SEXUAL DIFFERENTIATION IN MOLLUSKS

I. PELECYPODS

By W. R. COE

Osborn Zoological Laboratory, Yale University, and Scripps Institution of Oceanography, University of California

AMONG the more than 10,000 species representing the class Pelecypoda may be found every grade of sexual differentiation, from species that are strictly of separate sexes to those that are almost invariably functionally hermaphroditic. Even two species belonging to the same genus may differ considerably in sexuality. And in a relatively few species the functional sexual phase of some or of all the individuals may change during the animal's lifetime. Indeed there are some species in which nearly every normal individual experiences several alternating male and female sexual phases. Hermaphroditism may likewise be complete, partial or occasional.

Because of these wide variations in the expression of sexuality, not only in different species but in different individuals of the same species and at different periods of life, the bivalve mollusks promise to offer evidence of some importance toward an understanding of the general problem of sexuality in organisms. This evidence must be examined as to its bearing on embryology, physiology and genetics.

Except in those species that are strictly of separate sexes (unisexual, or dioecious) the gonad contains the antecedent cells of both eggs and sperm. As a general rule the male cells mature in advance of the female cells, whereby the initial phase of functional sexuality is male. Such individuals are said to be protandric. Less frequently the initial phase is female and the individual is proterogynous. In functional hermaphrodites the eggs and sperm develop concurrently.

When each normal individual experiences two or more alternative sexual phases it is quite illogical to treat the sperm-producing and egg-producing stages as males or females as if they were distinct categories of individuals. They are merely temporary phases in the life of the same individual. Furthermore the proportions of individuals in each of these phases after the first would have less significance than some investigators have supposed, since it is obvious that if twice as many individuals are found in one of these phases as the other, that may be merely an implication that one of the phases lasts twice as long as the other. It might also mean that some are older than others.

The sexual conditions in the pelecypods are sometimes further complicated by the occurrence of neoteny, or juvenile sexuality. In such cases the individual may form a small number of gametes when it is very young and only a small fraction of its definitive size. This juvenile phase also may be more or less aborted and, as mentioned in the following pages for *Venus* and *Ostrea* may be of a different sexual type than that found in the adult. Consequently the sexuality of a species can be determined only by the examination of numerous individuals at all periods of life.

At the outset it must be recognized that the process of changing from one sexual phase to another is usually, but not invariably, gradual, with an intervening phase during which the gonad presents all possible grades of hermaphroditism ambisexuality. Since in many species it is not possible to examine the gonad of any one individual at successive stages of its life, it becomes necessary to infer its history from an examination of other individuals at different ages. These individuals, if protandric, might be erroneously thought to be males if examined when young, females when older, and hermaphrodites during the stage of sexual transformation. They represent, on the contrary, merely the sexual phases experienced by each normal individual during its lifetime.

154

In some species, however, definite proof of the number of changes of sexual phase experienced by any individual can be obtained by marking the shell and observing the sexuality at succeeding spawning periods. This is likewise feasible with gastropods. But with other forms, as the wood-boring bivalves, that is not possible and recourse must be had to observations of the gonads of many different individuals of known ages. In the larviparous species the presence of embryos or larvae in the gill chambers or mantle cavities is sufficient evidence as to the character of the preceding sexual phase.

With these facts in mind a brief survey of the sexuality of the whole group of bivalves may be undertaken. For more than half the total number of described species the sexual conditions are quite unknown or undescribed. For the others, approximately 96 per cent are reported as of separate sex or that type of sexuality is inferred. Less than 400 of the 10,000 described species are known to deviate from a strictly dioecious, or unisexual, condition, except for an occasional case of hermaphroditism due to an abnormality of development. Of those species that are normally monoecious (hermaphroditic, bisexual, ambisexual) some are uniformly functionally hermaphroditic, producing eggs and sperm at the same time, while others show various grades of mixed sexuality or changes in sexuality at some period of life.

The pelecypods have no copulatory organs or other external sexual characteristics, with the exception that in certain dioecious species of *Unio*, *Astarte* and a few other genera the two sexes, when adult, can be distinguished by the shape of the shell. Otherwise it is necessary to examine the gonad unless it is possible to observe the individual during the act of spawning. Accessory sexual organs are absent with the exception of the seminal vesicles, found only in *Xylophaga* and *Cuspidaria*, and the brood chambers on the gills of various larviparous species.

DEVELOPMENT OF PRIMARY GONADS

The sexuality of any individual obviously depends primarily upon its hereditary characters and these first find expression in the gonads. Consequently the history of the gonads must be followed in order to obtain reliable information relative to the prospective sexuality of any individual.

The primary gonads arise from a pair of germinal primordia situated in the posterior portion of the body, ventral to the pericardium and in close proximity to the visceral ganglia and the renal organs. Each of the primordia develops into a profusely branching system of follicles surrounding the intestine in the mesosoma, or visceral mass. In the mussels the gonads extend also into the mantle lining both valves of the shell and in *Anomia* into the mantle on the right side of the body.

The constituent cells of each follicle soon become differentiated into nutritive follicle cells and primary gonia. If the individual is to become hermaphroditic the primary gonia soon give rise to cells of two types, clearly distinguishable as spermatocytes and ovocytes. But even in dioecious species there may be indications of ambisexuality in the primary gonad and some ovocytes are often formed in the early spermary. Such ovocytes undergo cytolysis during spermatogenesis.

A. AMBISEXUALITY: MONOECISM

For convenience of reference the various grades of ambisexuality may be considered as consisting of the four following categories: (1) functional ambisexuality (functional hermaphroditism), (2) consecutive sexuality, (3) rhythmical consecutive sexuality, and (4) alternative sexuality. Since the terms "bisexual" and "bisexuality" as currently used by different writers may indicate either a monoecious condition of an individual or a dioecious condition of a species they may well be avoided. The term "ambisexual," if used strictly with reference to individuals having gonads composed of both male and female sexual cells, is less ambiguous than either bisexual or hermaphroditic.

1. Functional ambisexuality (Functional hermaphroditism)

Functional hermaphroditism is found in nearly all phyla of invertebrates and in every phylum of plants. There is here no incompatibility between the male and female sex organs and both types of sexual cells may be produced simultaneously. Not infrequently, however, this condition is preceded by a brief period of maleness or occasionally of femaleness. In some of these species the extent of protandry is increased by unfavorable conditions and can be controlled experimentally.

(a) *Normal*. This implies a strictly monoecious, or ambisexual, condition, with the con-

current production of gametes of both sexual types by each individual. In some species the male and female elements are in separate gonads, with separate efferent ducts; in others there are male and female portions of a single gonad, while still others have both spermatogenic and ovogenic cells in the same follicle. A tendency toward protandry is general but some individuals discharge both types of gametes at the same time and some of the eggs are capable of self-fertilization.

Among the better known of the strictly hermaphroditic pelecypods are certain species of *Pecten*, *Gemma*, *Tridacna*, *Tivela*, *Anodonta*, *Kellia*, *Thracia*, and other genera of the suborder Anatinacea, *Cardium*, *Pisidium*, *Sphaerium*, *Musculium*, *Poromya*, *Silenia*, *Cyclas*, and at least one species (*Teredo diegensis*) of the wood-boring bivalves. In a considerable proportion of these the eggs are fertilized within the mantle cavity and the young complete development to the adult form in brood pouches on the gills of the parent.

The ribbed scallop (*Pecten irradians*) may be taken as a typical example of functional hermaphroditism. In this and other species of the genus the spermatic tissues occupy the dorsal portion of the gonad and the ovarian tissues the much larger ventral portion. The two portions are not demarcated morphologically but are readily distinguished in sexually mature individuals because of the differences in color, the spermatic tissues being creamy white and the ovarian tissues deep pink. Both eggs and spermatozoa are discharged through the same efferent ducts.

Both types of gametes mature at about the same time, although there is a tendency toward protandry, and a considerable portion of the sperm will usually be discharged before any of the eggs are spawned. In other individuals both eggs and sperm are discharged simultaneously and self-fertilization may sometimes occur (Gutsell, 1931). In nature the spawning of one individual, followed by the presence of gametes in the water presumably initiates a corresponding reaction in many other individuals in the vicinity. Simultaneous spawning and cross fertilization will then be general in the population. Furthermore, certain individuals appear to be self-sterile.

In other species of the same genus, as *P. opercularis* and *P. maximus*, some individuals are distinctly protandric at each spawning season, nearly all of the sperm being discharged before the eggs of the same individual become fully ripe. Other individuals are evidently proterogynous. There is thus a brief period when the individual morphologically hermaphroditic but functionally male and another period when it is functionally female, with an intervening stage when it is functionally hermaphroditic. A similar condition is found in *P. latiauritus* var. *monotimeris* on the coast of Southern California. Protandry is more frequent than proterogyny. This type of sexuality is consequently intermediate between such forms as are strictly hermaphroditic and those that experience consecutive male and female sexual phases.

In one species (*Teredo diegensis*) of the wood-boring mollusks most of the mature individuals are functionally hermaphroditic, following a primary protandric phase. The gonads at all seasons of the year on the coast of Southern California usually contain ripe sperm and mature ova. Experimental self-fertilization is often successful. Larvae are present in the brood chambers of the gills at all seasons of the year. In addition to the larviparous hermaphrodites the population contains a very small percentage of individuals known as true males. In the latter the ovocytes are aborted.

The successive sexual phases of this species may be indicated by the following schematic representation:

Primary male phase ... True male (exceptional)
Primary male phase ... Functional hermaphrodite

In a species of cockle (*Cardium corbis*), of the Pacific coast, the eggs and sperm are produced simultaneously in adjacent follicles of the gonad (Edmondson, 1920). This is the case also with *C. crassum* and *C. serratum*, although *C. edule* is dioecious. The Pismo clam (*Tivela stultorum*) is hermaphroditic, but as a general rule the sperm and eggs do not mature at the same time (Weymouth, 1920). Consequently the conditions are seldom favorable for self-fertilization. There is a general tendency toward protandry, for the hermaphroditic condition of the adult is frequently preceded by a primary male phase in young individuals.

(b) *Accidental or developmental.* Even in species which are otherwise strictly of separate sexes there may be an occasional individual with functional hermaphroditism. These can all be considered

resulting from deviations in the developmental processes due to the failure of the sex-differentiating mechanism to function normally. The proportion of spermatogenic and ovogenic tissues in the gonad is highly variable, some individuals having approximately equal parts of both sexual types, while others are principally of one sex, with but a few cells characteristic of the opposite sex. This type of sexuality is more common in the pelecypods than in most other groups of animals and it occurs most frequently in young individuals at the first reproductive season. In certain local races of oviparous oysters, clams, and mussels it is impossible to determine whether the initial sexual state is normal or accidental hermaphroditism.

2. Consecutive sexuality

This type of sexuality implies a single change in the functional sexuality of the individual, or a reversal. The male and female phases may intergrade or they may be sharply separated. Consecutive sexuality is of wide occurrence, being found not only in most classes of mollusks but in other phyla of invertebrates, including annelids, echinoderms, nemerteans and crustaceans, as well as in some plants and a few fishes. Most of the invertebrates of this type experience but a single change of sex, usually from male to female. An overlapping of the two sexual phases may produce a brief period of hermaphroditism, at which time self-fertilization may occur. Other representatives of the same groups may experience a rhythmical sexuality, with regularly alternating male and female phases.

The histological basis of this type of sexuality depends upon the presence of an essentially ambisexual gonad, associated with a hereditary mechanism which causes the activation of one of the two types of sexual cells in advance of the other.

Consecutive sexuality may become manifest during a single spawning season, one sexual phase following the other without an intervening period of recuperation, or there may be a more or less prolonged period of sexual inactivity between two successive phases. When there is but a single spawning period each year there is often a period of several months between the termination of one sexual phase and the beginning of the next.

The quohog (*Venus mercenaria*) may be taken as an example of consecutive sexuality. In this species, as determined by Loosanoff (1937), nearly all individuals experience a juvenile functional

male phase when only a few months of age and only a small fraction of the definitive size. Following this initial male phase the sexes are with few exceptions strictly either male or female, with approximately equal numbers of each sex. There is no indication of a further change of sexuality. The primary, juvenile gonads are invariably more or less distinctly ambisexual, forming a graded series from predominantly male to predominantly female, although about 98 per cent of all individuals are protandric. The remaining 2 per cent are proterogynic. The adults are thus dioecious, following (with few exceptions) a more or less aborted primary male phase.

The sexual conditions in different individuals of this species may be schematized as follows:

1. Juvenile true male phase (exceptional) ... Adult male (true male).
2. Juvenile ambisexual male phase ... Adult male.
3. Juvenile ambisexual male phase ... Adult female (protandric female).
4. Juvenile ambisexual male phase ... Adult hermaphrodite (exceptional).
5. Juvenile female phase (exceptional) ... Adult female.

This type of sexuality is also characteristic of the wood-boring mollusk *Bankia setacea*, although the protandric females may experience a second change of sexual phase if the length of life of the individual is sufficiently prolonged. Self-fertilization is sometimes possible in the juvenile phase of protandric females, due to the overlapping of male and female phases (Coe, 1941). The population contains a small proportion of true males as well as of juvenile females; in the latter the initial male phase is aborted or omitted.

Xylophaga dorsalis, another species of wood-boring pelecypod, is evidently protandric, for most of the smaller and younger individuals function as males and the larger as females (Pynchon, 1941). The change of sex is indicated not only by the hermaphroditic gonad but also by the appearance of the seminal vesicles, which are large in the male phase but diminish in size and eventually disappear in the female phase. This is one of the few pelecypods in which a specialized storage organ for the sperm has been found but there is no direct evidence that self-fertilization is possible.

3. Rhythmical consecutive sexuality

Some of the ambisexual species which are composed of individuals living long enough to undergo

several complete spawning periods experience an equal number of sexual phases. Such is the case with the larviparous oysters, *Ostrea edulis, O. lurida, O. equestris* and others. In all of these the initial phase is usually male, followed by a series of alternating female and male phases throughout life. The eggs are fertilized within the mantle cavity, usually with sperm which have been discharged into the water by other individuals in the vicinity. The fertilized eggs are carried through the gills to the pallial chamber of the parent, where they complete development to the adult form.

The European oyster (*Ostrea edulis*) is the best known example of this type of sexuality as the result of the extensive studies by Orton (1927, 1927a, 1933). In this species the initial stages of spermatogenesis are begun even before the ova of the female phase are set free from the gonad. Sperm formation then continues for a month or two. After the sperm have been discharged the production of ova for the following female phase is begun. This phase may become functional immediately or may be delayed until the following year, according to the season. As a general rule under favorable conditions each adult oyster completes one male phase and one female phase each year. Since some individuals function in the male phase early in the spawning season and change later to the female phase, while others have the reverse sequence of the sexual phases, the oyster population is provided with both sexual types during the entire reproductive season (Cole, 1941).

Orton (1927) found much variation in the proportion of the two types of sexual cells in the gonads of different individuals, with all gradations from "pure male" to "pure female." The "pure male" differs from the male-phase individual which has followed the female phase in lacking ovocytes in the gonad and in the profusion of sperm produced.

The Pacific coast oyster (*Ostrea lurida*) experiences a similar series of male and female phases. On the coast of Southern California those individuals which are hatched in early spring may sometimes have three phases of funtional sexuality during one calendar year. A brief male phase when the individual is 4 to 6 months of age is followed by a more lengthy female phase. The latter may be concluded in time for a second male phase in the autumn. Spermatogenesis is usually

in progress before the larvae of the female phase have left the mantle cavity.

Individuals which are hatched in the summer or early autumn complete only one or two sexual phases in that calendar year. This individual variation in periodicity provides the population with individuals in both sexual phases at all times during the spawning season (Coe, 1932a, 1941). Two separate spawning periods, resulting in two broods of young, may sometimes occur during a single female phase. Hopkins (1936, 1937) concluded that in certain years about half the adult oysters in the culture beds in Puget Sound produced two broods of young during the spawning season, while in other years only about three fourths of the adults produced even a single brood.

In addition to those individuals with regularly alternating sexuality the population contains a small proportion of true males which retain the male phase indefinitely and perhaps throughout life. Furthermore, some of the primary male phase individuals, the so-called ambisexual males, have a preponderance of ovocytes in the gonad and change to the female phase after a brief initial male phase. Others of the same age have relatively few and small ovocytes in the primary gonad and retain the male phase much longer. And finally, the primary male phase is occasionally aborted or omitted, the first really functional phase thereby becoming female.

The following scheme will illustrate the successive sexual phases of these different classes of individuals:

1. True male (exceptional) ... Second male phase ...

2. Ambisexual male phase ... First female phase Second male phase ... Second female phase ... Third male phase, etc.

3. Primary female phase (exceptional) ... First male phase ... Second female phase, etc.

Another species, *O. equestris*, with similar sexual phases, is found on the southeastern coast of the United States and on the shores of the Gulf of Mexico. Gutsell (1926) first called attention to its ambisexual nature.

Teredo navalis, one of the species of wood-boring mollusks, likewise has a closely similar sequence of sexual phases. The protandric nature of the sexual cycle and the distinctions between ambisexual males and true males were reported by C-

female phase

the summer or
or two sexual
This individual
the population
at all times
1933, 1934).
resulting in two
occur during a
1936, 1937) also
about half the
Puget Sound
the spawning
about three-
even a single

with regularly
tion contains a
which retain the
haps throughout
primary male-
bisexual males,
in the gonad
a brief initial
age have rela-
primary gonad
longer. And
is occasionally
ally functional

late the succes-
ferent phases of

second male phase

female phase ...
phase ... Third

tional) ... First
, etc.

with similar sexual
tern coast of the
of the Gulf of
alled attention to

of wood-boring
similar sequence
dric nature of the
between ambi-
reported by Coe

(1933). The sexual rhythm from the primary male phase to the female phase, sometimes followed by a second sequence of male and female phases, was described the following year (Coe, 1934a), with an account of the histological changes involved. In a brief summary of the sexual conditions of this species (Coe, 1935) it was shown that in exceptional individuals the initial sexual phase is female. Confirmation of this sequence with additional data was published by Grave and Smith (1936). A statistical analysis of the sexual phases of 2987 individuals examined at various ages and seasons of the year was reported by Coe (1936) as additional proof of the sexual rhythm and the presence in the population of a small proportion of true males and of a still smaller proportion of proterogynic individuals. These conditions were further emphasized in a subsequent paper by Coe (1941). In a study of young individuals only, Grave (1942) concluded that the proportion of proterogynic individuals was greater than previous observations had indicated. The data presented, however, do not seem to justify such a conclusion.

The following scheme will indicate the probable successive sexual changes in such individuals as live long enough to experience what may be considered as the normal sequence of sexual phases. Mortality during the second phase is usually very high and few individuals reach the fourth (female) phase.

1. True male phase (exceptional) ... Second male phase ...
2. Ambisexual male phase ... First female phase Second male phase ... Second female phase (if length of life suffices).
3. Functional hermaphroditic phase (exceptional) ... Male or female phase ...
4. Female phase (exceptional) ... First male phase Second female phase ...
5. Female phase (exceptional) ... Second female phase (?)

4. Alternative sexuality

In the oviparous oysters and perhaps in some other pelecypods the adults function seasonally as separate sexes, although it is impossible to predict during one reproductive season the sexual phase which the individual will assume at the next.

This type of alternative sexuality is illustrated by the commercial oyster of the North Atlantic coast, Ostrea virginica. At the first spawning season there is a strong tendency toward protandry, more than 70 per cent of the young oysters in some localities functioning as males at that time (Coe, 1938). After the second spawning season the number of individuals in each sexual phase is approximately equal, with a tendency toward an excess of females among the older individuals (Needler, 1936).

The adult Japanese oyster (O. gigas) was proved by Amemiya (1929) to be subject to a change in sexuality in the interval between two spawning seasons. A corresponding condition was found by Needler (1932), Burkenroad (1937), and Galtsoff (1937, 1938) in O. virginica.

Galtsoff (1937, 1938), using marked adult oysters, found that 8 per cent of those functioning as males changed to the female phase the following year, while 13.1 per cent of the females similarly reversed their sex. In the second year 11.2 per cent of the males of the preceding year showed a change of sex and 12.1 per cent of the females changed to the male phase. The total number of sex reversals in the first year was 9.7 per cent and in the second year 11.5 per cent. Hence this process of reversing the sexual phase seemed to be limited to a minority of the experimental animals. Moreover, 69 per cent of those that changed the sexual phase in the first year reversed again the second year. Some of the males which had changed to the female phase spawned in male fashion in the early part of the season but as typical females later.

In O. gigas, however, the proportion of the adult population experiencing changes in sexuality is evidently much greater, for Amemiya (1929) concluded that 25 per cent of the females and 60 per cent of the males in his experiment reversed their sex during one winter.

Protandry and change of sexual phase has been found in other species of oviparous oysters. Roughley (1933) concluded that nearly all the young of O. cucullata (commercialis) spawn first as males, although there appeared to be 2.7 times as many females as males in the adult population. Needler (1936) found more than 3 times as many females as males in an old population of O. virginica.

The primary gonad of O. virginica usually contains the antecedent cells of both sexes. Its protandric nature frequently becomes manifest when very young by the rapid proliferation of the spermatogonia and the formation of few or many ovocytes (Coe, 1932, 1934, 1938). In Long Island

Sound the process of gametogenesis often begins in the autumn when the animal is only 3 to 4 months of age; it is interrupted at the approach of winter and resumed the following spring. The first spawning occurs at the age of about one year.

The gonads of the young oysters previous to sexual maturity show all gradations between those of the so-called true males in which no ovocytes can be detected and those which develop directly into ovaries. Approximately 1 per cent (61 of 6315) of these young oysters become functionally hermaphroditic at the first spawning season. Most of the others function strictly as males or females during the first spawning season, although an occasional individual may produce sperm during the early part of the season and ova a few weeks later (Coe, 1938).

In those young individuals that are to function as males during their first spawning season, such ovocytes as may be formed in the early primary gonad are cytolyzed during the progress of spermatogenesis. Female sexual differentiation may be direct, with little or no indication of protandry, but it is more often indirect. In the latter case the primary gonad is more or less distinctly ambisexual during the autumn but takes on the appearance of an ovary the following spring by the proliferation of ovogonia and the cytolysis of the spermatogenic cells.

"A subsequent change from female to male phase evidently results from the later activation of some of the descendants of the primary undifferentiated gonia into spermatognia. 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" (Coe, 1938). The process by which this occurs is more fully explained by Loosanoff (1942).

The following scheme shows the presumable sexual phases of this species of oyster, based on the assumption that only those in which the primary gonad is ambisexual experience a change of sex in the second and later years.

1. Primary gonad male ... Male phase (first year) ... True male ...
2. Primary gonad ambisexual ... Male phase (first year) ... Male or female phase ...
3. Primary gonad ambisexual ... Functional hermaphrodite (first year) exceptional ...
4. Primary gonad ambisexual ... Male phase (early portion of first year); female phase (later portion of first year) exceptional ...
5. Primary gonad ambisexual ... Male phase (first year) ... Female phase (second year) ... Male or female phase ...
6. Primary gonad ambisexual ... Female phase (first year) ... Female (or male?) phase ...
7. Primary gonad female ... Female phase (first year) ... True female ...

Immediately after each spawning period the residual differentiated sexual cells undergo phagocytosis and cytolysis, leaving only undifferentiated gonia in the collapsed gonadal follicles. The individual thereby returns to a condition similar to that of sexual immaturity. Within a few weeks, however, the apparently undifferentiated gonia resume activity and they then show that they have, in reality, become uniformly differentiated into the cells characteristic of the one sexual phase or the other. It is not at present known to what extent, if any, environmental conditions determine whether this differentiation shall be in a male or female direction.

The percentage of females at the first spawning season varies with the locality and with the season. The females at this time average somewhat larger than males of the same age and the data obtained as to the sexuality of more than 6000 young oysters from various localities from Cape Cod to the Gulf of Mexico indicates that those conditions and situations most favorable for rapid growth have the largest proportion of females. Examination of the gonads of 4033 yearling oysters from Long Island Sound whose sex could be determined indicated that 3578 were in the juvenile male phase and 416 in the female phase, together with 39 which were evidently hermaphroditic. This is a ratio of 11.6 females to 100 males. Different samples varied from 3.34 to 25 (Coe, 1938).

There were in the same collections, however, individuals which were at the time sexually differentiated. Since female sexual differentiation commonly occurs at a later period than male differentiation, it seems probable that a still larger proportion of the sexually undifferentiated young would have developed into females than was found among those which were already sexually differentiated at the time of examination. The proportion of females is always largest toward the end of the spawning season, due in part to the protandric nature of seasonal hermaphroditism, especially to the delayed differentiation of females.

If allowance be made for these conditions it seems safe to assume from the data given by Coe (1938) that the yearling population of oysters

Long Island Sound consists in average years of approximately 20 individuals in the functional female phase to 100 in the male phase.

A sample of 751 yearlings from Delaware Bay had a ratio of 41.92 females to 100 males, while three samples from Beaufort, North Carolina, varied only from 37.09 to 48.84 females to 100 males.

Whether these differences may be due to differences in the hereditary characteristics of the populations in the localities mentioned or whether to responses to different nutritive and other environmental conditions, or to both, is not at present known.

Since there is usually found an equality in the ratio of the sexes or an excess of females after the second year, it is obvious that at least 25 per cent of the juvenile males in these localities must later function seasonally as females. It is not known whether any of the juvenile females later change to males. Nor is it known to what extent environmental conditions influence the sex differentiating mechanism.

B. UNISEXUALITY; DIOECISM; GONOCHORISM

The vast majority of pelecypods are believed to be strictly of separate sexes, except for an occasional hermaphrodite. Such was formerly the conclusion in regard to *Venus mercenaria* and *Ostrea virginica* until their juvenile sexual phases, as has already been explained, were discovered in recent years. Consequently it is now evident that both juveniles and adults must be examined in order to ascertain definitely the sexual conditions of a species.

This has now been done for *Volsella (Modiolus) demissus*, *Mya arenaria*, *Petricola pholadiformis*, *Barnea truncata*, *Mytilus californianus*, *M. edulis*, *Septifer bifurcatus*, *Donax gouldii* and *Anomia simplex* without finding evidence of ambisexuality except in the sexually undifferentiated young gonads and an occasional hermaphrodite. None of these species has a distinctly juvenile phase of sexuality in the localities in which the investigations were made. Males and females occur in approximately equal numbers.

DISCUSSION

The inquiry may now be raised as to the significance of the diversities of sexuality that have been mentioned. Is the sex-differentiating mechanism so labile that it responds to relatively slight environmental changes or are variable genetic combinations more directly responsible for the variability observed? The answer seems to be that both these factors are presumably operative in all cases, although the evidence as to the influence of the environment requires more direct experimental proof.

In general, it is evident that there is often an inherent tendency toward a change in functional sexuality at successive reproductive periods. This change may occur during the brief interval between two successive spawnings in one season, as in the larviparous oysters, in *Teredo* and in *Pecten*, or in successive seasons, as in the oviparous oysters. There may be a single change, affecting the primary male phase only, as in the consecutive sexuality of *Venus* and as is usual in *Bankia* or the changes may be numerous, as in the long-lived larviparous oysters.

Juvenile sexuality, or neoteny, as reported for *Venus* and oviparous oysters is of wide occurrence in both animals and plants, being characteristic of certain gastropods (as *Crepidula*), annelids (as *Ophryotrocha*), gephyreans (as *Bonellia*), echinoderms (as *Leptasterias*), nemerteans (as *Geonemertes*), and has been reported for crustaceans (*Pandalus*), fishes (*Sparus*), and amphibians (*Rana*). It is, of course, well known for the axolotyl. Some of these resemble the pelecypods mentioned in that the sexuality of certain individuals in the juvenile phase is opposite that of the adult. Both in the pelecypods and in the other groups mentioned the neotenous condition is often abortive, leading to the cytolysis of such gametes as may be partially or fully formed; hence spawning may not occur in the juvenile phase.

Particularly noteworthy is the variability in the sexuality of different individuals of the same species and in some cases in a single individual at different periods of life. This variability resembles in some respects the color patterns of certain mammals or of certain plants. Both are evidently the visible manifestations of various combinations of multiple modifying hereditary factors.

In the functionally hermaphroditic species, as *Pecten*, for example, the relative extent of the spermatic and ovarian tissues in the gonad is highly variable. Some individuals closely approach a unisexual condition, particularly a female condition, with only a very small amount of spermatic tissue or none at all. But occasionally the relations are reversed. Furthermore the extent of protandry or of proterogyny varies greatly in different members of a single population.

The importance of environmental influences in modifying these characteristics has not been satisfactorily shown experimentally for the pelecypods, although Amemiya (1935) found that groups of Japanese oysters having portions of their gills removed showed a larger percentage of males the following season than similar groups with uninjured gills. Awati and Rai (1931) also concluded that the disturbance in the nutrition of the Bombay oyster caused by the commensal crab *Pinnotheres* increased the proportion of males and Rosenwald (1926) found that the gastropod *Limax* became protandric or proterogynic according to the environmental conditions, particularly the amount of moisture. It was mentioned that the Virginia oyster has a larger proportion of females in the yearling population in those years and in those localities most favorable for rapid growth (Coe, 1936, 1938). At this age the functional females are of larger average size than those individuals that are functioning as males. Hence it seems quite possible that temperature and nutritive conditions within the body may influence the proliferation of one rather than the other of the alternative types of sexual cells in the early ambisexual gonad. Such influences are well authenticated among nematodes, annelids, amphibians and plants.

In several groups of animals, both invertebrates and vertebrates, as well as in some plants, there exist local races with differing hereditary types of sexuality. Such races are reported by Bloomer (1939) in the fresh-water pelecypod *Anodonta cygnea*, and have been thought to exist in the Virginia oyster (Coe, 1936, 1938). Additional information, particularly of an experimental nature, will presumably reveal them in many other species.

CONCLUSION

It may be concluded then that the pelecypods as a group are predominantly of separate sexes. Among the relatively few hermaphroditic species four intergrading types of ambisexuality are recognized: functional ambisexuality (functional hermaphroditism), consecutive sexuality, rhythmical consecutive sexuality and alternative sexuality. Some of the hermaphroditic species are characterized by multiple modifying hereditary sex factors which may produce all grades of ambisexuality from true males to true females.

There are also various hereditary physiological time factors which control the periodicities of the alternative sexual phases. The ambisexual species show a general tendency toward a rhythmical series of alternating male and female phases, of which the initial sexual phase is more frequently male. Some of the species appear to be composed of local races with differing sexual tendencies and these tendencies become manifest in differing degrees according to the environmental conditions.

The sexual conditions described on the foregoing pages are not peculiar to the pelecypods but have their parallels in other groups of organisms, both animals and plants. Even in the sponges there are species with incompletely separated sex, some individuals functioning for a time as male and others as females, while the later formation of eggs in predominantly male individuals suggest a general tendency toward protandry (Fischer 1940). Such is also the case with certain planarians, gephyreans, nematodes, annelids, branchs, gastropods, echinoderms, crustaceans, fishes. In some of these, as has been mentioned for *Venus*, the fully mature individuals are strictly of separate sexes, although nearly all the young first function as males. The genetic males (true males) continue to function as such throughout life, while the genetic females (hermaphroditic males, or protandric females) function as males when young and as females thereafter.

There is no direct proof of phenotypic sex determination in the pelecypods, although as a general rule in cases of alternative sexuality the male phase is often indicative of immaturity and unfavorable environmental influences, while the female phase is more often associated with optimum physiological conditions. This is in harmony with the evidence from other groups of animals and plants.

All these manifestations of sexuality are consistent with the view that the prospective sexuality of the individual is dependent upon the quantitative balance of the male as opposed to the female factors in the hereditary mechanism. They may all be referred to the homozygosity or heterozygosity of the primary sex factors, with their associated modifying factors for the activation or suppression of either the male- or female-determining components at certain stages of development or in harmony with different degrees of physiological maturity. They are also responsive to environmental conditions both within and without the body of the organism.

LIST OF LITERATURE

Amemiya, I. 1929. On the sex change of the Japanese common oyster, *Ostrea gigas* Thunberg. *Proc. Imp. Acad. Tokyo,* 5: 284–286.

———. 1935. Effect of gill excision upon the sexual differentation of the oyster (*Ostrea gigas*). *Rept. Jap. Ass. Adv. Sci.,* 10: 1023–1026.

Awati, P. R., and H. S. Rai. 1931. *Ostrea cucullata* (the Bombay oyster). *Indian Zool. Memoir,* No. 3, Lucknow.

Bloomer, H. H. 1939. A note on the sex of *Pseudanodonta* and *Anodonta. Proc. Malac. Soc. London,* 23: 285–297.

Burkenroad, M. D. 1931. Sex in the Louisiana oyster, *Ostrea virginica. Science,* 74: 71–72.

———. 1937. The sex-ratio in alternational hermaphrodites, with especial reference to the determination of sexual phase in oviparous oysters. *Jour. Marine Research,* 1: 75–84.

Coe, W. R. 1932. Sexual phases in the American oyster (*Ostrea virginica*). *Biol. Bull.* 63: 419–441.

———. 1932. Development of the gonads and the sequence of the sexual phases in the California oyster (*Ostrea lurida*). *Bull. Scripps Inst. Oceanogr., Tech. Ser.,* 3: 119–144.

———. 1933. Sexual phases in *Teredo. Biol. Bull.,* 65: 283–303.

———. 1934. Alternation of sexuality in oysters. *Am. Nat.,* 68: 236–252.

———. 1934a. Sexual phases in the pelecypod mollusk *Teredo. Science,* 80: 192–193.

———. 1935. Sequence of sexual phases in *Teredo, Ostrea* and *Crepidula. Anat. Rec.,* Suppl., 64: 81.

———. 1936. Sequence of functional sexual phases in *Teredo. Biol. Bull.,* 71: 122–132.

———. 1936a. Sex ratios and sex changes in mollusks. *Mem. Mus. Hist. Nat. Belgique* (2 ser.), fasc. 3: 69–76.

———. 1938. Primary sexual phases in the oviparous oyster (*Ostrea virginica*). *Biol. Bull.,* 74: 64–75.

———. 1940. Divergent pathways in sexual development. *Science,* 91: 175–182.

———. 1941. Sexual phases in wood-boring mollusks. *Biol. Bull.,* 81: 168–176.

———. 1943. Development of the primary gonad and differentiation of sexuality in *Teredo navalis* and other pelecypod mollusks. *Biol. Bull.,* 84: (In press).

Coe, W. R., and Harry J. Turner, Jr. 1938. Development of the gonads and gametes in the soft-shell clam (*Mya arenaria*). *Jour. Morph.,* 62: 91–111.

Cole, H. A. 1941. The fecundity of *Ostrea edulis. Jour. Mar. Biol. Ass'n.,* 25: 243–260.

Edmondson, Charles H. 1920. Edible mollusca of the Oregon coast. *Occ. Pap. Bishop Mus.,* 7: No. 9.

Fincher, John Albert. 1940. The origin of the germ cells in *Stylotella heliophila* Wilson (Tetraxonida). *Jour. Morph.,* 67: 175–197.

Galtsoff, Paul S. 1937. Observations and experiments on sex change in the adult American oyster, "*Ostrea virginica.*" *Collecting Net,* 12: 187.

———. 1938. Sex change and physiological intersexuality in *Ostrea virginica.* (Abstr.) *Anat. Rec.,* Suppl., 72: 42.

Grave, Benjamin H. 1942. The sexual cycle of the shipworm, *Teredo navalis. Biol. Bull.,* 82: 438–445.

Grave, Benjamin H., and Jay Smith. 1936. Sex inversion in *Teredo navalis* and its relation to sex ratios. *Biol. Bull.,* 70: 332–343.

Gutsell, J. S. 1926. A hermaphroditic viviparous oyster of the Atlantic Coast of North America. *Science,* 64: 450.

———. 1931. Natural history of the bay scallop. *Bull. Bureau Fish.,* U. S. Dept. Commerce, 46 (1930): 569–632.

Hopkins, A. D. 1936. Ecological observations on spawning and early larval development in the Olympic oyster (*Ostrea lurida*). *Ecology,* 17: 551–566.

———. 1937. Experimental observations on spawning, larval development and setting in the Olympic oyster (*Ostrea lurida*). *Bull. U. S. Bureau Fish.,* 23: 439–503.

Loosanoff, Victor L. 1937. Development of the primary gonad and sexual phases in *Venus mercenaria* Linnaeus. *Biol. Bull.,* 72: 389–405.

———. 1942. Seasonal gonadal changes in the adult oysters, *Ostrea virginica,* of Long Island Sound. *Biol. Bull.,* 82: 195–206.

Needler, Alfreda B. 1932. Sex reversal in *Ostrea virginica. Contr. Can. Biol. and Fish.,* 7: 285.

———. 1936. Sex ratios in oysters of known age. *Prog. Rep. Atlantic Biol. Sta.,* 7: No. 49.

Orton, J. H. 1927. Observations and experiments on sex-change in the European oyster (*O. edulis*). *Jour. Mar. Biol. Ass'n.,* 14: 967–1045.

———. 1927a. A note on the physiology of sex and sex determination. *Jour. Mar. Biol. Ass'n.,* 4: 1047–1055.

———. 1933. Observations and experiments on sex change in the European oyster (*O. edulis*). Part III, On the fate of unspawned ova. Part IV, On the change from male to female. *Jour. Mar. Biol. Ass'n.,* 6: 1–54.

Pelseneer, Paul. 1935. Essai d'ethologie d'après l'étude des Mollusques. *Pub. Fond. Agathon De Potter; Acad. Roy. Belgique, Cl. Sci.,* Bruxelles, 1–662.

PYNCHON, R. DENISON. 1941. On the biology and relationships of the lamellibranch *Xylophaga dorsalis* (Turton). *Jour. Mar. Biol. Ass'n.*, 25: 1–39.

ROSENWALD, K. 1926. Beeinflussung des Geschlechts von *Limax laevis. Zeits. f. ind. Abs. u. Verer.*, 43: 238–251.

ROUGHLEY, T. C. 1933. The life history of the Australian oyster (*Ostrea commercialis*). *Proc. Linnean Soc. New South Wales*, 58: 279–333.

WEYMOUTH, FRANK W. 1923. The life history and growth of the *Pismo* clam. *California Fish and Game*, Bull. 7: 1–120.