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In a recent paper on environment and sex in this species (Coe, 1936*a*), data were presented to show that the sex ratio at the first breeding season is correlated with age and size at sexual maturity. The proportion of individuals first functioning as females was found to be greatest in those localities and during those seasons most favorable for rapid growth. The mean size of the young females is larger than that of males of the same age, as Needler (1932*a*) had previously reported.

During the past year additional evidence has been obtained from other localities along the coast from Long Island Sound to the Gulf of Mexico. This evidence seems to justify certain more or less definite conclusions in regard to the factors which regulate the sexual changes which individuals of this species are known to undergo during their lifetime. Through the courtesy of Dr. Victor L. Loosanoff the writer has had the opportunity of examining additional extensive collections from Milford, Connecticut, while Professor T. C. Nelson has kindly furnished an abundant supply of young oysters of definitely known age from the culture frames of the New Jersey laboratory, on the shore of Delaware Bay, near Cape May. Dr. H. F. Prytherch, of the Bureau of Fisheries, has supplied additional material from Beaufort, North Carolina, and Dr. A. E. Hopkins has sent additional samples from the Gulf Coast laboratory at Apalachicola, Florida. The data concerning the sexual conditions found in this new material are included for comparison with those from other localities in Table I.

Examination of large numbers of fully adult oysters shows that there is usually an approximately equal number of individuals functioning at the time as males and females, or with an excess of females among the older members. The young oysters, on the other hand, always show a large excess of males at their first spawning season. This initial tendency toward maleness is the result of the more rapid growth and multiplication of the male than of the female constituents in the primary reproductive gland in a majority of the young individuals of this alternosexual species (Coe, 1932*a*, 1934).

From Cape Cod to the Chesapeake Bay, most of the young oysters may be expected to spawn at the age of one year. North of Cape Cod the first spawning is stated to occur more often at the end of the second year, as is the case with some individuals in certain years south of Cape Cod. On the coast of North Carolina and in the Gulf of Mexico, well nourished individuals of the early set spawn toward the end of their first summer, when only three to four months of age, while those of the later set do not become mature until the following spring.

TABLE I

Observed sex ratios during first breeding season at different localities and in different years

Locality	Date	No. undetermined	No. M.	No. H.	No. F.	Total	Ratio F. to 100 M.
New Haven Harbor	7-11-32	17*	389	4	13	423	3.34
" " "	7-27-33	1*	129	0	7	137	5.42
" " "	8-2-35	105*	521	4	40	670	7.66
" " "	7-28-36	3*	327	2	41	373	12.54
Milford (Bed)	8-1-34	2*	80	1	6	89	7.50
" "	7-20-35	2*	312	2	75	391	24.04
" "	7-3-36	77*	780	10	44	911	5.64
" "	8-13-36	7	410	4	58	479	14.15
" "	8-25-36	176†	92	0	21	289	22.83
Milford (Gulf)	7-7-37	0	116	3	25	144	21.55
Milford (Harbor)	7-7-37	10*	306	7	57	380	18.60
" "	7-28-37	6†	116	2	29	153	25.00
Great South Bay, L. I.	7-31-32	21*	197	0	7	225	3.55
West Sayville (float)	1932	0	154	4	48	206	31.17
Delaware Bay	7-7-37	0	520	13	218	751	41.92
Beaufort, N. C.	1933	0	43	0	21	64	48.84
" "	8-10 to 10-20-36	10	84	2	34	130	40.48
" "	10-20-37	73*	151	3	56	283	37.09
Apalachicola, Fla.	1936, 1937	81*	127	0	9	217	7.09
	Total	591	4854	61	809	6315	16.66

* Sexually immature.

† Spawned out.

The ratio of the two sexes at their first breeding season varies greatly from year to year and at different localities, as shown in Table I. It also varies at different parts of the season, not only because the males tend to become mature somewhat earlier than the females but also in consequence of an actual change in the sexuality of some of the individuals because a small percentage of the young oysters function as males in the early part of the season and as females immediately thereafter (Coe, 1936*a*). A few others are hermaphroditic and capable of self-fertilization.

This is well illustrated by three collections of yearling oysters from Milford, Connecticut, kindly supplied by Dr. Victor L. Loosanoff, of the Bureau of Fisheries, in 1936. A sample of 911 yearlings examined at the beginning of the breeding season contained less than 6 recognizable females for each hundred males, in addition to 10 hermaphrodites and 77 small individuals that were sexually immature. At the middle of the season, however, the ratio of females had increased to 14 and near the end of the season to nearly 23 (Table II). In 1937 there was likewise an increase in the proportion of females in the later of two collections from the same locality (Table II). Since the females are often somewhat later in maturing than are the males, they frequently spawn later. Hence, if it may be assumed that the immature individuals found early in the season contained a large proportion of potential females and that the majority of those spawned out when the last examination was made had previously functioned as males, the differences in the observed sex ratios of the several collections would have an adequate explanation.

TABLE II

Change of sex ratio during first breeding season

Locality	Date	No. undetermined	No. M.	No. H.	No. F.	Total	Ratio F. to 100 M.
Milford (Bed)	7-3-36	77*	780	10	44	911	5.64
" "	8-13-36	7*	410	4	58	479	14.15
" "	8-25-36	176†	92	0	21	289	22.83
Milford (Harbor)	7-7-37	10*	306	7	57	380	18.60
" "	7-28-37	6†	116	2	29	153	25.00

* Sexually immature.

† Spawned out.

An examination of some 4,500 young oysters from Long Island Sound during six successive years has shown a ratio of from $3\frac{1}{3}$ to 25 females for each hundred males, depending upon the local conditions and the season (Table I). The average size of the females exceeds that of the males and as a general rule those seasons and situations most favorable for rapid growth have shown the largest percentage of females.

Different culture beds in near-by situations may differ considerably in the ratio of the sexes in the yearling population. Those in shallow, warmer situations may spawn some weeks in advance of those less favorably situated. The first set may have a more favorable as well as a longer growing season than later ones, with a correspondingly larger proportion of females. Morphological sex differentiation may occur in the autumn in the one case or it may be delayed until winter in the later sets.

Since there is usually found an approximate equality in the ratio of the sexes or an excess of females after the second year, it is obvious that at least 25 to 45 per cent of the young males in these localities must change to females. It is not yet definitely known whether any of the yearling females change to males but there is conclusive experimental evidence that both males and females sometimes change their sex in the interval between two breeding seasons even at the age of four or more years (Needler, 1932*a*, Galtsoff, 1937). The stimuli which inaugurate these sexual transformations have not been determined, but it is suspected that both inherent tendencies and nutritional conditions may be involved.

The cellular mechanism involved in this change of sexual phase has been shown (Coe, 1932*a*, 1934) to depend upon the presence of gonia of both sexual characteristics or undifferentiated gonia through one sexual phase and their retention as residual cells after spawning. In some species of oysters and other mollusks an alternating rhythm of male and female phases is of regular occurrence (Coe, 1936*c*).

Summarizing the total collections from each area, as shown in Table I, it may be noted that the collections from New Haven Harbor for the four recent years in which suitable material could be obtained comprised 1,066 males and 101 females, a ratio of 9.6 females for each 100 males. From Milford, only nine miles distant, a total of 2,212 males and 315 females were contained in the eight collections during four summers. The sex ratio would there average 14.2 females for each 100 males, indicating a generally more favorable condition for the young oysters than in New Haven Harbor. This conclusion is supported by the fact that the average size of individuals of the same age was somewhat larger at Milford than at the latter locality.

From a natural bed in Great South Bay, Long Island, an exceptionally low ratio of females was found in 1932, while from culture floats not far distant, the ratio was nearly nine times as great (Table I) corresponding with a similar increase in the rate of growth.

Farther to the southward the ratio of females is still greater under favorable conditions, averaging more than forty for each one hundred males both on the culture frames in Delaware Bay and at Beaufort, North Carolina (Table I).

Correlation between Sexuality and Size at First Breeding Season

The more recent studies are quite in harmony with the evidence presented by Needler (1932*a*), Burkenroad (1931*a*), Coe (1932*c*), Roughley (1933) and others that the mean size of individuals functioning as females at the first breeding season is considerably greater than that of males of the same age. In an unselected sample consisting of 751 yearlings from the culture frames at the laboratory on the shore of Delaware Bay, 358 had a shell length of 50 mm. or more on July 7, 1937, while 393 were smaller. Examination of the gonads showed a ratio of 63.4 females for each 100 males in the group of larger individuals as compared with 26.6 females for each 100 males among the smaller ones. This correlation between sexuality and size for these 751 young oysters is shown graphically in Fig. 1. It will be noted that none of the females was less than 25 mm. in length and

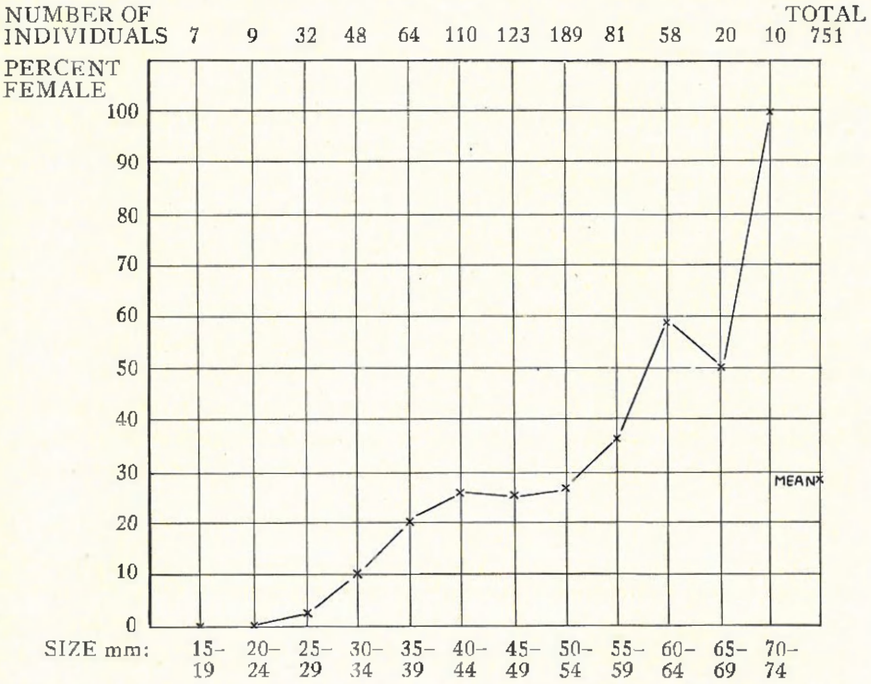

Fig. 1. Graph showing correlation between sexuality and size at first breeding season, based on an unselected sample of 751 yearlings from the culture frames at the New Jersey laboratory on the Shore of Delaware Bay near Cape May. The size of the class is shown at the bottom of the graph and the number of individuals in each class at the top. The mean percentage of females in the entire sample is also indicated.

none of the males more than 69 mm. The largest number of individuals, however, were between 40 and 55 mm. in length and in these groups there was little difference in the percentage of females.

DIRECT FEMALE DEVELOPMENT

The primary gonad may be either distinctly bisexual in appearance or it may become differentiated very early into either of the two sexual phases (Coe, 1932*a*). In the latter case there is a direct transformation of the primary undifferentiated gonia into the functional sexual cells. The female phase is then attained without indication of protandry.

In Long Island Sound the sexual phase of the first breeding season is sometimes distinguishable as early as October or November in exceptionally well nourished young at the age of only three or four months after setting. More frequently, however, morphological sex differentiation is delayed until mid-winter or later.

At Beaufort, North Carolina, direct female development appears to be the general rule, with a much higher ratio of young females than in more northern localities (Table I). Samples of well nourished individuals from Delaware Bay, known to have set July 9, 1936, likewise indicated direct development, when examined at the end of December. No evidence of incipient protandry was found at that time, although in many cases the gonads were sexually differentiated.

INDIRECT FEMALE DEVELOPMENT

It has been shown previously (Coe, 1932*a*), that the primary gonads of some young individuals develop directly into ovaries or spermaries, as the case may be, while those of other individuals of the same age are distinctly bisexual, or, to use a less ambiguous term, ambisexual.

The latter are characterized by a cortical layer of ovogonia and young ovocytes, with spermatogenic cells at one side or in the lumen. In many cases these ovocytes later degenerate or they may remain inactive during the primary functional male phase, to become activated and functional toward the end of that phase or some time thereafter. In this way the sexual phase may change from male to female either during the first breeding season or, much more frequently, during the following autumn.

A subsequent change from female to male phase evidently results from the later activation of some of the descendents of the primary undifferentiated gonia into spermatogonia. The propagation of such undifferentiated gonia may be continued year after year or even through a long lifetime, since some residual gonia always remain after spawning.

It was also previously reported (Coe, 1932*a*) that the developing gonads exhibit all intergradations between those of the so-called true males in which no indications of ovogonia or ovocytes can be detected and those which develop directly into ovaries. In a few individuals both types of cells in the ambisexual gonad multiply harmoniously, leading to the functionally hermaphroditic condition at the breeding season.

In northern localities some of the spermatogonia in the young ambisexual type of gonad may undergo transformations during the autumn comparable with those taking place in normal spermatogenesis (Coe, 1932*a*). These cells may later degenerate, followed by the activation of ovogonia, thus leading to the functional female phase at the first breeding season during the following summer. Such incipient protandry seems to be much less frequent than the direct development in which the initial dominant phase, either male or female, is retained during the winter and the following breeding season.

Sexual Conditions in Southern Localities

From North Carolina southward the initial sexual phase in the earliest set of the year becomes functional toward the end of the same season, when the young oyster is only three or four months of age, as Burkenroad (1931*a*) reported for the Louisiana oyster. Young of the later sets are similar to those of more northern localities in their sexual conditions, although protandry may be less frequent (Table I). Thus at Beaufort, North Carolina, and at Apalachicola, Florida, on the Gulf of Mexico, there may be two generations of this species in one year, individuals of the early set spawning some three to four months after setting while those of later broods do not become ripe until the following spring.

Cultures in both these localities are usually contaminated with more or less numerous individuals of the larviparous species, *O. equestris*, which matures at a still younger age and has a breeding season covering most of the year in warm situations. It is difficult to distinguish the two species externally when young, but the relations of the epibranchial chamber differ and the gonads are very different, since *O. equestris* has a sequence of overlapping male and female sexual phases and the spermatogenic cells are larger than in *O. virginica* and arranged in dense clusters.

A small collection of the over-wintered young at Beaufort in 1933, kindly supplied by Dr. H. F. Prytherch, showed a ratio of nearly 49 females to 100 males, while the first set of the season in 1936, stated to have occurred about June 1, showed a female ratio of about 40 when examined in August, September and October. The largest individuals had a shell length of 23 mm. in August, 40 mm. in September, and 70 mm. in October. In August there were only a few males with ripe sperm and toward the end of October most individuals of the early set were spawned out.

Collections of the early set taken September 20, 1937, about fifteen weeks after setting, were from 20 to 50 mm. in length and showed a female ratio of 37.09 (Table I). Those which exceeded 35 mm. in length had a female ratio of about 61 as compared with 16 for those of smaller size.

An unselected sample containing 88 individuals which were one year old, originating from the autumnal set during the last two weeks in September, 1936, on the other hand, showed a ratio of 75 females to 100 functional males. These were doubtless in their second spawning season, having previously participated in the spring spawning during June, 1937, when seven to eight months of age. The ratio of females was thus about twice as great as was found in the young at the first spawning period. This would indicate that the proportion of young males changing to the female phase for their second spawning period must have been about 22 per cent greater than the number of individuals, if any, which experienced a sex change in the opposite direction during the same time.

Small collections from Apalachicola, Florida, obtained through the courtesy of Mr. R. O. Smith, were examined three to five months after setting in 1936. These, combined with an additional collection kindly supplied by Dr. A. E. Hopkins from the same locality in 1937, showed a female ratio of 7.09 (Table I), corresponding closely to the ratios from northern localities under unfavorable conditions.

Discussion

The foregoing evidence concerning the sexual conditions in the Virginia oyster indicates that the sex-differentiating mechanism must be in a very labile condition since it responds so generally to environmental influences.

Because of the different sex ratios under different environmental conditions, it seems probable that the responses noted may depend upon the interaction of several associated factors, both genotypic and phenotypic. Among these it is evident that all these populations have at least the three following categories of hereditary and environmental influences.

(1). There is an inherent tendency toward protandry, as is the case with so many other mollusks, both pelecypods and gastropods, including the larviparous oysters (Orton, 1927; Coe, 1932*b*), *Teredo* (Coe, 1936*d*), *Venus* (Loosanoff, 1937), *Patella* (Orton, 1928), and *Crepidula* (Coe, 1936*b*, 1938), to mention only a few of the numerous examples. This tendency may or may not be realized, since direct female development so frequently occurs under favorable conditions.

(2). There appears to be an inherent tendency toward rhythmical change in the sexual phase during successive breeding periods, as in the larviparous oysters, *Teredo*, and certain gastropods. The fully adult Japanese oyster (*Ostrea gigas*) was proved by Amemiya (1929) to be subject to this change in sexuality in the interval between two breeding seasons. In the Virginia oyster a corresponding condition has been found by Needler (1932*a*, 1932*b*), by Burkenroad (1937) and by Galtsoff (1937). The proportion of the adult population in which this change occurred varied greatly in the different experiments, the discrepancies being due presumably both to varying environmental conditions and to comparatively small samples. The most extensive of these experiments is that by Galtsoff, who found that among 202 fully adult oysters of which the functional sexuality was reliably determined during the breeding season of 1936, only 167 retained the same sexual phase in 1937. Of the remainder, 14 had died in the meantime, while 9 or 14 per cent of the females had become functional males, and 11, or 8.9 per cent of the males were found to be in the female phase in 1937. The twenty individuals which experienced this change of sexuality during the interval between the two breeding seasons represented only 10.7 per cent of the total number of survivors.

It is evident, then, that sexual stability may predominate over any tendency toward rhythmical change much more definitely than is the case with the larviparous species. There is also considerable evidence which indicates that the sexual rhythm in the latter is associated with a metabolic rhythm (Coe, 1934). But the nutritive conditions are certainly modified by environmental influences. Change of sexual phase in *Crepidula*, like metamorphosis in other animals, has been shown recently (Coe, 1938) to be subject to environmental control, and this is presumably true to some extent in the oyster.

Immediately after spawning, the residual gonia in both the sexual phases show considerable variation. Some individuals which have spawned as males show residual small ovocytes in addition to apparently undifferentiated gonia. Others show only spermatogonia and undifferentiated gonia, while females may retain few or many or no young ovocytes after the cytolysis of the larger ovocytes. In both sexual phases cytolysis may proceed until only undifferentiated gonia remain and the individual has become essentially neutral.

Evidently relatively few fully adult individuals have gonads that are so evenly balanced that slight changes in the internal environment may activate either type of the residual gonia and thus determine the sexual phase of the following season. In the majority of both sexes, the same sexual phase is retained (Amemiya, 1929; Needler, 1932*a*; Galtsoff, 1937; Burkenroad, 1937).

(3). Since the primary gonads in the young oyster before morphological sex differentiation show great variability in the proportion of cells characteristic of the two sexual phase, it seems probable that these are to some extent at least dependent upon different heritable modifying factors (Coe, 1932*a*, 1934, 1936*a*). Functional males, hermaphrodites and females may then be expected under uniform conditions, the proportion of each depending upon the combination of factors present in each individual. But in the population of young oysters in any locality preceding the time or primary sexual differentiation, in the autumn, winter or summer, according to the locality, even a generally uniform environment will be highly variable in so far as each individual is concerned, since there are differences in age, in situation relative to other individuals and the like (Orton, 1936).

The larger average size of the females at the time of sexual differentiation and later and the higher ratio of females in those years and in those localities or situations most favorable for rapid growth may indicate either (*a*) differences in hereditary factors or (*b*) a direct response sexually to nutritive and other conditions, or (*c*) both. The evidence to date seems to imply that both these influences are operative.

The sexual conditions in other species of oviparous oysters are similar to those here discussed for the Virginia oyster. In the Indian oyster (*O. cucullata*) Awati and Rai (1931) found a corresponding tendency toward protandry, but with an excess of females in the adult population, which averaged 41 per cent males, 3 per cent hermaphrodites and 56 per cent females. Those which harbored the commensal crab *Pinnotheres*, however, showed 82 per cent males, 7 per cent hermaphrodites and only 10 per cent females, indicating a masculinizing influence on the part of the commensal, either nutritive or otherwise. Roughley (1933) likewise found a very strong tendency toward protandry in the Australian oyster (*O. commercialis*), but the adult population consisted of 54 to 88 per cent females, averaging about 2.7 times as many females as males. He considered that nutritive conditions were not influential in determining the sexual phase, since populations under poor conditions for growth showed about the same ratio of females as those having better nutritive conditions. The Portuguese oyster (*O. angulata*) is another species in which the females are more numerous than the males in the adult population, Orton and Awati (1926) reporting ratios of 60 to 88 per cent females. Indeed, in relatively few species of fully adult pelecypods are the males as numerous as the females (Pelseneer, 1935).

Amemiya's studies on change of sex in the Japanese oyster (1929) include later experiments (1935) in which a portion of the gills was removed from adult oysters immediately after spawning. The operation was performed on four groups of oysters, with an equal number of controls. The following year there was a slightly larger ratio of males in each one of the operated groups than in the controls. It was concluded that the proportion of individuals assuming the male sexual phase was influenced by the decreased facility of the operated individuals in obtaining food.

All these considerations emphasize the complexity of genotypic and phenotypic factors which may be influential in controlling the sexual phases of the Virginia oyster. The evidence here presented indicates that the populations along the coast from New England to the Gulf of Mexico consist of several local races living under widely different environmental conditions and differing in some respects in their inheritance. In the more northern localities a certain proportion of the young oysters function as females during their first breeding season at the age of about twelve months either by direct female development or indirectly after passing through an abortive or, less frequently, a brief functional male phase. In southern localities two generations, of which the second comprises a large percentage of females, may occur within the same twelve months period.

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