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Sex Reversal in *Ostrea virginica*

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Report for 1936.

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The work done in 1936 was a continuation of previous studies. Records were continued of the sex of a number of marked oysters and experiments were carried on designed to show the effect of crowding on sex determination.

Changes in individual oysters of known sex.

In 1930, 1931, and 1932 the sex was determined in a number of oysters by boring a small hole in the shell of each and extracting the contents with a pipette. Some of these were marked, some were in recorded groups and some were embedded in blocks of concrete in such a way that the valves could open freely. In these cases it was possible to keep track of the sex of the individual oysters over several years.

Of the oysters marked in 1930 only one lived until 1936 and it died before it could be examined.

In 1931 two hundred and sixty oysters were drilled, most of these were two or three years old. The drill used was too big so that the holes made in the oysters were rather larger than those made in the oysters drilled the next year. Then the oysters were placed in large salt water tanks near the Station. For some reason not clearly understood the conditions here were evidently very unfavourable and the mortality was high in all oysters placed there and very high amongst the drilled specimens. The experiment was set up in August. By October a great many oysters had died, none of them had grown and many were ailing. They were

then put in wires baskets and sunk under the water near the Station. Here they suffered rather badly from silt but were obviously healthier than in the tanks.

In 1932 and every year thereafter a landing stage was built at the station. This made a convenient place to hand the baskets containing the oysters so that they were free from silt but able to get plenty of food and were very much healthier. But there is reason to believe that they never overcame their bad start enough to do as well as the oysters drilled in 1932. As a result of all the poor conditions mentioned above only forty-one of the original two hundred and sixty oysters were still alive in 1936. The changes in sex of these oysters from 1931 to 1936 are recorded in the following table.

In 1933 and 1934 two or three oysters were discarded because, for some reason, it was impossible to be sure of their identity. These were entered as "dead". Where it was impossible to decide the sex of an oyster the sign "?" is used.

Table I. Changes of sex of oysters marked in 1931.

<u>Number of oysters</u>	<u>Sex from year to year</u>					
	<u>1931</u>	<u>1932</u>	<u>1933</u>	<u>1934</u>	<u>1935</u>	<u>1936</u>
	Male	Male				
4	Male	Male	Male	Male	Male	Male
4	"	"	"	"	Dead	
2	"	"	"	?	"	
2	"	"	"	dead		
5	"	"	dead			
2	Female	Female	Female	Female	Female	Female
1	"	"	"	"	dead	
4	"	?	dead			
1	"	Male	Male	dead		
7	Male	?	dead			
1	"	?	Male	Female	Female	Female
2	"	Female	"	"	dead	
1	Male	"	dead			
1	Female	Female	Male	Female	Male	Male
2	"	"	"	Male	"	"
1	Male	Male	Male	Female	Female	Female
1	"	Female	"	Male	Male	Male
1	"	Male	"	"	"	Female

In 1932 a number of oysters were drilled and kept. These were nearly all two years old but a few were three years old. The drill used was a dentist's drill which made quite a small hole and the oysters were kept in wire baskets hung from the landing stage. They grew normally and most of them appeared to be quite healthy. One of the baskets containing these oysters was lost during the winter of 1934-35, but eighty-eight oysters still remained. The changes in sex of these oysters from 1932 to 1936 are recorded in the following table.

Table II. Changes of sex of oysters marked in 1932.  
Sex from year to year

<u>Number of oysters</u>	<u>1932</u>	<u>1933</u>	<u>1934</u>	<u>1935</u>	<u>1936</u>
17	Male	male	male	male	male
1	"	"	"	"	dead
3	"	"	"	dead	
2	"	dead			
3	"	male	dead		
1	"	"	male	female	female
6	"	"	"	male	"
1	"	female	?	dead	
1	"	male	male	female	male
2	"	female	dead		
1	"	"	?	male	male
1	"	"	?	female	female
2	"	female	female	female	male
1	"	"	male	male	male
3	"	"	female	"	"
1	"	female	female	?	female
1	"	?	male	male	"
1	"	female	female	female	dead
2	Female	female	female	female	female
1	"	"	"	"	male
2	"	"	"	male	"
1	"	"	male	female	female
3	Female	dead			
2	"	male	male	dead	
2	"	female	female	male	female
1	"	"	"	"	dead
3	"	male	male	female	female
1	"	"	female	"	"
2	"	female	male	male	female
5	"	male	male	male	male
3	"	"	"	"	female
1	"	"	female	female	male
1	"	"	?	"	female

Table II Continued.

<u>Number of oysters</u>	<u>Sex from year to year</u>				
	<u>1932</u>	<u>1933</u>	<u>1934</u>	<u>1945</u>	<u>1946</u>
1	Female	?	?	dead	
1	"	male	dead		
2	"	female	dead		
2	"	male	male	female	male
1	"	"	female	dead	
2	"	male	male	Female	dead
1	"	"	?	"	male

Consideration of the last two tables shows that there is almost every variation from oysters which remain of one sex throughout the period to oysters which change sex almost every year. In spite of all this changing the ratio of males to females remains fairly constant among the 1932 oysters and becomes higher as the oysters get older among the 1931 oysters. This is unlike conditions on the natural beds where the ratio of males to females becomes lower as the oysters get older. But probably none of the oysters considered above were living under quite as good conditions as those on the beds and the 1931 oysters started under very poor conditions so that their behaviour cannot be taken as typical. However, these tables do show that in many of the oysters the sex is determined afresh each year and that even six and seven-year-old oysters are capable of changing sex.

It is also noteworthy that among both lots of oysters, but particularly among those drilled in 1932, there is a higher proportion of males that never changed their sex than is to be expected from chance variations. Since none of these oysters were less than 2+ years old when first examined they might have had one

breeding season in the other sex when only 1+ years old. There is no way of proving or disproving this but it is known that less than 20% of the oysters in this neighbourhood mature when 1+ and only about 2% produce eggs. Thus it is very improbable that an appreciable number of these steadily male oysters had ever produced eggs. If this is so it is possible that they have a different genetic constitution from those that change at intervals. A few oysters were consistently female but no higher proportion than the other variations. It is, therefore, suggested that there may be some "true" male oysters, while the others are genetically hermaphrodite. This has been shown to be the case in some other molluscs.

#### Effect of Crowding on Sex of Oysters.

In 1933 there were indications that crowding might affect the sex of the oysters. To test this a number of oysters of known sex were suspended in individual cages strung some distance apart on wires while control oysters were crowded together in baskets. These oysters were kept over winter and until the next breeding season. Unfortunately a number of the floats supporting the wires became water logged and sank so that the experiments were practically worthless. These experiments were repeated over the winter of 1934-35 and, of those kept in baskets, some were crowded with other oysters and some with mussels as well. In 1935 it was found that there was very little difference between the oysters that were kept individually and those that were crowded together in baskets without mussels. But the baskets that contained mussels had a higher proportion of females in each case. These experiments were repeated over the season 1935-36 except that quahaugs were

used in place of mussels. Since a quahaug has no byssus they cannot make the tight clusters formed by mussels and so should be better. In addition a number of unbored oysters were similarly placed individually and in baskets both with and without quahaugs with the idea of comparing the sex ratios in 1936. As there were not many females available in 1935 they were divided between those placed individually and those crowded with quahaugs and none were in baskets containing only oysters.

One basket of males alone was lost over the winter of 1935-36. The effects of crowding on the remainder of the drilled oysters are summarized in the following tables:

Table III. Effect of crowding on oysters female in 1935.

	<u>Number of oysters</u>	<u>% female in 1936</u>	<u>% male in 1936</u>
Oysters suspended individually	40	35	65
Oysters in baskets with quahaugs	40	40	40

Table IV. Effect of crowding on oysters male in 1935

	<u>Number of oysters</u>	<u>% female in 1936</u>	<u>% male in 1936</u>
Oysters suspended individually	62	23	77
Oysters in baskets, no quahaugs	37	22	78
Oysters in baskets with quahaugs	66	27	73

Consideration of table IV shows that there was very little difference between the oysters that were kept individually and those that were crowded together in baskets without quahaugs. But the baskets containing quahaugs had a slightly higher proportion of females.

Table III records no oysters in baskets without quahaugs

but shows that there was a higher proportion of females in the baskets crowded with quahaugs than among the oysters kept individually.

Before discussing this it will be well to show the effects of the same treatments on undrilled oysters. The oysters used were all 1+ years old taken from cardboard collectors in 1934 and kept in a suspended crate in 1935. Random samples were used. The results of the experiment are summarized in the following table.

Table V. Effect of crowding on undrilled oysters.

	<u>Number of oysters</u>	<u>% male in 1936</u>	<u>% female in 1936</u>	<u>% ♀ in 1936</u>
Oysters suspended individually	95	59	37	4
Oysters in baskets, no quahaugs	97	76	24	0
Oysters in baskets with quahaugs	100	74	26	0

Now here there is quite a different set of results from that found among the drilled oysters. There is a very much higher proportion of females among those kept individually than among those kept in baskets and, in the baskets, a slightly greater proportion of females among those mixed with quahaugs. It must be remembered that, as these oysters were undrilled, it was impossible to determine the sexes before the experiment was started so that the sexes were mixed in the baskets.

The results of the experiments summarized in the last three tables strongly suggest one thing - that the close proximity of oysters of the opposite sex has an influence on the change of sex. Among the drilled oysters the sexes were kept separate in the baskets and there was little difference in the sex ratios between those kept individually and those kept in baskets. In the drilled

oysters the sexes were mixed and there were many more females among those kept individually. But if crowding alone were the limiting factor there should be just as great an effect where oysters of the same sex are crowded together as where they are of both sexes. The slight tendency towards a higher proportion of females in the baskets with quahaugs is much harder to explain if it is significant. Possibly the oysters in baskets were, for some reason, in a slightly healthier environment than those suspended individually but the oysters with quahaugs got more food because the quahaugs were less effective competitors.

The idea that close proximity of sexes in oysters tends to retard the change from male to female is not a new one. The writer has herself attempted to prove it before but previous experiments did not seem to support it.

Over the winter of 1936-37 a number of undrilled oysters are again being kept much as before, as a check on the above results.

#### Discussion

The records of the sex of marked oysters have now been kept for five and six years and a considerable variation is shown in the number of times that the sex has been changed during this time. A suggestively high number of male oysters (about 28% of the total) never changed sex during this period and it is possible that they have a different genetic constitution from those that change at intervals. It is proposed to submit a paper on this subject before long.

The experiments on crowding suggest that crowding with oysters of the same sex has little effect but that the close proximity of oysters of the opposite sex may produce a high proportion of males. Some further experiments testing this have been set up.