sponding to the polymerizing runs are also presented in Fig. 1. The difference between the inhibited and polymerizing curves represents the temperature rise due to polymerization alone.

An inspection of the curves of Fig. 1 indicates that polymerization occurs at  $-20^{\circ}$  C but not at  $-80^{\circ}$  C. From a number of other such comparisons, it was found that the minimum temperature at which polymerization occurred was between  $-50^{\circ}$  C and  $-55^{\circ}$  C. which is also the range in which TEGMA hardens into a glassy solid. The apparent limiting temperature,  $-55^{\circ}$  C, is to the best of our knowledge the lowest temperature at which free radical polymerizations have been observed.

In TEGMA samples irradiated at temperatures lower than  $-55^{\circ}$  C, polymerization did occur on warming, but only after the material had reached a semifluid state. Such delayed polymerization was observed even after storing the sample at dry-ice temperature for several days.

The polymerization of TEGMA showed inhibition by both benzoquinone (Fig. 2) and oxygen (Fig. 3),



FIG. 2. Graph of temperature against time for the polymerization of TEGMA containing various amounts of added inhibitor. The polymerizations were initiated by 800 kvp electrons using a dose rate of  $5.2 \times 10^3$  R/sec.



FIG. 3. Graph of temperature against time for the polymerization of TEGMA initiated by 800 kvp electrons-3.75 µa, 10 cm from the tube window-indicating the effect of air (oxygen).

which is characteristic of free radical polymerization. On the basis that this is a free radical reaction, the number of radicals formed can be calculated from the induction period (Fig. 2) resulting from small amounts of benzoquinone (3). If it is assumed that one quinone molecule stops one free radical chain, the results indicate that  $5.4 \times 10^{18}$  radicals/ml TEGMA/

10<sup>6</sup>R are formed as opposed to calculated ion pair yields of  $1.8 \times 10^{18}$  ion pairs/ml TEGMA/10<sup>6</sup>R.

The polymerization of cross-linking monomers (e.g., TEGMA), initiated by high-energy electrons, was found to be dimensionally specific and could be used to form shaped objects in a pool of monomer. The lateral dimensions could be regulated by interposing a lead sheet, in which a design had been cut, between the monomer and the irradiation source. The thickness was determined by the electron energy and increased as the kvp was increased, indicating that the polymerization did not propagate beyond the region of ionization and radical formation.

Further work is being done to extend this method of polymerization to other monomers and co-monomers and, if possible, to still lower temperatures.

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## Early Sexual Development and Growth of the American Oyster in Louisiana Waters

### R. Winston Menzel<sup>1</sup>

### Texas A & M Research Foundation, College Station

Several investigators have noted the early sexual development of the American oyster, Crassostrea virginica (Gmelin) (1-5), in connection with sex reversal studies. Coe (2) states that from South Carolina southward young oysters of both sexes reach sexual maturity when only 10-12 weeks of age. In another publication (3) he says that well-nourished individuals of the early set on the coast of North Carolina and the Gulf of Mexico spawn when only 3 or 4 months of age. Burkenroad (4) found sexual development in some Louisiana oysters 20 mm or less in length.

The writer, working in the waters of Terrebonne Parish in Louisiana, has examined a total of 1,431 ovsters in which sex could be determined, during the period August 1947-March 1949. Several hundred other oysters were examined in which the gonads were not well enough developed to make determination of sex positive by the method used. All the data were obtained by examination of fresh unstained preparations. The oysters were preserved after study of fresh smears, and examinations of a number of prepared slides stained with Heidenhain's hematoxylin and eosin have corroborated the initial findings.

Of the total number of oysters examined during the period, 1,179 were of known maximum age, as they were attached to culch that had been planted at a

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AGE, NUMBERS, AND	PERCENTAGES OF MALES,	FEMALES, AND HERMAPHRODITES IN OYSTERS
	EXAMINED AUGUST	1947-MARCH 1949

Age (days)	Male		Female		Hermaphrodite			Length
	Number	Per cent	Number	Per cent	Number	· Per cent	Total	(Range in mm)
28- 42	101	61.5	49	30.0	14	8.5	164	9.0- 25.0
45- 60	295	63.5	160	34.5	9	2.0	464	15.0 - 35.0
65-170	139	67.5	67	32.5	0	0.0	206	20.0 - 75.0
240 - 450	160	46.5	185	53.5	0	0.0	345	40.0- 90.0
Over 700	91	36.5	161	63.5	0	0.0	252	100.0 - 150.0
Total	786	55.0	622	43.5	23	1.5	1431	

known date. Many of these oysters would be younger than indicated by this maximum possible age, as they did not all attach immediately after the culch was put in the water. The other 252 oysters were taken from natural reefs and were over 2 years old (estimated from the size).

Table 1 gives the age, size range (length), and the percentages of males, females, and hermaphrodites of the oysters examined. With one exception, the percentages of males, females and hermaphrodites are about the same as found by other investigators (2-5) of sex in American oysters. The exception is the high percentage of hermaphrodites (8.5%) among the oysters 4–6 weeks old. No report has been found of sex ratios of such young oysters. The high percentage of hermaphrodites at 28–42 days, when many of the oysters do not yet have fully developed gonads, followed by a drop to 2% at 45–60 days, seems to indicate that in some cases gonads of both sexes may develop before one sex becomes dominant.

Burkenroad (4) has stated that a higher percentage of young oysters will be male if they are associated with old oysters. The young oysters in Table 1 were all attached to planted culch and were not in close association with older oysters. On April 19, 1949, young oysters from a natural reef were examined. These oysters were attached to older oysters (over 2 years old), 100 of which showed a ratio of 36 males to 64 females. One hundred of these young oysters 8-20 months old (50-75 mm long) showed 76 males to 24 females. This ratio is significantly different from that of the culch oysters 8-15 months old (46.5% males; 53.5% females, Table 1). These data support Burkenroad's claim.

Paul (6) stated that Ostrea (Crassostrea) madrasensis attained sexual maturity in 21 days. In the present investigation no oyster as young as 3 weeks of age was found to be sexually mature. However, oysters 4 weeks old were found to be sexually mature. In one case, 10 spat were examined that were attached to culch that had been in the water for only 29 days. Of the 10, 2 were females, 4 were males, and 4 had gonads so undeveloped that sex could not be determined by the method used. The smallest sexually mature oyster was a male, 9.0 mm long and 7.5 mm wide. The gametes were functional, as ova from both females were fertilized *in vitro* with sperm from males in the same sample. These spat were from culch

planted June 29, 1948, and examined July 27, 1948. This precocious development continued later in the season also: Spat that attached to culch planted July 19, 1948, had functional gonads when examined August 16, 1948, after a period of 28 days. Thus it is possible for young oysters to produce functional gametes only 4 weeks after attachment. It is not known whether the resulting larvae are as viable as those in which the ova and sperm come from larger oysters. (Fertilization was made in vitro and the cultures were not kept after swimming larvae developed, several hours after the ova and sperm were mixed.) Nor is it known whether these young ovsters actually spawn at this early age or retain the gonadal products until they become larger. However, if it is assumed that 4-week-old oysters will spawn when the gonads are ripe, and if 12 days are allowed for maturation of the larvae before attachment, it would be possible to have a new generation every 40 days. The spawning season during 1948 lasted about 200 days. If 50 days are allowed after spawning for the development of the offspring to gonadal maturity, there could be 4 generations of oysters within one year in this area.

Ingle (7) gives some data on the growth of the American oyster at Apalachicola Bay, Florida. He gives the maximal growth at 31 weeks as 104.5 mm and states that this would equal a growth of about 4 years in Northern waters. During the Louisiana investigation numerous measurements were made on oysters of known age. In one case a few oysters that attached in late May were over 100 mm in length by the following January, or in a period of less than 9 months. These oysters were deep-cupped and broad in proportion and were good-sized market oysters. Measurements of shell size, weight, and volume indicate that oysters grow throughout the year in Louisiana. Newly attached spat grow actively during the hot summer months. The writer has found that young oysters will reach a length of 50 mm or more within 2 months and 75 mm or more in 4 or 5 months.

The data given above lead to the conclusion that oysters in the warmer waters of the Gulf live at a much faster rate than do those in more northern, colder waters. The writer has seen and measured oysters in both the Chesapeake Bay and Louisiana, and the maximum size is no greater in Louisiana. In Louisiana the oysters spawn earlier in the spring, grow faster (and during the entire year), and mature sexually at an earlier age than they do in the more northern waters. This faster rate of living in warmer waters has been reported often by investigators of other poikilothermous animals.

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# Direct Tissue Radioautography Technique Applied to Teeth

## Noel D. Martin and E. S. Slater

Department of Preventive Dentistry and Department of Photography, Faculty of Dentistry, University of Sydney, Australia

Radioactive isotopes have been used in the study of the permeability of the enamel and the dentine, and the pathways by which these isotopes penetrate the tooth structure have been recorded by the radioautographic method in which the radiograph of the tooth section after the penetration of the isotope is determined by comparison with the tooth section itself. In 1948 Amler (1) using this method studied the penetration of radiophosphorus in the dentine following the use of various medicaments, and more recently, Wainwright and Lemoine (2) illustrated the penetration of the enamel and dentine by urea con-



FIG. 1.



taining C<sup>14</sup>, by using the comparison of the radiograph to the ground tooth section.

In an investigation of the permeability of the dentine and enamel to P<sub>32</sub> labeled NaH<sub>2</sub>PO<sub>4</sub> following the treatment of the teeth by fluorides and other compounds, the authors thought that the application of the method of Evans (3) might provide a technique that would permit a direct viewing of the isotope penetration and, furthermore, microscopic examination of the sections that would give additional information as to the mode of penetration.

This technique consists essentially of placing a very fine grain stripping-film emulsion<sup>1</sup> on the surface of the ground tooth section and exposing to the radiation of the absorbed isotope. This emulsion is developed still in contact with the tooth section, and thus a direct view of the penetration is possible (Fig. 1).

In the specimen shown in Fig. 1, similar cavities were prepared on the mesial and distal surfaces of an anterior tooth and with the right cavity serving as a control the left was treated with 2% NaF and then 2% CaCl<sub>2</sub>, a precipitate of CaF<sub>2</sub> forming in the cavity. Into each cavity 0.02 ml of radiophosphorus in the form of  $NaH_2PO_4$ , with a specific activity of 30  $\mu$ c, was placed, and the cavities were sealed. After 24 hr a ground section of the tooth was prepared, and the film emulsion laid in contact with it. It was exposed for a length of time proportional to the radioactivity of the section and then developed.

The resultant composite radioautograph and section show a diffuse penetration of  $P_{32}$  in the control cavity, while the precipitation of calcium fluoride in the treated cavity has decreased the diffusion to a very

<sup>1</sup>Kodak-London Autoradiographic plates.