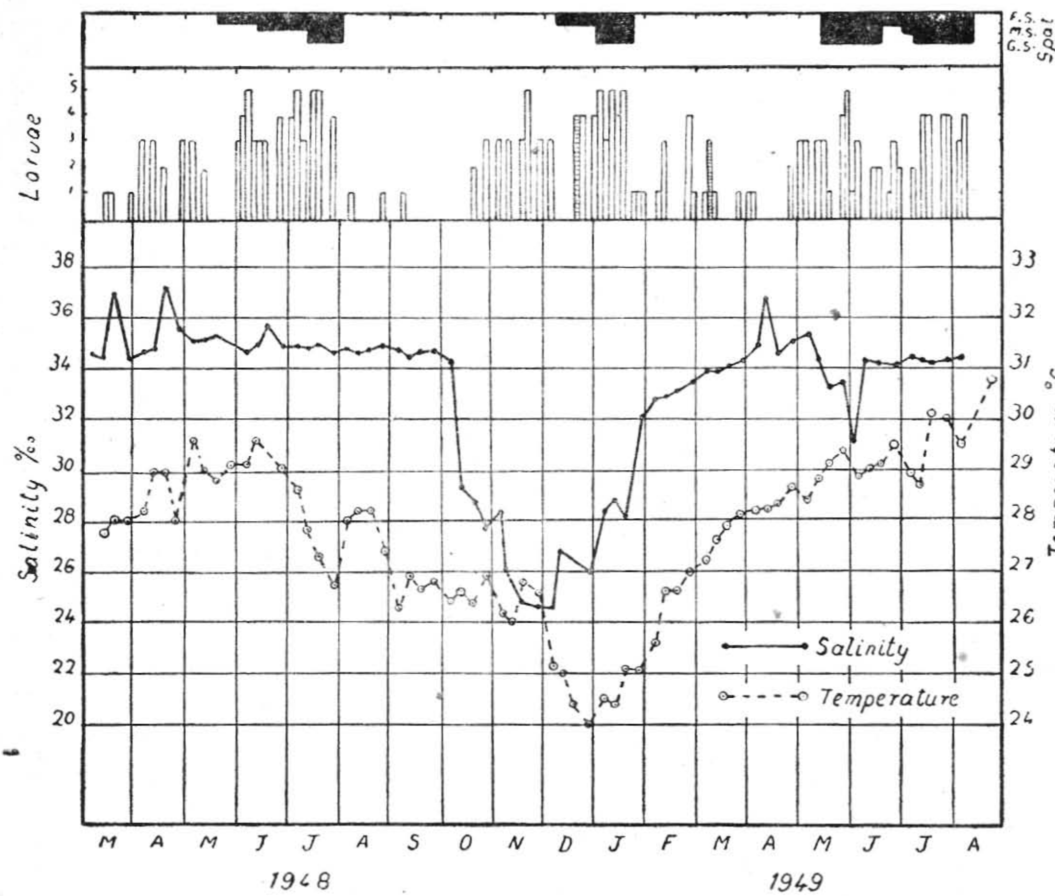

FIG. 2. Frequency of occurrence of larvæ and spatfall of *Ostrea madrasensis* in relation to variations in salinity and temperature in Madras Harbour. Data presented in Table IV. (Explanation of lettering is as for Table IV: histograms with cross stripes indicate larvæ of young size, and those without cross stripes represent larvæ of setting stage.)

spat. Thus there still seems to be an unascertained factor in the sea-water apart from salinity which induces spatfall.

8. General Considerations

What seems to be of the utmost importance in the cultivation of oysters is a knowledge of the factors that induce spawning, control larval development and promote setting. It is well known that a rise in temperature of the waters in the summer months is the chief stimulating factor in inducing the European and American oysters, *O. edulis* and *O. virginica* to spawn in temperate waters. Stafford (1913), Nelson (1921, 1928 *a* and *b*), Churchill (1920), Prytherch (1929) and Galtsoff (1930, 1932) have shown that spawning does not start in the American oyster, until the minimum or critical temperature of 20°·0 C. is reached. Even the maturing process of the gonads seems to be governed by the temperature factor: thus Nelson (1928 *b*) has shown that sperm production begins at about 10° C., deposition of yolk at 15° C. and maturation of the egg at 18° C. with wide individual variations. He (1921) has also recorded that if the temperature of the water fails to rise to the required minimum the reproductive elements are absorbed instead of being liberated. Prytherch (1929) has observed spawning at 20° C. at high tide when the pH factor is favourable, but not at low tide when, even if the temperature is high, the spawning reaction does not take place because of very low pH. Thus factors other than temperature seem to govern spawning. Galtsoff (1930, 1932, 1938 *b*) has shown that female oysters spawn not only spontaneously at 24°·5 C. but also by stimulation in the presence of sperm at a much lower temperature, *i.e.*, between 18°·6 and 20°·5 C. Hence in any locality with temperature of water at about 20° C. the females begin to spawn if the stimulus of male spawning is present. He also observed that males respond immediately to egg suspension in water as well as to sperm with a "latent period". Orton (1926 and 1937) has observed *O. edulis* breeding at 15° C. Changes in salinity have not been known to induce spawning in the American or the European oysters. Under tropical conditions of our coasts the water temperatures of the sea or the backwaters are maintained high throughout the year and do not even fall at any time below the optimum requirement of the oysters mentioned above. Hornell (1910 *a*) thought that a fall in density of the waters due to heavy rains of the north-east monsoon was the chief stimulating factor in the spawning of the Madras oyster in the east coast backwaters of South India. Experiments conducted by him in 1908 with spat collectors in Ennore during the north-east monsoon in October–December led him to the conclusion that the oysters spawned in October and November under the influence of low salinity conditions, but no records of salinity were maintained during the period of observations. He (1922) also observed that the chief spawning period was in August-September followed by supplementary spawning in March–April. My observations corroborate those of Hornell (1910, 1922) except that spawning after the north-east monsoon may extend up to about the first week of January, and that both the main and supplementary spawning periods are governed by the salinity factor in the environment. In the present investigations the salinity in the backwaters when spawning was observed, varied between 20·3 and 28·00‰, which is almost the same as the optimum requirement (22–26‰) for the developing eggs under laboratory conditions. This optimum may be attained either by a rise or fall in salinity of the backwater; if the salinity is very high due to evaporation of water when the bar is silted up, it is brought down by the influx of flood water due to rains; or if the salinity of the backwaters is considerably low for any length of time, it is raised to the optimum requirement either by evaporation or by the opening of the bar. When the optimum salinity is thus reached spawning takes place in the backwaters, provided the gonads are ripe.

Hopkins (1931) found correlation between the periods of setting and periods of high salinity in *Ostrea virginica* of Galveston Bay, Texas, and considered that the larvæ depended in some manner directly or indirectly, on a salinity of about $20 \cdot 0^{\circ}/_{oo}$ as a stimulus to develop to setting stage. Gaarder (1932, 1933) and Gaarder and Bjerkan (1934) found that a salinity of $24^{\circ}/_{oo}$ and above essential with $30–35^{\circ}/_{oo}$ as the optimum for the successful growth of the larvæ of *O. edulis*. The laboratory experiments and field observations in Adyar and Ennore show that not only higher salinities up to $29–30^{\circ}/_{oo}$, but also the mixture of the sea-water with the estuarine water as a consequence of the bar remaining open, are essential for successful larval development and spatfall in the backwaters. Higgins (1938), in the Long Island Sound, U.S.A., and Korringa (1941) in Oosterschelde, Holland, found the small salinity variations of no importance in determining success or failure of spatfall. In the small estuaries and backwaters of our coasts, however, periodical inflow and outflow of tidal and flood waters respectively with the tidal amplitude as small as three feet influence the fluctuations in salinity, and consequently of the spawning and setting of the oysters. Prytherch (1934) found larvæ of *O. virginica* undergoing complete fixation in 12 to 19 minutes in salinities between 16 and $18 \cdot 5^{\circ}/_{oo}$, but in salinities above or below this range, the duration of fixation was prolonged. He held that the alteration in the physical properties of the byssal fluid due to the changes in salinities of higher and lower grades as the cause of the delayed setting. The same author (1931 and 1934) observed in Milford Harbour abundant setting of the same larvæ in low water, which he attributed to slight variations in the copper content of the waters in the course of the tidal cycle, a view which received little support from Nelson (1931), Gaarder (1932, 1933) and Korringa (1941). All that can be said at present is that some unknown factor present in the sea-water has something to do with larval development and setting, but absent in the estuarine water which remains unconnected with the sea for a fairly long period when the bar is closed.

Butler (1949) observed inhibition of gametogenesis in 90% of *O. virginica* population in a low salinity area until the salinity rose to $6^{\circ}/_{oo}$. This suppression of the gonadic activity he attributed to the inability of oysters to feed under low salinity conditions. During the period of the present observations no fall in salinity in the backwaters was observed to such an extent as to cause any deleterious effect on the gonadic development.

The subject of periodicity in the breeding of marine animals in the tropics has attracted the attention of various workers, giving rise to the expression of a great variety of views. Semper (1881) and Orton (1920) held that the breeding behaviour of marine invertebrate animals is different under the tropical and temperate regions, with a tendency to be continuous in one and periodic in the other, respectively. Galtsoff (1933) thought that definite periodicity could be noticed in the breeding of animals both under tropical and temperate conditions. Anne Stephenson (1934) found in the animals of the Low Isles of the Australian Great Barrier Reef, continuous and discontinuous breeding, the latter sometimes favoured by lunar phases. Her observations find support in those of Paul (1942) on the breeding of the Madras Harbour animals. Panikkar and Aiyar (1939) in the brackish-water animals of Madras also observed different types of both continuous and discontinuous breeding, the latter in some cases determined by the rains. The present observations on the Madras Harbour oyster show that it breeds throughout the year, with two peak periods of maximum sexual activity in November–December and May–August as against the single peak in April-October observed by Paul (1942). The two peak periods seem to correspond to those of low salinity and high temperature of the coastal waters. These peaks of breeding activity under marine conditions seem to correspond with the restricted breeding periods in November-December and March–April in the backwaters.

9. Summary

1. A record of salinity of the waters of the Adyar estuary and back-water and of the Madras Harbour was maintained from March 1948 to August 1949 to study the effect of varying salinities on the breeding of the oyster, *Ostrea madrasensis*.

2. Regular examination of the gonads and observation of the act of spawning in the oyster show that under backwater conditions sexual activity is restricted to the November–December period usually followed by a supplementary spawning in March–April.

3. The occurrence of oyster larvæ of *Ostrea madrasensis* in the coastal waters of Madras near the Harbour throughout the year indicates that breeding is continuous.

4. Experiments with fertilised oyster eggs placed in varying prepared grades of salinity in the laboratory show that the optimum range of salinity necessary for early development up to the shelled veliger is from 22 to 26°/₀₀ at a temperature of 26°·0–28°·5 C. The maximum and minimum salinities required for the formation of the early veligers are 36°/₀₀ and 16°/₀₀ respectively. The ranges of salinity from 28–36°/₀₀ and 20–16°/₀₀ are described as the high and low salinity ranges respectively for the formation of the early veligers.

5. Successful spawning of both sexes seems to take place in the backwaters in almost the same range as the optimum requirement for the developing eggs.

6. In the Madras Harbour the oysters breed for most part of the year in the high salinity range for the formation of early veligers. Fall in salinity in October to about the optimum range is followed about a month later by increased number of larvæ in the plankton of the waters.

7. The difference in the breeding behaviour of the oysters in the backwaters in the two consecutive March–April seasons of 1948 and 1949 is attributed to the differences in salinity, only stray cases of male spawning having been observed under high temperatures and salinities in March to May 1949.

8. The incidence of oyster larvæ in coastal waters seems to depend on two different factors, *e.g.*, (i) high temperatures in summer months and (ii) fall in salinity after the rains of north-east monsoon.

9. The peaks in the occurrence of the larvæ in the sea seem to coincide with the restricted breeding periods in the backwaters.

10. After the early development of the eggs, a rise in salinity in the backwaters to 28°/₀₀ and above seems to be congenial for larval growth and spatfall.

11. The salinities which prevail in the sea seem to be always favourable for setting of spat judging from the fact that larvæ obtained from the plankton readily set in pure sea-water.

12. In the Adyar backwaters spatfall occurs only when the bar is open admitting sea-water into the estuary. There is marked difference in the intensity of setting of planktonic larvæ in two different samples of water of the same grade of salinity prepared (i) out of the sea-water from the open sea and (ii) out of the estuarine water when the bar is closed. This postulates presence of some suitable chemical or other factors in fresh sea water which promotes spatfall.

10. Acknowledgments

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11. References

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