While the method was devised primarily for teaching and demonstration purposes, it may be used in research, and in the physiological laboratory of this school Dr. Leland C. Wyman has carried on an investigation in which he made use of the window to observe vascular changes in the intestines of a rat.

The abdominal window herein described is simple, efficient, inexpensive, and has the added advantage that it maintains the interest of students because they are able to carry on their experiments easily and successfully.

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A METHOD FOR RIPENING HAEMATOXYLIN SOLUTIONS RAPIDLY

THE writers are calling the attention of biological workers to the ease in preparation of various haematoxylin solutions which ordinarily require a rather long ripening process. Within our knowledge, this method has not been reported heretofore. Ehrlich's or Delafield's haematoxylin solutions are prepared in the usual way. When ripening is to be brought about, the solution is placed in a very wide and somewhat shallow evaporating dish and exposed at a distance of approximately two feet, to any rather powerful quartz mercury vapor light. The rapidly darkening solution should be stirred frequently. Delafield's solution will be ready for use after an exposure of about two hours, and some three or four hours are necessary for ripening Ehrlich's solution. A very vigorous staining solution results. This method can also be applied to the ripening of a one half per cent. haematoxylin solution for use in Haidenhain's iron-alum stain except that the exposure to the quartz mercury arc is very much shorter.

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SPECIAL ARTICLES

SEXUAL RHYTHM IN THE CALIFORNIA OYSTER (OSTREA LURIDA)

IT has been known for many years[1] that the common edible oyster of our Pacific coast is hermaphroditic and viviparous but no definite information has been hitherto available as to the sequence of the sexual phases in this species. With the coöperation of Professor W. E. Allen, of the Scripps Institution of Oceanography, cement and wooden experimental blocks have been placed in the water at frequent intervals and at all seasons during the past five years. From these blocks, which were suspended from the Institution's pier at La Jolla, California, a fairly complete series of oysters of approximately known ages has been obtained.

Weekly or biweekly collections were made, at which time one face of a block was scraped free from attached organisms, including oysters. The block was then returned to the water to gather a new crop of the free-swimming stages of such organisms as were at that time ready for attachment.

Examination of such blocks shows that the oyster in that locality is in process of reproduction during at least seven months of the year,[2] or for a longer period in those years in which the water remains unusually long above the critical temperature of about 16° C. in the autumn or reaches this temperature earlier than usual in the late winter or spring. For it is found that spawning is inhibited when the water falls below this temperature, to be resumed when the critical point is again reached.

Microscopic sections of the gonads show that some members of the oyster population in that locality have ripe sexual products at all seasons of the year and that all possible combinations of sexual phases are represented. Immature individuals in the male phase, young hermaphrodites, inter-sexual forms predominantly of one sex or of the other, with all conceivable transition stages, are always to be found, as Stafford has long since reported.[1] Some of these are evidently young, others are mature and still others are obviously old, but they offer only vague testimony as to the sexual conditions appertaining to any one individual during its lifetime. By taking a series of oysters of definitely known ages, on the other hand, such as has become available from these experimental blocks, the sequence of sexual phases can be followed with little chance of error.

In the young animal the first trace of the gonad appears at the age of about eight weeks. The few cells composing this gonad show no distinguishing characteristics of sexual differentiation, but at the age of twelve to sixteen weeks each gonad in every animal studied shows that both primitive ovogonia and spermatogonia are present.

The spermatogonia, however, proliferate more rapidly than do the ovogonia and the gonad soon

[1] Jos. Stafford, The Canadian oyster. *Comm. of Conservation*, Canada, pp. 1–159, 1913.
[2] W. R. Coe, *Anat. Rec.*, 47: 359, 1930.

acquires the characteristics of a spermary, although ovogonia and ovocytes are always present. Spermatogenesis quickly follows if the temperature is sufficiently warm and the ripe spermatozoa are ready to be discharged when the oyster is about five months of age.

This is typical protandry, and this evidently occurs in all individuals, for no exception has been found. The spermatozoa are held firmly together in spherical masses, the so-called sperm-balls, each ball consisting of from about 250 to 2,000 or more ripe spermatozoa, all of which have been derived from a single primary spermatogonium. Before this initial male phase has been completed and before any of the sperm-balls have been discharged, the proliferation of the ovogonia and their transformation into ovocytes is in progress. Most of the sperm-balls are then discharged from the body, whereupon the animal assumes the first female phase, although some sperm-balls are always left in the genital ducts, and many spermatogonia for the subsequent male phase are present in the gonads.

In the early female phase the original primary gonads increase greatly in size by the growth of the ovarian tubules into the underlying connective tissue, forming the secondary gonads. In these tubules the ovocytes build up their yolk materials and at the age of about six months the climax of the first female phase is reached. Ovulation then occurs, the eggs being retained in the mantle cavity of the parent during fertilization and cleavage and through development until the embryos have become provided with a bivalved, straight-hinged shell—a period of approximately ten to twelve days, perhaps. It seems not improbable that ovulation takes place only when the animal is stimulated by the presence in the water of spermatozoa of other individuals, as Galtsoff[3] has found to be the case in other species.

In the process of ovulation the eggs frequently mingle in the genital ducts with the sperm-balls remaining from the preceding male phase, but self-fertilization can not occur, at least not until the gametes have reached the sea water in the mantle cavity, for the spermatozoa are only liberated by the solvent action of the water on the substance of the sperm-ball. Even then there is no evidence that self-fertilization takes place, for it is not known whether the gametes from the same individual are mutually reactive.

Not all the ripe ova are discharged at the first ovulation. Those remaining in the gonads may be absorbed by phagocytosis, or a second ovulation may occur as soon as the first crop of larvae has been spawned. Many other ova, evidently not quite ripe, are retained to form the basis for the second female phase.

While the embryos are developing within the mantle cavity the spermatogonia remaining in the gonads begin a rapid spermatogenesis and even by the time the embryos have been spawned the second male phase has been reached. The number of sperm-balls produced is now vastly greater than in the first male phase and a much greater proportion of them contain the maximum number of spermatozoa. If the animal is well nourished some hundreds of thousands of such sperm masses are formed, with upwards of 2,000 spermatozoa in each.

Given the suitable stimulus, most of the sperm balls will be discharged, a few remaining as before. The body of the oyster has then become soft, flabby and translucent, presenting a marked contrast to its plump whitish condition preceding the discharge of the gametes. A period of recuperation follows. The body grows larger and nutrient materials are then stored in the tissues in preparation for the following sexual phases. The time required will evidently depend upon the metabolic conditions of the animal.

Examination of the gonad during this resting period shows the preparation for a second female phase. When completed, if the environmental conditions are suitable, a new crop of embryos will appear in the mantle cavity, and coincidentally therewith the proliferation of spermatogonia for the third male phase. Following the ripening and discharge of the sperm will come another recuperation period. And, apparently, these alternating sexual phases may be repeated regularly throughout the remainder of the animal's life. But it is not at all improbable that in certain individuals, and possibly in some hereditary strains, one sexual phase or the other may be considerably reduced in older animals, with a corresponding tendency toward a dioecious condition. It is also not unlikely that in the colder portion of the range of the species a single annual rhythm or even a biennial rhythm may be found to occur, as is the case with the European oyster in some localities.[4,5,6]

Following the initial male phase, we thus find in the California oyster a regularly repeated sexual rhythm in (*a*) female, (*b*) male and (*c*) recuperation sequence for each individual. The conclusion follows that the fertilized eggs are all alike in regard to their primary sexual inheritance, with an associated hereditary mechanism, perhaps metabolic in nature, which is responsible for the rhythmical alternation of the sexual phases. Environmental conditions determine the rate of the sequence, and the

[3] P. S. Galtsoff, *Proc. Nat. Acad. Sci.*, 16: 555–559, 1930.

[4] P. P. C. Hoeck, *Tids. Nederl. Dierk. Ver.*, Deel 1, Suppl., 113–253, 1883–4.

[5] R. Spärck, *Rep. Dan. Biol. Sta.*, 30: 1–84, 1925.

[6] J. H. Orton, *Jour. Mar. Biol. Asso.*, 14: 967–1045, 1926–7.

time occupied by each of the successive phases, but there is as yet no evidence that the sequence is altered.

This statement needs to be qualified by the additional remark that the rhythm may be interrupted at any point by the advent of low temperature and presumably by any unfavorable metabolic conditions, to be resumed again when a suitable environment is restored.

It must be remembered that spawning occurs on the coast of Southern California during seven or more months of the year, so that on approach of cool weather the population will be represented by individuals which are in all stages of growth and reproduction, from larvae to old adults. Growth continues below the critical temperature but the discharge of sexual products is inhibited. The phase in which the animal enters this period will be retained throughout the winter unless the time of inhibition happens to coincide with the completion of one of the sexual phases.

A further complication results from the fact that not all parts of the reproductive system reach any one phase of sexuality at the same time, so that we shall find one portion in a somewhat different phase than is another area in the same animal. And finally, there are all grades of intersexuality as one phase passes into the next. This is particularly true of the younger animals, there being no exclusively male or female individuals among them at any season.

With increasing age, however, the intergrading stages become reduced and the sexual phase may be occasionally strictly male or female except for the minute gonia which anticipate the next phase. But in nearly all cases a few sperm-balls remain in some part of the system through the female phase and, conversely, a few ovocytes in the male phase recall the preceding part of the rhythm or foretell the next.

We may conclude, then, that *Ostrea lurida*, like the European *O. edulis*,[4,5,6] is a protandric hermaphrodite, with an alternating rhythm of female and male phases throughout life.[7,8]

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[7] Of some sixty species of the genus *Ostrea* ten are known to be monoecious. In some of the others hermaphroditism has been found in young animals or in some localities, while in one species, classed as dioecious, a considerable proportion of individuals have recently been shown by Amemiya (*Proc. Imp. Acad. Tokyo*, 5: 284–286, 1929) to change their sex during the winter.

[8] In a recent number of SCIENCE (74: 71, 1931) Burkenroad presents evidence that even *O. virginica* on the coast of Louisiana is protandrous, with an incidence of hermaphroditism of about 1 per cent. in the general population. The writer has also found a considerable percentage of intersexuality in small individuals of the same species at Woods Hole, Massachusetts.

RICE BRAN, A PREVENTIVE OF PEROSIS (DEFORMING LEG WEAKNESS) IN CHICKENS[1]

IN the fall of 1929 the animal husbandry division of the Bureau of Animal Industry and the departments of poultry husbandry of several Southern and Southwestern state agricultural experiment stations organized an informal cooperative project for the purpose of comparing, on a weight-for-weight basis, different feeding stuffs when included in a so-called "uniform" diet for growing chickens. During the spring and summer of 1930 the following experiment stations used the "uniform" diet in some of their feeding experiments with growing chicks: Kansas, Louisiana, New Mexico, Missouri, Texas, the U. S. Poultry Experiment Station, Glendale, Arizona, and the U. S. Animal Husbandry Experiment Farm, Beltsville, Maryland.

Several of the stations reported that when the "uniform diet" was fed to chicks kept in confinement a high percentage of them became afflicted with perosis (deforming leg weakness). One of the writers (W. M. G.), observed, however, that when 10 and 20 per cent. of rice bran replaced equivalent amounts of wheat in the "uniform" diet, no cases of perosis occurred. Soon after this observation was made, the other writer (H. W. T.) started a series of experiments for the purpose of studying the cause of the beneficial effect of rice bran in preventing this condition.

The results of these experiments are now being written up for publication. Since perosis, or leg weakness, has been a serious obstacle to the rearing of chickens in confinement, it seems to be advisable to call attention, at this time, to some of the conclusions drawn from these experiments.

Perosis, or deforming leg weakness, in chickens is a condition of dietary origin, in which the legs become deformed in various ways. The first observable symptoms are a slight puffiness of the metatarsal-tibia joints and a marked tendency on the part of the chicks to rest for long periods of time in a squatting position with the legs doubled up under them. As the condition becomes worse, the joints become more enlarged and a pronounced bending of the tibiae and tarsometatarsi may occur. Union of the diaphyses and epiphyses at the distal end of the tibiae is greatly delayed, or fails to occur. Sometimes there is a marked separation of the epiphyses from the diaphyses and the large tendons slip from their condiles leaving the metatarsal-tibia joints permanently deformed.

[1] Published with the permission of the director of the Louisiana Agricultural Experiment Station and the chief of the Bureau of Animal Industry of the U. S. Department of Agriculture.