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PROBLEMS IN ANALYSIS OF MORTALITIES

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The interpretation of mortality data presents a variety of pitfalls that may trap the investigator into making erroneous predictions about the results of a change in mortality rates. The essence of managing and harvesting a crop is to replace natural mortality by human consumption. But unfortunately a mere reduction of the mortality due to one cause is no guarantee that this part of the crop will become available for human use, because another cause may assume importance and compensate for the previous losses. Thus compensatory mortality may defeat efforts to reduce the number of deaths.

The determination of mortality rates is naturally the first step in obtaining data for interpretation. First we shall define the various kinds of mortality and briefly show the types of calculations. Then we shall discuss the relation of these rates to populations and the compensatory effects. The definitions of mortality unfortunately are badly confused. The following agree with those suggested by Ricker (1958), and with demographic use (Spiegelman 1952). The basic necessity is to be sure that the population under consideration is clearly defined. Thus a crude rate is based on a population containing a miscellaneous assortment of ages or sex while a specific rate refers to a particular character. A rate could be specific with respect to age (i.e. not indicate sex). Age and sex are the characteristics most commonly specified but others (habitat, genetic strain, etc.) could be used. The term "mortality rate" is often used to refer to two fundamentally different kinds of populations. In one definition (here called q, the probability of dying) the number of deaths in a time interval is divided by the initial population or cohort. In the other (here called d, the death rate) the number of deaths is divided by the average population and should be restricted to stationary populations. As examples let us consider a group of 100 oysters on Jan. 1. Suppose that during a month 60 of these die, then the probability of dying in this month is $60/100 = 0.6$. Now suppose that at the instant each oyster died it was replaced by another so that the population instead of declining to 40, always remained at 100. The death rate is $60/100 = 0.6$ which is numerically the same as above. But note that probability of dying can never be greater than 1.0 (certainty) while death rate can be more than 1.0 due to replacements. Thus it may happen that a population by instantaneous replacements remains at 100 even though say, 200 oysters die, giving a death rate of 2.0.

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The collection of data for determination of mortality rates requires a knowledge of the number of deaths. Certainly the simplest method is to determine the age of death of a sample of the population. When suitably arranged as a frequency distribution, these records will give a life table (Ricker 1958, Deevey 1947). Unfortunately this method requires some assumptions about randomness of the sample and also a method for determining the age of death.

Other methods require a knowledge of the population. One procedure depends upon recapture of marked individuals. Perhaps the most generally useful method is to estimate the number in a particular group at two different times. If emigration is negligible, then the difference is due to deaths. All of these procedures are laborious and require fairly complicated calculations.

Assuming that by some method we have some information on numbers of deaths, the next step is to compute the values. Obviously the probability of dying (q) is complementary to the probability of surviving (p) so that $q + p = 1$. Now the relation of d, the death rate, to q, the probability of dying, is $1-q = e^{-d}$, where e is the base of natural logarithms. (This relation is not satisfactory for values of q greater than about 0.7 because of discrepancies in the time intervals). The advantage of this relationship is that one can change types of data that are relatively easy to get (probability of dying), to the type that is hard to obtain (death rate). An advantage of death rates is that, being exponents, the death rates due to various causes can be added. Thus $1-q = e^{-(d_1 + d_2 + d_3)}$. Now, if the death due to d_1 or d_2 or d_3 can be determined, then a prediction can be made of the value of q when this cause is removed. The difference between this prediction and the observed death rate results from compensation. Before indicating the nature of compensation it is desirable to explore some other fundamentals.

The effect of disease and predation on a population spans a wide spectrum from a level inadequate for the welfare of the species to extinction. Persons familiar with sport fishing know the many examples of stunting and reproductive failure that occur in overpopulated ponds. Here an increase in mortality rate would permit greater growth and reproduction and it is hoped that fishermen rather than disease will increase the mortality. In other situations the effect of a disease or predator is not measurable. By this we mean that whatever effect may occur is so small that our methods of estimation cannot detect it. In still other cases the disease or predator restricts the population since it can be shown that without the disease the population will be higher in number or mass. Cases of overfishing belong in this category (except where the habitat has been altered). Finally the end of this wide spectrum is reached in the examples of extermination of the population. It is vital to recognize that this spectrum is continuous; only four points on a continuous scale have been singled out for mention here.

The numerical relationship depends upon local circumstances. At one time and place a disease may have one effect but at some other time or place have another level of effect. Thus local conditions may

permit a disease to be harmless at one time and to be catastrophic at another time. This variation in effect is bewildering to investigators as well as practitioners.

Another fundamental principle deals with the concept of density-dependence. (Nicholson 1954). This notion considers the effect of a mortality factor at various densities of the population. If the effect increases percentagewise as the population increases, the relation is said to be density-dependent. If, on the other hand, the effect remains the same percentagewise, the relation is said to be density-independent. Unfortunately, in true biological fashion, animals refuse to be neatly pigeon-holed into our categories but show a complex of intergading conditions. Nevertheless the essence of the concept is important because it shows that a density-independent factor by itself cannot restrict the population. Thus if 95% of the recruitment to a population is killed each year, the population will increase by about 5% each year forever. In contrast if 100% of the recruitment are killed then the population will be limited. A factor whose effect can increase with density is able to govern the population level. Disease by the very nature of transmission is a classic example in theory of a density-dependent factor. To determine in practice what quantitative relations exist requires collection of certain data. To understand the relation to density, the number that die must be related to the number of new individuals (births and immigration). This recruitment is usually defined on a realistic basis such as the number reaching a certain size or age. It is at once apparent that the task of obtaining information is great. One must know at several densities the deaths, the recruitment and, of course, the population. Each of these statistics requires a formidable effort. As might be expected few studies are sufficiently complete to meet these exacting standards. Some programs have determined the percentage of the population killed by a disease or predator. Others have calculated the amount consumed and others list the food habits of the predator or the diseases found in the population. None of these studies is sufficiently profound to indicate the effect on the population.

To complicate the problem still further is the principle of compensation. The essence is very simple. Every animal dies from something. If disease X is prevented then parasite Y may cause the deaths. The important point is whether the time between these events is sufficient to permit growth in size or number. Thus if a hawk catches today a mouse that was destined to die of starvation tomorrow, then no measurable effect on population occurred. But if 6 months intervenes between these potential events, a measurable change may occur. To manage a resource the causes of death must be determined and the compensatory aspects analyzed (figure 1). Suppose that cause X and cause Y each produce a probability of dying of 0.60. This means that the probability of survival for each is 0.40 which must be multiplied to give the joint survival (0.16). Hence the probability of dying from either X or Y is 0.84. Suppose further that X is eliminated but Y now alone causes a probability of 0.84. Obviously no change has occurred due to the compensation by Y. If, however, the total is now only 0.70 then a change

has occurred and more individuals survive. These rates may vary numerically with changes in density.

The procedure for analysis of a problem of mortalities is tedious and involved. First the causes capable of producing death must be

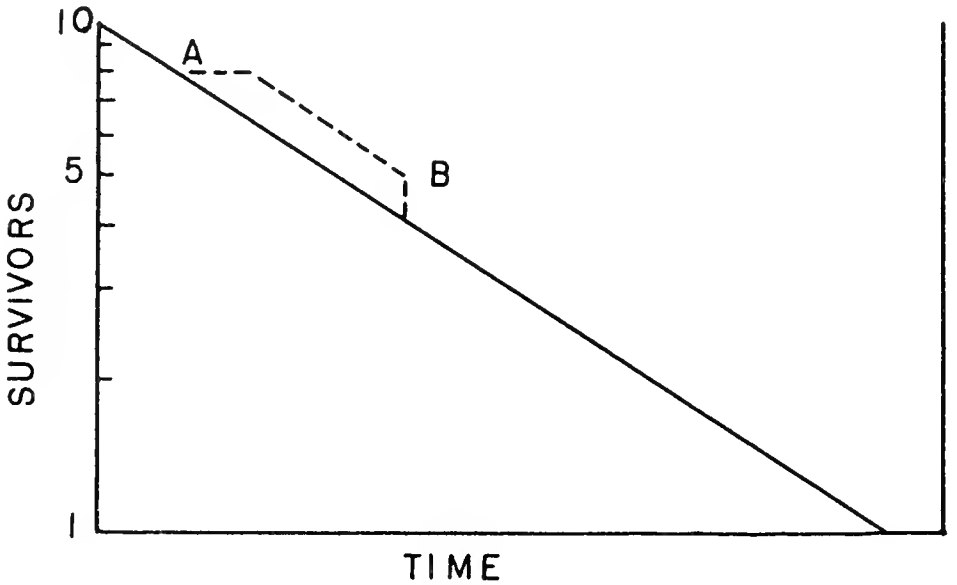

Figure 1. Diagrammatic representation of compensatory mortality. The rate is constant (solid line) and hence is a straight line on arithlog paper. At A a cause of mortality is removed, permitting some individuals to survive as indicated by the horizontal dotted line. Then other causes operate until the point B where some cause has an increased effect and compensates for the lack of mortality at A. If the time intervals between A and B are very small then nothing is gained by removal of cause at A. However, if interval is great, permitting breeding or harvesting, then removal of cause A is worthwhile.

determined. Then they should be eliminated (one by one if feasible) on an experimental basis in small areas to determine the effect on the death rate. If elimination of one or more causes does not alter the probability of dying it is obvious that other causes are taking the place and more causes need to be eliminated. Extensive eradication programs are not justified till this point has been established experimentally. Failure to reduce the death rate may result from compensation or from density-independence, since a factor that removes a constant proportion has little chance to be flexible.

This article has not cited specific examples partly because data on shellfish are scarce and partly to emphasize the principles. The reference cited will provide additional information and examples of these principles.

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IMMUNITY IN INVERTEBRATES, WITH SPECIAL REFERENCE TO THE OYSTER

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INTRODUCTION

There have been several excellent reviews of the field of invertebrate immunology and the author acknowledges his great indebtedness to men like Metchnikov (1905), Metalnikov (1920,1921), Chorine (1931), Cameron (1934), Huff (1940), and Bisset (1947) for the job they have done in collating the large literature. Other reviews, like those of Liebman (1946), George (1941) and Wagge (1955) discuss the kinds and functions of cells playing a role in invertebrate physiology, including immunity. These and Steinhaus' books on insect pathology (1949) and insect microbiology (1946) have been helpful also. This article lays no claim to being an exhaustive review but is intended as a statement of principles with selected examples of what is considered to be of possible special interest to shellfish biologists.

In examining much of the literature in this field several points are soon recognized: 1) Most of the work was done 20 or more years ago; 2) most of it refers to observations on insects, especially insect larvae; and 3) aside from reports of mortalities and descriptions of individual parasitic organisms, almost none of it refers to molluscs. Michelson, in his recent review of predators and parasites of fresh water molluscs, for example, cites only a few articles of possible interest in our discussions here. Even the fine work of Cuenot on the phagocytic organs of molluscs apparently stimulated none of the continuations and extensions so easily and logically deduced from his findings. The oyster, surprisingly because of its economic importance, has been one of those so neglected. Effects of starfish, oyster drills, hurricanes and freshets and the enormous problems of seed production and management have probably led to the neglect of the development of oyster pathology. Also some of the difficulties of working in the laboratory under reasonably controlled conditions with oysters, portions of whose shells must be removed to expose the heart for injection, are quite evident and need not be stressed, though they probably have contributed to the delay. Our interest in immunology and in the oyster led us to enter the field and some of the findings have already been reported (Stauber 1950, Tripp 1958 a,b,c, Feng 1959a).

Fortunately, Dr. J. G. Mackin also entered the field at about this time and, with his associates, has made great contributions to the field of oyster pathology for which we are deeply indebted. In spite of these, however, our lack of specific knowledge concerning susceptibility and resistance in the oyster will be disappointment to most of you.

DEFINITIONS

In the earlier literature the so-called resistance of a host to infection with another organism was considered as the reciprocal of its susceptibility to that organism (Falk 1928). A highly susceptible animal was thus one lacking in resistance. More recently Schneider (1951), and especially Read (1958) have shown that this is much too simplified an expression of the problem and that susceptibility and resistance "are separate and distinct biological attributes" (Schneider).

If we begin the discussion with host insusceptibility, two kinds are readily discernible. In the one case, the potential invader present in the surroundings of the host cannot gain entry. Whether this be because of the protective shell of the host (like Limulus), the integrity of its exposed epithelia or the normal secretions (like mucus) of its food-gathering, digestive or other systems is of no moment. The fact is that the "invader" cannot effect entrance. In the other case, even if entry is effected, the organism cannot survive in the host since, as Read defines insusceptibility, the "physiological state" of the host is such that "the life needs of the parasite are not satisfied" in it. This could mean, for example, for an intercellular parasite, that a required free amino acid is not available, that a growth factor is present in too low a concentration or that an inhibitor is present in too high a concentration for the parasite to grow and develop. For an obligate intracellular parasite, it might mean that the characteristic host cell is not present in the species of host animal under study. If it is presumed that entry by the parasite is possible under natural conditions or is obtained through experimental manipulations, then susceptibility is "a physiological state of the host in which the parasite is supplied with its life needs," and, therefore, does grow and develop (Read 1958).

Read defines resistance as "those alterations of the physiological state of the host which represent a response of the host to previous or present experience with the parasite or a chemically related entity" (italics mine). The emphasis is on host response. Under such definitions as given above, a host may be highly susceptible and show no resistance, barely susceptible and show high resistance or even be "a highly susceptible host which is also a highly resistant one" (Read). Trypanosoma rhodesiense, in the mouse, satisfactorily exemplifies the first case since the parasites seem to accumulate in numbers exponentially until the death of the host, with no indication of any response to the parasite by the mouse. On the other hand, the rat seems almost as highly susceptible to T. lewisi as the mouse in the above example is to T. rhodesiense, but the response (or resistance) of the rat brings about its eventual recovery with complete destruction of the accumulated parasites (Taliaferro 1929).

Resistance defined as host response implies that it takes time to accomplish the end results, namely, the manifestation of the resistance. It has been customary to divide resistance into two categories, innate and acquired. Acquired resistance is an individual host response which develops after a primary exposure to the parasitic agent or its

products and after survival from this experience, and it may persist for a very long time once developed. It is usually manifested as a heightened response to the parasitic agent upon subsequent contact with it, and in vertebrates like men, it is most often associated with the presence of antibodies. Innate resistance relates to those responses of the host appearing soon after its first contact with the invading organism. It is usually a racial or species characteristic, though it may be an individual one. It is obvious that, in the present state of our ignorance, insusceptibility and innate resistance are probably often used interchangeably since adequate evidence is not available for decision; this would be the case when deciding whether a phagocytized micro-organism, is ingested before or after its death. In this paper, we will retain the words "innate" and "acquired" as categories for the remainder of this discussion and will attempt to illustrate them by discussing the relative resistance of a series of rodents to intracardial infection with a protozoan parasite, Leishmania donovani (Grun 1958, Stauber 1958). It has been shown that the golden hamster and the cotton rat show no innate resistance, parasites continuing to increase in number in these hosts as long as the hosts live. By contrast, almost from the moment of inoculation parasite numbers decrease when introduced into the rat and rabbit. It might be argued that this is mere insusceptibility in the rabbit and rat, but some increase in parasites does seem to occur in one of the organs (spleen) of these animals and normal-appearing parasites persist for appreciable lengths of time. Until further evidence is available we are calling this innate resistance. In the mouse, guinea pig and gerbil, the early rate of increase of parasites is less than that seen in the cotton rat and hamster. We have been calling this an effect of innate resistance, although we do not know whether it means that some of the parasites produced are being destroyed by the host from the beginning or that the needs of the parasite are less ably met by these hosts. In the latter instance, it would be more appropriate perhaps to call this a lesser degree of susceptibility. Guinea pig, mouse and gerbil are able later to reduce the parasite numbers significantly enough essentially to recover from this infection. This is acquired resistance and the heightened resistance so produced has been demonstrated by following the course of infection after a second injection of parasites.

INNATE RESISTANCE

Two points need introductory consideration in a discussion of innate resistance in invertebrates; namely, the portal and mode of entry of the parasite and the size of the infecting dose. These factors are applicable also to the phenomena of acquired resistance, but since there is so little evidence for naturally acquired resistance in invertebrate hosts they are more likely to apply to innate resistance under the conditions of infection of invertebrates in nature.

Any epithelial surface might be penetrated for entry into deeper tissues including preliminary entrance into natural cavities such as mouth, anus, and genital and reproductive canals. While men-

tion has already been made of the possible role of secretions, such as mucus or digestive juices, as impeding entrance, it is a fact that examples exist of parasitic organisms using every conceivable portal of entry into their hosts. Some might enter a host like the oyster passively, swept along in the incurrent water to gain the digestive tract or gill surface where ingestion by a phagocyte might carry them into the deep tissues. Possibly Nematopsis is in this category. Or they might enter the deep tissues only later from the gut, perhaps like Hexamita, some bacteria and the commonly observed oyster ciliate. Others might be able to penetrate by means of either their own motile efforts or their digestive powers, like the miracidium of the flatworm Bucephalus. Still others might require the assistance of a third organism to break this initial line of defense. Bucephalus thus might carry in bacteria, fungi or even viruses. The mantle erosions of the boring sponge or the Dutch shell disease fungus and the gill erosions produced by Pinnotheres are also breaches which might admit the entry of microorganisms and the so-called pustule or "maladie du pied" of oysters may arise in some such way. That infection of oysters could occur in these ways is entirely logical from our knowledge of infectious processes in higher animals.

Mode of entry is also important because it might lead to effective host resistance or none at all. Entry directly into the blood stream, as in our injections of foreign substances and microorganisms into the oyster heart, may lead directly to phagocytosis and destruction or directly into those very cells in which an obligate intracellular parasite must eventually find itself in order to grow and develop. The same organism entering a tentacle of the mantle would find a considerably different set of circumstances facing it.

The numbers of the infecting organisms gaining entry is also of prime importance. An excellent example may be studied in the work of Hewitt, Richardson and Seager (1942) with an avian malaria parasite, Plasmodium lophurae, in the duckling. Per cent mortality, time to death, numbers of parasites at the height of the infection, and even whether parasites reach a density detectable by the counting procedures used, are all directly correlated with the size of the infecting dose of parasites injected. Even under more natural conditions of infection, McConnell and Cutkomp (1954) have shown recently that the median lethal spore concentration of Bacillus thuringiensis necessary for 100% kill of the first instar larva of the corn borer, Pyrausta nubilalis, is approximately 50,000 spores per ml. The plants were dipped in the spore suspensions and the larvae exposed to the plants for 36 hours to produce this result. The important point is that significant reductions of mortality occur when less concentrated spore suspensions are used. The analysis has not been carried farther, but presumably it takes these conditions either for one spore to reach the place of penetration into the host tissues or, what is more likely, to permit entrance of sufficient numbers of infecting organisms to render ineffective the innate resistance of the host.

Innate resistance is usually discussed under the categories of cellular and humoral mechanisms of response, depending on whether host cells or body fluids are chiefly involved. My studies and those of my students have largely concerned the cellular aspects, more specifically phagocytosis and some of its consequences. We have injected into the oyster's heart and adductor muscle such materials as India ink, starch grains, erythrocytes (red blood cells of fish, mammals and birds, especially cells of the duckling infected with a malaria parasite, Plasmodium lophurae, with its relatively indigestible malaria pigment), bacteria (as both vegetative cells and spores), soluble starch, bovine hemoglobin, human serum albumin and diptheria toxoid (Stauber 1950; Tripp 1958 a,b,c; Feng 1959 a,b). Most of the living organisms used are non-pathogenic for the oyster (except possibly Adelson's A-3 bacterium isolated from gaping oysters). We may briefly summarize the results as follows: 1) The injected "particles" are quickly phagocytized or pinocytized (unless arterial blockage occurs as when very large numbers of particles are injected) by cells which we call leucocytes or phagocytes and which ordinarily are found to be abundant, especially in the smaller blood vessels and sinuses. 2) The "particle"-laden phagocytes soon become distributed widely throughout the oyster. 3a) If the "particle" is digestible by the oyster leucocyte (vegetative bacteria, erythrocytes, malaria parasites, hemoglobin), digestion will proceed toward completion. 3b) If the "particle" in indigestible (India ink, bacterial spores and malaria pigment), leucocytic migration will carry it eventually across an epithelial surface to be voided in feces or in the mucus masses ejected from the oyster. The time required for the removal of approximately 90% of the material from the blood varies with the material injected and the method of determination, being longest for indigestible material, like bacterial spores and India ink, where migration seems to be the only mechanism for removal, and shortest for soluble organic substances like hemoglobin.

Another manifestation of host cellular response in invertebrates is leucocytosis, the mobilization of phagocytic cells in the blood stream. In the work of Feng and Canzonier (1959) even the intracardial injection of sterile sea water may cause an increase in abundance of cells in the heart blood within 24 hours. The proportions of the various types of blood cells may also change after the injection of foreign material (ink, bacteria) or even after hemorrhage. This has been well described by Cameron (1934) for the larvae of the wax moth caterpillar. The shift is toward greater proportions of cells called lymphocytes by Cameron and others, and is similar to some of Feng's (1959b) observations for the oyster. It is believed by Cameron and others that the lymphocytes differentiate into phagocytic cells like the ordinary leucocytes. That phagocytosis often leads to destruction of the organism engulfed may have been best demonstrated by Shirodkar et al. (1958) with whole Limulus blood in roller tissue culture when as many as 24 million bacteria, of a species isolated from oysters, were destroyed in six hours at room temperature.

Encapsulation is another cellular aspect of innate resistance and usually appears as a response either to a large mass of foreign material or to a special type of indigestible organism or material. The former is well described by Salt (1955, 1956) as host response to the introduction of the whole egg of a parasitic insect into the body cavity of another species of insect larva. In innately resistant hosts the phagocytes or hemocytes come together in large numbers around the foreign body, sometimes even differentiating into a fibrous-like connective tissue capsule around it. Dr. Mackin (1951) has described and has permitted us to examine abscesses in oysters infected with Dermocystidium, the outer margin of which conforms to this type of response. In the oysters injected by Tripp with avian red cells there is evidence for encapsulation of large masses of the cells and also for the fact that lymphocytoid as well as leucocytic cells are involved in the encapsulation. Newton (1952) reports cellular infiltration and fibrotic reaction in certain snails to invading flatworm larvae. In the case of the special type of indigestible organism, acid-fast bacilli are most often involved, though nodules may even be formed around India ink (Metalnikov and Chorine 1930, Cameron 1934). A similar situation has recently been described for freshwater gastropods by Michelson (1958) where very large masses of acid-fast bacilli are surrounded by a kind of fibrous cell capsule. Huff's review indicates that this type of capsule has been found around encysted gregarines and nematodes in crickets, a flatworm in a beetle and a sporozoan in a mealybug. That encapsulation, or phagocytosis, does not necessarily result in the death and destruction of the inciting agent should be emphasized (for recent studies in rabbits see Rogers 1958). This is especially true of the acid-fast bacilli, which several workers have shown may remain viable (Cameron) and may even increase in numbers within the nodules (Michelson, 1958).

In considering humoral aspects of innate resistance, the assumption must be made that cellular fragmentation or secretion (Wagge 1955) or some biochemical alteration confers bacteriostatic, lytic or other properties on the body fluid. If these properties are present even before the introduction of the microorganism then they more properly should be called insusceptibility factors. Some insect bloods do have striking bacteria-dissolving properties and many have agglutinating activity on microorganisms which may facilitate phagocytosis and encapsulation. In insects, melanin deposition, which may enshroud large objects or be deposited within small nodules containing India ink or bacteria, is also a typical innate host response. As for detoxification of toxic products of organisms much more work is required to resolve the difference between Feng's finding of the almost immediate removal of diphtheria toxoid and bovine hemoglobin from oyster plasma and the reports of Metchnikov (1905) and Bengston (1924), e.g., that tetanus or botulinum toxin may remain for several weeks in the blood of injected insects without losing toxicity even through metamorphosis to the adult winged insect.

The oyster has been little investigated for humoral factors though Tripp (1958) and Feng (1959) have found some indications of both agglutinating and properdin-like properties in oyster blood.

One aspect of innate resistance (or insusceptibility) deserves emphasis, namely, the many reports of racial and even individual resistance among invertebrates to specific infectious agents. Pasteur in 1870 became the savior of the silkworm industry by observing that all the larvae were not infected by the microsporidian of pebrine and that selection of the uninfected larvae led to the establishment of resistant stocks. Huff's (1935) demonstration, by selection of culicine mosquitoes, that susceptibility to the avian malaria parasite Plasmodium cathemerium behaved as a simple Mendelian recessive, is another striking example of hereditary influence on susceptibility or resistance. In the molluscan field, although little has been done experimentally, the resistance of the local survivors of the several epizootics of "Malpeque disease" in Canadian waters is evidence of similar individual differences among oysters and of the effects of rigorous selection on the susceptibility of the population (Logie 1958, Needler and Logie 1947). The observations of Andrews and Hewatt (1957) on oysters from different areas exposed to Dermocystidium marinum should also be mentioned here. Newton's (1952,1953) work on the inheritance of susceptibility of a freshwater gastropod, Australorbis glabratus, to the flatworm Schistosoma mansoni is an excellent beginning in the molluscan field. Exposure of the Puerto Rican strain to the parasite yielded infections in 95% of the snails whereas in similar exposure of Brazilian snails none became infected. In the resistant snails the invading parasites were destroyed in 24 to 48 hours, with marked cellular infiltration and a fibrotic walling off. Matings between the two strains were accomplished and three generations of progeny studied. The findings show that resistance is a heritable character, but that the picture is complicated, several genetic factors being involved.

It is probable that many factors influence innate resistance. The most obvious for the infected oyster are temperature, salinity, nutritional state and the flora and fauna of its environment. Little has been done along this line experimentally, though the studies of Andrews and Hewatt (1957) with Dermocystidium illustrate what can be done. We have conducted but one experiment so far, with the A-3 bacterium, to determine the role of temperature in the oyster's response to the intracardial injection of this organism. We can report at this time only that environmental temperature does influence the rate of initial clearing and subsequent fate of the organisms in the oyster. This phenomenon is well-known to those interested in the biological control of insects where percentage kill by infection with a microorganism may be directly correlated with environmental temperatures (Heimpel 1954).

The influence of nutrition (metabolites and their inhibitors)

I believe is best illustrated by the experiments of Terzian, Stahler and Ward (1952) on mosquitoes infected with avian malarial parasites. Before and after the infecting blood meal the mosquitoes were fed on sugar alone or on sugar containing vitamins or inhibitors. It has been shown not only that specific compounds may have significant effects on resistance, but that the maximum effects depend upon a specific optimal range of concentrations above which the specific effects of the compounds are depressed or eliminated. These findings suggest a fundamental relationship between such substances and innate resistance (or susceptibility).

As for the influence of other living microorganisms found in and about a given animal, it first should be noted that infectious agents can be isolated regularly from the bloods or tissues of many otherwise normal animals. This is especially true of arthropods (Steinhaus 1946, Cameron 1934) and indeed is a necessity if those arthropods are to transmit infections like malaria parasites, bacteria, rickettsiae and viruses to vertebrates and even to their own offspring (through the egg). Some of these organisms isolated from tissues may be true pathogens of the host under study, others may be mere secondary invaders unable to invade by their own capabilities, but whose inherent pathogenicity may seal the fate of the host if they do get in. For example, Cameron states, with respect to virulent strains of certain bacteria in the alimentary tract of larval moths, that they "tend to invade the body cavity if for any reason the defensive mechanism is disturbed."

Drs. Cort, Hussey and Ameel have given me the privilege to report their unusual and as yet unpublished findings with certain freshwater gastropods. These snails, if infected with flatworm larvae, may then be infected with a microsporidian protozoan which invades and damages not the snail but the flatworm larvae within the snail. It seems probable that the microsporidian hyperparasites are regularly invading these snails, but can be established only when the snails contain a suitable tissue for their growth, namely, the trematode larvae. An analogous situation, also in snails with trematode larvae, was reported much earlier (Cort, Olivier and Brackett 1941). The point I wish to emphasize from these examples is that the epithelial surfaces of animals are being penetrated regularly, and perhaps most often, by adventitious non-pathogenic organisms present in relative abundance at points where entry can most frequently occur. It is believed by some that this explains in part the so-called normal agglutinins in man and other animals to so many essentially non-pathogenic organisms (Wagner 1959, Springer, Horton and Forbes 1959).

ACQUIRED RESISTANCE

In this section naturally acquired resistance will be discussed chiefly; there will be only casual reference to that artifically induced by injections of dead organisms. If thus restricted and if

defined as a heightened response of a host as the result of previous contact with the infecting agent, then the following statement from Steinhaus is pertinent: "It is somewhat surprising that so few observations have been made on naturally acquired resistance in insects. We know practically nothing, e.g., concerning the residual immunity in insects that have survived an epizootic wave" (italics mine). Huff (1930), with avian malaria parasites in mosquitoes, clearly indicated no change in susceptibility with reinfection, but there is a large literature showing that injections, especially of old cultures of bacteria, into the body cavity of caterpillars (Metalnikov and others) have immunized the caterpillars in as short a time as 24 hours to doses of virulent organisms lethal to unvaccinated controls. Huff (1940) writes that "insects have poor powers of overcoming parasitic protozoa, fungi, and insects once these organisms have invaded their tissues." Concerning the oyster we know even less. The apparent decrease in the numbers of Dermocystidium in oysters during the winter (Mackin 1953, Ray 1958, Andrews and Hewatt 1957) or of Nematopsis when infected oysters are transplanted to clean water (Feng 1958) furnish us with instances which could be investigated for heightened resistance to subsequent infection.

That so few examples of acquired resistance are known among invertebrates may even be quite logical. Because of their relatively short generation times, their usual small size and often enormous reproductive capacities, subsequent epizootics would be much more likely to be circumvented by the appearance of resistant stocks through natural selection, as in "Malpeque disease." Even with very high mortality rates a residual stock of animals under favorable conditions later might repopulate an area. Indeed, this seems to be our chief hope in the present catastrophic mortalities of oysters in New Jersey and has been given consideration in the discussions of mortalities caused by Dermocystidium (Andrews and Hewatt 1957).

If this reasoning is adequate to explain the lack of evidence for the occurrence of acquired resistance in most of the invertebrates, perhaps those invertebrates with a long life span, like Limulus, should be investigated more fully as likely hosts capable of demonstrating acquired resistance.

Since acquired resistance must yet be demonstrated for the oyster it seems unnecessary to discuss this topic further in this paper.

CONCLUSIONS

It is fair to state that although invertebrate immunology has been under study for a long time, little precise knowledge is available for the oyster and its relatives. Since some of the mechanisms of resistance reported here for invertebrates may not even occur in molluscs and more specifically in oysters (like melanin deposition),

I am concerned that I should not mislead you. The only satisfactory conclusion to draw is that much painstaking effort is needed to furnish the information we so greatly need at this time.

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MORTALITIES OF OYSTERS

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INTRODUCTION

Mortality may mean many things. It can be either the basic annual mortality, below which losses never drop, or the peak waves of mortality which periodically sweep the oyster beds and which account for losses of high percentages of the total population. Or it may mean an increasing annual loss resulting in complete extinction of oysters over considerable part of the original range. It is hoped to discuss all of these types, and as far as possible to analyze the factors which produce the mortalities. Additionally it is aimed to point out recognition characteristics of each of the general types to be discussed. There is ample illustrative material. As pointed out by Gross and Smyth (1946) decimation of oyster populations is one of the most important biological phenomena of the first half of the twentieth century, and is worldwide in scope.

Studies of mortality are essentially studies of one phase of population ecology. On a broad basis there are only two phases of population studies. One is the examination of all of those forces which tend to build up and maintain a population, the other focuses attention on all agencies which tend to reduce populations (produce mortalities) or to prevent them from exploding. Ideally these two classes of forces should balance each other to maintain a static population. Actually any population, at any one time, is either crescent or it is declining, the two processes alternating and progressing by a series of waves. These waves may average out, over a long period of time, in an increase, or in a decline. For oysters, decline has been the rule.

In the following sections, the most important types of mortalities classified as to origin are discussed. Emphasis is placed on those types which are generally classed as of "unknown origin." The literature is full of these enigmas, and it is this type which has produced the most spectacular declines in population of oysters, sometimes extending over many years, and destroying entire industries over certain areas. Mortalities deriving from predation are subordinated in this paper, not because they are unimportant, but because symposiums of rather exhaustive nature have been organized and completed in the

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past, which dealt with the principal predators. Oyster biologists quite generally have dedicated a very high percentage of their efforts to study of problems of predation, and the author believes that there is less reason for extensive treatment of this phase of oyster population studies at this time, important as it is.

TYPE I. MORTALITIES CAUSED BY EXTREMES OF THE NATURAL PHYSICAL ENVIRONMENT

Examples are extremes of cold and heat, low salinity caused by floods, and hurricane damage. Cases of mortalities of this type are numerous and nearly everyone can recall several. I chose the mortality in the Mississippi Sound caused by flood of the Mississippi in 1945 as a prime example (Gunter 1950, Owen 1950). Flood crests of the Mississippi rose so high that the Army Engineers opened the Bonne Carre Spillway locks in early 1945. Floodwater from the Mississippi was shunted through Lake Ponchartrain and thence into Lake Borgne and Mississippi Sound. Water was fresh or near-fresh over the oyster beds of Mississippi Sound from Grand Island (not the same as Grand Isle) eastward for about 10 miles. The great oyster-producing areas of the Louisiana marsh were similarly affected in the northernmost part. The kill was 100 per cent through much of the area but graded off to zero in the more easterly and southerly parts of the Sound and marshes. Many other cases could be cited: The Santee floods of South Carolina, (Lunz 1936) upper Chesapeake area affected by floods of the Susquehanna (Beaven 1946), and many others. Details of such losses are too well known to need further description.

In some areas, mortalities by flood and low salinity are not so drastic. Marginal areas which lie on the landward side (or riverward side) of all estuaries are annually threatened with loss to freshwater kill. In some years loss is 100 per cent, in others zero, but in most years a certain percentage of the oysters are lost, but the number fluctuates between the maximum and the minimum. Oysters planted in marginal low-salinity areas are spared most sources of mortality other than low salinity.

Characteristics of losses to extremes of physical environments are as follows:

(1) The duration of the mortalities is apt to be sharply limited.

(2) Recovery is rapid and complete and the oysters rebound to a level of population higher than that prior to the flood (because the floods also destroy predators and foci of disease, and reduce fouling).

(3) The effects of extremes of physical environment extend to many marine forms other than oysters, and the oyster community as a whole is affected to greater or lesser extent.

(4) The mortalities are density independent.

(5) The mortalities are quite apt to be seasonal, since extremes of temperature, floods, etc., are usually seasonal.

There may be difficulty in some instances in distinguishing between mortalities caused directly by heat and cold, or other natural environmental extremes, and those caused by disease. However, the application of other yardsticks should eliminate confusion. Disease is selective and spotty in occurrence, and is density dependent, while the opposite is true of environmentally induced mortality due to physical extremes.

TYPE II. MORTALITIES DUE TO DISEASE

Of the greatest interest to present studies are those mortalities known to have been caused by disease or in which disease is suspected to be the cause. These cases are fairly numerous and the mortality waves attributed to disease may affect wide areas over the host range. Disease is known to have caused high rates of mortality of oysters in Europe, Australia, Japan, and North America for well over a half century. Generally the study of diseases of oysters in the United States has lagged far behind other studies. In spite of that, much has been accomplished in the past ten years. This has been the result of development of method, and a better understanding of the nature of the diseases of oysters. In earlier years, with few exceptions, workers assumed that etiologic agents of disease in oysters must be bacteria, and that these bacterial parasites could be isolated by the time-honored methods used in human diseases. Crude methods were used in testing for pathogenicity, with little or no regard for epidemiological factors. The crudity of the methods was primarily responsible for failures to establish etiological relationships. Often, even when disease was suspected, no efforts at isolation of a pathogen were made. The science of the study of diseases of marine invertebrates is lagging at least a half century behind disease studies in insects and crop plants.

It seems certain at the present time that all oyster-producing bays are endemic areas for one or more diseases. It would be exceedingly strange if this were not true. It has been the general opinion until recent years that oysters were somehow unique in that diseases are rare. A great preponderance of evidence now indicates that not only are bivalve molluscs frequent hosts for pathogens, but that they are regularly parasitized by a unique group of low fungi, which are so far off the beaten path of scientific inquiry that knowledge of taxonomy, relationship, life cycles, physiology, and epidemiology is only beginning to be accumulated. An entire new field of research is being opened in oyster biology. This is not intended to suggest that only this low group of fungi is important. Diseases involving bacteria and protozoa are known, and the oyster is afflicted with a wide variety

of diseases. It is predicted that viral diseases will be found in the near future.

It is proposed to examine here several of the mortality waves attributed to disease and to analyze the factors peculiar to these waves of high mortality. It is believed that a study of representative types may be of value.

A. The Mortalities of 1919-1925 on the coast of Europe

Beginning in 1919, a series of mortalities decimated oysters (Ostrea edulis) in various parts of Europe. Orton (1924) investigated this mortality wave and wrote two voluminous reports. Mortalities occurred in Italy, Atlantic coast of France and Holland, Ireland, and south England. The time of deaths is believed by Orton to have been mainly summer and spring, but a careful reading of accounts shows that the data given reflect periods when the mortalities were noted and not when they actually occurred. There are frequent mentions of mortalities continuing into the winter. This part of the accounts is confused, as are the reports of the numbers of oysters dying. It is my opinion that "unusual" mortalities were recorded which were far from being out of the ordinary, and were observed mainly because everyone was, at the time, mortality conscious. Be that as it may, it seems certain that the mortalities continued through several years; they began in either 1919 or 1920, and in some areas had not subsided until the mid-1920s. Most of the deaths appear to have been in warm weather, but some may have been in the fall, winter, or spring. The mortalities in Italy probably had no real relation to those of the Atlantic coast of Europe. Spottiness of locale of mortalities was marked.

It was at first believed that munitions dumped into the sea after World War I were responsible for the mortalities. Oil from sunken tankers was also considered as a possible cause, as was arsenic. These hypotheses were eliminated by experimentation, and the pollution hypothesis generally discarded. There were also those hypotheses which involved weather extremes as directly responsible for the mortalities, and lastly, disease was considered. Study of weather data led Orton to conclude that extremes of weather were not responsible for the mortalities and he was not able to find parasites in the oysters. As stated, crude experiments with bacteria were negative, but it is certain that there was nothing in the results which actually ruled out a bacterial parasite. Incidentally, some of Orton's figures show what appear to the author to be intracellular stages of Hexamita in the tissues. However, if Orton was correct, and the mortalities were concentrated in summer months, Hexamita probably was not a cause of the mortalities. He was handicapped in that his investigations were always several months behind the mortalities. It seems probable that he made most of his studies of disease on some other than the one operative when oysters were dying at the greatest rate.

Although Orton stated that he failed to find a cause of these mortalities, it is clear that he believed they were due to disease. He listed a number of histopathologies characteristic of "hockley" (sick) oysters and in his book on oyster culture (1937) referred to the mortalities as an "epidemic". Dollfus (1922) listed these mortalities as caused by a disease of unknown origin. Korringa (1947) referred to the mortalities in France and England in 1920-1921 as "mysterious and catastrophic", and later (1952) stated that what is known indicates that they were caused by a disease. The mortalities are so treated here, with the reservation that it is probable that there was more than one disease, and that in the efforts aimed at completeness of data, deaths from causes other than disease were introduced to compound the very evident confusion. For example, Korringa, although he accepted the disease hypothesis so far as mortalities of French oysters were concerned, believed that the Dutch mortalities occurring at the same time were due to low salinity. Gaarder and Alvsaker (1941) further befogged the issue by suggesting that all of these oysters starved to death.

Groping through the interminable non-pertinent details, the author believes that the following things are probably true of the European oyster mortalities of 1919-1925:

(1) A great mortality of oysters occurred on the Atlantic coast of Europe and the south coast of England, and the mortalities peaked in at least two years, 1920-1921.

(2) The mortalities were concentrated in summer. The two summers involved were warmer than usual, but not as warm as some years in which no "abnormal" mortalities occurred.

(3) Affected oysters showed strongly marked histopathologies, the most important of which was probably myolysis. However, oysters tended to die fat. Other histopathologies included severe cellular reactions, reduction of liver pigmentation, reduction of Leidig cell tissue, excess development of mucoid glands in epithelia, abscesses, and degeneration of the gonads.

(4) Mortalities were spotty in distribution, affected areas and apparently unaffected areas sometimes lying close together.

(5) The mortalities followed a long period of neglect of oyster beds during World War I.

(6) The mortalities failed to affect organisms other than oysters. Even the Portuguese oyster (Crassostrea angulata) was not affected.

(7) The mortalities were superposed on a background of regularly occurring "normal" mortality variously estimated at 10 to 25 per cent. Certain studies cited by Orton tend to indicate that the

background mortalities were grossly underestimated, as they nearly always are in every great mortality.

(8) There are data which indicate that there were earlier mortalities which at least approached the severity of the 1920-1921 wave. Circumstances indicate very similar conditions for some of these. Others were definitely winter mortalities which probably should be kept separate from those of high temperature periods.

(9) Oyster stocks apparently never recovered completely from the 1920-1921 mortalities, suggesting a higher level of endemic disease following the epidemic.

B. The Australian "winter disease"

Roughley (1926) described a disease of oysters (Crassostrea commercialis) occurring in the George's River of New South Wales, Australia. Oysters in this estuary had for 8 or 9 years previous to 1924 (the year of Roughley's study) died in varying numbers. From Roughley's account the following facts relating to the mortalities may be stated:

(1) The mortalities occurred in late winter and spring, and were accentuated by unusually cold weather.

(2) Although mortality was associated with cold weather Roughley showed by several studies that cold was not a direct cause of death, and indeed those oysters exposed to lowest temperatures were not affected to the extent that others were.

(3) The mortalities were associated with cessation of feeding activity which occurs in C. commercialis at 10° C.

(4) The sick oysters showed various histopathological conditions when sectioned and stained. Diapedesis was marked, the digestive gland was pale, and some oysters had severe ulcerations and abscesses, especially in the gonadal region. Myolysis was marked, and in some oysters the gills disintegrated.

(5) Most mortalities were in the lower half of the intertidal zone although not confined to that level.

(6) Winter disease was not confined to the George's River, but appeared intermittently elsewhere in southeast New South Wales.

(7) Plankton studies showed that a normal fauna and flora was present while oysters were dying nearby in large numbers. No mention was made of mortalities of animals other than the one species of oyster. Ostrea angasi inhabits the same region but is not considered to be a commercial oyster.

(8) Oysters died "fat" in most cases.

(9) Mortalities were "spotty." They might cover only a small section of a bed, or occur on one side of an estuary and be absent on the other, or involve only some sections of one part of an estuary. Mortality rates also varied greatly in different beds.

(10) Different years differed radically in the extent of the mortalities. Death rates might be high in one winter, and quite low in others.

Roughley failed to find the cause of these mortalities, but believed that they were caused by disease of bacterial origin. It has been my privilege to study Orton's slides of diseased oysters as well as slides made from oysters dying of winter disease in Australia in recent years. Some of these latter (and perhaps all) were infected with Hexamita. The histopathologies were identical to those described for oysters (O. edulis) from Holland with "pit disease" (Mackin, Korringa, and Hopkins 1952). Oysters in holding basins in Holland are subjected to low temperatures (5° C). The histopathologies in these oysters also correspond with those observed in Ostrea lurida from Puget Sound in Washington.

Winter disease of Australian oysters was chosen as an example of an intermittently recurring mortality producer which apparently is world-wide in distribution. While Hexamita is associated with the disease, there is some evidence that there may also be another simultaneously operative disease, or there are phases of Hexamita attack which have not as yet been definitely associated with the parasite. There may be several species of Hexamita involved.

A careful reading of much of the European literature dealing with the decline of the Ostrea edulis industry indicates that devastating losses of oysters associated with cold winters have played a considerable part in elimination of the species from certain areas as, for example, parts of Scotland. Epidemiological data suggest that cold was a factor only when coupled with other agencies. A very similar picture is found in Washington (State), where O. lurida has been eliminated from certain areas for no known cause, except overfishing. Overfishing may explain radical reduction on natural beds only, but cannot explain failure to recover when fishing ceases over a period of years.

C. Fungus disease caused by Dermocystidium marinum.

This is by far the best documented of the diseases. Because the details are relatively well known, only a very brief analysis is here presented. The major factors are as follows:

(1) The causative agent is a low fungus, Dermocystidium marinum,

closely related to the Synchytriaceae. General outlines of the life cycle are known with some details of the biochemistry of the parasite.

(2) The disease caused by this fungus produces annual summer epidemics in its host (Crassostrea virginica), the severity of which increase southward in its range, sometimes reaching high peaks, e.g., death rates of 90 per cent in a single summer in Louisiana. Various environmental factors modify these death rates in a quite regular manner.

(3) The known range is apparently from New Jersey to Texas, with some areas of low concentration or even complete absence scattered along the coast within the range.

(4) Scouring of bays by fresh water, and low salinity generally may control the disease in certain years. Conversely, a build-up of high salinity and high temperature over several years results in decimation of oyster populations.

(5) Dense planting of large numbers of susceptible seed oysters produces maximum conditions for development of the fungus. Under conditions of high temperature, high salinity, and dense populations of susceptible oysters, the maximum losses occur.

(6) Immature oysters under field conditions are resistant to infection, and susceptibility increases with age. In Louisiana, market oysters two years old and older are most often victims of attack. Resistance of young oysters probably is due to lesser feeding volumes (Andrews and Hewatt 1957) and possibly a tendency of young oysters to reject infective cells. Epidemics sweeping beds of older oysters and leaving freshly planted young oysters barely touched are often observed in Louisiana.

(7) There is evidence that resistant strains of Crassostrea virginica exist.

(8) There is also some evidence of acquired immunity.

D. Characteristics of mortalities caused by disease.

A study of the conditions surrounding mortalities of oysters produced by disease permit the formulation of some general characteristics of this type of mortality. There are as follows:

(1) Mortalities due to disease are almost always specific, that is, one species of oyster, or possibly two, may be affected, but the oyster community as a whole is unaffected. It is, of course, not impossible that a disease-producing organism might attack a variety of related hosts, but the community as a whole will be unaffected.

(2) Mortalities due to disease are almost always seasonal. Peaks of mortality are more or less sharply defined in limited periods of the year, while low-level losses may spread into other seasons.

(3) Peaks of mortalities may be cumulative over several years, sometimes building up to a peak year and then declining. These cyclic effects may sometimes be correlated with climatic cycles.

(4) The rates of mortality in epidemics nearly always are density dependent. That is especially true if there is only one host (the oyster) and no free-living stages. In cases where alternate hosts are involved, abundance of the parasite may be controlled by the alternate host, or by conditions obtaining during free-living stages. Shell disease of Ostrea edulis is such a case, where the abundance of the fungus causing the disease is controlled by the abundance of dead shell of molluscs other than oysters. But most disease-producing parasites of oysters seem to have only one host and free-living stages exist as spores which may not reproduce, but simply bridge an intervening ecologically unfavorable period for the parasite.

(5) Recovery from the effects of disease is slow. Introduction of new host susceptibles as seed in an area, may largely counteract the natural agencies working toward reduction of peaks of mortality. Density reduction and elimination of imports will speed up recovery from disease-produced mortalities.

(6) Mortalities from disease are "spotty", i.e., they affect different beds in one locality in a seemingly haphazard manner, especially in the beginning of a cyclic wave of mortalities. Unequal effect on different plantings in the same locality will continue because of difference in the local rates of elimination of susceptibles and because of varying densities of plantings, variations in susceptibility due to different origins of seed, and perhaps other factors.

(7) Survivors of an epidemic generally are found to be in good physiological condition. They survive either because of chance escape or because of individual resistance. In either case growth and reproductive capacity will be unimpaired. However, those oysters attacked by disease but not becoming fatalities may be found to be variously affected.

(8) Sections of oysters dying of disease will show characteristic histopathologies which will contrast markedly with the normal tissues of survivors in general. Some of the survivors, infected but not so heavily as to cause death, will inevitably show developmental stages in the histopathological conditions, and often the parasite itself will be found, also in developmental stages of infection. But most survivors will be largely free of characteristic histopathologies.

The author believes that, of all causes of mortality, disease

ranks first. Disease not only produces spectacular major declines in production, but also accounts for much "background" mortality. Studies of disease in invertebrates have lagged behind those of commercially valuable vertebrates, with the exception of those diseases of insects, which form a segment of research well worth review. Best general works on insect diseases are the volumes by Steinhaus (1946, 1949).

E. Some basic principles of epidemiology

The development of epidemics (technically epizootics) is dependent on three things. These are (1) variance in virulence and infectivity of the pathogen, (2) variance in the susceptibility of a population, and (3) the effectiveness of the methods of transportation of the parasite. All three of these are influenced by variation in the physical and chemical environment, and all vary with time. Thus epidemics develop, or fail to develop, develop partially, develop fully, or terminate because of an almost unlimited interaction of variables, which progressively change from the beginning to the end of an epidemic. Without going into those factors having to do with virulence, infectivity, and immunity, the population composition factors effective in epidemic development are discussed briefly.

Any population of animals is made up of several well defined categories of individuals so far as disease is concerned. These are as follows:

(1) Susceptible individuals, i.e., those which can be infected by a pathogen, and which will develop typical disease following infection.

(2) Immune non-carriers, those individuals which, if they can be infected, do not develop disease, and which rid themselves completely of the pathogen.

(3) Immune carriers, which do become infected and harbor the pathogens but do not react with the typical disease syndrome.

(4) Infected individuals, which will later develop disease (latent infections).

(5) Cases with typical disease.

(6) Atypical cases. (For a full discussion of these six categories, see Topley and Wilson 1936).

If a case of oyster mortality is considered in which the disease is a new import, and has not been endemic in the area in question, there are only two types of individuals in the host population: the susceptibles and natural immunes. The degree of development of an epidemic under such conditions would depend on the relative numbers

of these two categories, with the likelihood that immunes will be scarce. Given proper density of the host population, epidemics under these conditions are apt to be very severe.

However, after a disease has become endemic, all six categories will appear and as the epidemic progresses, their relative numbers change. Susceptibles become fewer, and with development of induced immunes, the percentage of the population capable of becoming infected decreases. With significantly high death rate, the population of susceptibles becomes more and more thinly scattered with proportionately greater difficulty in transmission of infective elements. The epidemic is thus self-limiting.

Introduction of a new host population into the area, either by natural accretion of spat, or by planting, will again tip the scales in favor of the disease. As a result, in the face of an endemic disease, an oyster population is itself selflimiting. When density of the population of susceptibles reaches a point where transmission becomes easily accomplished, a new epidemic is triggered.

The matter of host susceptibility varies very greatly with changes in the external environment. For example, in recent studies on Dermocystidium marinum in Louisiana it was found that deaths per thousand cases increased from 23 in April to 207 in August, a result of increased temperature; this was an overall increase of more than 900 per cent (Mackin and Sparks 1959).

The effect of the introduction of non-immunes into an endemic area also was shown in the study cited above. One thousand oysters from non-endemic territory for Dermocystidium marinum were placed beside 1000 oysters in the endemic area. Culture tests of samples showed that 690 out of the thousand endemic oysters were in one or the other of the groups of carriers, ranging from a few typical cases to numerous lightly infected oysters. The non-endemic oysters, in the following summer epidemic, all became infected with fungus disease and developed the highest rate of mortality to disease ever observed, 470 deaths per 1000 population in one month's time at the peak period, while the endemic oysters attained only a rate of 185 deaths per 1000 population in the peak month. Just what part acquired immunity played in this is not certain. Other studies have showed that a remnant population left after a severe epidemic may develop as high death rate due to disease in the following summer as the original population attained in the initial subjection to disease.

TYPE III. MORTALITIES DUE TO STARVATION

This category is discussed more because the literature contains supposed cases, than because of a personal belief that mortalities due to starvation occur on a large scale. There are no cases supported

by conclusive data. On the basis of certain studies and observations made in past years the author believes that it is almost impossible to starve an oyster to death in the natural habitat.

Hoek (1902) studied mortalities of oysters in Holland which occurred in the latter years of the past century. The mortalities were accompanied by failure of oysters to fatten and grow properly. Hoek concluded that the oysters had been starved to death, and that for any given area there would be a maximum number of oysters which could successfully be grown without starvation. For the limited area of Dutch ground under study, the number was stated to be 100 million. Korringa (1947) agreed with Hoek that certain mortalities of Dutch oysters were due to starvation.

The bases for the decision that Dutch oysters starved to death when the populations became too great were (1) the large population itself, and (2) the fact that the oysters failed to fatten properly. Other than these two reasons there seems to be no basis for this theory. But mortality following over-population can be caused by disease. Thinness, and even death of oysters can be a result of over-concentration of food (Loosanoff and Engle 1944), or a result of disease. The possibility of initiating a significant mortality by means of plankton blooms due to overfertilization, as shown for the Great South Bay area, is more impressive than is the starvation theory. Phytoplankton has been shown to have toxic properties in some cases, and may control a habitat by means of metabolites. In any event, if oysters are starved to death, the mortalities must necessarily involve other plankton feeders and cannot be so selective that the oyster is the only organism affected. No data showing a similar effect of the hypothetical lack of food causing starvation of Dutch oysters on other general plankton feeders was presented by Korringa or Hoek, and the theory must be considered to be unproved.

In the early part of the century, there were two years (1905 and 1906) in which oysters in Louisiana were very poor, and in fact largely unmarketable. This condition was attributed to lack of food (Oyster Commission of Louisiana 1906). H. F. Moore of the U. S. Bureau of Fisheries investigated this matter, but failed to find a deficiency of food plankters in the water (Moore and Pope 1910). The periodic failure of oysters to fatten properly is characteristic in many parts of the world and is not a result of so simple a factor as lack of food. It is believed that lack of metabolites, or over-concentration of metabolites is a better hypothesis, but this too remains to be demonstrated.

TYPE IV. MORTALITIES RESULTING FROM SPATIAL COMPETITION

Korringa (1947) described a case of oyster mortality due to competition with the slipper limpet, Crepidula fornicata, in Dutch

waters. This Crepidula was an import from the United States, and shortly after importation developed tremendous populations on old cockle shells. They were so numerous that space normally utilized by spat for setting was preempted, resulting in setting failure. Korringa ranked the crisis in the industry produced by the slippers along with that caused by shell disease. Apparently both crises were met by cleaning all beds in the affected area down to the bare mud. Use of Crepidula on a commercial basis during World War II completed the counter measures against the limpet.

Space competition between young oysters and various foulants is common everywhere. Barnacles are perhaps the most important of these competitors, but in some areas encrusting Bryozoa, serpulids, or others may become important. All this usually may be classed as background mortality to be expected in average years. Such competition may sometimes be helpful when set of young oysters is so plentiful as to be embarrassing, as it is in some parts of the Gulf Coast. New Englanders will find that difficult to understand, but it is a very real handicap in the South.

TYPE V. MORTALITIES DUE TO PREDATORS

Along with mortalities due to disease, predator-produced losses take front rank in importance. Oysters have an unusual number of the most effective kinds of predators. At least several of these are effective in mortality in any area where oysters are grown. Most of them are well known, but a few have only recently been described. There are five major groups of animals which prey an oysters:

(1) Fishes. Most important of the fishes are the drum, sheepshead, and skates or rays. Locally any of these may produce major damage, especially to young oysters on newly planted beds. Fishes as oyster predators seem to be more common in subtropic areas than they are in temperate zones. Predation due to fishes is apt to be very local. In Louisiana, oystermen, until very recently, were in the habit of fencing oyster beds against this type of predation. It is believed that much of the damage attributed to fishes actually may be due to other causes, especially crabs.

(2) Crabs. Any of the larger crabs may destroy oysters, depending on the size of the oysters, size of the crabs, environment, etc. However, the large Cancers and their relatives are most effective in destruction of oysters. On the Gulf Coast the most active is Menippe mercenaria, a crab with very heavy and strong claws, which can crack large, heavy shelled oysters (Menzel and Hopkins 1954). These authors found densities of crabs of 3500 to an acre of oyster reef in parts of Louisiana. They showed that these crabs could kill spat at the rate of 10 per day, and all sizes of oyster were killed in experimental tests at the overall rate of 219 oysters per crab per year. Extended,

these data could be taken to show that it is possible for an average population of stone crabs (Menippe) to destroy 766,500 oysters per acre per year. If only 1/3 of these oysters were market-size this would mean around 800 Louisiana sacks, or about the maximum capacity of oyster bedding grounds to hold oysters. Experimental figures may not be projected directly to field conditions and the normal natural kill is unquestionably only a fraction of the experimentally demonstrated possibilities; nevertheless, losses to the large crabs are unquestionably very much heavier than generally recognized. The extent of damage by crabs is very difficult to measure because of fragmentation of the shell by the predator.

(3) Predaceous snails. Mortalities of oysters due to predaceous snails are probably better known, and certainly have had wider publicity and have been subjected to more research than mortalities of any other kind. On the Atlantic Coast, for many years, intensive researches have been directed at problems of the drill (Urosalpinx cinerea). Carriker (1955) has recently summed up these researches and there have been recent seminars directed at the predaceous snail problem alone. Because of these thorough reviews, it is not thought necessary to attempt to add anything here. However, a high percentage of the background mortality of oysters on the Gulf and Atlantic coasts, and in the Pacific northwest, is caused by various species of predaceous snails, which are present on certain oyster grounds in all parts of the country.

(4) Echinoderms. Starfishes, where they are present, constitute the cause, both of continuing background mortality in years of normal abundance, and of catastrophic mortalities in those years when the starfish cyclically produce enormous populations. Burkenroad (1946) predicted a peak of abundance of starfish in Long Island Sound for 1957, a remarkably accurate forecast. Burkenroad studied 185 cases of sub and super-normal abundance, which he found to alternate at about 7-year intervals. The most interesting point in the study of the intermittent "plagues" of starfish is that the peaks of abundance are in no way dependent on abundance of oysters. Just what cyclic changes are operative is not known with any degree of certainty. Indeed, a study of the predators of oysters indicates that their abundance may not be based on oyster abundance, since most oyster predators have alternate prey and may prefer some food source other than the oyster.

(5) The predaceous flatworms. The predaceous flatworms make up the last major group of oyster predators. Some genus of polyclad is present in most oyster producing areas. Extensive mortalities have been attributed to these worms in various places. In the United States, the outstanding examples have been Florida and the Puget Sound area of Washington. In the latter case, the flatworms actually drill a hole through the shell (Woelke 1957). This author estimated the population of flatworms (Pseudostylochus ostreophagus) in one area at 600,000 per acre, and indicated a loss of 88 per cent of spat in a one-year period (1953-54). The worms were found on nearly all oyster beds in South Puget Sound.

Generally speaking, the characteristics of mortality waves due to predators are of the same nature as those outlined for diseases. However, the less pronounced dependence of predators on any one "host" species or taxonomic group of prey species, makes their population variation less density-dependent, and it is often completely independent of numbers of oysters. Herein, the classic picture of predator-prey population interdependence breaks down. Otherwise mortalities due to predators are generally simple to detect, because of the large size of the predator itself, and the more or less obvious attack of the predator. The factors of (a) seasonal development, (b) non-physiological effect on escapees, (c) non-involvement of the community as a whole, parallel the same characteristics as given for diseases.

TYPE VI. MORTALITIES DUE TO TOXINS

The literature is full of studies dealing with the effect of suspected toxic substances on oysters. Most of them stand in the category of the so-called pollutional toxins, and nearly every recent mortality that had no readily ascertainable cause has been claimed to be of pollutional origin, irrespective of whether the characteristics fit or not. But the number of proved cases of pollutional damage to oysters is surprisingly small, and all such are local in nature, and the facts obvious to all concerned.

The best examples of destruction of oysters by toxins are those caused by red tide organisms in the Pacific area. There are several reports of such cases from Australia, and they appear to be common in Japan. There have been claims that oyster mortalities due to red tide have occurred in the northeast Pacific (i.e., Willapa Harbor), and on the west coast of Florida. Gonyaulax poisoning appears to affect humans more than it does the oyster, and mussel poisoning is well known.

A. Characteristics of mortalities of toxic origin.

(1) The mortalities are non-specific. Considerable numbers of animal species other than the oyster affected are also destroyed. These are not necessarily related molluscs. Industrial wastes are generally toxic rather than specifically toxic and would be expected to destroy a great part of a fauna, irrespective of taxonomic relations of the species, if oysters are affected. At the same time that most of the fauna is being destroyed, a few species may be stimulated to develop larger populations. One would then expect profound changes in the community of organisms associated with oysters. These changes would not take the form of reductions in numbers of individuals but would appear as wholesale complete eliminations of dominant and subdominant species, genera or families.

(2) When a mortality of oysters is caused by toxic substances, and that mortality destroys any considerable part of the oyster

population, a continuation in time of subjection to the toxin will destroy the entire population. It is not possible, for example, to reach the LD_{50} for oysters which is then followed by a revival of the oyster population and cessation of mortalities if the pollution is continuing. Continuation in time of such lethal concentration must result in destruction of the population, since the LD_{50} is essentially that level of toxicity which is lethal threshold to the population as a whole. Additionally, when a level of toxicity is reached which will destroy a significant part of an oyster population, an increase in concentration of the pollutant will, in the same time, destroy the whole population.

(3) A level of toxicity which can directly destroy a part of a population will so affect survivors that the individuals of the population will be physiologically altered. The two most basic of physiological yardsticks, growth and reproduction, may be used to test for sub-lethal levels of pollution. It is not possible for a mortality due to pollution to be followed or accompanied by normal gonadal development, spawning, and setting in the face of continued pollution. Neither can survivors of pollutional mortality continue to grow and fatten as long as pollution continues. Before lethal levels of pollution are reached these basic functions will be destroyed and both growth and reproduction will cease.

(4) Mortalities due to pollution are non-seasonal. While it is obvious that resistance to toxic effect may vary with the metabolic level of the oysters, which in turn varies with seasonal temperature changes, such variation only modifies in some small degree the amount of the lethal dosage. In the face of such drastic effect as death from toxin, the small threshold modification produced by seasonal temperature or other change is hardly measurable. Certainly a concentration of toxic substance effective to the point of producing death of oysters in one season cannot be so ineffective in a succeeding season that deaths and physiological depression do not occur in any degree. Physiological damage is a yardstick often overlooked in measurement of effect of pollution.

(5) The effects of pollutional damage are greatest at the source of the toxin and the effects on oysters diminish with distance away from the source. This criterion would seem to be self-evident. The decrease in effect is due to two factors, the most important being dilution. The other is biological and chemical modification of the pollutant which tends to reduce toxicity.

(6) Deaths from toxic effects are not density-dependent. Concentration of the oysters or population densities have no effect on death rates.

(7) In a given area of approximately equal pollution, all oyster beds will be found to be affected. It is not believed to be

possible for beds with high mortality from toxic substances to alternate or be interspersed with beds in good condition and with little or no mortality. Extremes of variation with respect to mortality rate within a limited area are not consistent with natural effects of pollution.

Pollution may not involve a toxin, but instead may modify the habitat in some indirect manner. The usual non-toxic effect is to exert a strong oxygen demand. Deaths are due to asphyxiation rather than to toxic effects. All of the characteristics mentioned above apply equally well irrespective of whether or not the action is direct or indirect.

VII. MORTALITIES DUE TO METABOLIC COMPETITION

It is not known for certain that mortalities due to metabolites actually exist, but, because of the developing interest in this field, the matter is explored briefly. For a recent discussion of researches on metabolites in the sea, see Lucas (1955). It has become increasingly apparent that dominant organisms produce substances, which, excreted or secreted into the sea, exercise control over other organisms. More to the point, large and rapidly growing organisms may suppress growth of smaller, less rapidly growing organisms of the same or closely related species (Rose 1959). Thus, when a large fast-growing species of oyster is introduced into an area originally populated by a small, slow-growing species it is apparent that there may develop a competition unrelated to competition for either food or space. Modern oyster production requires populations of oysters of such density that artificial dominance is set up and sometimes maintained. In some cases attempts have been made to maintain two such oyster dominants in the same area. There are three such cases, in which species of Crassostrea appear to be in direct competition with species of Ostrea. The first of these is the Crassostrea angulata - Ostrea edulis combination on the French coast. C. angulata was introduced to the French coast originally in the middle of the 19th century in the basin of Arcachon. In 1868 another introduction was made in the Gironde estuary. Natural reefs developed from these introductions, and the conditions seemed to be well suited to the imported species. It successively became numerically dominant over estuaries farther and farther north on the French coast. Oystermen found it easier and more profitable to cultivate than was the native O. edulis. Lambert (1946) described the reduction in numbers and importance of O. edulis through the years. He believed that C. angulata will take over the entire French coast and replace the native.

In Australia a similar situation exists. Crassostrea commercialis originally coexisted with the flat oyster, Ostrea angasi, which is very similar to O. edulis. O. angasi disappeared from the north part of its range and is now restricted to the colder waters of the

south of Australia where C. commercialis does not grow.

In the South Puget Sound area of Washington, the introduced Japanese oyster, Crassostrea gigas, is now preempting the area formerly occupied exclusively by O. lurida, the excessively small native species. The C. gigas population has increased rapidly in late years, with a corresponding decrease in O. lurida. Oystermen have increasing difficulty raising the latter, while at the same time the C. gigas industry has grown enormously.

In all of these cases, there is reason to believe that economics may play a part in the substitution of a fast-growing, large oyster for a slow-growing, smaller oyster. Also there is the possibility that introduction of predators and diseases may have had some influence. Certainly, in the case of O. lurida, the introduction of the flatworm Pseudostylochus has played a part. Such a species would certainly be more effective against a small, slow-growing species such as O. lurida than against the large, fast-growing C. gigas. In Australia it has been reported that O. angasi was decimated by the "worm" disease (Polydora ciliata or P. ligni).

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A METHOD OF ESTIMATION OF MORTALITY RATES IN OYSTERS

J. G. Mackin[1]

In Louisiana, and sometimes elsewhere, it has been customary to use the "box count" method of estimation of mortality. It has been widely used in short-term studies of planted beds and natural growths of oysters. Where more elaborate studies are possible, with sufficient periods of time, the tray method is highly accurate, and has been extensively used by the author and others (Hewatt and Andrews 1954, Beavin 1949). Otherwise productivity studies may be based on analysis of mortality and growth as interacting factors (Hopkins and Menzel 1952, McHugh and Andrews 1955, Andrews and McHugh 1957). Basically this method compares the number of oysters planted per unit measure with the number harvested per unit measure taking into consideration also the number of units harvested per unit planted. This also is a highly efficient method.

Estimation of mortality rates on natural reefs and planted beds when it must be done quickly and without knowledge of planting and harvesting data, and without long-term use of trays, has been mostly based on the "box count" method. In using the method, random samples of oysters are collected and percentage of hinged shells in the total of live oysters and hinged shell is usually reported as "mortality". It has been my contention that this method is highly inaccurate, misleading, and, as a method of population investigation, unacceptable. There are legitimate uses for box counts and studies of condition of boxes and shells when it is necessary to make rough calculations of the extent of very recent and cataclysmic mortality. But box counts are a source of gross error in any study of oyster populations or productivity. To show the inadequacy of the system, two illustrations are given. Nine samples of oysters from natural reefs in one limited area of Louisiana were pooled to make a total of 3252 oysters, of which 14.1 per cent were boxes. This normally would be reported as "mortality." But there is no hint as to the time required to produce the 14.1 per cent of boxes found; neither is there any method of determining just how many "boxes" lose their right valves in a given length of time and hence cease to be boxes. The 14.1 per cent boxes cannot in any sense be a rate, and only rates are useful in productivity or population calculations. In the case cited above, a careful analysis indicated that the population did not contain any oysters older than four and one-half years. This being true, the mean annual loss could not be less than 50 per cent and actually must have been greater. Menzel and Hopkins (1952), in studies of bottom plantings made very near the area from which these samples were taken, also found that boxes were a poor indicator of mortality rates and demonstrated, using the tray method,

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that mortality rates ranged from 25 to 31 per cent annually in oysters two months old (at start) to 96 to 100 per cent annually in oysters 16 months old or older. This indicates that the 14.1 per cent boxes in the nine samples mentioned above gave a grossly unrealistic indication of mortality rates.

A second check was made using samples of South Carolina oysters from Wadmalaw Island's We Creek. Through the courtesy of Dr. Robert Lunz, a sample of 1291 oysters was analysed. This sample contained eight per cent boxes, but the oldest oysters were roughly about four years of age. The mean annual mortality was, therefore, in excess of 60 per cent.

Because increasing data indicate that oyster mortalities are generally much higher than the estimates found in the literature, a study of the matter using statistical methods borrowed from fin fisheries investigations, has been projected. This study has only begun, but it is believed that the approach may be helpful in population analyses. It is remarkable that so few studies of natural oyster populations have been made. This is more to be wondered at since it has been assumed by most researchers that oysters are ecological dominants in the oyster reef community, both as to numbers and as to community control.

STUDIES ON MORTALITIES IN LOUISIANA OYSTERS USING THE TRAY METHOD

Prior to beginning this study, all available data bearing on mortality rates in Louisiana oysters were studied. A considerable number of studies using the tray method were examined. In these, oysters were held in trays or cages for varying lengths of time and in different areas. Results of these studies are presented here as background material. These tray (or cage) studies showed unexpectedly high rates of mortality at widely scattered places in Louisiana. In Table 1, the data are summarized and reduced to an annual basis. This, in some cases, was difficult to do because of different rates which pertain to different seasons, but they are believed to be within ± 5 per cent of the true rates. Most studies reported for Menzel and Hopkins are round-number approximations of means for several studies in each area, as is true also for some studies by Mackin and Wray. The data from Owen (1955) were difficult to assess, since he ran his tray studies only during the summer, for seven and one-half months. However, winter mortalities are generally light, so Owen's figures were only raised to the next higher figure which rounded to the nearest 5 per cent. The same applies to figures from Mackin and Sparks (1959). These authors reported on tray check of mortality from March to September in 1957. St. Amant et al. carried one group of caged oysters (equivalent to tray) for six months, with a mortality of 44 per cent. This one would have been about 60 per cent if extended to one year, but the data are reported for the six months only in this case, because of uncertainty of the locale of the study.

Table 1. Annual rates of mortality of oysters in Louisiana as shown by tray studies (Oysters one year old or older).

Period of Study	Author	Place	Per cent Mortality
1947-48	Mackin and Wray 1949	Bayou Rigaud	80
1947-48	Mackin and Wray 1949	Sugar House Bend, Barataria Bay	80
1947-48	Mackin and Wray 1949	Bassa Bassa Bay	70
1947-48	Mackin and Wray 1949	Chene Fleur Bay	30
1948-49	Mackin and Wray 1950	Bayou Rigaud	80
1948-49	Mackin and Wray 1950	Lower Barataria Bay	75
1948-49	Mackin and Wray 1950	Bassa Bassa Bay	60
1948-49	Mackin and Wray 1950	Chene Fleur Bay	30
1948-49	Mackin and Wray 1950	Lake Grande Ecaille	70
1947 to 1949	Menzel and Hopkins 1952	Bayou Bas Bleu	65
1947 to 1949	Menzel and Hopkins 1953	Bay Ste. Elaine	60
1947 to 1949	Menzel and Hopkins 1951	Lake Barre	70
1947 to 1949	Menzel 1950	Lower Terrebonne Bay	75
1947 to 1949	Menzel 1951	Lake Pelto	80
1949	Owen 1955	Bayou Pierre	60
1949	Owen 1955	Quarantine Bay	45

Table 1 (Continued)

Period of Study	Author	Place	Per cent Mortality
1949	Owen 1955	Grand Bay	50
1949	Owen 1955	Sandy Point Bay	50
1949	Owen 1955	Bayou Scofield	60
1949	Owen 1955	Bay Adam	65
1949	Owen 1955	Lake Grande Ecaille	70
1949	Owen 1955	Northern Barataria Bay	45
1949	Owen 1955	Bassa Bassa Bay	40
1949	Owen 1955	Lower Barataria Bay	75
1949	Owen 1955	Lake Felicity	45
1949	Owen 1955	Sister Lake	30
1948-49	Mackin, Welsh and Kent 1950	Bay Adam	85
1948-49	Mackin, Welsh and Kent 1950	Bayou Cook	75
1948-49	Mackin, Welsh and Kent 1950	Bastian Island	80
1948-49	Mackin, Welsh and Kent 1950	English Bay	90
1948-49	Mackin, Welsh and Kent 1950	Bay Pomme D'or	80
1948-49	Mackin, Welsh and Kent 1950	Skipjack Bay	75
1948-49	Mackin, Welsh and Kent 1950	Bay Jacque	65
1948-49	Mackin, Welsh and Kent 1950	Bay Tambour	55

Table 1 (Continued)

Period of Study	Author	Place	Per cent Mortality
1957	Mackin and Sparks	Billet Bay	65
1957	Mackin and Sparks	Northern Grande Ecaille	55
1957	Mackin and Sparks	Southern Grande Ecaille	65
1957	Mackin and Sparks	Dredged cut Freeport Sulphur	30
1957	Mackin and Sparks	Southern Barataria Bay	45
1957	Mackin and Sparks	Southern Barataria Bay	80
1957	Mackin and Sparks	Sugarhouse Bayou	55
1956-57	St. Amant, et al.	(not stated)	44*

* Six months only; estimated about 60 per cent for a year.

Data in Table 1 indicate that annual mortality rates of oysters more than a year old are usually in excess of 60 per cent annually. St. Amant et al. (1958) believed that 20 per cent is a "normal" rate for bedded oysters "over the growing period." This latter, in Louisiana, could be any period from three months to two years. It was indicated that in most bedded oysters, the growing period might be about nine to ten months. If that is so, 20 per cent is much too low an estimate. It is believed that there has never been a mortality rate consistently that low in Louisiana. St. Amant et al. (1958), in several mortality studies, failed to find any that low, and one study by these authors showed about 80 per cent annual mortality. It may be that these authors consider all mortality rates in Louisiana to be abnormal, and that the "normal" is a desired low rate of mortality, never to be actually attained.

MORTALITY RATES BY YEAR-CLASS ANALYSIS

Because of the objections to box counts stated above, it was decided to apply the method of year-class analysis to determine whether or not such a method used with natural oyster populations would corroborate figures derived from the tray method as shown in Table 1. It was believed that if a natural population of oysters could be made to yield data from which the number of year-classes represented in the population could be determined, and also the approximate number of oysters in each year-class, the problem could be resolved with the simplest kind of calculation.

As a beginning, calculations were made to determine the time necessary to bring any year-class population to practical extinction, using various rates of mortality. "Practical extinction" was arbitrarily decided to mean that less than 5 per cent of the original population would remain. This procedure was adopted because the time required to eliminate a population completely, when the percentages of annual loss are very low, is a very long time. For example, even at 50 per cent annual mortality, a population may be reduced to less than 5 per cent of its original size in four to five years, but to eliminate the remnant 5 per cent requires another six to seven years. Since sampling methods cannot be depended on to detect remnant populations of very small size, and because mortality rates become excessively erratic when dealing with small numbers of oysters, the compromise reduction to 5 per cent of original size was adopted, and found to be satisfactory.

In Table 2 are figures showing the time required to reduce a population of oysters to less than 5 per cent of its original size at various annual mortality rates.

Table 2. Time required to reduce a population of oysters to less than 5 per cent of its original size with different mean annual rates of mortality.

Annual mortality rate, per cent	Years required to reduce to less than 5 per cent
10	28-29
20	about 13
30	8-9
40	about 6
50	4-5
60	3-4
70	2-3
80	nearly 2
90	1 plus

This table shows that in any natural undisturbed population of oysters, there should be about 28 recognizable year-classes if the mean annual mortality rate is 10 per cent, and a small part of the population would be 28 to 29 years old. If the rate was 20 per cent, one should find oysters about 13 years old, etc. These preliminary calculations emphasized the fact that commonly-quoted low annual mortalities of 10 to 20 per cent in Louisiana oysters have no validity, since oysters attaining an age of five years are certainly rare, if they exist at all.

Analyses were made of a considerable number of natural populations to determine whether or not it could be determined with a fair degree of accuracy how many year-classes were represented in a sample and approximately how many oysters made up each year-class. If this could be done, it would greatly simplify determination of annual death rates. Oysters in large samples were carefully measured for length of the right valve, and size distribution diagrams were made. Most of these showed clearly that the approximate age of the oldest oysters could be determined, and that fused modes of the year-classes could be recognized. This can be done only if recruitment is reasonably constant. One of the diagrams in block form is shown in Figure 1. The process of separation of year-classes was aided because oysters in their first year could be recognized by form and color. Other year classes were clearly indicated by sub-modes, and corroborated by growth data. The histogram in Figure 1 showed that the 1954 year-class was the oldest in the population and made up only about one per cent of the total.

Where difficulty in recognition of year-class modes is encountered, aid is found in a knowledge of normal growth rates. Menzel and Hopkins have made extensive studies of growth in Louisiana oysters (see any of several references given in the bibliography). These data indicate that oysters in their first year have a modal length of about 30 mm in three months, 55 to 60 mm in one year, 85 to 95 mm in two years, and 105 to 115 mm in three years. Depending on the area, these growth rates are greater or less. Oysters which are older than four years are rare and usually form a remnant population on the right extremity of a size-distribution diagram.

Estimates of numbers in each year-class may be made by reconstructing the normal curves for each year-class. Accuracy in this is not necessary, so long as the numbers in the youngest and oldest year-class are approximately correct. An error in estimation of numbers in the second, third, or fourth year-classes must necessarily be compensated for by opposite error in the next older or younger class. After numbers are estimated, the approximate mortality from one year-class to the next is easily computed. It is convenient to plot the numbers or per cent in each year-class as the logarithm of the percentage of the population in each, as shown in Figure 1. This type of plot is the same as the fishery statisticians' "catch curve" (Ricker 1948). Considerable information can be derived from these curves.

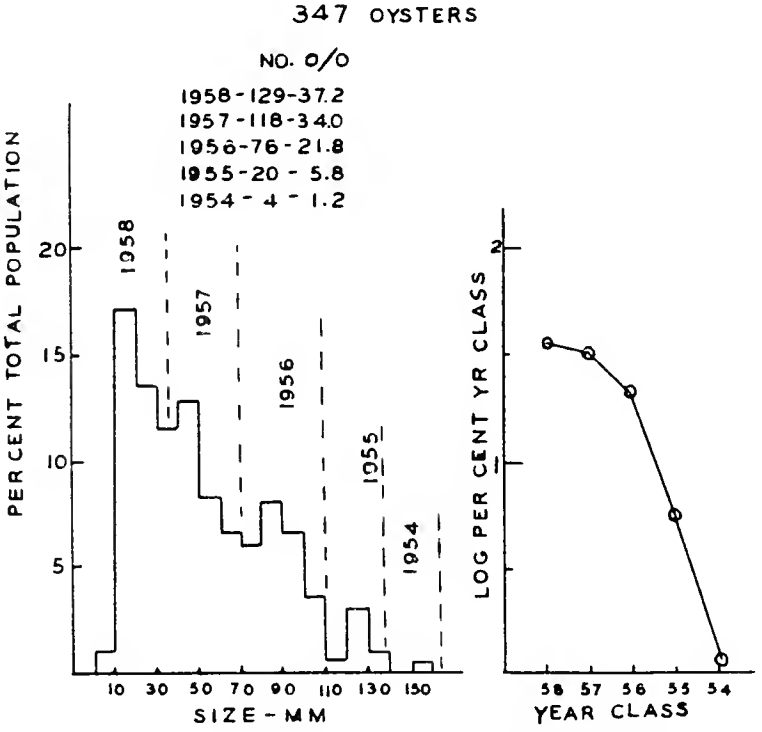

Fig. 1. Size distribution of oysters from Sandy Point Bay, August 1958.

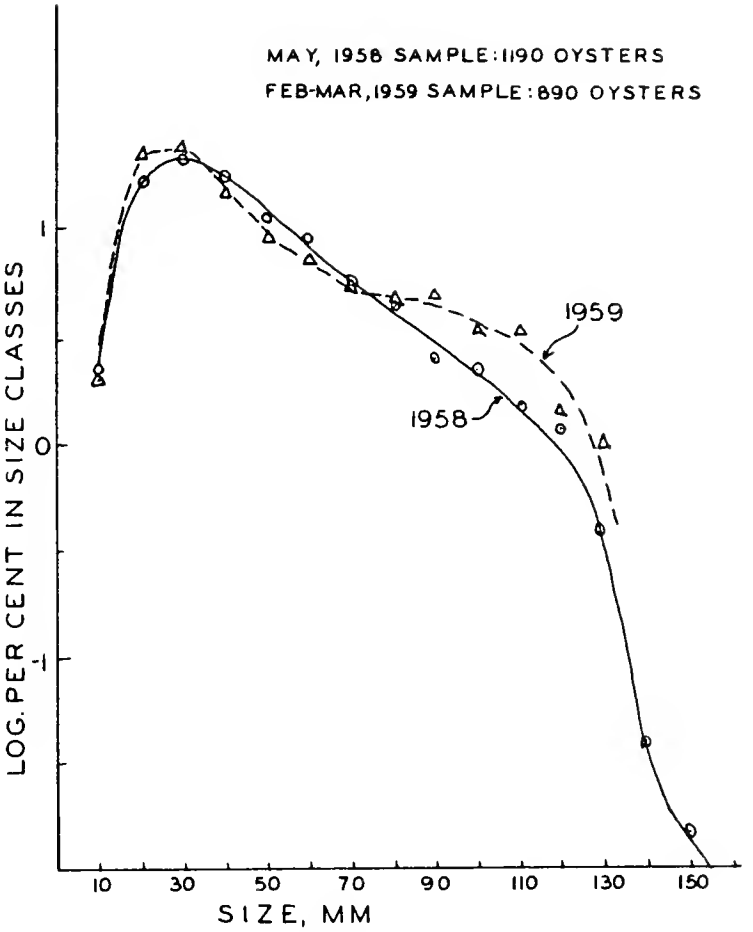

Fig. 2. Size distribution of oysters from We Creek, South Carolina.

First, the mean slope of the right hand limb of the curve represents the survival rate for the period represented by the year-classes plotted, and hence also the mortality rate. To some degree also, if the slope is not a straight line, the various segments of it indicate those year-classes in which most mortality occurs, and those in which the least mortality occurs. In Figure 2 (1959 curve) the youngest oysters in two South Carolina samples are shown to have the highest mortality rate, and rates in older year-classes are less. In Louisiana (Figure 1) just the opposite is true.

Since size is a function of age, it was believed that the necessity for determination of year-class numbers could be eliminated by plotting the logarithm of the percentage numbers in size-classes rather than in estimated year-classes. It was found that this method was reasonably accurate. In using such curves it is convenient (1) to use a standard graph scale for all plots and (2) make up a group of comparison graphs based on assumed mortality rates in populations with constant recruitment rate. These comparison graphs, plotted to the same scale as are the natural populations, give a ready estimation of mortality rates by comparing slopes. The use of graphs facilitates comparison with limited parts of a "survival curve." Two samples of oysters from South Carolina were plotted in this manner (Figure 2). These two samples came from about the same area, but were taken nearly a year apart.

It is believed that, with refinement, this method of approach to mortality estimations has promise. It is suggested that the measurement of shell length may not be the best available variate. Volume might be better, or total weight, or product of length, width, and thickness. It is recognized that in areas of slow growth and erratic set the method may be difficult to use. Nevertheless, the need for improvement in methods of estimating mortality rates warrants a trial.

SUMMARY

(1) Tray studies of mortalities made by various authors show that in Louisiana the usual annual loss in oysters one year old and older is between 50 and 70 per cent.

(2) These same studies show that annual mortality rates of from 70 to 90 per cent are not unusual.

(3) A few very favorable areas have showed low annual rates of around 30 per cent. This is believed to be about the minimum in Louisiana.

(4) A method of rough calculation of mortality rates in oyster populations has been tried in Louisiana with promising results. This method involves identification of the number of year-classes in the population, and calculation of the mean annual rate necessary to reduce

a population to near extinction in the indicated number of years represented by year-classes in the population.

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PACIFIC OYSTER CRASSOSTREA GIGAS MORTALITIES WITH NOTES ON COMMON OYSTER PREDATORS IN WASHINGTON WATERS

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ABSTRACT

Laboratory studies are reported giving approximate predation rates of oyster predators found in Washington waters. Data on seasonal and annual mortality rates of experimental oyster plots are presented. Mortalities on an area and statewide basis by age are reported as determined through annual surveys of the commercial beds in 1956, 1957 and 1958. Syndromes of mass mortalities encountered are outlined.

INTRODUCTION

Mortalities and predator-oyster relationships of most species of oysters have been widely studied and reported. Reference to predation on the Pacific oyster, Crassostrea gigas, has been made by Elsey (1933), Glud (1947), Galtsoff (1932) and Kincaid (1951). In his survey of the Japanese oyster literature, Cahn (1950) refers to predation and mortality. None of these authors present data on mortalities or predation rates. Thomson (1952) on the other hand reports approximately 60 per cent mortality of this species in the first year after planting and 55 per cent during the second in Australian waters. From his report it is assumed that predation was not a factor in the losses recorded. Chew and Eisler (1958), in a report on the feeding habits of the Japanese oyster drill (Ocinebra japonica), make reference to oyster predation. Woelke (1957) noted Pseudostylochus ostreophagus predation on juvenile Pacific oysters.

Review of the literature provides no scientific information on Pacific oyster mortalities or predation in Washington waters. This report deals with predators, mortality by age, seasonal mortality and annual mortality as a continuous interrelationship.

Extensive studies have been conducted by this laboratory on predation rates, predator habits and predator control; however, only one series of predation experiments is discussed in this report. Data from a series of field experimental plots are presented to demonstrate seasonal and annual mortalities experienced in the first and second years after planting (Pacific oyster seed caught in Japan in the summer is imported and planted during the following spring when 5-15 mm in

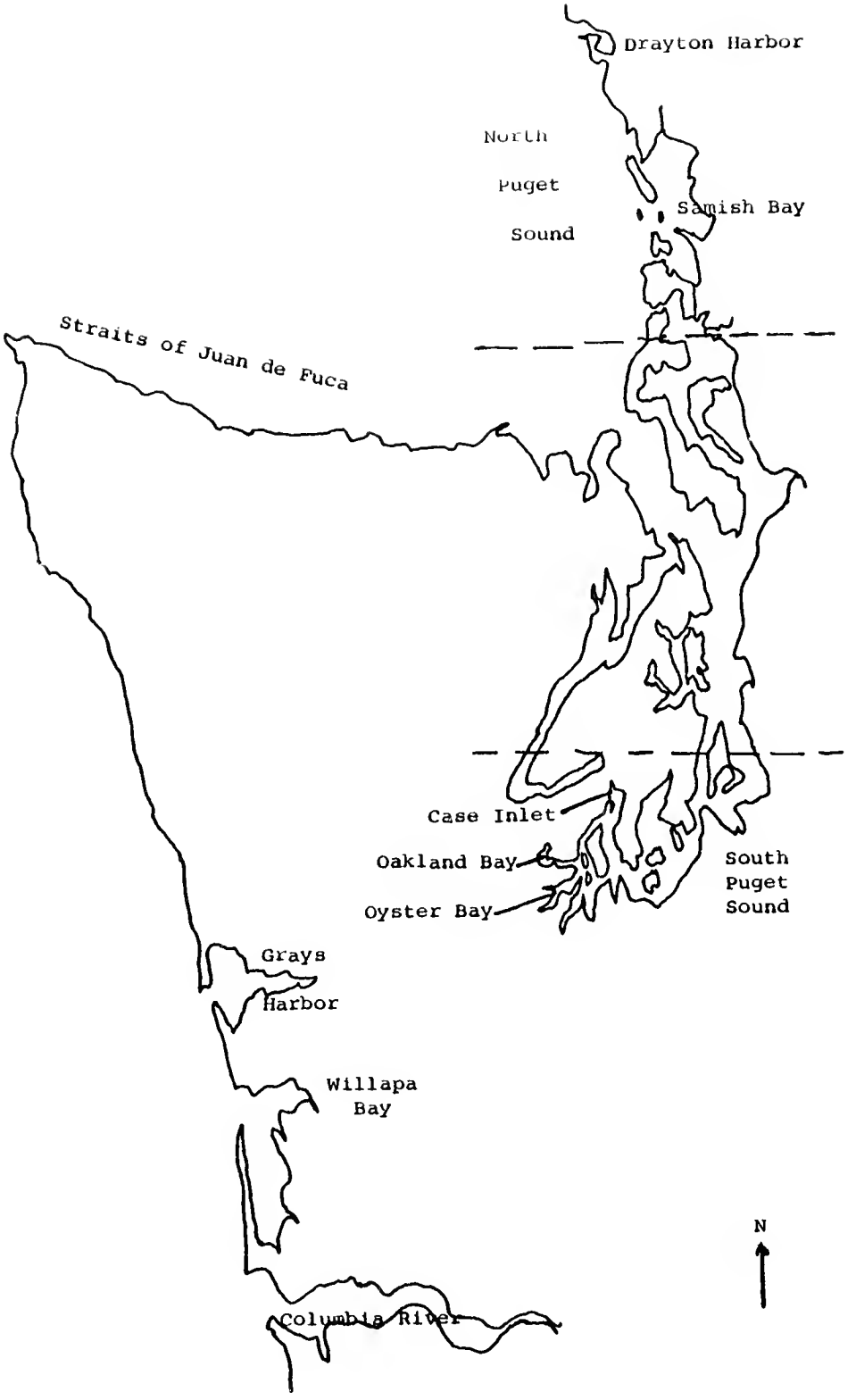

Fig. 1. Principal Oystering Areas in Washington State.

length - less than 1 cc in volume).[1] Pacific oyster culture is restricted almost entirely to the intertidal zone. The intertidal nature of the plantings makes identification of age groups and representative sampling of the beds much simpler than where oysters are subtidally cultivated. Oystering areas are shown in Figure 1.

PREDATION STUDIES

To determine which of the potential predators would attack Pacific oysters, aquaria studies were conducted. Limited numbers of seven species of suspected predators were placed in seperate aquaria with four-month-old oysters for 20 days. During the study, temperature of the flowing water ranged from 14-20°C and the salinity from 24-26 o/oo. No potential macroscopic food other than oysters was available in the aquaria.

Results of these studies are summarized in Table 1. Number of "predators," number of oysters (48-70), mortality at 8 and 20 days as well as "20-day predation rates per specimen" are shown. While these data have many readily recognized limitations they demonstrate the ability of the species considered to attack and destroy young oysters in the absence of other food. The eighth day mortalities may indicate that neither Cancer gracilis nor Cancer magister (the commercial crab of Washington) readily attacks oysters, while Cancer oregonensis and Cancer productus probably do. After 20 days' exposure all species except Cancer magister had caused appreciable mortalities. Except for Cancer magister, crabs caused the greatest losses followed in order by starfish, Japanese oyster drill and flatworm. Observations in the field have confirmed these findings in all instances except Cancer gracilis.

SEASONAL AND ANNUAL MORTALITY RATES

Field studies were conducted during 1952-53 in three of the four principal oyster-growing areas to ascertain the variation in mortality rates by age, area and season. Lots of 20 or 40 individually marked spat and yearling oysters of common stocks taken from our experimental oyster beds were utilized in this study. Replicate lots of spat and oysters were placed inside the boundaries of commercial plantings. All marked oysters were checked each time a mortality observation was made.

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[1] As a result of the unique method by which the Pacific Coast oyster industry replenishes the bulk of its oyster stocks, i.e., annual importation of seed from Japan, both industry and researchers designate successive year classes by year of planting. Both age and year class as referred to in this report follows this precedent.

Table 1. Predation on four-month-old Pacific oysters in laboratory aquaria

"Predators"	Number of oysters	After 8 days exposure		After 20 days exposure		20-day predation Rate per specimen
		Live	% Dead	Live	% Dead	
Controls	55	55	0.0	52	5.5	
Cancer gracilis(4)*	50	46	8.0	5	90.0	11.3
Cancer magister(3)	50	50	0.0	49	2.0	0.3
Cancer oregonensis(1)	68	47	30.5	0	100.0	68.0
Cancer productus(3)	48	0	100.0	0	100.0	16.0
Evasterias troschelii(3)	45	35	22.0	6	88.7	13.0
Pseudostylochus ostreophagus(10)	70	56	20.0	49	30.0	2.1
Ocinebra japonica(10)	50	39	22.0	6	88.0	4.4

*Number of specimens used

Table 2. Mortality of Pacific oysters in control plots during their first year after planting

	5/52	6/52	7/52	8/52	9/52	11/52	1/53	4/53	Total	Per cent
Live (N=220)	220	174	159	149	137	119	116	113		
Dead - drill		16	10	5	4	12	0	2	49	22.3
crab		3	1	0	2	1	0	0	7	3.2
unknown		27	4	5	6	5	3	1	51	23.2
Totals		46	15	10	12	18	3	3	107	48.6
% Mortality by time period		20.90	8.62	6.28	8.05	13.13	2.52	0.86		

Table 3. Mortality of Pacific oysters in control plots during their second year after planting

	5/52	6/52	7/52	8/52	9/52	11/52	1/53	4/53	Total	Per cent
Live (N=85)	85	83	77	77	74	73	71	66		
Dead - drill			1	0	1	0.	1	2	5	5.9
crab			1	0	0	0	0	1	2	2.4
unknown		2	3	0	2	1	1	2	11	12.9
Totals		2	5	0	3	1	2	5	18	21.2
% Mortality by time period		2.35	6.02	0	2.59	1.35	2.74	7.04		

Table 4. Summary of Grays Harbor Pacific oyster mortality data

Year of observation	Planting year	Total oysters checked	Dead oysters	Cause of death				Mortality in per cent		
				Drill	Crab	Other	Unk.	Total	Crab	Unk.
1956	1954	519	80		20		60	15.5	3.9	11.6
	Per cent	100	15.5		3.9		11.6			
1957	1957	449	19		10		9	4.2	2.2	2.0
	1956	1,258	111		24		87	8.8	1.9	6.9
	1955	958	71		14		57	7.4	1.5	5.9
	Totals	2,665	201		48		153			
	Per cent		7.5		1.8		5.7			
1958	1958	519	40		19		21	7.7	3.7	4.0
	1957	2,713	340		66		274	12.5	2.4	10.1
	1956	1,958	309		15		294	15.9	0.8	15.0
	1955	616	98		3		95	15.9	0.5	15.4
	Totals	5,806	787		103		687			
	Per cent		13.6		1.8		11.8			

Table 5. Summary of North Puget Sound Pacific oyster mortality data

Year of observation	Planting year	Total oysters checked	Dead oysters	Causes of death			Mortality in per cent			
				Drill	Crab	Unknown	Total	Drill	Crab	Unknown
1951	1951	187	21	6		15	11.2	3.2		8.0
	1950	129	25	11		14	19.4	8.5		10.9
	Totals	316	46	17		29				
	Per cent		14.6	5.4		9.2				
1952	1952	224	8	3		5	3.6	1.3		2.2
	1951	194	16	11		5	8.2	5.7		2.6
	Totals	418	24	14		10				
	Per cent		5.7	3.3		2.4				
1953	1952	118	18	2		16	15.3	1.7		13.6
	1951	108	8	1		7	7.4	0.9		6.5
	Totals	226	26	3		23				
	Per cent		11.5	1.3		10.2				
1954	1954	184	36	7	27	2	19.6	3.8	14.7	1.1
	1951	453	75	22	39	14	16.6	4.9	8.6	3.1
	Totals	637	111	29	66	16				
	Per cent		17.5	4.6	10.4	2.5				
1956	1956	1,104	62	26		36	5.6	2.4		3.3
	1955	666	122	14		108	18.3	2.1		16.2
	1954	936	186	37		149	19.8	3.9		15.9
	1953	363	56	10		46	15.4	2.8		12.6
	1952	338	142	34		108	42.0	10.0		32.0
	Totals	3,407	568	121		447				
	Per cent		16.7	3.6		13.1				
1957	1957	661	14	1	1	12	2.1	0.2	0.2	1.8
	1956	1,140	67	16	3	48	5.9	1.4	0.3	4.2
	1955	560	35	1	3	31	6.3	0.2	0.6	5.5
	1954	458	82	2	1	79	17.9	0.4	0.2	17.3
	1953	145	19	6	4	9	13.1	4.1	2.8	6.2
	Totals	2,964	217	26	12	179				
	Per cent		7.3	0.9	0.4	6.0				
1958	1958	291	13	3	1	9	4.5	1.1	0.3	3.1
	1957	1,897	444	54	1	389	23.4	2.8	0.1	20.5
	1956	2,978	426	8		418	14.3	0.3		14.0
	1955	1,562	222	5	5	212	14.2	0.3	0.3	13.6
	1954	579	112	15		97	19.4	2.6		16.8
	Totals	7,307	1,217	85	7	1,125				
	Per cent		16.7	1.3	0.1	15.3				

Table 6. Summary of south Puget Sound Pacific oyster mortality data

Year of observation	Planting year	Total oysters checked	Dead oysters	Cause of death			Mortality in per cent			
				Drill	Crab	Unknown	Total	Drill	Crab	Unknown
1956	1956	2,442	71	17	4	50	2.9	0.7	0.2	2.1
	1955	1,687	173	3	1	169	10.3	0.2	0.1	10.0
	1954	1,967	190			190	9.7			9.7
	1953	56	8			8	14.3			14.3
	Totals	6,152	442	20	5	417				
	Per cent		7.2	0.3	0.1	6.8				
1957	1957	737	22	12	1	9	3.0	1.6	0.1	1.2
	1956	1,085	99	16		83	9.1	1.5		7.6
	1955	834	55	18		37	6.5	2.1		4.4
	1954	164	20	1		19	12.1	0.6		11.5
	Totals	2,820	196	47	1	148				
	Per cent		7.0	1.7	0.1	5.2				
1958	1958	1,133	54	14	6	34	4.8	1.2	0.5	3.0
	1957	2,790	502	19	35	448	18.0	0.7	1.3	16.0
	1956	3,492	599	16	34	549	17.2	0.5	1.0	15.7
	1955	151	80			80	53.0			53.0
	Totals	7,566	1,235	49	75	1,111				
	Per cent		16.3	0.6	1.0	14.6				

Table 7. Summary of Willapa Bay Pacific oyster mortality data

Year of observation	Planting year	Total oysters checked	Dead oysters	Cause of death		Mortality in per cent		
				Crab	Unknown	Total	Crab	Unknown
1956 (Autumn)	1956	913	22		22	2.4		2.4
	1955	558	39		39	6.9		6.9
	1954	969	72		72	7.5		7.5
	Totals	2,431	133		133			
	Per cent		5.5		5.5			
1957 (Autumn)	1957	499	10	5	5	2.0	1.0	1.0
	1956	1,152	48		48	4.2		4.2
	1955	1,164	58		58	5.0		5.0
	Totals	2,815	116	5	111			
	Per cent		4.1	0.2	3.9			
1958 (Autumn)	1958	933	34		34	3.6		3.6
	1957	1,183	79		79	6.7		6.7
	1956	664	54		54	8.1		8.1
	Totals	2,780	167		167			
	Per cent		6.0		6.0			
1958 (Summer)	1958	531	8		8	1.5		1.5
	1957	762	90		90	11.8		11.8
	1956	746	68		68	9.1		9.1
	Totals	2,039	166		166			
	Per cent		8.1		8.1			

Table 8. Summary of statewide Pacific oyster mortalities

Year of observation	Age in years	Per cent total mortality				Per cent unknown mortality			
		North Puget Sound	South Puget Sound	Grays Harbor	Willapa Harbor	North Puget Sound	South Puget Sound	Grays Harbor	Willapa Harbor
1956	0+	5.6	2.9		2.4	3.3	2.0		2.4
	1+	18.3	10.3		6.9	16.2	10.0		6.9
	2+	19.8	9.7	15.5	7.5	15.9	9.7	11.6	7.5
	3+	15.4	14.3			12.6	14.3		
	4+	42.0				32.0			
	Average all ages	16.7	7.2	15.5	5.5	13.1	6.8	11.6	5.5
1957	0+	2.1	3.0	4.2	2.0	1.8	1.2	2.0	1.0
	1+	5.9	9.1	8.8	4.2	4.3	7.6	6.9	4.2
	2+	6.3	6.5	7.4	5.0	5.5	4.4	5.9	5.0
	3+	17.9	12.1			17.2	11.5		
	4+	13.1				6.2			
	Average all ages	7.3	7.0	7.5	4.1	6.0	5.2	5.7	3.9
1958	0+	4.5	4.8	7.7	3.6	3.1	3.0	4.0	3.6
	1+	23.4	18.0	12.5	6.7	20.5	16.0	10.1	6.7
	2+	14.3	17.2	15.8	8.1	14.0	15.7	15.0	8.1
	3+	14.2	53.0	15.9		13.6	53.0	15.4	
	4+	19.3				16.8			
	Average all ages	16.6	16.4	13.6	6.1	15.3	14.6	11.8	6.1

Table 9. Summary of mortality by cause from annual statewide surveys

	Oysters checked	Dead			
		Crab	Drill	Mass mortalities	Unknown
Totals	48,370	272	348	1,155	3,734
Per cent		0.6	0.7	2.4	7.7

Data collected are summarized by age of oysters (0-1 year and 1-2 years) in Table 2 and 3. In view of the small number of oysters per area (60-80) no intra-area comparisons are attempted. Total mortality during the first year of life was 48.6 per cent with drills causing 22.3 per cent, crabs 3.2 per cent and "unknown causes" (neither valve of dead oysters damaged) 23.2 per cent. Over 20 per cent of the total loss and over 50 per cent of the annual loss from unknown causes occurred during the first month after planting. Losses were largest between May and November. From November to April slightly over 3 per cent mortality was recorded.

Considerably lower loss was recorded on second year oysters with a total annual mortality of 21.2 per cent. Drills caused 5.9 per cent, crab 2.4 per cent, and 12.9 per cent died from causes not determined. Loss of 6.0 per cent was recorded during June with all other observations less than 3.0 per cent per time period except for 7.0 per cent during the four months from January to April. In general, decreased mortality with increased age, increased mortality during summer, and a predominance of losses from undetermined causes summarize this study.

ANNUAL MORTALITY SURVEYS

Statewide mortality surveys begun in 1956 cover approximately 70 per cent of the major oyster beds in the state and 90 per cent of the general oyster producing areas. The surveys are conducted on the last two or three daylight low tide series of the year (August and September). All age groups present on the beds are sampled. Mortality values are generally derived from random samples of 200 or more oysters per planting. Mortalities are the shells of dead oysters held together by the hinge ligament at the time of sampling. These samples have been demonstrated to be statistically valid at the 5 per cent level. In collection of data, mortality causes are assigned as drill, crab, "mass mortalities" or unknown. Mass mortalities, i.e., sudden, excessive losses (over 20 per cent) are recorded only when observed in action, since starfish, sea weed and siltation can also cause heavy losses but may not be operating at the time of sampling. This method of recording results in a large proportion of the losses being classified as unknown. On occasion, losses are due to burying of oysters (especially spat) by mud shrimp (Callianassa and/or Upogebia). Decomposition of large "mats" of Enteromorpha and other sea weeds which settle on oyster beds cause moderate to serious mortalities.

Tables 4 through 7 present summaries of mortality data from the four principal oyster areas by year and age. In the northern Puget Sound summary, data from 1951-1954 (primarily from Samish Bay) are also shown. Mortalities by cause are given in percentages. In Table 8, percentage mortalities only are shown by area, age, and year. To demonstrate the relative importance of the categories into which the mortalities fall, Table 9 summarizes all data collected in the annual

surveys. In the absence of information on decomposition rate of the hinge ligament of Pacific oysters, these data in no sense can be interpreted as true annual mortalities but rather relative from year to year and area to area at the time surveyed. A second limitation of the annual survey data is its failure to measure losses of any given age group when they are greatest, i.e., the first month or two after planting.

No extensive analysis of these data is presented; however, the following tentative conclusions are drawn:

1. Mortality from unknown causes generally increases with age, especially after the third year on the beds.

2. Based on 48,730 oysters checked in the three annual surveys, 88.7 per cent were live at the time evaluated.

3. Willapa Bay has best survival, southern Puget Sound second, Grays Harbor third, and northern Puget Sound poorest.

4. Except in localized instances, none of the known predators appear to have a major influence on the industry.

5. From the annual survey data, on an industry-wide basis, unknown mortalities account for the bulk of the losses followed by mass mortalities, drills and crabs.

MASS MORTALITIES

Pacific oysters, on occasion, have heavy losses for which no causative agent has been found. Nearly every year at least one mass mortality may be encountered and in some years they may be quite common. In terms of overall industry plantings, they do not seem to be serious; however, to the individual grower they are often important. These mortalities usually follow a common pattern. In most cases they are localized to a single bed or planting and may result in over 50 per cent loss in a 2-4 week time period. The loss is nearly always on the yearling (1+) oysters which are in their second growing season after planting, though both seed and older oysters are occasionally affected. They are without exception fast-growing, fat (or spawny) oysters. Usually the areas of loss will be in coves, at the head of a lagoon, or other type of relatively "dead water" area (in terms of new water replacement only - often good localized currents exist with rise and fall of the tide). Heaviest losses are in the summer when water temperatures are in excess of 18° C. Type of bottom, tidal level of planting, presence or absence of pollution, salinity (20-30 o/oo) and number of degrees above 18° C seem to have little relationship to the losses. Occasionally "red tide" is associated with the losses. Losses occur in both diked and open bed plantings. Level of planting

relative to tidal height seems to have no bearing on the occurrence of the mortalities. Fidalgo Bay, Case Inlet, Henderson Inlet, Eld Inlet, Totten Inlet (Gallaghers Cove and Burns Point), Grays Harbor (Damon and Alder Points) and Willapa Bay (Stackpole area) seem to have these losses quite regularly. Neither Dermocystidium nor Hexamita are associated with the mortalities. Limited work by this laboratory has uncovered no potential pathogens. Usually these mortalities are ascribed to either "red tide" or "heat kill." Neither of these suggested causes appear to fit the circumstances associated with the mortalities.

SUMMARY

1. At least two species of crab, one species of starfish, the flatworm and the Japanese oyster drill are predators of the Pacific oysters.

2. Mud shrimp and seaweed, while not predators, will cause Pacific oyster mortalities.

3. Annual oyster mortality rates decrease with age during the first two years after planting.

4. Summer is the period of greatest mortality.

5. In control plot studies, mortalities from unknown causes made up the largest portion of oyster losses.

6. Annual survey data indicates increasing mortality from unknown causes after the third year on the beds.

7. Based on 48,730 oysters checked in the three annual surveys, 88.7 per cent were live at the time evaluated.

8. In comparing annual survey oyster mortality data from the four major oystering areas, Willapa Bay has the lowest mortality followed by southern Puget Sound, Grays Harbor and northern Puget Sound.

9. Except in localized instances none of the known predators appear to have a major influence on the industry.

10. From the annual survey data, in order of magnitude, unknown mortalities account for the bulk of the losses followed by mass mortalities, drills and crabs.

11. Certain conditions surrounding mass mortalities are described.

12. No causative agent for the mass mortalities has been discovered.

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HEXAMITA SP. AND AN INFECTIOUS DISEASE IN THE COMMERCIAL OYSTER OSTREA LURIDA[1]

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ABSTRACT

Recent mortalities (1958-59) of the Olympia oyster, Ostrea lurida, have occurred in southern Puget Sound, Washington. The mortalities were generally associated with cold water conditions. Examination of the tissues revealed the presence of Hexamita sp. and bacteria. Consequently, experiments were set up to ascertain if dead oysters infected with Hexamita and bacteria could transmit an infection of Hexamita or bacteria and cause mortalities. Healthy oysters were therefore exposed to oysters infected with Hexamita and bacteria. For controls, healthy oysters were exposed to autoclaved oyster tissues. Continuously running water was used and the experiments were carried out at two temperature levels, 6 and 12° C. At 6° C, 70 per cent of the healthy oysters exposed to diseased tissues died within 76 days, whereas only 14 per cent of the controls died within the same period of time. All of the dead oysters had moderate to heavy infection of Hexamita. Bacteria were not apparent at light levels of Hexamita infection and were either present or absent at higher levels of infection. Tissue damage was severe. Hexamita was found in survivors, but no bacteria were in evidence. In the warm water experiment, there was no significant mortality difference between experimental and control aquaria.

INTRODUCTION

Hexamita is a flagellated protozoan having two anterior nuclei, six anterior flagella and two posterior flagella (Figure 1).

Members of the order Polymastigina, to which Hexamita belongs, have been found to be parasitic in trout, salmon, turtles, toads, pigeons, and man (Mackin et al. 1952).

In 1950 and 1951, oyster mortalities in Holland, commonly referred to as "pit disease", were associated with the presence of Hexamita, (Mackin, et al. 1952). More recently, the protozoan has been

[1] Contribution No. 51 from the Research Department of Rayonier Incorporated.

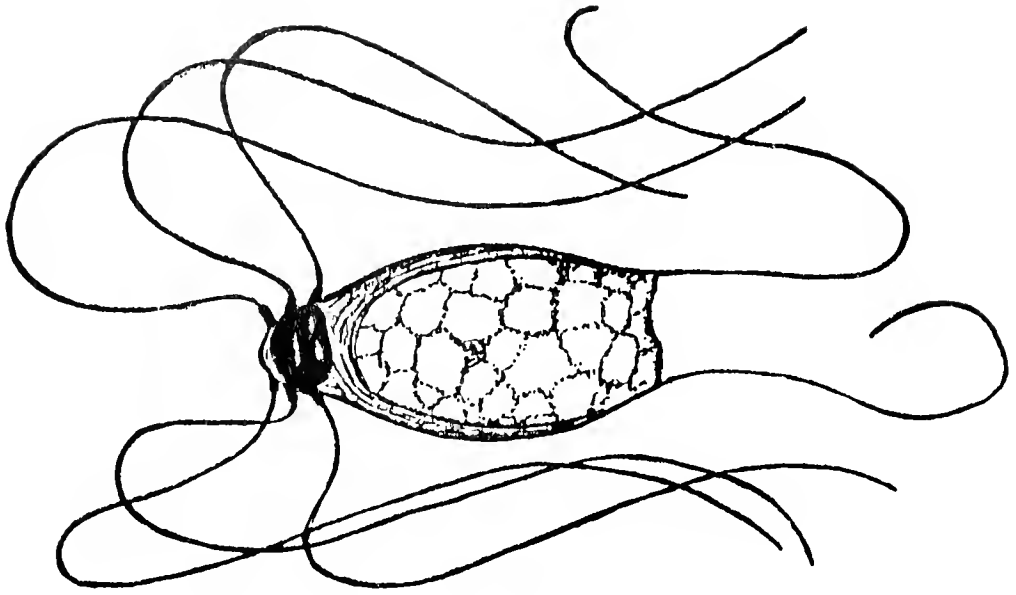

Fig. 1. Semi-diagrammatic sketch of a trophozoite of Hexamita showing flagella and axostyles (Mackin, et al., 1952).

reported present in mortalities of introduced Ostrea edulis in Prince Edward Island, Canada (Medcof 1959). The authors have observed similar mortalities of the small commercial oyster Ostrea lurida in the southern Puget Sound area of the Pacific Northwest. These mortalities occurred in Little Skookum Inlet in April of 1958 and 1959, and Hexamita was found in the tissues of dying oysters.

Cold temperature, poor circulation over the beds, and overcrowding appear to be optimum conditions for an epizootic associated with the presence of Hexamita (Mackin et al. 1952).

From external appearances the oyster generally died fat, but histological examinations revealed protozoan-occluded blood vessels, necrosis of the gastro-intestinal tract, and histolysis of supporting connective tissues. Specimens dying in the field frequently had massive concentrations of Hexamita and bacteria.

Preliminary experiments by the authors (unpublished) involved the insertion of Hexamita-infected oyster tissue in the mantle cavity. The insertions were made by wedging the valves apart approximately 1 to 2 mm. For controls, autoclaved tissues were inserted. No mortalities resulted from the mechanical separation of the valves. After the tissues were inserted, all oysters were held out of water at 3° C for approximately 12 hours; this increased the exposure time by preventing the oyster from immediately ejecting the tissues.

Within 24 days, 78 per cent of the experimental oysters died, while in the control group there was only a 10 per cent mortality. These experiments were carried out in 2½-gallon glass aquaria, each containing 4 liters of standing, aerated sea water. The water temperature varied from 4 to 10° C, with an average for the 24 days of 7° C.

The following experiments were designed to test the effect of Hexamita on healthy oysters under more natural conditions, to explore the role of temperature, and to determine whether or not Hexamita can be transmitted through running water.

METHODS AND PROCEDURES

Materials

1. Standard 15-gallon aquaria were used. Each aquarium had a hole in one end to facilitate a constant level of running water. Throughout the course of these experiments, a 3-inch water head was maintained over the oysters (total volume = 13.8 liters).

2. Running water was supplied by a salt water circulating system which pumped directly from Hood Canal (chlorinity 14-16 parts per thousand).

3. Ostrea lurida (2 to 3 year class) were used after all barnacles, spat, and epiphytic plants had been removed. These oysters were obtained from Little Skookum Inlet on January 22, 1959 and kept in Hood Canal until the start of the experiment.

4. Proximity tissues, i.e., tissues heavily infected with Hexamita and bacteria, were obtained by the simple expedient of exposing O. lurida to overcrowding in non-circulating 3° C water. Adequate levels of infection were thus obtained in 30 to 40 days.

5. Control proximity tissues were obtained by autoclaving healthy O. lurida for 15 minutes at 120° C and 15 lbs pressure.

Experimental Design

In order to test the possibility of Hexamita and bacteria transmission through water, proximity experiments were designed in the following fashion:

A. Cold Water Experiment:

1. All test oysters (O. lurida) were held in running water aquaria for a one-week period of observation. Each aquarium contained approximately 100 oysters. In this manner, any weakened oysters would probably be eliminated before the start of the experiment.

2. Ten oysters heavily infected with Hexamita and bacteria were selected for a source of infecting elements in the experimental aquarium. Hereafter, these oysters will be referred to as proximity-oysters. The right valve of each proximity-oyster was removed and the tissue held in place with rubber bands. This was done to assure a more effective circulation of water over the tissues of the proximity-oysters, and to prevent them from floating away from the shell and possibly blocking the aquaria outlets.

3. In the control aquarium, ten autoclaved oysters were used for proximity-oysters. In each case, one valve was removed as described above.

4. In the experimental and control aquaria, the oysters were arranged so that five healthy oysters surrounded each proximity-oyster. In this manner, 50 oysters were located in each aquarium.

5. The aquaria were set up in a cold room and aeration was provided for approximately 6 hours until the aquaria water temperature equaled that of the cold running water system (6.0° C). At that time water circulation was started in each aquarium. Oysters received an average of 5.04 liters per day at the start of the experiment. However, as the dead oysters were removed, the remaining oysters received proportionately increasing amounts of water, e.g., on the 76th day, the remaining 15 experimental survivors were receiving 16.8 liters per day.

6. Since this experiment was started in July, it was necessary to design a system that would lower the incoming Hood Canal water to approximately 6° C. Toward this end, six 50-gallon barrels were placed in a cold room. These barrels were connected by a series of siphons such that water flowing into one end of this system would be progressively cooled to 6° C. It was found that the maximum rate of flow could not exceed 21 liters per hour. Accordingly, two lines were metered to provide a flow of 10.5 liters per hour through the experimental and control aquaria, (Figure 2).

7. Both experimental and control proximity-oysters were periodically renewed to prevent fouling of the aquaria.

8. Each aquarium was examined once every 24 hours, at which time gapers were removed.

9. When a gaping oyster was removed, the tissues were immediately examined and the levels of Hexamita trophozoites were determined. These slides were prepared by lightly smearing the entire oyster, gills down, on a slide and approximating the level of Hexamita infection. When using a wide field 12.5X eyepiece and 10X objective, the following criteria were employed:

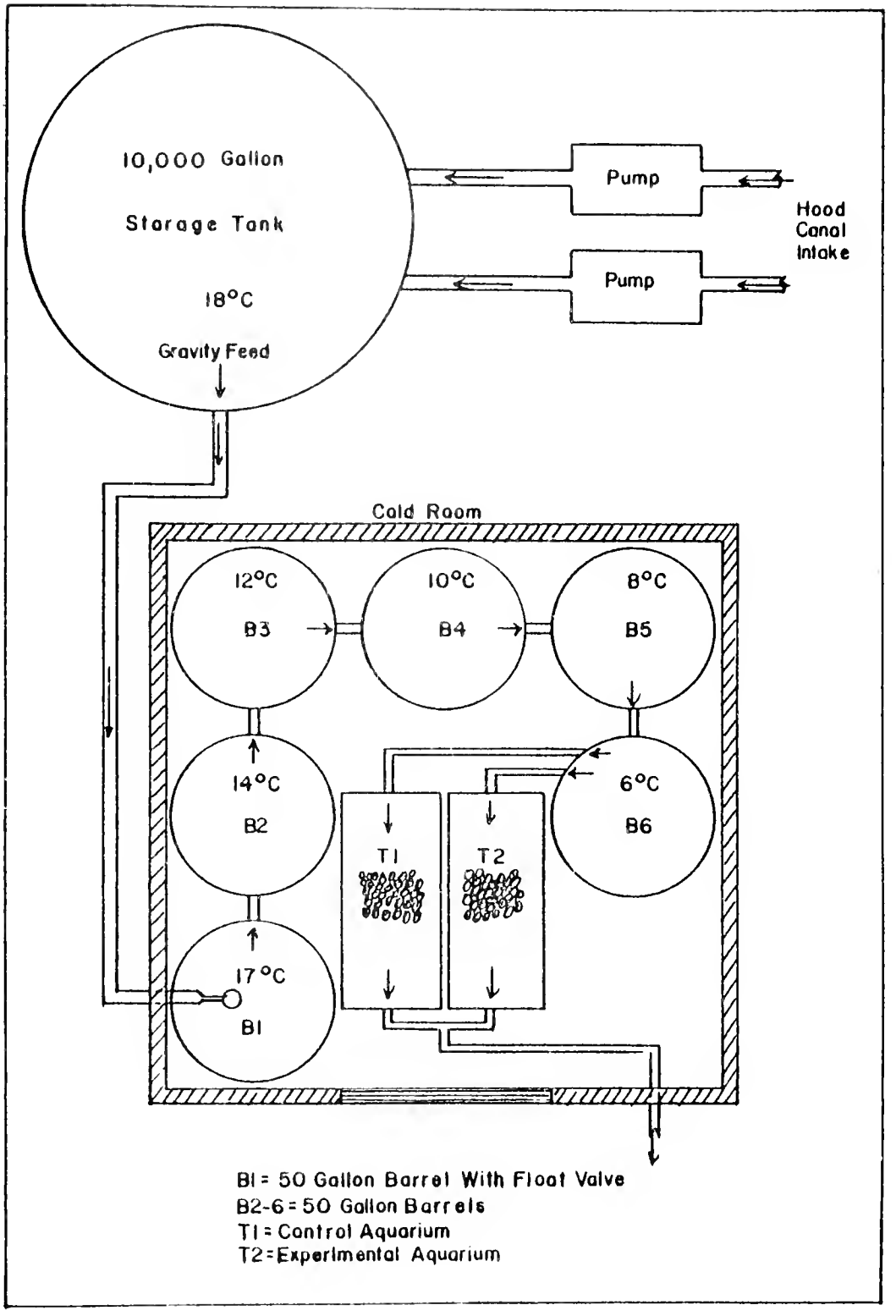

Fig. 2. Schematic diagram illustrating the experimental set-up of the cold water experiment.

Levels of trophozoite infection		Number of trophozoites per microscopic field
Heavy	=	over 100
Moderate	=	50 to 100
Light	=	10 to 50
Very Light	=	1 to 10
None	=	None

The above criteria apply only to freshly prepared, wet slides.

10. After each oyster was examined, sections were made for histological study. Giemsa, Heidenhain's iron-hematoxylin, and periodic acid Schiff (PAS) stains were used. For the PAS stain, tissues were predigested with diastase and, following the Schiff reaction, were counterstained with hematoxylin (Lillie 1954).

11. At the termination of this experiment, all remaining survivors were examined in the manner described above.

B. Warm Water Experiment:

1. On March 26, 1958, a similar experiment was conducted in which the running water temperature ranged from 8° C in March, to 17° C in June with an average of 12° C for the 76-day period. In this study, running water was provided directly from a 10,000-gallon storage tank without preliminary cooling. The rate of flow per oyster averaged 7.2 liters per day. In all other respects this experiment was the same as the cold water investigation.

RESULTS AND DISCUSSION

General Discussion

In both the cold and warm water proximity experiments the same source of infected tissues was used in the experimental aquaria. In the control aquaria, autoclaved tissues were used. The frequent replacement of proximity-oysters for both control and experimental aquaria increased the probability of having higher levels of Hexamita and bacteria in the experimental aquaria as compared to the controls.

At the end of the cold-water experiment there was a decided difference between the mortality rates occurring in the control and

experimental tanks. The mortality for the control aquaria was 14 per cent over a period of 76 days and for the experimental tank it was 70 per cent for the same period of time. No difference in rate of mortality was found for the control and experimental aquaria of the warm water experiment. In the 76-day period of the warm-water study, both the control and experimental tank had a 6 per cent mortality. These data indicate that in the presence of Hexamita and bacteria there is an interaction between temperature and mortality.

Examination of tissues from Giemsa-stained sections revealed variable levels of bacteria in dead and surviving oyster tissues. Although no counts were made, the level of bacteria in the surviving oysters from both the experimental and control aquaria was similar to that observed in tissues of healthy oysters removed from the field. In most cases extensive searching was required to find evidence of bacteria in the tissues of surviving oysters - even when moderate levels of Hexamita, as determined by wet slide preparation, were found. In the mortalities of the experimental aquarium, 36 per cent of the oysters having moderate to massive levels of Hexamita also had very evident bacterial infections. The remaining 64 per cent had no apparent bacterial infections, nor were bacteria obvious in the tissues of oysters having light to very light infections of Hexamita. Hexamita was consistently present in the experimental and control mortalities, while bacteria were frequently absent even at elevated levels of the protozoan infection. Furthermore, no developing bacterial levels were observed in the survivors, thereby depreciating the role of bacteria as a factor in the cause of death. The consistent presence of Hexamita and the frequent absence of bacteria in freshly dead oysters points to Hexamita as a prime suspect for the cause of death.

Figure 3 shows the level of infection of each oyster at the time of death in the cold-water study and the survivor levels of infection at the termination of the experiment. A pooled Chi-square analysis (Cochran and Cox 1957) was carried out to determine if the levels of Hexamita infection in the survivors of the experimental and control aquaria were significantly different. A Chi-square value of 9.35 was obtained and found to be significant on the .05 probability level.

In order to locate the cause of this significant difference, the respective levels of infection for the control and experimental aquaria were compared separately, e.g., moderate level of Hexamita infection of control survivors against moderate levels of infection for experimental survivors. None of the separate tests were found to be significant. As a consequence, a Chi-square test of significance for linear regression was carried out (Cochran and Cox 1957). The linear regression Chi-square was found to be 8.60, which is very significant for the one degree of freedom associated with regression. Therefore, the meaning of the significant "pooled" Chi-square is that the level of Hexamita infection was building up at a greater rate in the experimental than in the control aquarium.

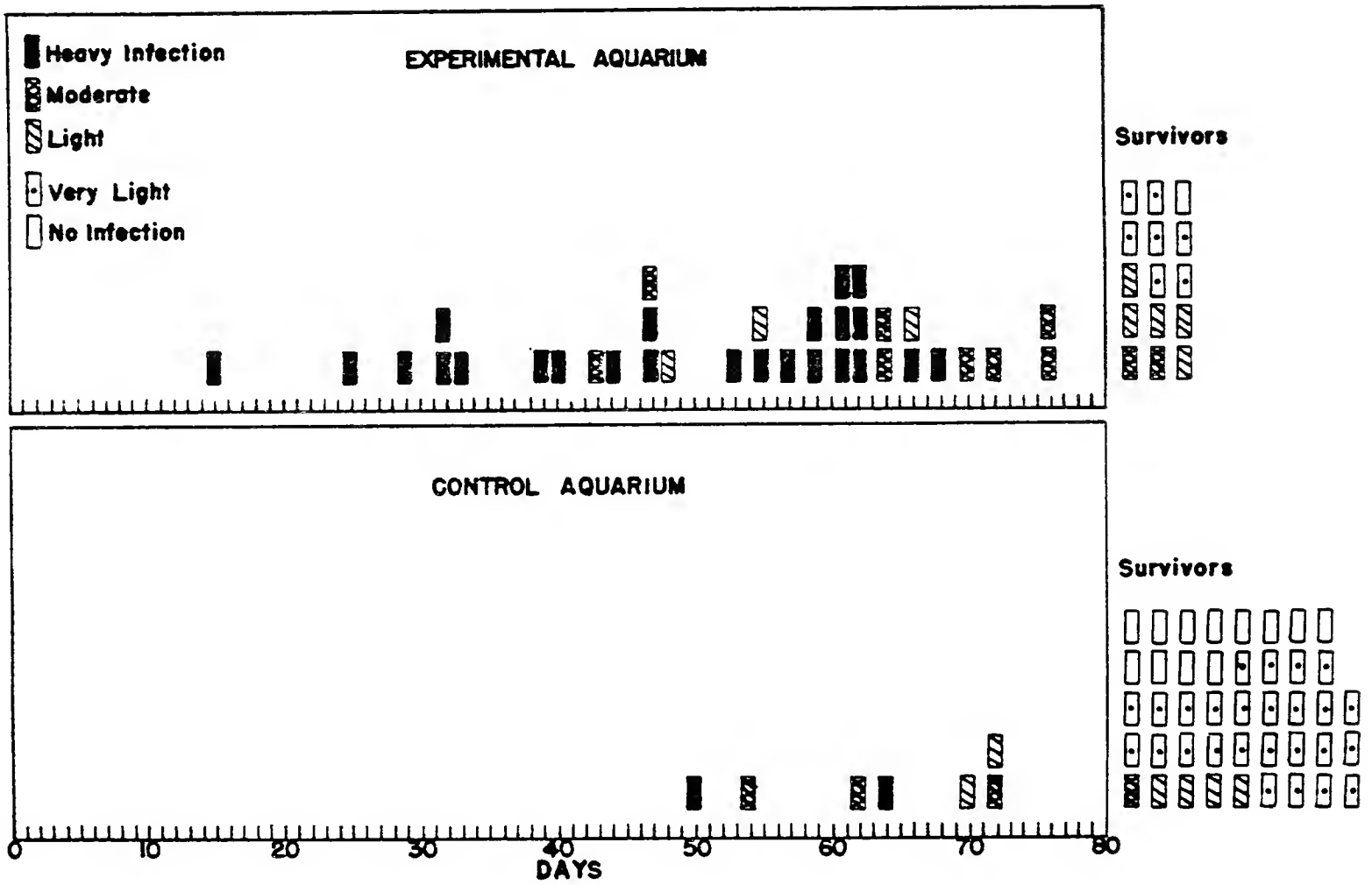

Fig. 3. Day-by-day mortality of experimental and control oysters and levels of infection for dead and surviving oysters.

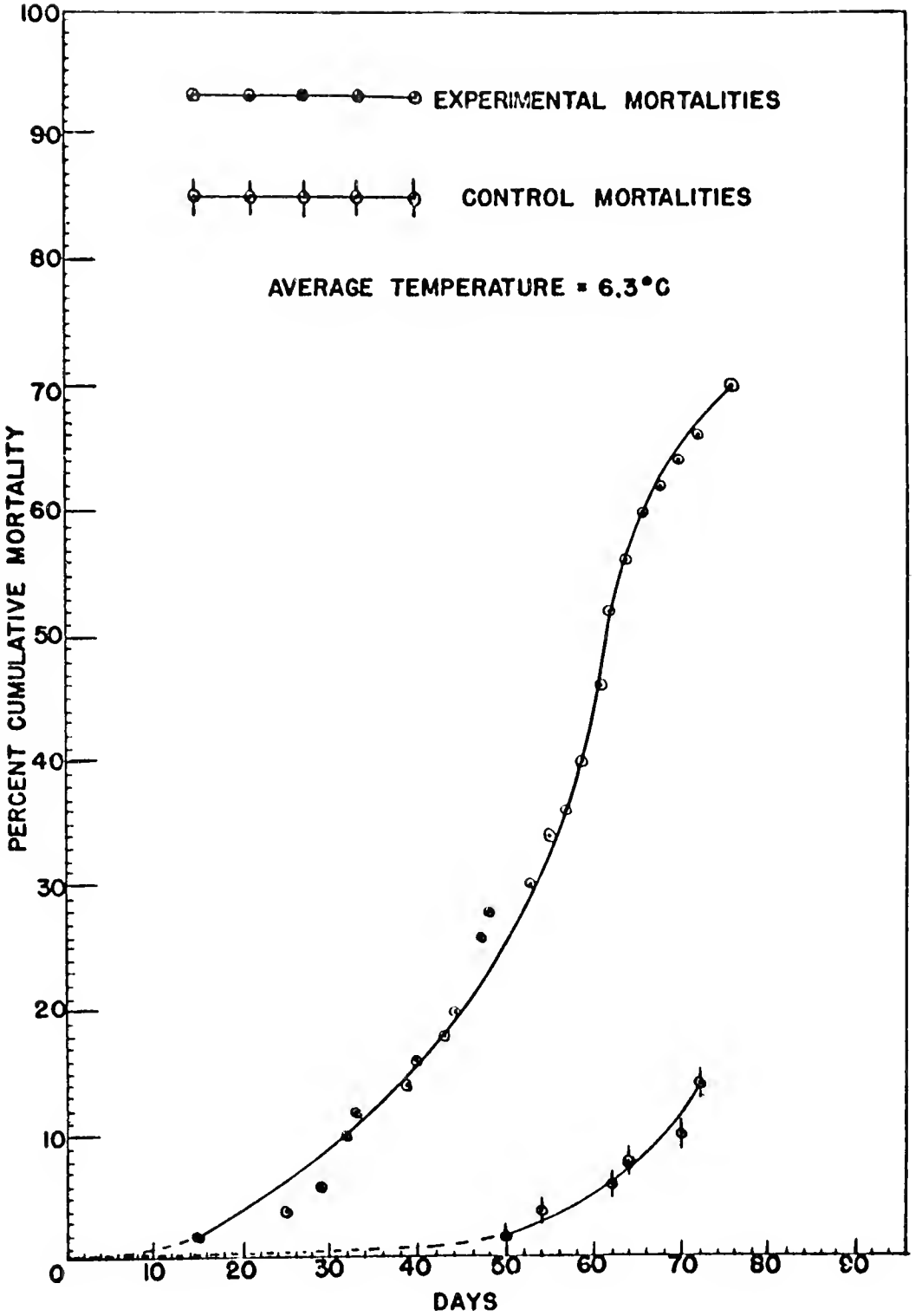

Fig. 4. Compares per cent cumulative mortalities of the experimental and control oysters.

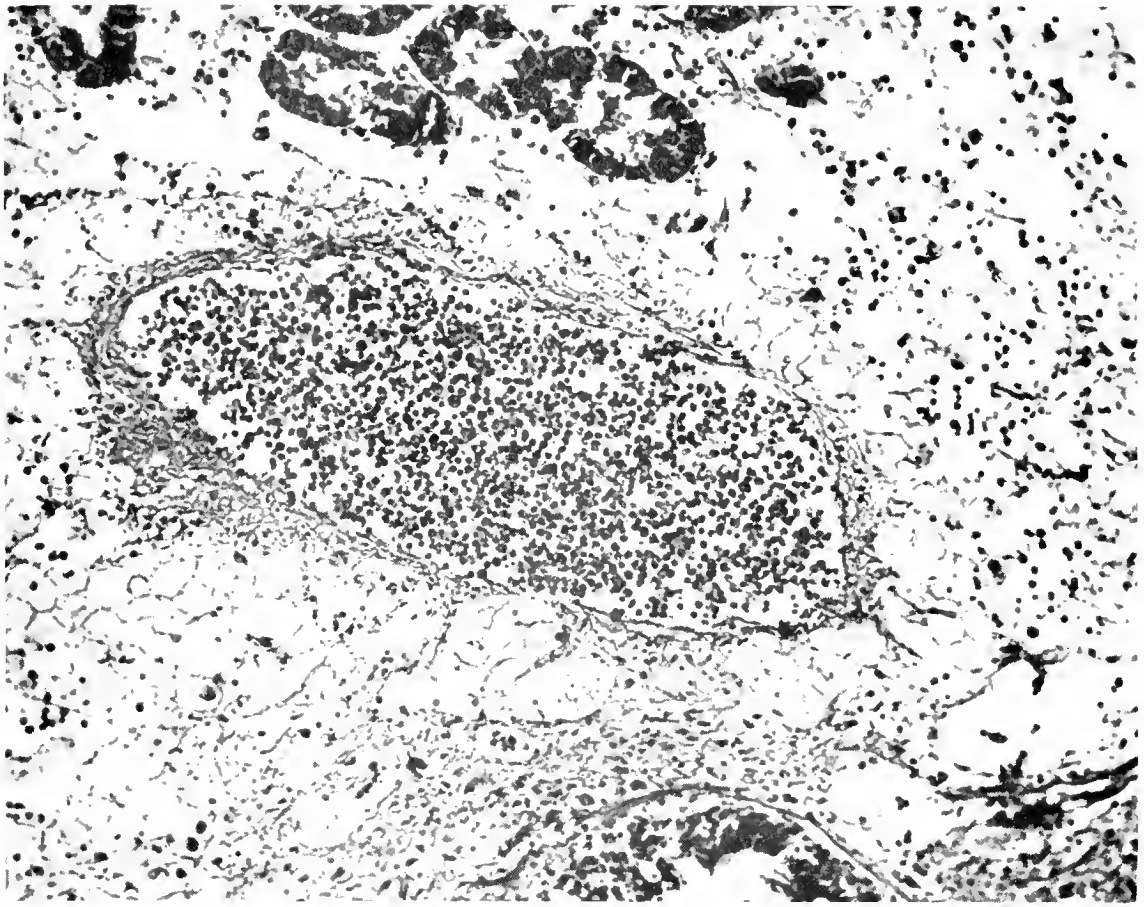

Fig. 5. Occlusion and inflammation of a blood vessel. This photograph shows the results of vascular involvement by Hexamita. (Approximately 100X)

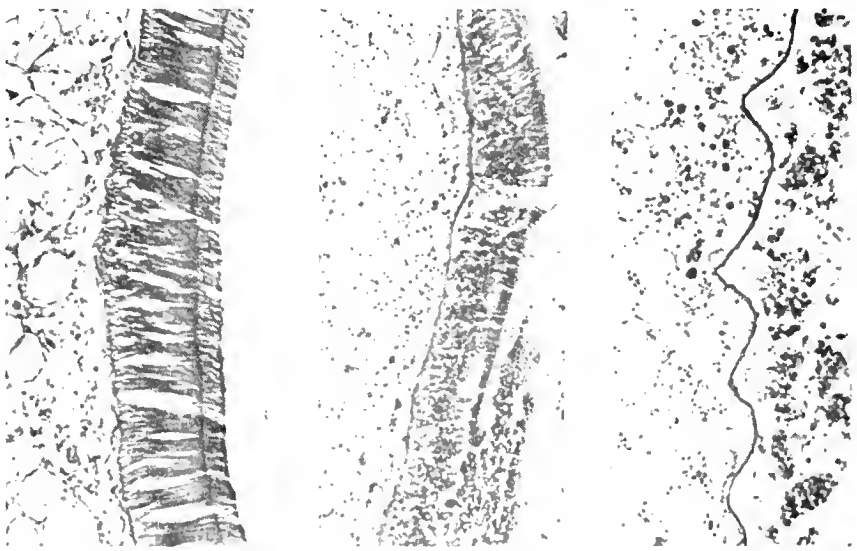

Fig. 6. Destruction of intestinal mucosa. The photograph on the left shows the normal intestinal mucosa. The middle and right-hand photographs represent varying degrees of destruction seen in oysters having moderate to heavy infections of Hexamita. (Approximately 100X)

Table 1. X^2 for pooled date of survivor level of infection in cold running water proximity experiment.

	Survivor Levels of Infection				
	None	Very Light	Light	Moderate	Total
Experimental	1	7	5	2	15
Control	12	26	4	1	43
Total	13	33	9	3	58

$X^2 = 9.3512$ A significant difference at the 0.05 probability level. (3 d.f.)

The occurrence of Hexamita in dead and surviving oysters of the cold-water control aquaria indicates that the protozoan is endemic to southern Puget Sound. Had the experiment continued for another 70 days it is conceivable, under the conditions of this experiment, that a substantial number of the control oysters would have died due to hexamitiasis (Figure 4).

Although there was no difference in the number of dead oysters in the control and experimental aquaria of the warm-water experiment, the few that died (6 per cent) were heavily infected with Hexamita. This indicates that the protozoan may well be a saprozoite at warmer temperatures and takes on the role of a parasite in colder temperatures. This may be due to the decreased pumping rate and lowered metabolism of O. lurida when exposed to water temperatures at or around 6° C. It could be speculated that decreased pumping and metabolic activity permits the accumulation of large Hexamita populations which, directly or indirectly, cause extensive tissue damage and death.

Histopathology

Whenever moderate to heavy levels of Hexamita were observed in wet slide preparations, stained slides prepared from these tissues revealed one or more of the following pathological aspects:

1. Blood vessels--frequently the involvement was characterized by inflammation of the vascular epithelium and at times trophozoites were numerous enough to occlude the vessel (Figure 5).

2. Gastro-intestinal tract--necrosis of the intestinal mucosa was frequently observed when trophozoites were common in the intestinal tract (Figure 6).

3. Leydig cell connective tissue--in the presence of trophozoites, lysis and/or disarticulation of the connective tissue was often

evident (Figure 7).

4. Gill branchia--gill tissues frequently manifested various stages of decomposition when trophozoites were common in this tissue. Moreover, wet slide preparations indicated that the gills appeared to be the favorite site of trophozoites.

Blood vessels occluded by hypertrophic leucocytes were also encountered in many of the stained slides prepared from the cold-water experimental mortalities. These leucocytes frequently contained an intracellular parasite thought to be a reproductive stage of Hexamita (Mackin et al. 1952). These intracellular forms appeared in 20 per cent of the experimental mortalities, while few were observed in either the experimental or control survivors of the cold-water investigation. Figure 8 is a photomicrograph of the parasitized, hypertrophic leucocytes described above. While it has been assumed that parasites found in leucocytes and free in blood vessels are Hexamita, it has not been demonstrated that that interpretation is the correct one and these parasites may represent an unrelated infectious agent.

1. A lethal disease can be transmitted from Hexamita infected tissues to healthy oysters.

2. Bacteria were depreciated as a causative factor in the death of oysters infected with Hexamita. The "intracellular stages" of Hexamita may be an associated but independent parasite.

3. The accelerated mortality rate of the cold-water experimental aquarium (6° C) and the absence of acceleration at warmer temperatures (12° C) suggest that there is an interaction between oyster mortality and parasitization at the colder temperature.

4. There was no difference between the mortality rates of the experimental and control aquaria of the warm-water experiment; however, the few oysters which did die had heavy levels of the protozoan and bacteria. This indicates that under these temperature conditions, Hexamita may act as a saprozoite.

5. The accelerated mortality rate in the cold water experiment suggest that under these conditions Hexamita and the associated intracellular stages act as parasites. This may be due to the decreased metabolic activity of O. lurida at low-water temperatures.

6. Severe tissue damage accompanies infections.

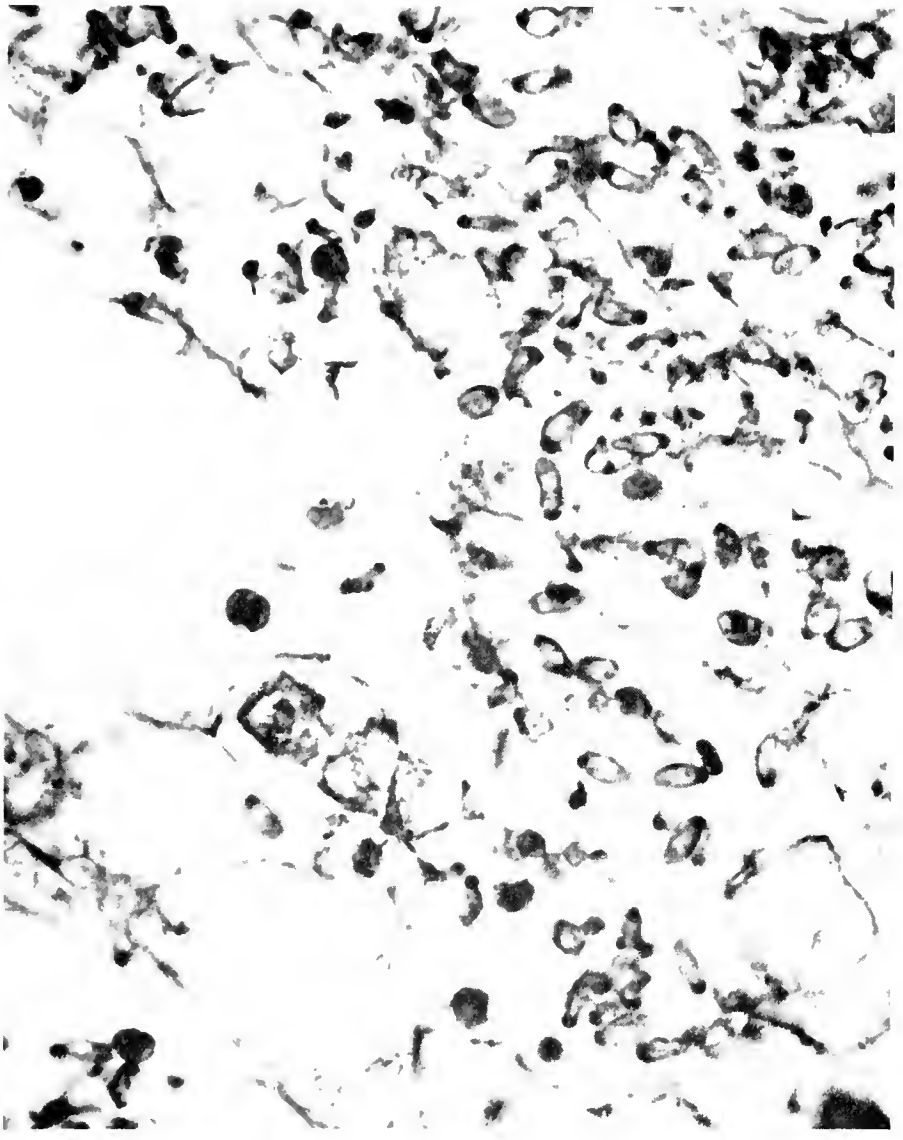

Fig. 7. Trophozoites in Leydig cell connective tissue. Note the histolysis and disarticulation of the connective tissue cells. (Approximately 450X)

Fig. 8. Intracellular parasites found in leucocytes associated with Hexamita infected tissues. (Approximately 2500X)

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EFFECTS OF THE FLATWORM STYLOCHUS ELLIPTICUS (GIRARD) ON OYSTER SPAT IN TWO SALT WATER PONDS IN MASSACHUSETTS

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ABSTRACT

During the summer of 1957 the larvae and juveniles of the flatworm Stylochus ellipticus occurred in great abundance in two salt water ponds on Martha's Vineyard Island. Setting rates of flatworms were as high as one per shell per day. Data over a period of three years indicate that such larvae and post-larvae occurred in these ponds only when salinities were below 20°/oo. Mortality of newly set oysters due to depredation by these flatworms was severe, approaching 100% in some samples. Dipping infected oyster spat in a concentrated salt solution proved an effective method of ridding the set of flatworms, but this is an effective remedy only if the treated spat are protected from reinfestation. Because of the widespread distribution of S. ellipticus in many oyster-growing regions in New England and elsewhere the need for a more effective control of its predation on oyster spat is obvious.

The U. S. Fish and Wildlife Service Marine Biological Laboratory at Milford, Connecticut, in cooperation with the Oyster Institute of North America, is engaged in a study designed to develop the principles of shellfish culture in salt water ponds. Two of the ponds under study, West Tisbury Great Pond and Oyster Pond on Martha's Vineyard Island, Massachusetts, are periodically open to the sea and contain populations of soft clams and oysters of economic importance. These ponds are well suited for oyster seed production since they are remarkably free of such oyster enemies as drills, starfish and sponges. However, observations during 3 years of study indicate that the flatworm Stylochus ellipticus is an important predator on oyster spat in these ponds and that the future utilization of such ponds for production of seed oysters may depend on effective control of this pest.

The potential of some polyclad flatworms as oyster enemies has long been known and certain aspects of the biology and predatory nature of related species have been published by Pearse and Wharton (1938), Dawson (1953), and Woelke (1957). Hopkins (1955) ascribed some oyster mortality to Stylochus and Menzel and Hopkins (1956) included Stylochus ellipticus in their list of predators on Louisiana oysters. Loosanoff

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Table 1. Occurrence of larval and post-larval flatworms in Tisbury Pond, 1957.

Date	In Plankton	Salinity o/oo	On biweekly cultch samples #/20 shell faces
20 June	no tow	18	53
21	a few	18	no sample
24	a few	17	24
26	present	17	57
28	present	14-18	no sample
1 July	present	16-17	22
3	present	16	no sample
4	no tow	16.5	4
5	present	16.5	no sample
8	many	15.5-16.5	18
10	present	13	12
12	present	16.5	no sample
15	present	16.5-17.5	6
17	present	15.5-16.5	2
18 Tisbury Pond Opened			
19	present	10.5-13.5	no sample
22	no tow	11.0-11.5	22
25 July - 18 August	absent	21-28	absent
18 August Pond closed		24	
30 September	1 indiv.	15	

(1956) was the first to publish an account of predation on oyster spat by Stylochus ellipticus in the laboratory.

The present paper is a record of observations on the numbers of Stylochus and their predation on oyster set, during 1956, 1957, and 1958 in the two ponds on Martha's Vineyard. During the summer of 1956 very few S. ellipticus, either adults or recently set individuals, were seen. Again in 1958 the population was very sparse. In 1957, however, large numbers of larvae, recently set individuals and adults were observed.

The first indication that this was to be an unusually large year class was the discovery on June 20, 1957 of large numbers of what appeared to be recently set flatworms on Tisbury cultch examined for oyster spat. Continued observations on the development of these animals confirmed their identity. Plankton samples were being taken three times a week with a #20 silk net. On June 21 large numbers of what were assumed to be Stylochus larvae or post-larval stages were noted in the plankton of Tisbury Pond. These forms appeared as miniature flatworms, oval in shape, and measured about 82 x 71 micra. During the first week of July such numbers occurred that they constituted a major component of the zooplankton. Flatworm larvae were also found in the plankton of Oyster Pond, but in smaller numbers. In Tisbury Pond, flatworm set were found in considerable numbers until July 18 when this pond was opened to the sea. Some flatworm larvae were found in Tisbury plankton samples until July 22 but only occasional specimens were found on the biweekly set bags after the pond was opened.

Data for three stations in Tisbury Pond were similar with respect to dates of occurrence of the larvae and approximate numbers of flatworm set per shell. Values for one of the sampling stations are presented in Table 1. The disappearance of flatworm larvae and cessation of flatworm setting just after the pond opening was striking. While it is possible that this would have occurred regardless of whether or not the pond was opened, it is more likely that the disappearance was due to large numbers of larvae being swept out of the pond at the initial opening, for at least one quarter of the pond water was estimated to have escaped within 24 hours of the opening, and additional losses of plankton and pond water occurred with each tidal cycle. Oyster Pond, which was closed most of the summer, continued to show a few flatworms in the plankton but none were found on cultch until July 29 when pond salinity had dropped to 20 o/oo. A few flatworms continued to set during August as salinity continued to fall.

Whether the rapid change in salinity which followed the opening of Tisbury Pond was detrimental to the young flatworms can only be conjectured at this point. Available data from 3 years' observation indicate that planktonic and recently set flatworms occurred in these ponds only when salinities were below 20 o/oo. Menzel and Hopkins (1954) found adult Stylochus ellipticus in abundance only in waters of "relatively low salinity." In several cases in the literature other

species of flatworms were found associated with high rather than low salinities (Dawson 1953, Pearse and Wharton 1938). Apparently each species has its own requirements which need not be similar to those of other members of the same or a related genus. Some adaptability to salinity is to be expected. Woelke (1957) showed that despite a preference for 28 o/oo by Pseudostylochus ostreophagus, that species could tolerate 10 o/oo for as long as a week and even lower salinities for a shorter time.

Predation by Stylochus on oyster set became apparent when on August 7 some cultch bags from Tisbury Pond were examined and in half an hour several hundred flatworms measuring form 1/4 to 1/2 inch in length were found. In some cases as many as 15 worms were found on the two sides of a single shell. Dead oyster spat were common, but at this time there were still some live spat. A number of times flatworms were observed entering live oyster spat up to 1/2 inch in greatest diameter. When the top valves were lifted from recently dead spat, 1-3 flatworms usually were found, occasionally one of them in the act of eating the young oyster. In one instance a small recently set flatworm was observed closely appressed to a two-day-old oyster. The flatworm was slightly smaller than the spat.

To estimate mortality due to flatworms and other causes, samples of broadcast cultch were secured on August 13. These samples yielded 590 dead spat, 33 live spat and 8 flatworms. Only those spat were counted which had grown large enough to leave a scar or be seen with the unaided eye. This meant that a mortality of at least 95% had occurred since setting. There are no drills, and no starfish in either pond. There was no evidence of crab damage and no reason to suspect epidemic disease. Silting was responsible for some of the mortality, for portions of the cultch with spat scars showed blackening from a mud coating over the dead spat. In contrast to the mortality on broadcast shell, a sample of bagged cultch showed 303 dead spat, 179 live spat and 9 flatworms, or a total post-setting mortality of at least 50%. In this case there was virtually no evidence of silting and nearly all mortality could be ascribed to flatworm predation alone.

In Oyster Pond a sample of spat examined in September 1957 and again in October, showed a mortality of 62% for a six-week period. These spat were bagged and had been washed by wave action, so silting was not a factor and there was no evidence whatsoever to indicate causes for mortality except for the presence and behavior of flatworms among the living and dead spat. Survivors had grown well in the interval between examinations.

It is obvious from the above that an outbreak of the nature described would be very serious in a pond culture operation. In an effort to find a method for field control in the summer of 1957, the Milford laboratory began a series of experiments immediately. One suggested method which had been tried experimentally in earlier years

was the use of concentrated salt solution as a dip for infested seed. The method was field tested by immersing bags of cultch with spat into pond water saturated with rock salt. Flatworms curled and after one minute immersion, none recovered. The treatment also killed algae, polychaetes and other organisms associated with the cultch. Eels and toadfish trapped in the bags reacted violently and soon died. After 15-30 minutes draining following a one-minute dip, the bags were divided into two lots. Some bags were placed in a floating raft to keep them off the worm-infested bottom. Although there were no longer large numbers of flatworm larvae in the plankton, a few Stylochus were found on the suspended bags several weeks later. These may have resulted from a few pelagic larvae remaining in the water and setting late, from a few flatworms surviving the dip treatment inside spat boxes or may have been carried to the suspended cultch by water currents. Mortality on the treated bags was arrested but bags replaced on the bottom became reinfested and again began to suffer mortality.

On December 11, 1957 a control sample of untreated bagged cultch was taken from Tisbury Pond. Shells which in August had shown 50% post-setting mortality now yielded 600 scars, 7 live spat and 7 flatworms, a total mortality of at least 99%.

The treatment with salt was effective in killing the flatworms but to be commercially practical in these ponds a method of handling the cultch and seed oysters will have to be developed and methods of preventing reinfestation found.

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FLATWORM DISTRIBUTION AND ASSOCIATED OYSTER MORTALITY IN CHESAPEAKE BAY

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ABSTRACT

The marine flatworm Stylochus ellipticus (Girard) is reported from 73 widely scattered localities in Maryland Chesapeake Bay and its tributaries. It is the only polyclad turbellarian so far identified in dredgings from oyster beds within that area. Occasionally found in fresh spat boxes, the worm is considered to be a predator of oyster spat in the Bay. Evidence for worm predation on small oyster spat is presented. Of the 567 worms found in 90 seed-oyster samples, about 95 per cent were recovered from fresh spat boxes. Statistical treatment of the data concerning worm incidence and recent spat mortality indicated the probability that such mortality resulted from predation by S. ellipticus.

INTRODUCTION

Although the marine flatworm, Stylochus ellipticus (Girard), has been known to occur in Chesapeake Bay for some time, little attention has been given to its potential as a predator of oyster spat. In view of this, a systematic collection of this species was started in 1958 to determine its distribution and possible association with oyster spat mortalities.

DESCRIPTION OF THE WORM

The largest specimens of S. ellipticus collected in Maryland Chesapeake Bay measure about 25 mm. The worm is oval, leaf-like, flat and thin with undulating margins. The most common color is brown, but dark pink, orange and olive drab worms have also been observed. The ventral surface is usually lighter than the dorsal. There is commonly a median dorsal stripe of lighter color athwart which are paired tentacles near the anterior end. There are numerous ocelli (eye spots) around the anterior margin and on the tentacles. The mouth, with its short pharynx, is situated about 1/3 back from the anterior end of the body on the ventral side. The pharynx may be everted to a considerable distance. The intestine has many branches and terminates in a fine network along the edges of the worm. Male and female genital pores, situated close together near the ventral posterior margin, pro-

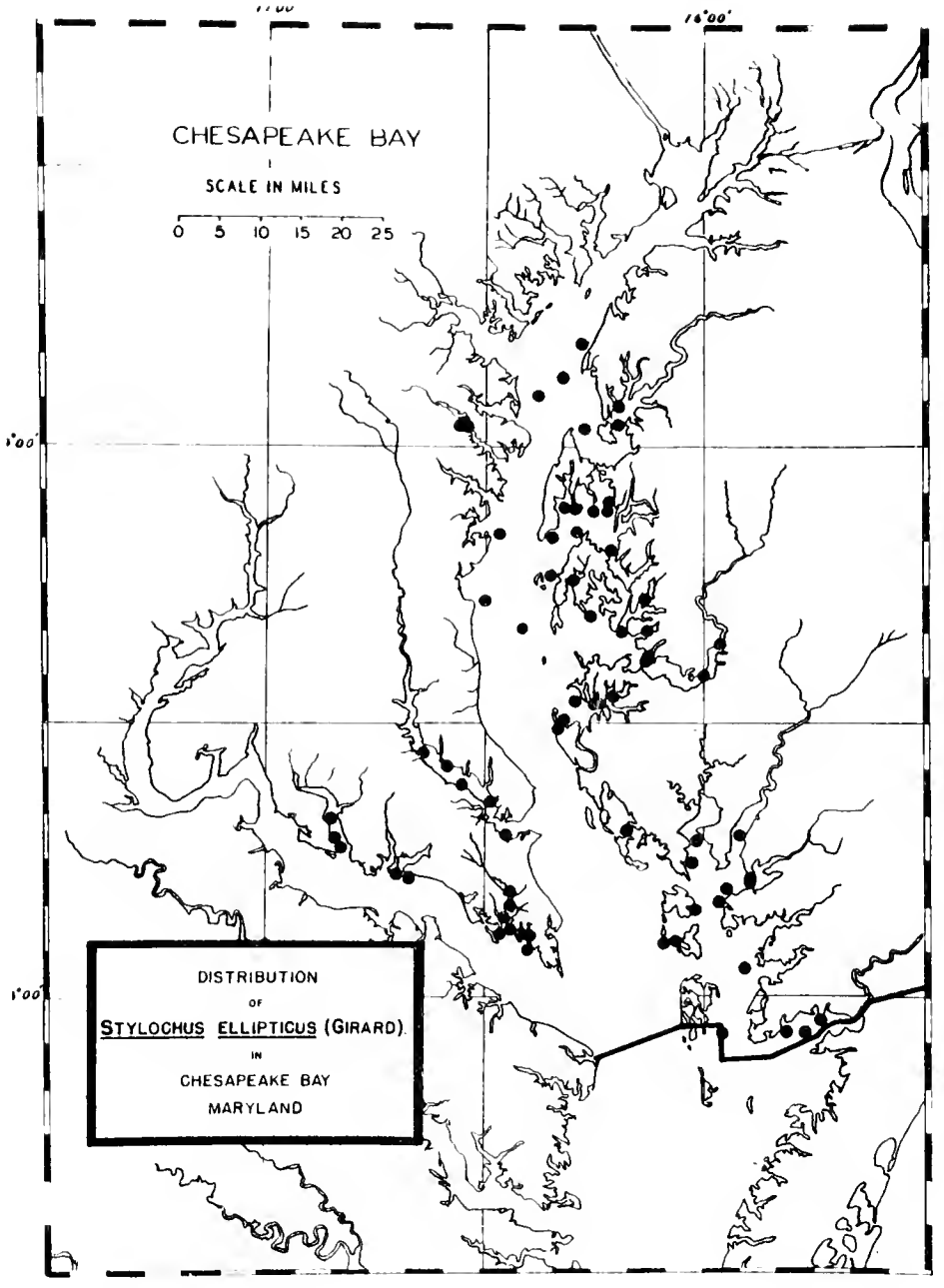
CHESAPEAKE BAY
SCALE IN MILES
0 5 10 15 20 25
DISTRIBUTION
OF
STYLOCHUS ELLIPTICUS (GIRARD)
IN
CHESAPEAKE BAY
MARYLAND

Fig. 1.

vide for sexual reproduction. Hyman (1940) gives a detailed taxonomic description of this species.

DISTRIBUTION OF STYLOCHUS ELLIPTICUS IN MARYLAND CHESAPEAKE BAY

The geographical range of this species, as listed by Hyman (1940), is from Prince Edward Island to Texas. Pearse (1938) records the presence of S. ellipticus from a number of localities in Chesapeake Bay and Engle (unpublished field data) reported this species fairly abundant on oyster beds in the Bay as early as 1944.

Our present collection was started in 1958. Most specimens were found in samples of bottom material dredged during spring oyster-population surveys. These surveys included dredgings on some of the public oyster beds in Maryland Chesapeake Bay and most of its tributaries. The actual finding of worms in these dredgings was incidental to counts bearing upon the age composition of oyster populations. Thus, while the collection data are not quantitative, the survey records show that flatworms were present at 73 widely scattered localities in the Maryland Chesapeake Bay (Figure 1).

The total number of worms collected on the baywide surveys since 1958, about 150, is small in comparison with their broad distribution. Within limits of our collection methods, however, Stylochus has been found in greatest numbers on oyster beds in the lower Potomac River and its immediate tributaries. Hyman (1940) reports that the habitat of this species is littoral and that it is generally found among oysters and old shells, barnacles, and under rocks. The data presented in this paper relate only to flatworm distribution on oyster beds in the Bay.

EVIDENCE OF PREDATION

Sometimes during oyster-bed surveys flatworms were found in fresh oyster spat boxes (hinged valves with clean inner faces but no meat). Pearse and Littler (1938) and Hyman (1940) consider S. ellipticus to be an oyster predator. Pearse and Wharton (1938) demonstrated that a related species, Stylochus frontalis (=inimicus), is an active oyster enemy in Florida. Loosanoff (1956) has shown in laboratory experiments that S. ellipticus feeds on oyster spat. In one experiment, 10 worms consumed 21 spat in less than one month. Although other members of the genus Stylochus may be present in Chesapeake Bay, S. ellipticus appears to be the dominant species of polyclad turbellarian common to oyster beds in the Maryland part of the Bay. It was the only species present in our collections as identified by Dr. Libbie H. Hyman of the American Museum of Natural History. Thus, while attacks by flatworms against healthy oysters were not specifically observed, our finding of worms in fresh spat boxes certainly suggests that predation of

oyster spat by S. ellipticus does occur in Chesapeake Bay.

The contention that predation of oyster spat by flatworms may be common in the field is supported by the relationship of worm distribution and spat mortality. The data were obtained in conjunction with an oyster-seed production experiment conducted in 1958 at Smith Creek, a tidal estuary of the Potomac River.

Wire bags, each containing one-quarter bushel of oyster shells, were suspended in vertical strings at 10-foot intervals along a 240-foot transect running outward from the shore across grounds planted with shell. Depths ranged from 3 feet at the inshore end to about 8 1/2 feet at the offshore end of the transect. Most strings accommodated 4 bags arranged at various depths between surface and bottom. The entire transect was represented by 90 bags.

After removal of bags in the fall, they were examined and counts were made of living spat (a measure of survival after setting) and spat boxes (an approximate measure of recent spat mortality). A search was made for flatworms on shells, in spat and in all spat boxes. Some bags were free of worms; in others the count ranged as high as 19. In all, 567 worms were found for an average of about 6 per bag. Nearly 95 per cent of the worms were found in fresh spat boxes, the remainder occurring elsewhere on culch shells or in the debris on the examining table. In most cases, worms were not present in spat boxes whose inner faces were covered with fouling growth or silt.

RELATIONSHIP BETWEEN SPAT MORTALITY AND WORM INCIDENCE

Spat-box counts were limited to material from 26 bags distributed throughout the transect. These counts showed a positive correlation between the percentage of boxes in the total of spat and boxes together and the numbers of worms per bag. The coefficient of this correlation was + 0.58. Linear regression variance was summarized as follows:

	DF	SS	MS	F	1% Point
Explained by regression	1	1544	1544	12.1	7.82
Unexplained deviations	24	3056	127		
Total	25	4600			

Apparently the relationship between spat mortality and worm incidence was not a consequence of chance alone. A scatter diagram of the correlation points, with the line of regression determined by the method of least squares, is shown in Figure 2.

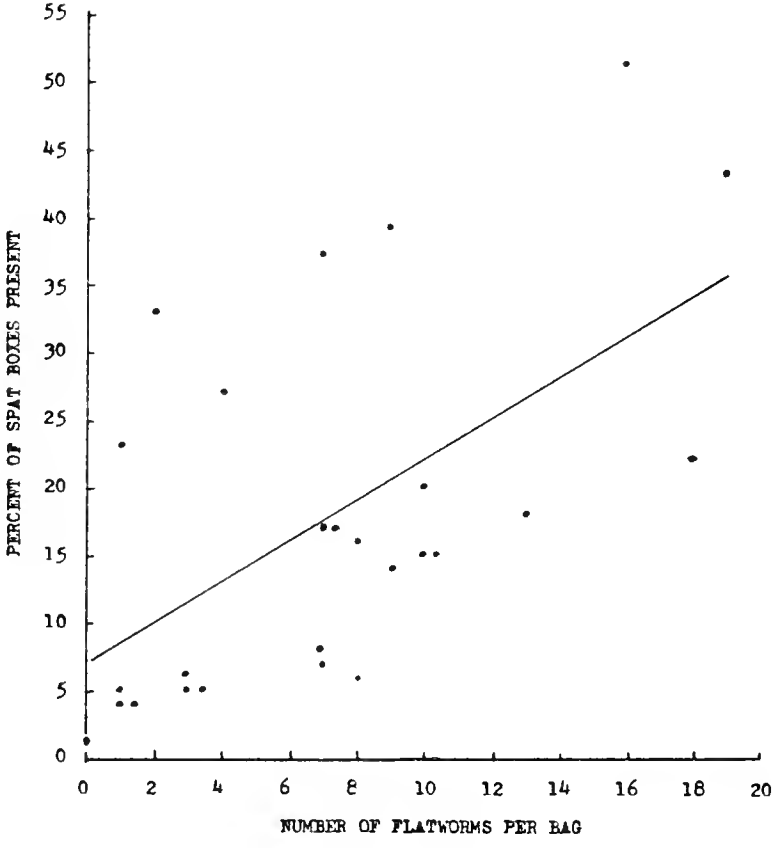

Fig. 2. Correlation of Flatworm Incidence and Recent Spat Mortality.

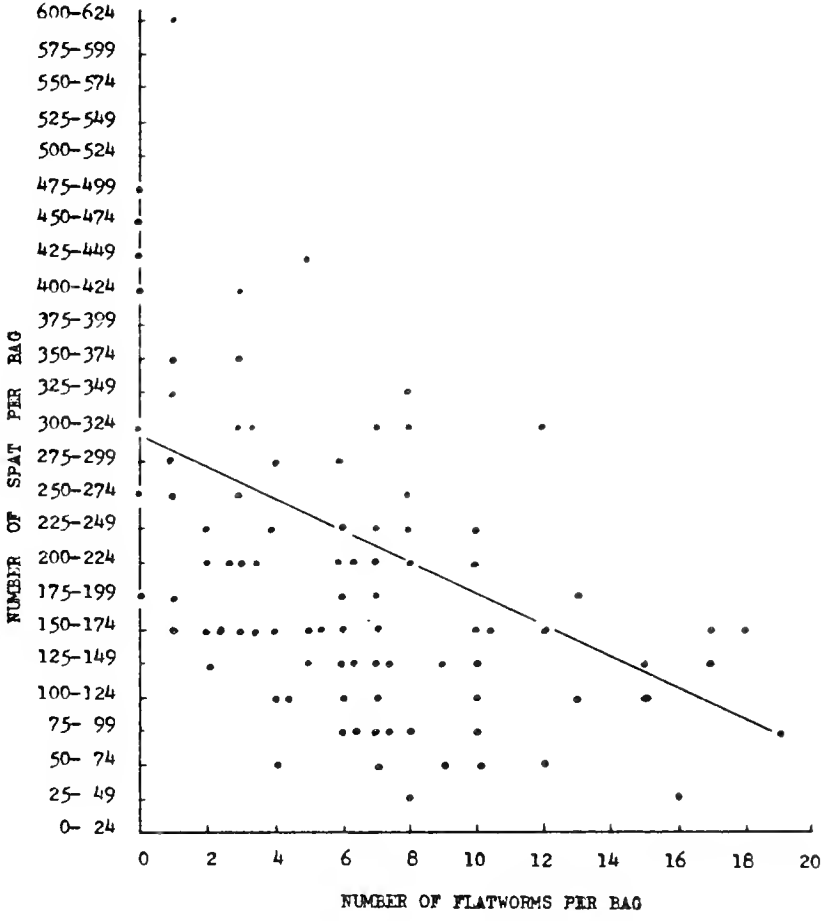

Fig. 3. Correlation of Flatworm Incidence and Spat Survival.

RELATIONSHIP BETWEEN SPAT SURVIVAL AND WORM INCIDENCE

Counts representing spat survival and worm incidence for all 90 bags were treated without regard to bag position in the transect. Here, a coefficient of correlation of approximately - 0.49 was calculated. Despite this low coefficient, a simple analysis of variance showed high probability that the relationship was not a consequence of chance. In this instance:

	DF	SS	MS	F	1% Point
Explained by regression	1	264256	264256	28.2	6.96
Unexplained deviations	88	824883	9374		
Total	89	1089139			

A scatter diagram of the ralationship between spat survival and worm incidence, with the line of regression calculated by the method of least squares, is shown in Figure 3.

CONCLUSIONS

Our field collections prove that the turbellarian flatworm, Stylochus ellipticus, occurs widely in the Maryland Chesapeake Bay and its tributaries. Recovery of worms from fresh oyster boxes, mostly spat, suggests a predatory relationship in explanation of many oyster mortalities. The evidence is circumstantial but corroborates reports of predation by investigators elsewhere. Returns from a seed-oyster production experiment in southern Maryland show that the vulnerability of oyster spat to flatworm predation could be real and markedly detrimental to seed production. The possibility is substantiated by the statistical association between three sets of numbers, one representing flatworm incidence, another representing spat mortality and the third representing the rate of spat survival during the summer.

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THE EFFECTS OF SALT SOLUTIONS OF DIFFERENT STRENGTHS ON OYSTER ENEMIES

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ABSTRACT

Boring sponges (Cliona celata), starfish (Asterias forbesi), and tunicates (Molgula manhattensis) were treated in salt solutions of different strengths for 1, 3, 5, 10 and 15 minutes. Oyster drills (Urosalpinx cinerea) were exposed to a saturated solution for 30 minutes, one hour and three hours. Sponges were killed by a 5-minute exposure in a saturated solution and by a 10-minute exposure in solutions 90, 70 and 60 per cent saturated. If sponges were dried following exposures, greater mortalities occurred. A one-minute exposure, followed by a one-hour drying period, was sufficient to cause complete mortality in solutions 100 and 90 per cent saturated, three minutes in 80 per cent saturated, ten minutes in 70 and 60 per cent saturated, and fifteen minutes in 50 per cent saturated. Starfish were killed by a 3-minute exposure in solutions 50 per cent or more saturated. Tunicates were killed by a 5-minute exposure in saturated and 90 per cent saturated solutions, 10 minutes in solutions 80, 70 and 60 per cent saturated, and 15 minutes in solutions 50 and 40 per cent saturated. Although drills, Urosalpinx and Eupleura, are not killed readily by salt solutions, embryos of both can be killed by treating them in a saturated salt solution. Caution should be exercised in exposing seed oysters (Crassostrea virginica) with damaged bills to salt solutions, since they are killed by high salt concentrations.

INTRODUCTION

Of more than a thousand chemical compounds tested at Milford Laboratory for their effects on shellfish enemies, none killed the boring sponge, Cliona celata, at concentrations low enough to be non-injurious to oysters. In the past, certain workers, including Dollfus (1921), deLaubenfels (1947) and Warburton (1958), recommended that sponges be killed by placing infested oysters in fresh or brackish water. Loosanoff (1945) reported that starfish can also be killed in sea water of reduced salinity. With both sponges and starfish, however, periods of lethal exposure to fresh water are too long to be practical.

Since death in fresh water is the result of upsetting the isotonic balance between body fluids of the organism and its environment (Prosser et al. 1950, and many others), it was suspected that death

could be achieved much more rapidly in waters of greatly increased salinity. In a highly hypertonic solution, an aquatic animal's body fluids might be lost resulting eventually in destruction of cells and organs.

Loosanoff (1957) conducted experiments in which he killed several oyster enemies by exposing them to saturated salt solutions (300 parts per thousand) for certain periods followed by drying them in air (Table 1). The present study was undertaken to determine the effects of less than saturated salt solutions on these enemies.

METHODS

In these experiments a saturated solution of "rock salt" was prepared and then diluted with sea water to give 90, 80, 70, 60, 50, 40 and 30 per cent saturated solutions.

Because it had previously been found that oyster enemies were killed within 15 minutes in a saturated salt solution (Table 1), the periods employed in this study were 1, 3, 5, 10 and 15 minutes.

Table 1. Minimum exposures in a saturated salt solution and drying times required to cause 100 per cent mortality of six species of oyster enemies (Loosanoff 1957).

	Exposure in minutes	Drying time in minutes						
		None	5	10	15	30	45	60
Asterias forbesi	3	X						
Molgula manhattensis	5-10	X						
Cliona celata	3-5							X
Crepidula fornicata	5				X			
Urosalpinx cinerea and *Eupleura caudata* egg cases	5	X						

Drying, which prolongs the contact of animals with salt and concentrates unsaturated solutions by water evaporation, was used only where it had proven necessary before. Starfish and drills were kept for 14 days in running sea water in the laboratory following salt treatments. Other animals were suspended for the same period in Milford Harbor in perforated plastic boxes which allowed adequate circulation of water.

Boring Sponges

Two samples of ten marketable-sized oysters infested with Cliona celata were exposed to salt solutions for each immersion period. One sample from each was dried for one hour. Drying caused much higher mortalities (Tables 2 and 3).

Sponges, extended with their oscula open in normal sea water, contracted during exposure. Dying and decomposing sponges pass through several color changes. From a deep, rich yellow they change to tan, black and then to white.

Starfish

All starfish (10 per sample) were killed by an exposure of one minute in 100, 90, 80 and 70 per cent saturated solutions. In 60 and 50 per cent saturated solutions three-minute exposures killed them. Starfish were not killed when exposed 15 minutes in solutions less than 50 per cent saturated. Water temperatures during this experiment ranged between 13 and 17.2°C.

During the drying periods, red pigment was observed to leach slowly from the starfish's integument. If starfish were placed in normal sea water immediately following exposure, much red pigment was lost from their ruptured body cells and organs, and their rays shrank and curled at the tips. Soon the starfish flattened out, and autotomy of the rays and rapid disintegration of the animal followed.

Tunicates

Twenty-five unattached Molgula manhattensis were used in each sample. Salt solutions 100 and 90 per cent saturated killed tunicates within five minutes. Solutions 80, 70 and 60 per cent saturated killed them within ten minutes and 50 and 40 per cent saturated solutions, within 15 minutes (Table 4).

Dead tunicates usually remained rigid for several days after their return to sea water, but then became flabby and often eviscerated themselves.

Table 2. Per cent mortalities of sponges 14 days after their exposure to salt solutions of different strengths and immediate return to sea water. Sea water temperature 9 - 11°C

Immersion in minutes	Per cent saturation							
	100	90	80	70	60	50	40	30
1	0	0	*	0	0	0	0	0
3	10	0	*	0	0	0	0	0
5	100	50	50	0	20	0	0	0
10	100	100	90	100	100	50	0	0
15	100	100	100	100	100	0	0	0

*Sample lost

Table 3. Per cent mortalities of sponges 14 days after their exposure to salt solutions of different strengths and drying for one hour. Sea water temperature 9 - 11°C

Immersion in minutes	Per cent saturation							
	100	90	80	70	60	50	40	30
1	100	100	50	60	75	25	0	0
3	100	100	100	80	100	80	0	0
5	100	100	100	90	50	50	0	0
10	100	100	100	100	100	50	25	0
15	100	100	100	100	100	100	50	0

Table 4. Per cent mortalities of tunicates 14 days after exposure to salt solutions of different strengths. Sea water temperature 5.2 - 11.5°C

Immersion in minutes	Per cent saturation							
	100	90	80	70	60	50	40	30
1	8	12	8	8	16	4	0	0
3	72	40	20	52	24	4	8	8
5	100	100	64	84	20	12	12	0
10	100	100	100	100	100	96	80	4
15	100	100	100	100	100	100	100	0

Drills

Urosalpinx cinerea is an extremely hardy animal capable of isolating itself from unfavorable environmental conditions by tightly closing its operculum (Carriker 1955). Therefore, it was decided to try a saturated solution for periods as long as 30 minutes, one hour and three hours before proceeding to weaker solutions and shorter exposures. Groups of 50 drills were exposed to these conditions. Following exposures to salt solutions one-half the drills in each group were returned to normal sea water and the others were dried for one hour. Only three of the six treatments caused any mortality. Eight per cent of the drills died after three hours of exposure and immediate return to sea water; 12 per cent died after one hour of exposure and one hour of drying; and 32 per cent died following three hours of exposure and one hour of drying.

Although drills are not readily killed by this method, it was found to be quite successful in killing embryos of U. cinerea and Eupleura caudata, regardless of their stage of development (Table 1).

Oysters

Eight to 15 Crassostrea virginica, 10 to 20 millimeters in length, were used in each sample. Few oysters died after exposure to any salt treatment, provided the edges of their thin shells were not chipped (Table 5). Chipped oysters were killed. While exposed to salt solutions, oysters remained tightly closed, except when they opened their shells slightly, probably to test the water. There was evidence that oysters were adversely affected by salt solutions because some of them did not produce true feces for several days follow-

ing a treatment; however, all produced pseudofeces.

Table 5. Per cent mortalities of young oysters, 10 to 20 millimeters in length, 14 days after exposure to salt solutions of different strengths. Sea water temperature 9 - 11°C

Immersion in minutes	Per cent saturation							
	100	90	80	70	60	50	40	30
1	0.0	0.0	0.0	1.9	0.0	0.0	0.0	0.0
3	2.1	11.1	3.0	0.0	0.0	0.0	0.0	0.0
5	0.0	10.2	0.0	0.0	0.0	0.0	3.1	4.0
10	4.3	3.5	0.0	9.0	2.3	0.0	1.8	0.0
15	0.0	3.3	*	0.0	0.0	0.0	0.0	0.0

*Sample lost

DISCUSSION

Several oyster enemies can be killed readily by exposing them to partially saturated salt solutions. Effective concentrations can be easily maintained by constantly stirring salt crystals into solution.

In practice, if several enemies are involved, the salt treatment should be administered to kill the species most resistant to it.

Since water temperatures during these experiments ranged from 5 to 17.2°C, repetitions of them are planned for water temperatures ranging between 15 and 25°C. At higher temperatures, minimum exposures for these enemies may be considerably shorter.

ACKNOWLEDGMENTS

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CHEMICAL CONTROL OF POLYDORA WEBSTERI AND OTHER ANNELIDS INHABITING OYSTER SHELLS

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ABSTRACT

A number of chemical compounds were tested and found either to compel Polydora websteri and nereid worms to emerge from oyster shells or to kill them directly within the shells. Compounds which caused more than 50 per cent of both groups to emerge from shells within three hours were considered effective vermifuges. Compounds which killed more than 50 per cent of worms within the same time were considered effective vermicides. The most efficient vermifuges for P. websteri were benzene and ethylene compounds. Common salt was the most practical vermicide tested because it is simple to use, inexpensive and kills worms quickly.

Worms of the genus Polydora, commonly called mud-blister worms, are serious pests. They not only form unsightly black areas on the inner faces of oyster shells, rendering infested oysters undesirable for half-shell trade, but also make oyster shells brittle. There are several published reports of attempts to use chemicals to control these worms. Among them is that of Korringa (1951) who reports that P. hoplura and P. ciliata can be killed by immersing infested oysters for three hours in sea water containing di-nitro-ortho-cresol at 500 parts per million or by placing oysters for 16 hours in fresh water; and Mackin and Cauthron (1952) observed that a solution of phenol at 500 ppm in sea water causes P. websteri to emerge. These authors do not state the rate at which worms leave the shells.

During the screening of a large number of chemical compounds for possible use in combatting shellfish enemies, a study carried on at Milford since 1946, several compounds have been found which are more effective against P. websteri, the species which occurs in southern New England waters, than those proposed by earlier workers. Two categories of compounds could be used in control of these worms: (1) vermifuges, chemicals that cause worms to emerge from their tubes and leave the oyster shell, and (2) vermicides, chemicals that kill worms within their tubes, but do not ordinarily cause emergence.

Oysters heavily infested with P. websteri were gathered from uncultivated beds in West Tisbury Great Pond on Martha's Vineyard Island. They were kept in wire baskets in Milford Harbor until 24 hours prior to their use when they were brought into the laboratory and allowed to become acclimated gradually to room temperature. For each

experiment two oysters, each in a separate finger bowl, were submerged in a nine-liter container of the solution to be tested.

In initial tests each compound was tried at a concentration of 100 ppm. Following each test, oysters were kept in warm running sea water for five to eight days to allow any living worms to recover from the effects of the compound tested. They were then placed for 24 hours in a solution of O-dichlorobenzene at 100 ppm which was found to cause almost 100 per cent emergence of both Polydora and nereid worms. Thus, a group of eight oysters which yielded an average of 88.7 P. websteri and 10.7 nereids per oyster upon initial treatment gave only one or two worms per oyster on the second treatment with this chemical. Any worms which survived the initial test could be collected and counted by this method. Because oysters immersed for 24 hours in a 100 ppm solution of O-dichlorobenzene may die, this treatment was used merely to insure 100 per cent emergence of worms. As many as 332 P. websteri were recorded for a single oyster.

The effectiveness of vermifuges was calculated directly by comparing the number of worms emerging in the experimental vermifuge with the number emerging in the subsequent treatment with O-dichlorobenzene. Since no simple method exists for quantitatively removing from oyster shells worms that have been killed in situ, it was necessary in evaluating vermicides to use the average number from the eight oysters mentioned above as the total worm population and the number emerging on subsequent treatment with O-dichlorobenzene as survivors of various chemicals. Obviously, the data for effectiveness of vermicides are less accurate than those for vermifuges.

Over 30 compounds caused some P. websteri to emerge, but only those causing 50 per cent of worms to emerge within three hours are considered effective vermifuges (Fig. 1). Of these, O-dichlorobenzene was selected as the most practical vermifuge because at concentrations below 100 ppm it is more effective than monochlorobenzene, trichloroethylene and tetrachloroethylene. Moreover, the latter two compounds are quite volatile and may be somewhat dangerous to people handling them on a large scale. One compound more effective than O-dichlorobenzene, 4-nitrobenzene-azo-resorcinol, is difficult to dissolve in sea water and is expensive. Phenol is not an effective vermifuge at concentrations much below 500 ppm, e.g. at 100 ppm it caused emergence of only about 33 per cent of the worms. Naphthalene might be effective, but is difficult to dissolve in sea water. When added to O-dichlorobenzene, however, enough of it goes into solution to increase the effectiveness of the O-dichlorobenzene. Certain nereid vermifuges, such as emulsifiable rotenone, Bulan crystalline, and Tris (acetoxy methyl) nitro methane, caused P. websteri to retract into their tubes but did not cause emergence.

Usually it required about five or six minutes for the first P. websteri to emerge, probably because it takes about that long for the chemical to penetrate the worm's tube. Worms usually back away from

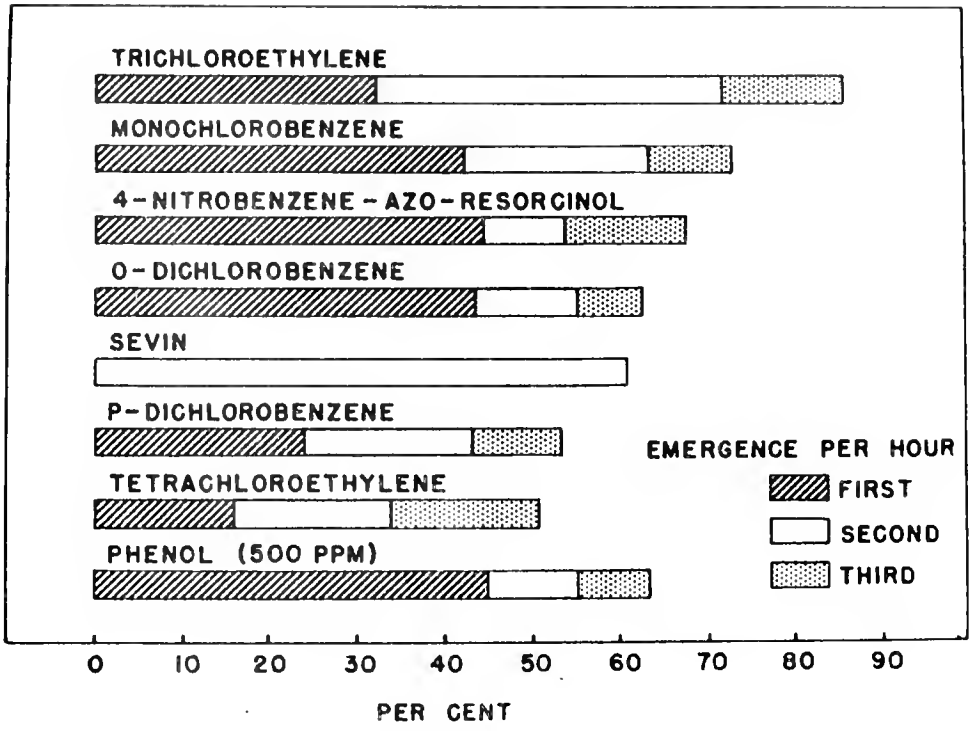

Fig. 1. Rate of emergence of P. websteri from oyster shells during exposure to 100 ppm solutions of various chemicals for three hours. Only those compounds that were more than 50 per cent effective are shown. Rate of emergence was not determined for Sevin.

chemicals and emerge posterior end first. Since they do not swim, worms drop to the bottom of the pans and, if removed to another vessel containing untreated sea water and some bottom detritus, they soon begin building new mud tubes.

Nereid worms appear to be more sensitive to certain chemicals than P. websteri and react to them more quickly (Fig. 2). Since they do not live in long narrow tubes and contact a chemical with their whole bodies, nereids usually begin to emerge as soon as oysters are placed in the solution.

Several compounds killed P. websteri within their tubes (Fig. 3). Di-nitro-ortho-cresol at 100 ppm killed few worms. At 500 ppm, however, the concentration which Korringa (1951) used, 81.4 and 90.5 per cent were killed by exposures of one and three hours, respectively. A 500 ppm solution of 2-chloro-1-nitro propane killed 96.6 per cent of the worms within three hours. Victoria Blue at 200 ppm killed 82 per cent with only a 5-minute immersal; 89 per cent were killed within 10 minutes; and 97 per cent, within 15 minutes. Actually, the lethal effects of this dye on annelid worms were discovered at this laboratory before the

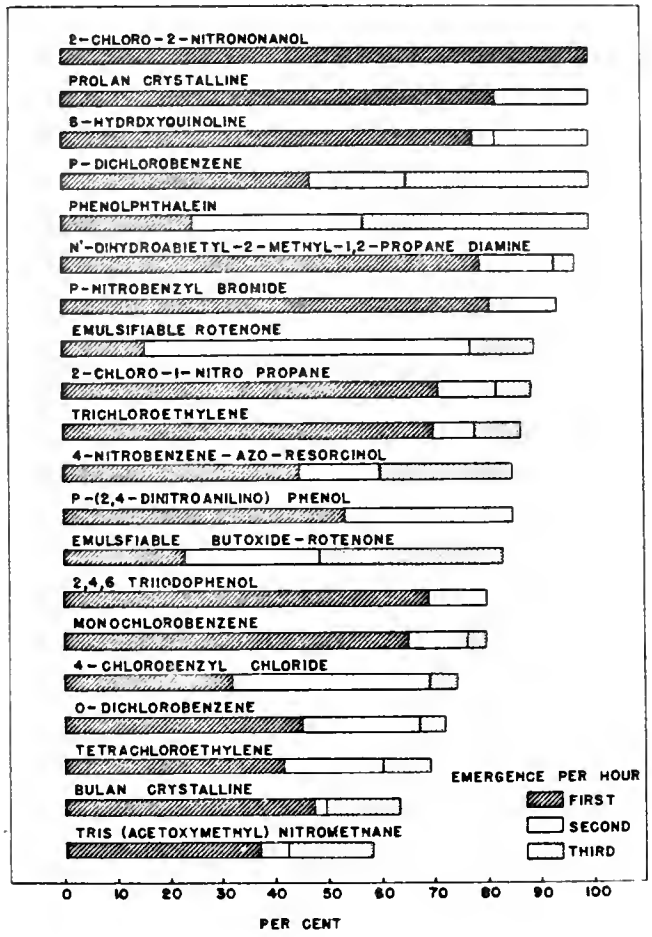

Fig. 2. Rate of emergence of nereids from oyster shells during exposure to various chemicals at 100 ppm for three hours.

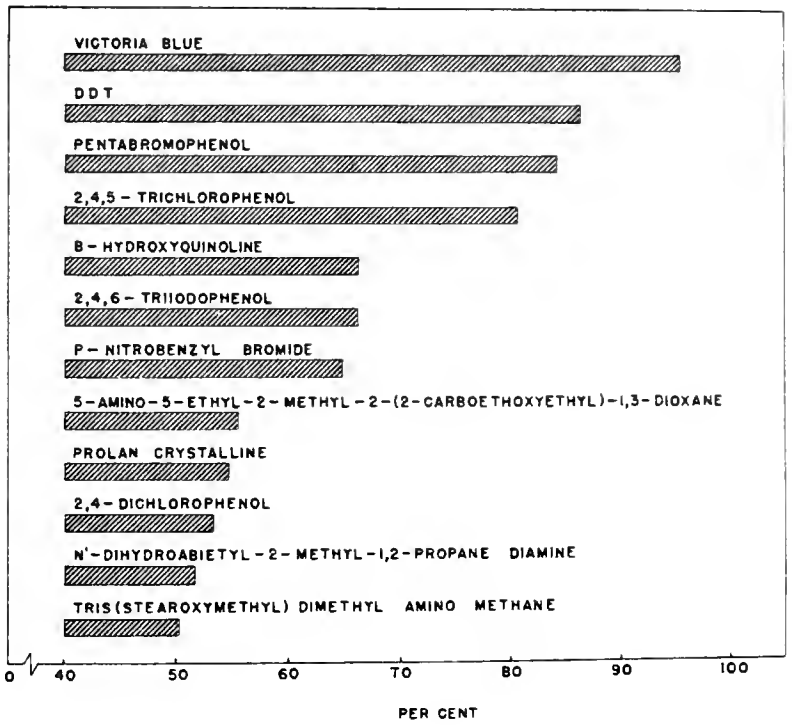

Fig. 3. Mortality of P. websteri in oyster shells after exposure to various chemicals at 100 ppm for three hours.

present study, and this compound has been used routinely during the last four years to exterminate worms in clam hatchery troughs. The above compounds, except 2-chloro-1-nitro propane, were also lethal to nereids as were other compounds listed in Figure 4.

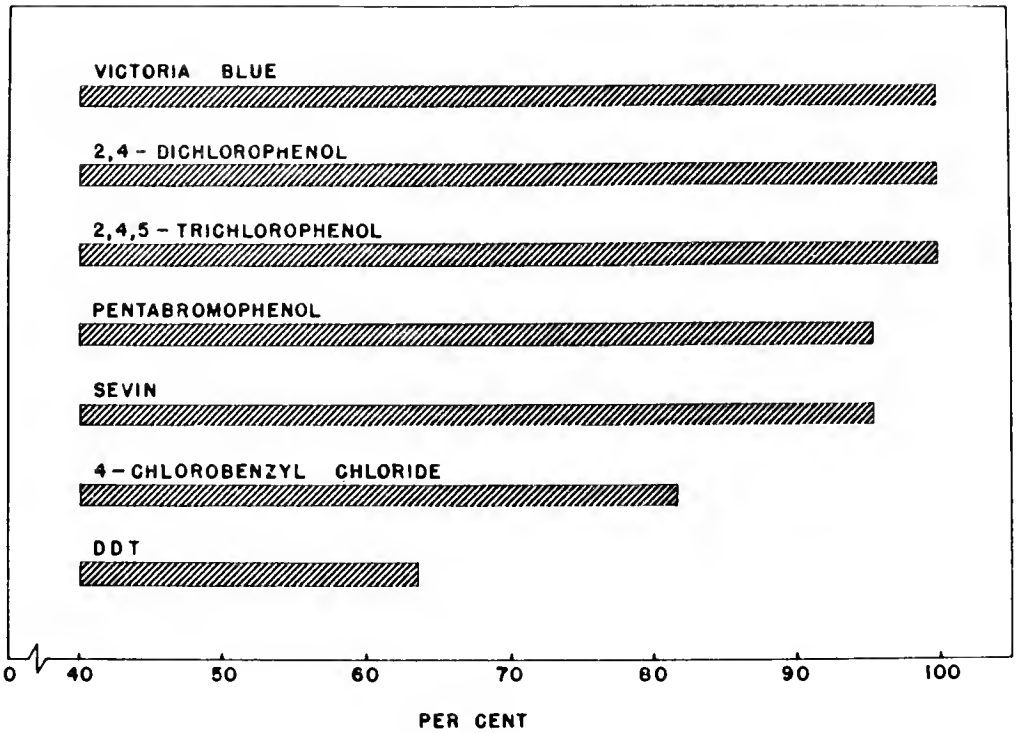

Fig. 4. Mortality of nereid worms in oyster shells after exposure to various chemicals at 100 ppm for three hours.

Perhaps the most practical compound for worm control is common salt. From 87 to 98 per cent of P. websteri were killed by a 10- to 15-minute dip in a saturated salt solution followed by 15 or more minutes of drying in air. Shorter dips required a longer period of drying to kill worms, but even a 1-minute dip was 89 per cent effective when combined with a drying period of at least two hours. These studies showed that it is almost impossible to kill every P. websteri in an oyster shell within three hours.

At low concentrations several compounds apparently acted as inhibitors to P. websteri. For example, in 2-chloro-1-nitro propane the worms neither extended their tentacles as in feeding nor emerged. When combined with vermifuge 4-nitrobenzene-azo-resorcinol, worms emerged only as the concentration of the inhibitor was decreased (Table 1).

Table 1. Comparison of numbers of P. websteri emerging within three hours from shells of two oysters in a 10 ppm solution of vermifuge 4-nitrobenzene-azo-resorcinol in the presence of an inhibitor, 2-chloro-1-nitro propane, at various concentrations.

Concentration of inhibitor in ppm	Number of worms emerging	Number remaining alive in shells
50	37	103
20	63	48
10	77	78
5	112	67
0	158	35

Inasmuch as oysters stay closed while immersed in solutions of most chemicals, they are usually not harmed by them. They pump intermittently, however, in benzene and ethylene compounds; hence, these compounds may be lethal if the immersal is too long. In O-dichlorobenzene at 100 ppm, for instance, an immersal of three hours caused little mortality of oysters, but one of 24 hours killed almost all of them. At the end of this period oysters gaped widely and did not recover after being placed in running sea water. They also pump in a solution of Victoria Blue, which, even if the concentration is low, accumulates in their tissues and eventually kills them. Consequently, in these experiments we used Victoria Blue in fresh water which kept oysters closed and therefore prevented them from absorbing the dye. Oysters with chipped bills (7 of 40) could not keep the dye out and eventually died.

We believe these vermicides can be applied in commercial oyster culture. Oystermen could use them to kill worms in shells of oysters in spring, thus giving oysters a chance to deposit layers of white shell over the black mud blisters by fall. This treatment, however, would not prevent setting of young P. websteri which could form small blisters at the periphery of shells. A saturated solution of salt appears to be most practical for oystermen, because it is simple to use, inexpensive, requires only a short exposure to kill worms, and kills some other fouling organisms as well (Loosanoff 1957). The other compounds would be difficult to maintain at precise concentrations. Vermifuges could be used in various ways in studies of the biology of worms and of oysters. To cause all worms to emerge without harming oysters, infested oysters could be alternately immersed in O-dichloro-

benzene for not longer than three hours in which the worms will emerge and then in sea water for recovery of the oysters, until all worms have emerged.

ACKNOWLEDGMENTS

The authors express their gratitude to Dr. V. L. Loosanoff for suggesting these experiments and for his guidance in planning them, and to Mr. Harry C. Davis and Miss Rita S. Riccio for editing the manuscript.

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TRIAL INTRODUCTION OF EUROPEAN OYSTERS (OSTREA EDULIS) TO CANADIAN EAST COAST

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ABSTRACT

In the spring of 1957, 1958 and 1959, seed oysters beginning their second and third growing seasons were imported from the United Kingdom oyster breeding tanks at Conway, North Wales. They were examined for parasites and extraneous organisms, carefully cleaned and planted in screen-bottomed trays in Passamaquoddy Bay near St. Andrews, New Brunswick. Some were taken in late 1958 to Ellerslie, Prince Edward Island, and held in Malpeque Bay water. Growth was good in Sam Orr Pond, a warm inlet from Passamaquoddy Bay, but poor in the cool open Bay. The oysters brought in by steamer in 1957 suffered a 95% mortality within a month after arrival. The 1958 lot was brought in by air freight and showed a post-shipment loss of only 35%. The 1959 lot, also air-shipped, suffered only 9% loss up to July 16, 1959, but mortalities rose to 53% by August 6. Overwinter survival varied greatly and the flagellate Hexamita was found in most moribund oysters. It was found in Ellerslie aquarium stock which died after three weeks' exposure to below-zero water temperatures and in St. Andrews aquarium stock which survived reasonably well at water temperatures that remained above 2°C. It was also found in native oysters taken directly from their beds and in a sea scallop which was held in an aquarium with European oysters but not in quahaugs. The oysters in trays under the ice in Sam Orr Pond survived the relatively mild winter of 1957-58 but died during the severe winter of 1958-59 showing heavy Hexamita infestation.

INTRODUCTION

Since 1950 there has been a drastic decline in the production of soft-shell clams (Mya arenaria) from the Canadian east coast, with a consequent serious economic effect on shore communities. Efforts at clam farming have been discouraging. Clams can be farmed in some areas but the expenses are too high to make the operation profitable.

In an attempt to replace this loss in clam production, the approval of the Minister of Fisheries was obtained for a trial introduction of the European oyster (Ostrea edulis).

ACKNOWLEDGMENTS

I wish to acknowledge my dependence on data collected by my colleagues at this Station, Miss Joan Mortimer (unpublished MS reports), Mr. Peter J. Downer (unpublished MS report), and Dr. Louis Lauzier. Mr. R. E. Drinnan, of our Ellerslie Sub-Station, has generously furnished the information reported here on his studies of oysters we sent to him. I am indebted to Dr. J. E. Stein and Dr. Marshall Laird, who examined specimens for Hexamita infection, and to Dr. E. L. Bousfield, and Dr. J. P. Harding for identification of invertebrates encountered in this work and for permitting me to refer to their findings.

I would like to thank Dr. H. A. Cole, Dr. N. Reynolds, Mr. B. T. Hepper and Mr. P. R. Walne, officers of the United Kingdom Ministry of Food, Agriculture and Fisheries, for their co-operation in supplying the oysters.

I am also grateful to Dr. J. L. Hart for his encouragement and assistance, and to Mr. Drinnan and Mr. Downer for assistance in preparing this paper.

CHOICE OF CONWAY OYSTERS

The European oyster was selected for trial introduction because it is known to thrive in places where summer water temperatures resemble those recorded in our soft-shell clam areas. It is readily marketable and practical culture methods for it have been worked out.

This choice was also influenced by the establishment of a breeding population of this species from the spawning of a relatively small stock which Dr. Loosanoff brought to Boothbay Harbor, Maine, in 1949 (personal communication).

The choice of the Conway hatchery stock was influenced by its relative freedom from oyster diseases and parasites. This was considered to be particularly important in view of the danger of introducing undesirable species. In this case, care was taken to guard against the Dutch shell disease, the mussel parasite, Mytilicola intestinalis, which occurs in some European oysters, and the barnacle, Elminius modestus, a recently-introduced pest of oyster beds in western Europe.

At the same time it was necessary to obtain oysters which could withstand the low winter water temperatures in eastern Canada. All things considered, the oysters bred at the Conway hatchery and subsequently reared in the Menai Straits seemed to be the best prospect.

IMPORTATIONS

In May 1957 about 5,000 oysters of the 1955 set were packed,

with damp seaweed (Fucus) in wooden boxes and shipped in the chilled vegetable compartment of a passenger steamer. These oysters were out of water for 11 days during transit.

In April 1958, approximately 5,000 of the 1956 set were placed in plastic bags, surrounded by "Vermiculite" as an insulating medium, and packed in cardboard boxes. They were shipped in a pressurized, temperature-controlled compartment of a freight aircraft. They were out of water for approximately 4 days.

In April 1959, approximately 1,000 of the 1956 and 5,000 of the 1957 set were shipped in the same way and were out of water for 4 days.

REMOVAL OF UNDESIRABLE ORGANISMS

There were considerable numbers of barnacles on the oysters imported in 1957. In case these should include specimens of Elminius modestus, it was decided to remove them prior to planting the oysters. Subsequent identification of samples by Dr. E. L. Bousfield of the National Museum of Canada, and Dr. J. P. Harding of the British Museum of Natural History, showed that Elminius was present.

Trial batches of oysters were immersed in seawater solutions of Lindane (benzene hexachloride) in an attempt to kill the barnacles. The solutions were made up from a commercial powder, which contained 25% by weight of this poison, to give concentrations of the active ingredient of 1:50,000, 1:225,000, and 1:500,000. This poison is known to be toxic to several species of crustacea (lobsters and green crabs) but proved ineffective on the barnacles at the concentrations used. After overnight exposure, many were still active although a few appeared to be paralyzed. In case prolonged exposure to Lindane might harm the oysters, it was decided to abandon this method of destroying barnacles.

It was finally decided that individual cleaning by hand was the only satisfactory method. Each oyster was therefore scrubbed clean of mud and debris and then barnacles, tube worms and other encrustations removed with a scalpel. This proved effective as no barnacles were found on the oysters in subsequent handling. This method was again used in 1958 and 1959.

Examination for Dutch shell disease and Mytilicola gave negative results.

During 1958 the protozoan Hexamita appeared in the oyster stock during the late summer and following winter. Inspection of the 1959 import immediately after arrival showed none of this protozoan. As Hexamita had been previously reported from our native oysters (Crassostrea virginica) by Mackin et al. (1952) and by Logie (personal

communication) and was encountered again by Dr. Laird in 1958, it is concluded that our European oysters were infested after their arrival in Canada.

PLANTING

The oysters were planted out in trays with 8-inch legs to raise them off the bottom, and 1/4-inch mesh wire bottoms and lids. Periwinkles (Littorina littorea) were placed in the trays to keep down epiphytic algal growth.

Plantings were made in several locations. Some oysters were retained in the Station tanks, and some on the beach below the Biological Station in the cool water of Passamaquoddy Bay, but most of them were set out in Sam Orr Pond, a warm, salt-water inlet of Passamaquoddy Bay.

GROWTH

During their first year in Sam Orr Pond, where temperatures rise at times to 25°C, the oysters showed excellent growth (Table 1). This was greater in 1957 than in 1958, presumably because 1957 was warmer (Table 2). The survivors of the 1957 stock which lived through 1958 grew well in their second summer in the pond. By August their mean length was 80 mm.

Table 1. Growth of oysters during their first year in Sam Orr Pond trays.

Import Year	Shell Length mm		Annual Growth mm
1957	May	35	39
	Nov.	74	
1958	May	34	26
	Nov.	60	

In both years the oysters in Station tanks and in Passamaquoddy Bay grew less than a third as much as those in the pond. This is

attributed to the coolness of the Bay (Table 2) where the highest temperature recorded in the two years was 15.8°C.

Table 2. Two-year comparison of summer water temperatures in Sam Orr Pond and Passamaquoddy Bay.

	Sam Orr Pond		Passamaquoddy Bay
Year	No. of days temperature was above 15°C	20°C	No. of days temperature was above 15°C
1957	67+	26	4
1958	44	6	6

SURVIVAL

The oysters imported in 1957 seemed vigorous. When placed in water immediately after arrival, many appeared to begin pumping. However, next morning gapers appeared and steadily increased. Approximately 90% died during the first week (Fig. 1) and by the end of June 95% were dead. The 5% remnant of the stock showed no further losses, grew well during the summer and survived the 1957-58 winter 100% in Sam Orr Pond. This encouraged belief that Sam Orr Pond was a safe wintering place.

The heavy mortality on arrival was attributed to prolonged air exposure during transit. This conclusion was supported by Korringa's (1956) experience and by observations on storage of our native oyster, Crassostrea virginica, which shows a similar mortality if placed in water after long air exposure (Medcof 1959).

Survival and growth after the initial mortality was over, encouraged the 1958 importation by air which reduced air exposure to approximately 4 days. This shipment was made in April before commencement of the growing season. As an added precaution, the oysters were held in the intertidal zone in Menai Strait for several days prior to shipment to accustom them to prolonged air exposure. The Japanese regularly condition spat of Pacific oysters in this way before shipping them to the west coast of North America (Glude and Lindsay 1947).

The precautions taken in 1958 did not avert a heavy initial loss but they seem to have delayed it until some 8 weeks after arrival and to have reduced it. Approximately 40% of the stock survived until winter (Fig. 1) as compared with 5% in 1957.

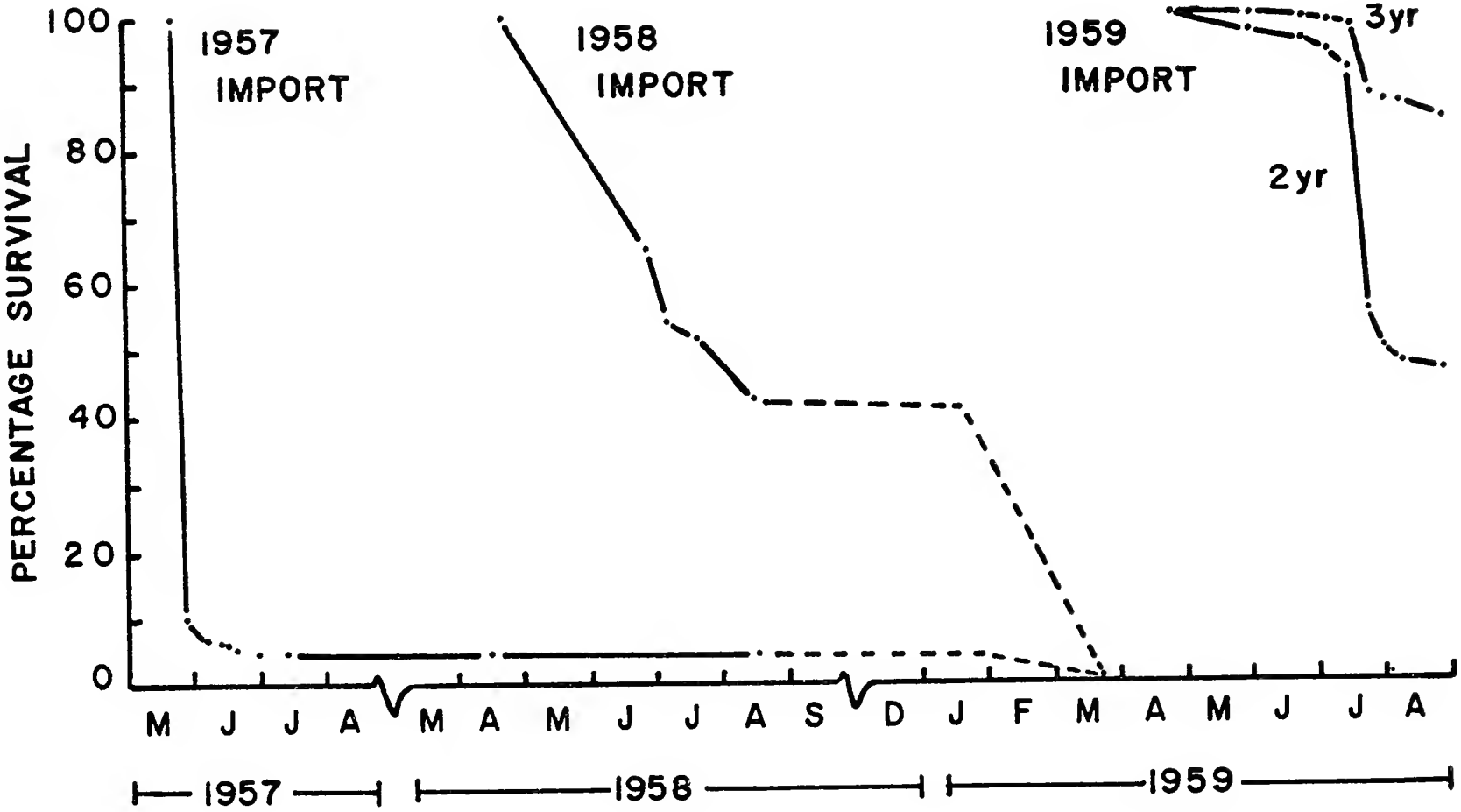

Fig. 1. Survivals in Sam Orr Pond of three lots of imported European oysters.

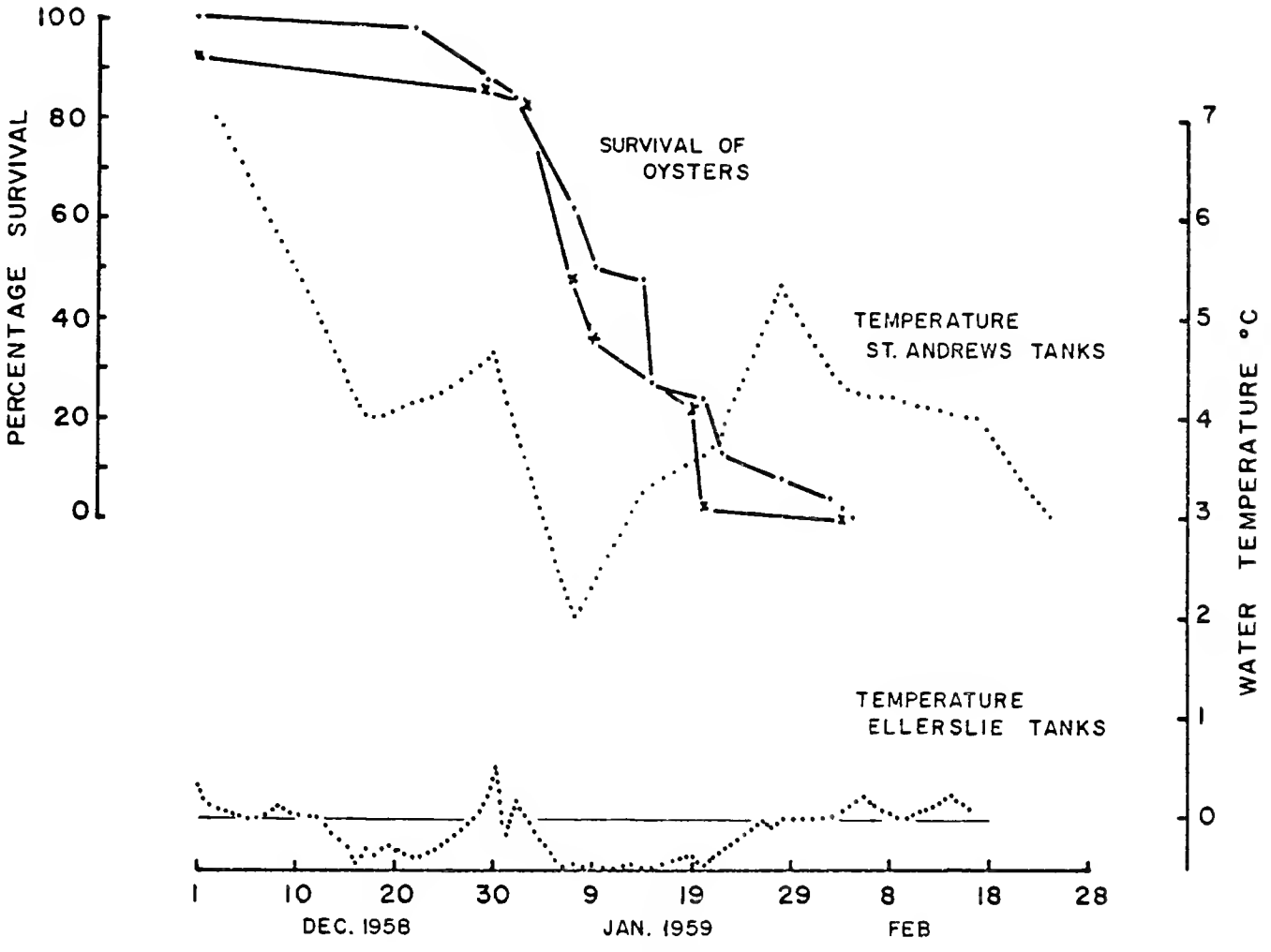

Fig. 2. Survival in two lots of oysters transferred from St. Andrews to Ellerslie laboratory tanks July 11 (lower curve) and November 6 (upper), 1958. And winter water temperatures in laboratory tanks at Ellerslie (recorded daily) and at St. Andrews (recorded weekly).

In July 1958, Dr. Marshall Laird, a protozoologist from the Institute of Parasitology, McGill University, examined samples of the 1957 and 1958 imports. In the 1958 import he found ciliates on the mantle and gills of all specimens and one oyster showed trophozoites of the flagellate Hexamita inflata in the intestine. All these oysters were in poor condition, thin and watery. No ciliates and no Hexamita were found at that time in oysters imported in 1957. These oysters were in excellent condition, fat and creamy-white.

During their second (1958) summer in the pond the survivors of the 1957 importation showed almost no mortality. This was encouraging.

On July 11 and November 6 samples of 150 and 200 oysters, respectively, of the 1958 importation were sent to Mr. Drinnan at Ellerslie, Prince Edward Island. He held some of these in trays in Bideford River and some in laboratory tanks. The oysters transferred in July grew well and were quite fat by autumn. The November transfers grew little and were still thin when cold weather set in. All oysters in both lots died in early winter. Drinnan's records show that the mortality began about 3 weeks after water temperatures dropped to 0°C and below, and that it reached 100% within 2 months (Fig. 2). After the mortality began Mr. Drinnan made many examinations of living, moribund and dead oysters. Most but by no means all proved to be heavily infected by trophozoites of Hexamita.

Mr. Drinnan's observations at Ellerslie stimulated sampling of the oysters wintering in Sam Orr Pond. On March 5 the ice, which was 28 inches thick, was sawed through and a tray containing oysters of the 1958 importation was located and raised. Only 2% were sound. The remainder were either weak gapers or dead. On examination, all of them, living and dead, proved to be heavily infected with trophozoites of Hexamita. Samples were sent to Mr. Drinnan, Dr. Laird and Dr. Stein, all of whom confirmed these findings. On April 22 when the ice had cleared from Sam Orr Pond, all the oysters from both importations were found to be dead.

In contrast, about two-thirds of the oysters held in the laboratory at St. Andrews survived the winter. The water temperatures in these tanks are shown in Figure 2. They were consistently higher than those in Ellerslie tanks and higher than those in Sam Orr Pond for at least part of the winter of 1958-59. This is shown by two readings at the pond with a reversing thermometer lowered through holes in the ice which were +0.1°C on February 5 and -0.4°C on March 6, 1959. These St. Andrews tank temperatures were probably higher than the pond temperatures in the winter of 1957-58, but there are no direct observations to support this assumption. Passamaquoddy Bay hydrographic records supplied by Dr. Lauzier show that the winter of 1958-59 was more severe than that of 1957-58 (Table 3).

Table 3. Comparison of monthly means of Passamaquoddy Bay water temperatures (°C) taken daily during the two winters oysters were held at St. Andrews, N. B.

Winter	December	January	February	March
1957-58	5.4	3.5	2.1	2.7
1958-59	3.4	1.1	-0.1	0.4

The oysters imported in 1959 arrived by air on May 2. They were given the same intertidal hardening treatment in Menai Strait before shipment as those imported in 1958. The survival since planting seems to be following the regular pattern (Fig. 1). Up to July 16, 1959, 10 weeks after arrival, 97% of the 3-year-olds (1956 set) and 91% of the 2-year-olds (1957) were still alive. By July 24, however, these values had fallen to 88% and 55% and by August 6 to 86.5% and 47.3%. During the peak of the mortality all the moribund and dead oysters were heavily infected with Hexamita trophozoites. Healthy-looking oysters have been consistently free.

ADDITIONAL OBSERVATIONS ON HEXAMITA

The quahaug (Mercenaria mercenaria) is native to Sam Orr Pond and at no time has Dr. Laird found it to harbour Hexamita. Even when held in tanks at the Biological Station alongside heavily infested oysters, the quahaugs have not been contaminated.

A scallop (Placopecten magellanicus) which had been living in a St. Andrews tank with infected oysters was found dead on May 30, 1959, and as heavily infected with Hexamita trophozoites as the oysters.

Dr. Laird found the flagellate in samples of mud taken April 6, 1959, from the bottom of Sam Orr Pond at the place where the oyster trays were wintered.

DISCUSSION

Apparently the European oyster may thrive on our coast in sheltered inlets in summer but may or may not survive our winters depending on their severity. Mr. John Hurst of the Maine Department of Sea and Shore Fisheries reports (personal communication) that one winter the European oysters (Netherlands strain) growing in Maine showed substantial mortality, when severely cold weather coincided

with extreme low tides. But only intertidal animals were affected. Gaarder and Bjerkan (1934) report that the Norway strain of European oyster does not withstand severe winters very well even when submerged. Korringa (1957) also recognizes this but suggests that there are racial groups within the species which show differences in cold-hardiness. In his opinion the Netherlands strain is quite hardy. Our laboratory and field experiments with Conway oysters clearly indicate that at temperatures about and slightly below 0°C they suffer some kind of stress. Under present conditions they may or may not survive, depending on the severity of the winter.

McLellan and Lauzier (1956) report sea water temperatures for the Bay of Fundy and the outer coast of Nova Scotia and predict more severe winter conditions on this coast for the next 25 years. Their statements discourage hope that this species can be established here. We find that present conditions are marginal--perhaps too risky to justify further trial introductions of Conway oysters. If conditions were less favorable they could rule out any chance of success with any strain of European oyster.

The role of Hexamita in oyster mortalities is by no means clear. It lives freely in the mud in Sam Orr Pond and is probably present in the water supply to laboratory aquaria at St. Andrews and Ellerslie. But it does infect oysters with various effects under various conditions. Some of these are clear; others can be only vaguely outlined.

Oysters can tolerate Hexamita infection for long periods without unusual mortality at low temperatures, about 4°C. This is shown by the St. Andrews tank experiments.

Hexamita may cause death of oysters that are in stasis (hibernating with low metabolic rates) or that are suffering stress from still lower temperatures (approaching the lower lethal temperature) Our observations of low-temperature mortalities in Sam Orr Pond and at Ellerslie illustrate this and parallel those by Stein (1960) for Olympia oysters.

Hexamita will also attack at high temperatures when oysters are experiencing difficulty (stress) in adjusting to new conditions or in obtaining enough food. This is illustrated by Dr. Laird's 1958 summer studies of the thin-meated Sam Orr Pond stock.

Intermediate temperatures which prevented normal summer growth of oysters (e.g. in St. Andrews aquaria) apparently favored chronic Hexamita infection. Food shortage may have been involved in this situation.

Hexamita does not ordinarily infect quahaugs, which live well in aquaria and show great cold-hardiness. But it may attack sea scallops which do not thrive in aquaria, although they are tolerant

to low temperatures (Dickie 1958).

From this variety of evidence our Hexamita could be considered a facultative parasite. It seems to have taken the first evolutionary step toward parasitism. If it were a well-adjusted parasite, our oysters might have survived better. On the other hand, the occurrence of the flagellate in slow-growing, weak or dying oysters and in scallops living in aquaria may be purely coincidental with approaching death. Hexamita may be simply an opportunist.

PLANS FOR FUTURE

At the moment we are still convinced that we need more species of shore molluscs to stabilize our shellfish fisheries. We would like to establish some exotic species in our depleted clam areas. We are not quite satisfied that the European oyster is not adaptable to this coast. We have tested the Conway strain without very encouraging results but Dr. Loosanoff's experience and the experience of the Maine Department of Sea and Shore Fisheries with the Netherlands strain are more heartening. There are several strains, and one or more of these may have the degree of low-temperature tolerance that would make the European oyster adaptable to our coast. Even though hydrographers warn us to expect colder winters in the next few years, search for a suitable strain may be justified.

Aside from this possibility there are other species of shore molluscs which we have considered as possibly adaptable, for example, the Pacific oyster (Crassostrea gigas), the Japanese littleneck clam (Tapes philippinarum), and the Atlantic bay scallop, (Aequipecten irradians). This last species has been reported from the Canadian Atlantic coast and the second last is already established on the British Columbia coast.

At this stage no decision has been made to go on and make an exhaustive study of the possibilities of finding an adaptable strain of European oyster or to try out what seem to be the hardiest strains of some of these other species.

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PRELIMINARY REPORT ON GROWTH AND SURVIVAL OF THE PACIFIC OYSTER IN WASHINGTON WATERS[1,2]

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INTRODUCTION

The Pacific oyster, Crassostrea gigas, has become increasingly important as a fishery resource in the Pacific Northwest of America. Yet, we have relatively little information on its growth and mortality rates in local waters, information basic to the utilization of this valuable resource.

Chapman and Esveldt (1943), in their study of spawning and setting of the Pacific oyster in Washington waters, gave a brief account of its growth. Woelke (1955) discussed the growth of a small group of the Kumamoto variety of this species and graphically compared its growth with that of the typical C. gigas seed; both were grown on the same bed. Quayle (1951), working on the Pacific oyster in British Columbia waters, followed the increase in length, width, and thickness of a group of 6 to 8-year-olds for one year. We are not aware of other published accounts dealing with growth and mortality of the Pacific oyster in our local habitat. Clearly, more information is desired and needed.

In March 1959, we started a field study, with emphasis on growth and mortality, of some 3,000 yearling Pacific oysters (1957 year class) originally imported from Japan. Results through January 1960 on growth, condition index, and mortality rates at three field stations are presented in this report.

MATERIALS, METHODS, AND FIELD STATIONS

The Western Oyster Company at Purdy, Washington, provided more than 3,000 yearling Pacific oysters, which were imported as seed from Miyagi Prefecture, Japan in the spring of 1958. These oysters were culled to singles in the laboratory of the College of Fisheries, and length, height, and thickness of each individual were measured to the nearest millimeter. The condition index was determined from 30 oysters, and 10 specimens were fixed for histological examination.

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[2]Contribution No. 65 from the College of Fisheries, University of Washington.

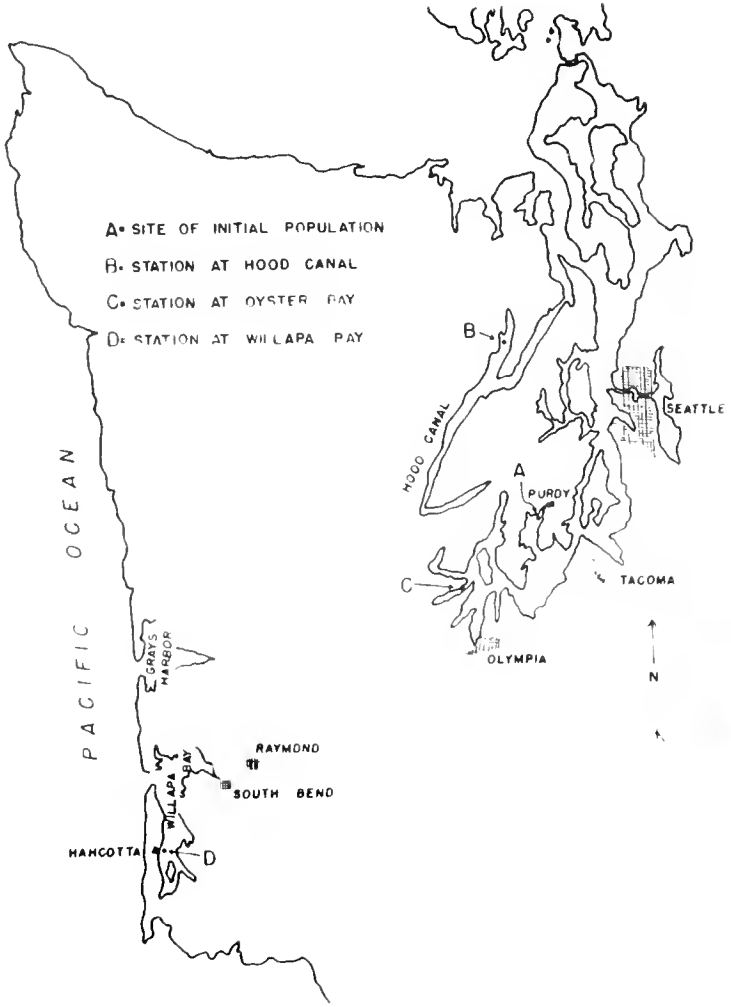

Fig. 1. Location of experimental stations in the State of Washington.

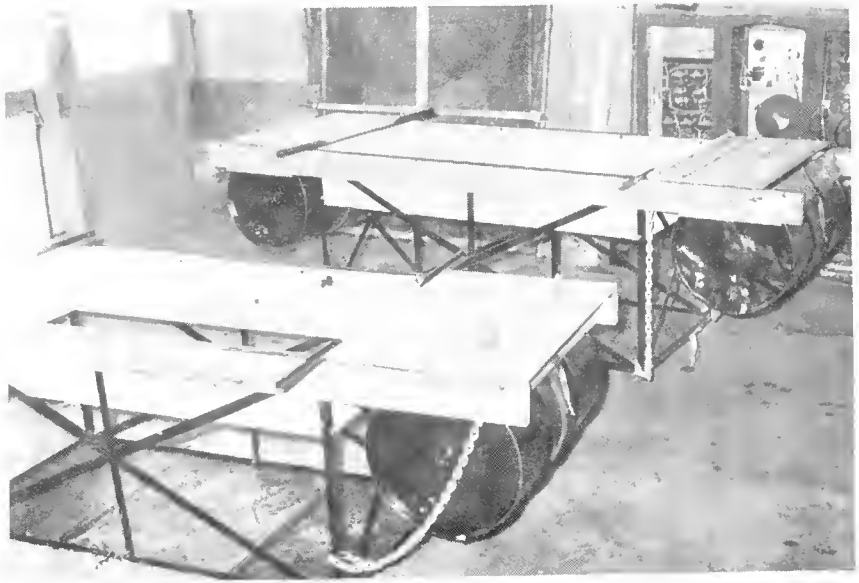

Fig. 2. Experimental floats.

Then 1,000 oysters were distributed during the first week of March, 1959, to each of three field stations: Oyster Bay in southern Puget Sound, Pt. Whitney in Hood Canal, and Nahcotta in Willapa Bay (Fig. 1). Each station consisted of a float (Fig. 2) much like that used by Collier (1953). Four woven-wire baskets, each measuring 35.5 by 17.0 by 7.0 inches, were contained in the float, and in each basket were placed 250 oysters.

Three baskets at each station were designated as experimental; with a few exceptions, they were examined each week from March until September and every other week thereafter. During these weekly and biweekly examinations, measurements of length, height, and thickness were taken on 100 randomly selected oysters, 33 each from two baskets and 34 from the third. The random sample was obtained by thoroughly stirring all oysters in each tray, then removing a portion of the pile estimated to contain approximately 33 oysters and measuring from this randomly selected group. Dead and dying oysters were also measured; the latter were fixed in Zenker's solution for subsequent histological study. Boxes were measured and discarded. Associated fauna was removed, enumerated, and preserved. Every other week one live specimen from each basket was fixed for histological study and three specimens from each basket were measured for condition index, making a total of three normal oysters fixed from each station at each sampling, and a pooled total of nine oysters for condition at each sampling. Condition index (C. I.) of oysters was determined by a method outlined by Medcof and Needler (1941) who used the ratio of the dry weight of oyster meat to the volume of shell cavity, multiplied by 1000. In this study the ratio is multiplied by 100.

The fourth basket at each station was designated as a control. Oysters contained therein were left undisturbed except that in July and October 1959, and January 1960 they were measured once along with those in experimental baskets. This was to determine if frequent handling of oysters in experimental baskets had any effect on growth and survival.

Water temperature, salinity, dissolved oxygen, and p^H were determined by standard methods at each station on each visit.

RESULTS

Growth of oysters was measured in terms of increases in length, height, and thickness of shells. Data on growth in shell length from the beginning of the experiment (first week of March) through January 1960, are shown in Table 1. Only the mean values of each set of measurements are given, and the final cumulative percentage increases are calculated.

Table 1. The mean length in mm. of oysters in experimental and control baskets at each station from March 1959 through January 1960

	Hood Canal		Oyster Bay		Willapa Bay	
	Exp.	Control	Exp.	Control	Exp.	Control
Beginning of Experiment	59.8	55.0	56.6	58.2	60.1	59.3
March	61.0		57.6		60.5	
April	64.9		61.2		62.0	
May	65.5		64.8		64.8	
June	70.9		73.7		73.7	
July	76.6	91.2	82.4	99.2	80.1	90.2
August	78.0		85.6		82.4	
September	81.5		92.1		89.2	
October	82.0	98.4	95.1	117.3	89.5	107.4
November	82.2		97.0		86.2	
December	81.2		96.2		88.6	
January	82.8	97.6	92.7	125.6	87.6	104.1
Cumulative percentage increase	38.5%	77.5%	63.8%	115.8%	45.8%	75.5%

As shown in this table, the experimental oysters at the Oyster Bay Station revealed the greatest percentages of increase in shell length. The percentage of shell growth of experimental oysters was next highest at Willapa Bay, followed closely by Hood Canal. At all stations growth shown by increase in shell was greater in the control trays than in the experimental ones. This was probably due to breaking off of fragile new extensions of shell margins by handling.

At all stations, growth ceased by the end of October, after decelerating greatly during that month. From the middle of September until the end of October water temperatures had gradually dropped from approximately 16.0°C to about 12.0°C (Table 3).

Condition indices of the experimental and control oysters are presented in Table 2. Condition indices of oysters at the three stations were virtually the same in March when the study began; Oyster Bay, however, was soon superior to the other two stations. By April 30, the condition index of Oyster Bay oysters reached 13.9 compared to 8.8 at Willapa Bay and 8.7 at the Hood Canal station. During the summer months the C. I. of Oyster Bay oysters remained high, between 13.1 and 15.5, while the Hood Canal and Willapa Bay oysters improved to a level of 10.0 to 11.0. In October the condition indices of the oysters at Oyster Bay and Willapa Bay began to decline while those at Hood Canal remained high, with the year's highest values for this station, 13.1 and 13.0, occurring at the end of October and the middle of December. Temperature and other hydrographic data showed no obvious reason for the C. I. remaining high at Hood Canal.

Table 2. Condition index (C.I.) of experimental and control oysters from three stations

Hood Canal			Oyster Bay			Willapa Bay		
Date	Exp.	Control	Date	Exp.	Control	Date	Exp.	Control
3-18-59	5.2		3-18-59	5.4		3-18-59	5.5	
4- 4-59	6.3		4- 5-59	7.6		4- 5-59	8.7	
4-16-59	7.5		4-16-59	9.6		4-18-59	7.8	
4-30-59	8.7		5- 3-59	13.9		5- 3-59	8.8	
5-28-59	11.1		5-30-59	14.6		5-30-59	9.8	
6-18-59	10.9		6-18-59	15.5		6-16-59	11.0	
7- 2-59	10.8		7- 2-59	13.6		6-30-59	10.5	
7- 7-59		9.1	7- 7-59		15.2	7- 9-59		8.3
7-29-59	9.8		7-29-59	13.4		7-30-59	9.5	
8-12-59	11.3		8-12-59	14.6		8-14-59	10.5	
8-25-59	9.8		8-25-59	13.5		8-26-59	11.0	
9-10-59	11.5		9-10-59	13.1		9-11-59	10.0	
10- 3-59	11.8	8.7	10- 3-59	11.5	9.0	10- 1-59	9.0	6.2
10-15-59	13.1		10-15-59	10.6		10-17-59	7.6	
10-29-59	11.9		10-29-59	10.8		11- 1-59	7.1	
11-11-59	12.3		11-11-59	12.6		11-14-59	8.1	
11-29-59	12.1		12- 1-59	10.0		11-29-59	7.5	
12-17-59	13.0		12-16-59	11.4		12-20-59	9.8	
1- 8-60	11.7	8.5	1- 9-60	10.1	7.3	1-12-60	7.1	5.6
1-22-60	11.0		1-22-60	9.5		1-25-60	7.3	

Table 3. Temperature data for three experimental stations[1] from March to December 1959

Date 1959	Temperature °C		
	H C	O B	W B
March			
1- 4- 9	8.9	9.4	9.4
18-18-21	10.0	8.9	9.4
27-27-28	8.3	8.9	9.4
April			
4- 5- 5	9.4	9.4	10.3
9-11-11	12.2	11.1	11.1
16-16-18	10.6	10.6	11.7
23-23-26	12.8	11.7	12.2
30- 0- 0	11.7		
May			
0- 3- 3		12.2	12.2
7- 7- 9	13.3	14.4	14.4
21-21-23	14.4	13.9	18.3
28-30-30	12.8	17.2	16.7
June			
10-10- 8	15.6	15.6	16.7
18-18-16	15.6	15.6	17.2
24-24-25	15.6	16.7	18.3
0- 0-30			18.8
July			
2- 2- 0	19.4	16.7	
7- 7- 9	17.7	16.1	18.8
21-21-0	23.3	20.6	
29-29-30	18.8	16.9	20.6
August			
5- 5- 7	19.4	20.0	18.8
12-12-14	20.0	17.7	18.3
18-18-20	18.3	17.2	18.3
25-25-26	19.7	18.3	17.7
September			
10-10-11	16.4	16.4	16.1
19-18-17	13.1	16.1	16.1
October			
3- 3- 1	13.6	15.3	15.3
15-15-17	12.2	14.2	14.4
29-29- 0	11.1	12.8	
November			
0- 0- 1			12.8
11-11-14	10.0	10.6	8.6
28- 0-29	9.4		9.4
December			
0- 1- 0		10.0	
17-16-20	8.3	8.6	8.9
January			
8- 9-12	6.4	6.7	5.0

[1] H C, Hood Canal; O B, Oyster Bay; W B, Willapa Bay

The C. I. of controls was approximately the same as that of the experimental oysters in July. In subsequent measurements, however, control oysters showed much lower condition index than the experimental ones. This was probably due to overcrowding of oysters in the control baskets, as a result of rapid growth, low mortality, and lack of removal of oysters for condition index and histological study. Sixty oysters were removed from the control basket at each station during the first week of October to alleviate crowding. These were placed on the bottom in nearby areas and will be compared in subsequent periods to the float oysters for growth, condition, and survival.

Mortalities of both experimental and control oysters were surprisingly low at all stations. A Chi-square test indicated that the survival of the experimental oysters between stations was not significantly different at the 5 per cent level. The same result was obtained for the control oysters between stations, but a further Chi-square test revealed that the mortality was significantly greater at the 5 per cent level in the control tray at the Oyster Bay Station than in the experimental trays at the same location. No significant difference was found when the total survival data for the experimental oysters were pooled and tested by Chi-square against the pooled data for the controls. Adjusted cumulative mortalities, based on mortalities less those oysters removed from the trays for condition index determination and histological studies, in the experimental trays were 2.6 per cent at Willapa Bay, and 4.1 per cent at both the Oyster Bay and Hood Canal stations. The pooled cumulative mortality of the experimental oysters at all three stations was 3.5 per cent. Mortalities in the control trays were 2.8, 4.7, and 7.1 per cent at the Willapa Bay, Hood Canal, and Oyster Bay stations, respectively.

DISCUSSIONS AND CONCLUSIONS

From the first year of this study it has been demonstrated that growth in Crassostrea gigas in Washington waters occurs largely during the summer months; mortality rates are extremely low under the conditions of the present study. The growth data contrasts with that reported by Woelke (1959) who found growth to be more or less continuous during the year at Case Inlet. Of the three locations studied, growth was best at Oyster Bay and next best in Willapa Bay; survival was greatest in the Willapa Bay station. Woelke (1959) found growth to be better in Willapa Bay than in southern Puget Sound, but condition index at Hood Canal has been superior to the C. I. in the Willapa Bay station throughout most of the study and better than in Oyster Bay from October through December. It should be pointed out, however, that parts of Willapa Bay are suitable for growth, and other areas for fattening, with the Nahcotta station selected as intermediate between the two extremes. Because of this, these data probably do not reflect either best condition or best growth for Willapa Bay.

The relationship between growth and handling is of particular

interest because of possible use to the industry. Although handling was shown to affect growth as measured by increases in shell length, it did not appear to increase mortality.

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GROWTH OF THE PACIFIC OYSTER CRASSOSTREA GIGAS IN THE WATERS OF WASHINGTON STATE

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ABSTRACT

Seasonal growth, year to year growth variation in a single area, growth relative to tidal height, and growth between areas of Crassostrea gigas as determined from experimental plantings are reported. The possibility of predicting the market size of oysters after six months growth is suggested. Growth on commercial beds is described by area from data collected in annual industry-wide growth surveys.

INTRODUCTION

Growth of the Pacific oyster, Crassostrea gigas, in Washington state has been systematically studied on an industry-wide basis only since 1956. Seki (1937) followed monthly growth of Crassostrea gigas in three areas of Japan. Quayle (1951) reported seasonal growth of stunted specimens of Crassostrea gigas moved to a more favorable environment. Thomson (1952) followed the growth of this oyster in experimental plantings in Australia. Woelke (1955) described growth of a variety of Crassostrea gigas (the Kumamoto oyster) in Washington waters in a report on the introduction of this oyster to the Pacific Coast.

This report summarizes data collected from two sources on growth of Pacific oysters in Washington. First is a series of studies designed to evaluate seasonal growth, year to year growth variations in one area, growth relative to tidal level, and growth variation between areas. The second source is data collected in annual industry-wide surveys measuring growth, mortality and "fatness" of oysters on commercial beds.

METHODS

Measurement of oyster size is made by two methods, linear and volumetric. Quayle (1951) measured length, width, depth and volume in studying oyster growth. He observed, "Because of irregular shape of the Pacific oyster and the high degree of shell fluting, consistently accurate linear measurements are difficult to make." Beaven (1952) recognizes the simplicity of volume measurements but objects to their use because of problems arising from small oysters, spat, mussels etc.

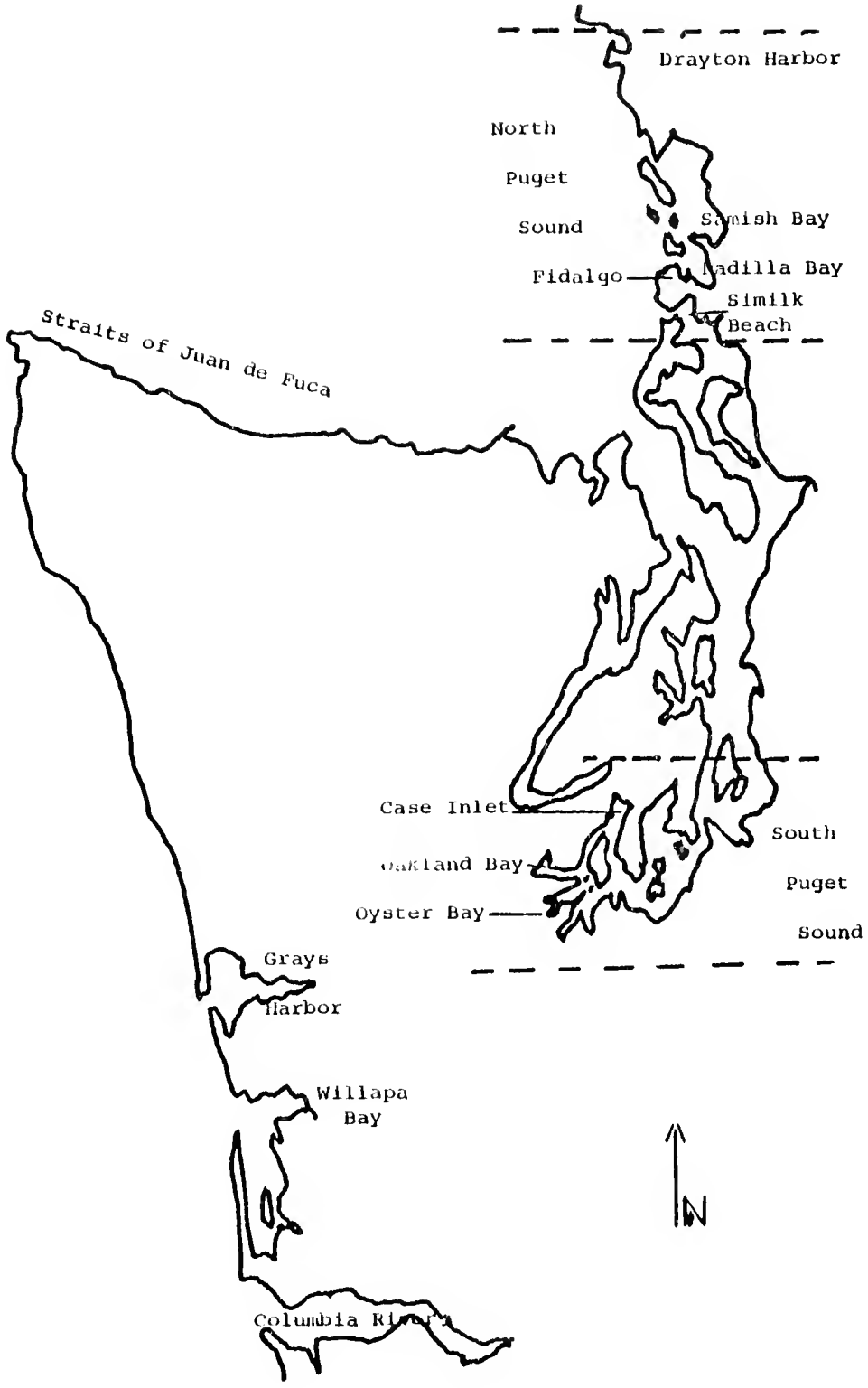

Fig. 1. Principal oystering areas in Washington state.

attached to the oysters. Hopkins and Menzel (1952) stress the value of a volumetric measurement which can be related to net yield. Butler (1952a, 1952b, 1953) uses volume measurements in growth studies and points out (1952b) the need of using oysters of common heredity and known age and avoiding mixed year classes.

All data in this report are from volume measurements. After removal of all organisms, volume is measured in the laboratory by subtracting weight in water from weight in air and in the field by water displacement. Increased speed and reduced measuring error (less than 0.5 cc per oyster in samples of 10 or more) are achieved by processing an entire sample in one measurement rather than measuring individual oysters.

Intertidal cultivation and limited reproduction in most Washington oystering areas eliminate the serious fouling problems found objectionable by Beaven. As a result of the unique method by which the Pacific Coast oyster industry replenishes most of its oyster stocks, i.e., annual importation of seed from a common stock in Japan, the problems of known heredity or mixed year classes are absent.

The data reported have been collected from oysters grown on the bottom in the intertidal zone. The diurnal tide range in most Puget Sound areas is about 14 feet. In coastal areas (Grays Harbor and Willapa Bay) diurnal tide range is about 10 feet. Experimental plantings, as well as most commercial beds, are located between one foot below and five feet above mean lower low water. All sampling is done at low tide when the beds are exposed and are easily accessible.

EXPERIMENTAL PLANTINGS

Seasonal growth as determined by repeated measurement of the same oysters (less mortalities) over a 2+ year time period in Case Inlet is presented in Table 1. Average temperatures and salinities for the time period preceding each growth observation are also included in the table. While the time intervals between data collection were variable (generally 2-3 months), growth occurred within each interval. The greatest per cent growth occurred during periods of warmer water (average of about 18°C), and higher salinities (27.5 - 30.6 o/oo). Less growth occurred during lower temperatures (down to 6.4°C) and reduced salinities (20.2 o/oo). While these data disagree in part with Quayle (1951), who indicates cessation of shell growth at about 9°C, the same general trends are evident in both studies. From Australia, Thomson (1952) reports growth during all seasons (temperature range 1.1 - 25.6°C) though at a reduced rate during periods of low temperature.

To measure growth variations on a long-term basis, representative samples are collected quarterly from our experimental beds and brought to the laboratory for volume measurement. Growth at the same

Table 1. Seasonal growth of the Pacific oyster in Case Inlet - 1952 planting

Date	Age in months	Ave. size in cc (*)	Growth increment in cc	Per cent increase	Ave.temp ° C**	Ave. sal. o/oo **
4/22/52	0	1.2 (100)				
9/12/52	5	8.5 (91)	7.26	590.2	18.5	27.9
11/2/52	7	14.4 (89)	5.89	69.3	12.5	30.6
1/14/53	9	16.6 (87)	2.22	15.4	9.6	20.2
4/30/53	12	20.6 (85)	4.03	24.2	8.4	20.5
7/29/53	15	38.8 (49)	18.16	89.1	18.6	27.5
11/22/53	18	64.0 (61)	25.22	65.0	12.6	30.2
1/31/54	21	77.2 (57)	13.16	20.5	6.4	22.9
6/30/54	26	92.5 (40)	15.33	19.8	13.6	22.1

* Number of oysters measured.

** Average of samples collected during time period preceding date of growth observation.

tide level in Case Inlet from 1952 to 1958 by year and year class shows wide fluctuations (Table 2). In the seven years covered, the average size of oysters at 15 months has varied from 27.3 cc to 80.7 cc. During excellent growth years the size at 15 months has been greater than that of other years' plantings at 27 months of age.

This point is further emphasized by comparing the per cent growth of the various plantings. First year growth has ranged from 192% to 2,126%, second year from 114% to 164%. Limited data collected on 2- to 3-, and 3- to 4-year-olds do not permit comparison of per cent growth on the older oysters.

It is demonstrated that a poor growth year is reflected by per cent growth of all year classes on the beds; therefore, routine sampling of a single age class adequately detects changes that occur, younger oysters being the more sensitive measure of change. Annual growth declined between 1952 and 1955, and increased markedly from 1955 to 1958.

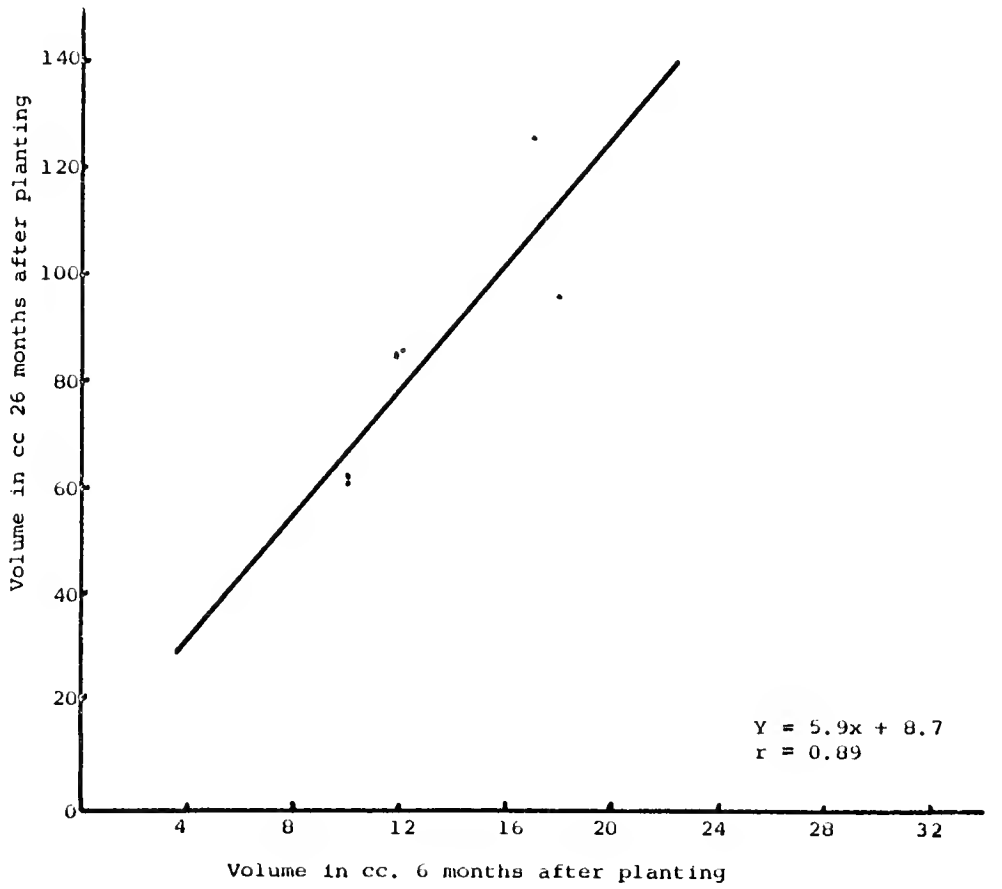

Fig. 2. Six month vs. 26 month size of Pacific oysters.

Table 2. Annual growth variation of Pacific oysters in Case Inlet 1952 thru 1958

Year of observation	Year class	Age	Ave. size cc (*)	Per cent growth
1952	1952	0 +	8.5 (91)	
	1951	1 +	80.7 (20)	----
1953	1953	0 +	15.7 (42)	
	1952	1 +	38.8 (49)	357
	1951	2 +	--	----
1954	1954	0 +	--	
	1953	1 +	45.7 (31)	192
	1952	2 +	90.0 (25)	132
	1951	3 +	166.8 (20)	
1955	1955	0 +	5.3 (41)	
	1954	1 +	52.0 (22)	
	1953	2 +	103.5 (35)	127
1956	1956	0 +	2.0 (75)	
	1955	1 +	29.3 (15)	413
	1954	2 +	111.3 (35)	114
1957	1957	0 +	3.0 (59)	
	1956	1 +	27.3 (20)	1,263
	1955	2 +	74.7 (15)	155
	1954	3 +	179.1 (17)	61
1958	1958	0 +	6.5 (31)	2,126
	1957	1 +	65.9 (22)	164
	1956	2 +	71.9 (21)	77
	1955	3 +	132.1 (21)	23
	1954	4 +	221.1 (19)	

* Number of oysters measured.

From industry's standpoint a very practical application of growth data is indicated in Figure 2. In this figure the 6-month size is plotted against the 26-month size of oyster seed planted on our experimental beds for six successive years. These data suggest the possibility of using the size at six months to predict the size of oysters at harvest age. With an r value of 0.89, a strong correlation is indicated at the 5 per cent level. Within general parameters these data may provide a useful tool for oyster farmers. Whether this pattern is peculiar to our experimental beds has not yet been determined.

It seems reasonable to assume that where oysters are intertidally cultured the feeding time of the population is limited by the tidal level at which they are planted, which in turn might be expected to materially affect growth of the oysters. Data in Table 3 were collected from seed of a common stock planted at the +2.8, +1.3 and -0.9 foot tide levels in Case Inlet. At 26 months after planting, the average sizes were 80.4 cc, 104.7 cc and 118.8 cc in order of decreasing tidal height. Oysters planted only 3.7 feet lower in the intertidal zone were nearly 50 per cent larger at 26 months.

Table 3. Pacific oyster growth relative to tidal height in Case Inlet

Tide level*	Date planted	Date harvested	Age in months	Ave. size in cc (**)
+2.8	4/23/52	6/30/54	26	80.4 (17)
+1.3	4/23/52	6/30/54	26	104.7 (14)
-0.9	4/23/52	6/30/54	26	118.8 (31)

* Refferred to mean lower low water
** Number of oysters measured

In 1952 a study was begun to measure the growth of a common seed stock in several of the principal oyster growing areas of Washington at about the same tidal level (plus 2.5 feet). In Table 4 the accumulated growth at 28 months of age is presented for the various areas. Sizes range from 57 cc to 131 cc. The poorest growth was in Oakland Bay and the best in Willapa Bay

Table 4. Growth of 1952 year class Pacific oysters in five different areas

Area	Size in cc at 28 mos. (2+ years)
Case Inlet	90 (62)*
Oakland Bay	57 (22)
Oyster Bay	109 (17)
Samish Bay	79 (26)
Willapa Bay	131 (23)

* Number of oysters measured.

GROWTH ON COMMERCIAL BEDS

Extension of growth data collection from experimental plots to industry-wide measurements was begun in 1956. These data were collected on an annual survey basis covering about 70 per cent of the major oyster beds and 90 per cent of the general oyster-producing areas of the state. Surveys have been conducted on the last two or three daylight lower low tide series of the year (August and September). All year classes present on the beds in each bay were sampled and evaluated. Between 45 and 50 separate beds in the four principal oyster-growing areas of the state (norther Puget Sound, southern Puget Sound, Grays Harbor and Willapa Bay) have been sampled each year. The same areas on the same beds were sampled in each survey to provide continuity of comparable data.

Each sample consisted of 20 or more randomly selected oysters of each year class. In areas of natural reproduction, "wild oysters" were excluded from the samples. Volume measurements were made in the laboratory.

The results of these surveys are summarized by year, year class and area in Table 5. Grouping of data by areas in this table masks the internal variations; however, the average values in general (with the exception of Drayton Harbor in northern Puget Sound and Oakland Bay in southern Puget Sound)* demonstrate oyster growth in the four areas.

*Because of reduced growth and fattening, many northern Puget Sound growers did not plant seed in 1957 and 1958. The absence of plantings on much of the poorer ground gives an apparent improvement in growth in these years. Oakland Bay, in southern Puget Sound, has been a poor growing area throughout the period covered by this study.

Table 5. Growth of the Pacific oyster as determined by annual statewide surveys

Year observed	Age	Average vol. in cc				Statewide average	
		North Puget Sound	South Puget Sound	Grays Harbor	Willapa Bay	N	Ave. Size cc
1956	0+	6.2	4.8	4.4	8.9	2,125	5.3
	1+	22.9	37.0	36.0	71.7	985	38.1
	2+	70.2	104.7	89.0	120.8	745	97.9
	3+	105.5		129.9		173	116.8
	4+	127.8				103	124.3
1957	0+	5.6	5.8	6.3	21.0	1,244	11.9
	1+	33.1	45.4	44.2	77.6	633	47.4
	2+	66.2	80.4	95.6	134.6	345	103.2
	3+	105.9	179.1			64	118.5
	4+	112.5				34	112.5
1958	0+	6.8*	5.8	8.1	12.9	1,350	8.9
	1+	58.9*	52.5	81.2	75.6	719	64.0
	2+	80.6	104.3	122.8	133.1	816	104.9
	3+	122.3	132.1			216	108.2
	4+	143.1	221.1			78	154.9

* These data reflect the planting of 1957 and 1958 Japanese seed on optimum ground available in the area.

Using the 2+ age oysters for comparison, Willapa Bay has consistently had the best growth and northern Puget Sound the poorest. Grays Harbor and southern Puget Sound have been alternating at second place. Using 1+ oysters for comparison, since they seem to provide the best short-term measure of growth, Willapa Bay seems to be staying about the same, with northern Puget Sound, southern Puget Sound and Grays Harbor improving. It must be remembered that improvement in northern Puget Sound reflects the cessation of seed plantings on all except the two best beds. During three years of observation there appears to be a general increase in growth in all areas except northern Puget Sound from 1956 to 1958.

In Table 6, data on size of oysters in all surveys to date are summarized by year class. Since the Pacific oyster is generally harvested between the second and third year after planting, the average market size lies between 102 and 113 cc.

Table 6. Pacific oyster average size by age as determined in statewide surveys, 1956-1958

Age	Average size in cc
0+	8.1
1+	48.6
2+	101.8
3+	113.0
4+	133.5

SUMMARY

1. Growth of the Pacific oyster in Case Inlet appears to be a continuous process with no evidence of winter hibernation.

2. Between 1952 and 1958, year to year growth fluctuations have been such that the size at 15 months has varied from 27.3 cc to 80.7 cc in Case Inlet.

3. Percentage growth for the first year after planting has ranged from 192% to 2,126%, and from 114% to 164% in the second year.

4. In our Case Inlet study area growth declined between 1952 and 1955 and has been steadily increasing during the 1955-1958 period.

5. There are indications that it may be possible to predict size of the Pacific oyster at 26 months from their size 6 months after planting.

6. Growth of oysters planted intertidally between +2.8 and -0.9 feet increases inversely with tidal level.

7. Accumulated growth on oysters 28 months of age at about the same tide level in different areas has varied between 57 cc and 131 cc.

8. Based on annual industry-wide oyster growth surveys, Willapa Bay is the area of best growth, northern Puget Sound the poorest, with Grays Harbor and southern Puget Sound alternating between second and third.

9. With the exception of northern Puget Sound, growth generally increased between 1956 and 1958.

10. Based on the age-size relationship, the Pacific oyster is usually harvested when 102 cc to 113 cc in size.

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SELECTION AND EVALUATION OF A METHOD FOR QUANTITATIVE MEASUREMENT OF OYSTER CONDITION

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ABSTRACT

Fatness is one of the most important considerations of oyster culture. The "Grave method of condition factor index determination" was used initially but found to lack reproducibility. Two improved measuring techniques are presented plus an evaluation of the modified procedure as used by the Washington State Department of Fisheries.

Fatness is one of the most important considerations in evaluation of oyster culture by either commercial operators or biologists. Researchers, recognizing the need, have devised various methods for evaluation of oyster fatness. This paper presents and evaluates the method found most suitable by the Washington Department of Fisheries.

Grave (1912) describes a method for measuring oyster condition. This method consists of dropping unopened oysters into an overflow container and measuring the water displaced. The same procedure is followed with the empty shells and the volume of the shell cavity is then calculated. Volume of the drained meats is measured by water displacement. "Condition factor index" is the ratio of meat volume to volume of the shell cavity:

$$C.F. = \frac{\text{Vol Meat}}{\text{Vol. Shell Cavity}} \times 100$$

In monitoring oyster condition in Washington state, the "Grave method" was initially used. Subsequently it was found to lack reproducibility in volumetric measurement and in estimation of meat quantity.

Quayle (1950), measuring volumetric growth of the Pacific oyster (Crassostrea gigas), utilized a method borrowed from J. C. Medcof involving an application of Archimedes' principle. Oysters are weighed first in air, then in water. The difference between the two weights equals volume. To eliminate errors present in wet-meat volume measurements, Higgins (1938) suggests oven drying of meats at 100°C, and Galtsoff et al. (1947) propose initial drying at 50°C and final drying at 100°C. Haydu (personal communication) found variation in drying at 100°C due to removal of varying amounts of chemically-bound water that

did not occur with initial drying at 50°C. More recently, Korringa (1955) suggests further improvement in drying meats by use of toluene distillation.

By combining the weight-in-air, weight-in-water method with oven drying of the meat, we have evolved a procedure for measurement of fatness of Crassostrea gigas and Ostrea lurida in Washington state. Sample size is based on size of adult oysters, reproducibility of measurements, and variation in meat quality in oysters. In the case of the large Pacific oyster (average adult size 125 cc volume) 20 oysters are used, giving a total sample size of 2500 cc. In the case of the small Olympia oysters (average adult size 4.0 cc volume), 50 oysters were used giving a total sample size of 200 cc. In both cases, amount of variation is only about ± 0.6 condition index unit at the 95% confidence level (Table 1).

The following procedures* are followed:

1. Oysters are carefully cleaned. Those gaping or having chipped edges are rejected.

2. The oysters are held in running sea water for at least one hour prior to weighing to insure that no air is trapped between the valves.

3. Volume is determined and oysters are opened, care being taken not to break the shells or leave meat attached to the shells. Then the volume of the shell cavity is determined by subtracting volume of shell from total volume.

4. The meats are placed in a tarred aluminum foil tray and dried in a forced air oven for two days at 50°C and two days at 100°C (producing constant weight) and weighed.

5. Calculations:

   A. $\frac{\text{Dry weight of meats in grams}}{\text{Volume of shell cavity in cc}} \times 100 = \text{Condition Index}$

In measuring oysters, considerable error can be introduced if there is air between the valves of the whole oysters (reason for step 2 in the procedure). The weight of the whole oysters in water should be equal to, or slightly greater than, the shells in water if there is no air trapped in the whole oyster.

* When routinely sampling the same age oysters from an established station, this procedure provides data which can be used for growth evaluation. The procedure and calculations can be shortened if growth data are not desired.

Table 1. Results of replicate analyses of multiple lots of oysters

Crassostrea gigas

Area: Oakland Bay
Date: July 5, 1956
Ave. vol. per oyster: 100 cc

C.I.	
7.8	S.D. = 0.40
7.2	t for N-1 95% C.I.
8.0	7.8 ± 0.6
8.1	

Area: Oakland Bay
Date: July 12, 1956
Ave. vol. per oyster: 100 cc

C.I.	
7.6	
7.5	
7.1	S.D. = 0.64
7.1	t for N-1 95% C.I.
6.2	7.1 ± 0.7

Area: Oakland Bay
Date: July 19, 1956
Ave. vol. per oyster: 100 cc

C.I.	
7.3	
7.0	S.D. = 0.20
7.1	t for N-1 95% C.I.
6.7	7.0 ± 0.3

Area: Oakland Bay
Date: July 25, 1956
Ave. vol. per oyster: 100 cc

C.I.	
7.1	
7.7	S.D. = 0.66
6.3	t for N-1 95% C.I.
7.8	7.0 ± 1.0

Area: North Bay
Date: August 8, 1957
Ave. vol. per oyster: 125 cc

C.I.	
13.3	
15.0	S.D. = 1.10
12.3	t for N-1 95% C.I.
13.4	13.5 ± 1.7

Ostrea lurida

Area: Oyster Bay
Date: July 11, 1957
Ave. vol. per oyster: 4.0 cc

C.I.	
7.2	S.D. = 0.30
6.7	t for N-1 95% C.I.
6.6	6.8 ± 0.5
6.6	

Area: Oyster Bay
Date: August 8, 1957
Ave. vol. per oyster: 4.0 cc

C.I.	
17.3	
17.4	S.D. = 0.60
16.9	t for N-1 95% C.I.
16.1	16.8 ± 0.6
17.2	
16.0	

Reliability of the method is affected by two factors, accuracy of techniques of measurement and sampling error, and variation of oysters on the beds. To check the accuracy of the measuring method repeated measurements of the same oysters and objects of known volume were made, which demonstrated that measurements are both accurate and reproducible. The problem of sampling error, or adequacy of sampling, is more complex and has no easy solution. To minimize this, field samples are collected at set locations. Table 1 presents data from several sets of Pacific and Olympia oyster samples. Each set was collected from the same area (bed) on the same date. The various sets were collected from known areas of good and poor oysters. These data demonstrate the combined effect of accuracy of method and sampling error.

The standard deviations of Crassostrea gigas samples indicate consistency of results when oyster condition is poor with increased variation in better oysters. In the case of Ostrea lurida the deviation is low for both poor and good oysters. The failure of the method to distinguish whether a high condition factor index is due to fatness or spawn poses no serious problem as presence of spawn is easily determined by eye. To date the relationship between condition factor index and commercial yield has not been determined.

The method provides a rapid and reproducible way of evaluating oyster condition. The actual time required to process a sample is less than any method using the overflow technique, although the introduction of oven drying creates a four-day waiting period. The procedure has been utilized for five years by the Washington Department of Fisheries for measurement of oyster condition and has proven to be a valuable tool (Westley 1959). Information collected permits detection of seasonal variation, long range changes, and effects of extreme weather conditions. The method is also being used in assessing oyster condition in laboratory and field bio-assays.

The writer wishes to gratefully acknowledge the help and advice of Dr. D. B. Quayle in setting up this procedure.

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EFFECT OF HYDRAULIC ESCALATOR HARVESTER ON UNDER-SIZE SOFT-SHELL CLAMS

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ABSTRACT

A modified Maryland-type hydraulic escalator shellfish harvester was used at high tide on intertidal beaches in Nova Scotia to determine its effect on soft-shell clams less than 2 inches long. The boat was equipped with a propeller guard to prevent bottom scouring and three types of experiments were carried out with small, marked clams: (1) Dead clams were released in the scoop to determine their scatter pattern after passing through the harvester. (2) Plots of planted dead clams were dug through to discover breakage rates and distribution. (3) Plots of planted living clams were dug through to see how the harvester affects their distribution and ability to re-establish themselves. Results were observed on the dry beach at low tides.

Most of the small clams sifted through the mesh of the escalator belt before they reached the surface of the water. In spite of strong currents from harvester jets and the boat propeller, 90% of the clams were returned to the harvester track within 75 or 100 feet of the place where they entered the harvester. Soil is heavy and settled first in the track. Clams are lighter and were deposited on the soil surface, not buried and smothered. The harvester broke 7 to 10% of the living clams but the rest dug in again quickly. Because damage was so small compared with that caused by conventional clam hoes we think production would be improved if hydraulic escalator harvesters were used.

INTRODUCTION

Since 1954 the Fisheries Research Board of Canada, with support from Industrial Development Service of the Department of Fisheries, has been modifying the Maryland-type hydraulic escalator clam harvester (Manning 1957). This machine works under water from a boat containing a pump supplying powerful jets of water to wash shellfish from the soil into the harvester's digging head, or scoop, and then onto the escalator belt which brings them up to the boat at the surface. We were looking for ways of increasing productivity of stocks of soft-shell clams (Mya arenaria) which are depressed by intensive fishing and by wasteful methods of fishing.

Our early work with the harvester was described by Dickie and MacPhail (1957). Further modifications have been made in design of

the scoop, and a larger pump installed (750 gallons per minute at 40 pounds pressure per square inch). Harvester performance has greatly improved. Now it takes more than 95% of marketable-size, soft-shell clams in its path and, when fine-mesh escalator belts are used, it takes small clams (3/4" to 1") with this same efficiency. It has also been used successfully in harvesting quahaugs and bar clams from beds up to 6 feet deep. In 1959 a new-type head, designed by Mr. J. S. MacPhail and Mr. H. Y. Brownrigg of our Station, proved highly efficient for fishing bedding-size and adult oysters from shallow grounds and for cleaning oyster beds.

The Department of Fisheries has approved use of harvesters for fishing quahaugs and bar clams from public beds and for fishing oysters from leased grounds, but has hesitated to legalize it for fishing soft-shell clams. We knew too little about how it might affect productivity of clam grounds and economics of industry. The Fisheries Research Board carried out experiments described here to provide a partial basis for regulatory policy.

The work was done in May 1958 with M. B. Cyprina (Skipper Earl Durkee). I am grateful to Mr. Durkee and Mr. MacPhail for co-operation, to Dr. L. M. Dickie who initiated work with the harvester, to my Director, Dr. Hart, who fostered the project, and to Mr. L. S. Bradbury, Director of Industrial Development Service, Department of Fisheries, for providing funds.

PROCEDURE

General

Three types of experiments were carried out with sub-legal size clams--release of dead clams in the harvester scoop to determine scatter patterns of clams and guide designing of other experiments, diggings through planted plots of dead clams to discover breakage rates and distributions of under-size clams that might be expected in ordinary digging and, finally, diggings through planted plots of live clams to see how harvesters affect ability of under-size clams to re-establish themselves.

Clam Harbour, Nova Scotia, was chosen as a work site because it has wide expanses of nearly level, uniform-textured, sandy flats. In 1958 these flats supported only sparse populations of clams, which was an important advantage because it reduced catch-culling efforts.

Minimum legal length for market clams in eastern Canada is 2 inches. Our interest was focused on smaller sizes because harvester effects on them would influence recruitment of marketable stocks. Accordingly, we used only clams 1 1/4 to 1 3/4 inches long in all experiments. It is difficult to do marking experiments with smaller animals and this is the sole reason for choice of this size. Shells were marked with Volger's ink for identification.

The harvester was equipped with an escalator belt with 1-inch-square mesh, which is commonly used for commercial harvesting, and the scoop lip was adjusted to dig 15 inches into bottom as in regular fishing for commercial-size clams. A propeller guard (Manning 1957) was used to prevent bottom scouring and in other ways the harvester was run as in commercial fishing. M. B. Cyprina draws 2 feet of water and the shallowest depth she will work in satisfactorily is 28 inches. This limits periods of operation on intertidal flats.

Scatter Patterns

Clams were killed by soaking them overnight in a 10% solution of formaldehyde. Next day at high tide the harvester was set on a straight course and run for 200 feet. Then 110 clams were released deep in the harvester scoop just above and behind the lip by dumping them through a 6-foot pipe (diameter 5 inches) whose upper end was at gunwale level. A buoy was cast overboard when clams were dumped to mark the release point and the harvester was kept in operation for another 100 feet. Experimental clams probably experienced full effects of propeller blast and hydraulic jet currents as do under-size clams in normal commercial fishing. This same release operation was carried cut at three water depths (30, 40 and 50 inches) over intertidal flats.

About a third of the animals came up on the escalator belt and dropped off over the end. The rest apparently passed through belt meshes and dropped back to bottom before reaching the water surface. In contrast, many large native clams were brought up.

At the next low tide, plots were visited and distribution of marked clams on the dry beach was simply recorded because, being dead, they did not burrow in. Gulls were everywhere and some were feeding on damaged native clams left exposed in shallow trenches or tracks which marked harvester paths. So far as could be judged they had not touched experimental animals. These retained a strong odour of formaldehyde but it is doubtful that gulls would detect this.

Recovered marked clams were classified as buried or exposed on beach surface and as occurring in harvester track or on shoulders of undisturbed flat beside tracks. Besides this, clams were classified as having broken or intact shells. They were also classified according to distance transported from point of release. For this classification, 25-foot-long zones were marked off along digger tracks in front of and behind release points which were identified by buoys.

If any part of a clam's shell was showing above sand that animal was classed as surfacing. We searched for buried clams only in harvester tracks where soil was loose. Elsewhere the surface was firm and undisturbed and without soil deposits that could bury clams. Track soil was usually soft enough to probe with the hands for the first two hours after tidal exposure. When it was too firm it was turned with conventional clam hoes. Both methods of search could have damaged

Table 1. Distribution counts of small, dead clams released through a shoot into harvester scoop and recovered on dry beach at next low tide. "T" indicates clams found in harvester track. "S" indicates clams found on the shoulders beside the track.

Water depth when clams were released (inches)		30				50			
Disposition of clams		Surface		Buried		Surface		Buried	
		T	S	T	S	T	S	T	S
Distance behind point of release (feet)	-50-0	2	0	0	0	3	0	0	0
	0-25	11	0	0	0	10	5	0	0
	25-50	7	1	0	0	64	11	0	0
	50-75	15	3	0	0	1	3	0	0
	75-100	22	10	0	0	0	0	0	0
	100-125	30	1	0	0	0	0	0	0
No. Recovered		87	15	0	0	78	19	0	0
Distribution of recoveries		85%	15%	0%	0%	80%	20%	0%	0%
No. recovered / No. released		(102/110)		93%		(97/110)		88%	
No. broken / No. recovered		(3/102)		3%		(2/97)		2%	

Table 2. Distribution counts of small, dead, planted clams recovered on beach at next low tide after harvester cut through plots. "T" indicates clams found in harvester track. "S" indicates clams found on shoulders beside track.

Water depth when plot was dug (inches)		40				40			
Disposition of clams		Surface		Buried		Surface		Buried	
		T	S	T	S	T	S	T	S
Distance behind plot (feet)	-50-0	3	0	0	0	1	0	0	0
	0-25	84	8	1	0	14	7	1	0
	25-50	41	10	4	0	163	7	10	0
	50-75	111	0	1	0	0	5	0	0
No. recovered		239	18	6	0	178	19	11	0
Distribution of recoveries		91%	7%	2%	0%	86%	9%	5%	0%
$\frac{\text{No. recovered}}{\text{No. in path of digger}}$		$\frac{(263)}{(158)}$		166%		$\frac{(208)}{(158)}$		132%	
Washouts (difference)		105		66%		150		32%	
$\frac{\text{No. broken}}{\text{No. recovered}}$		$\frac{(14)}{(263)}$		5%		$\frac{(18)}{(208)}$		9%	

some of the recovered clams but very few were buried and most were so close to surface that numbers broken in searching must have been negligible. Looseness of track soil persisted for more than a week.

Results of tests conducted at 30 and 50 inches appear in Table 1. Those for 40 inches were essentially like those for 50.

Digging Planted Dead Clams

Formalin-killed clams were planted, 9 per square foot, in plots measuring 7 by 15 feet. Long axes of plots were set at right angles to prevailing directions of tidal currents across the flats. For precision in density of planting we used a grid frame, about 3 by 5 feet, strung lengthwise and crosswise with cod-line to give 4-inch squares. In planting, this frame was laid on the beach, a hole was made for each square with a wooden spike and a clam placed, siphon-end up, in the hole. Upper ends of clams were 2 to 3 inches below surface, which is normal for clams of this size (Medcof 1950). Corners of plots were marked with stakes.

Next day at high tide the harvester was set in operation on a straight course 200 feet from each plot. Digging was continued up to and through each plot and 100 feet beyond. Few planted clams were brought to water surface. At next low tide clams that could be found on the dry flat were classified as before.

Harvester tracks measured 50 to 75 inches wide and their surfaces averaged 4 to 6 inches below levels of adjacent beach. Apparently crumbling of track shoulders and erosion by ebb-tide currents extended track widths sometimes to twice or more the original widths (digger scoop 30 inches wide). Where tracks had cut through plots, several clams were found in various stages of being washed from planted positions along shoulder edges. Approximate numbers of washouts were estimated roughly from differences between the expected number of clams (158) in direct path of harvester and the numbers actually recovered (Table 2). In spite of erosion there were few buried clams in the track.

Results of two diggings at intermediate depths (40 inches) appear in Table 2. Meats were missing from four of the 18 broken clams found in the second plot (Table 2) as though they had been eaten by gulls.

Digging Planted Live Clams

Procedures were as in previous tests except that living clams were used. These were dug manually at low tide, marked, and kept in flowing water until next day's planting. Beach soils were firmer in some plots than in others. Depths of water over plots, when they were dug, varied but were mostly less than 40 inches. Few marked clams were brought to water surface by the escalator belt.

After digging one plot we rowed a small boat over the track. Tidal currents cleared the water soon after harvester operation ceased and marked clams could be seen lying on sandy bottom. All had closed shells but in a few minutes many extended siphon and foot and within a quarter of an hour from the time they had been disturbed most had buried or partly buried themselves. This observation agrees with others, for example Mead's (1901), that clams of this size re-establish themselves quickly.

We visited the flats at following low tides before gulls could attack the experimental clams.

Table 3 reports results of four tests. By surface-picking and digging it was impossible to recover all marked clams disturbed by the digger. Sometimes more than 158 (the number directly in harvester path) were recovered, sometimes fewer. This is understandable because we know from experience that careful digging of a plot with hand tools seldom recovers more than 80% of market-size clams in it.

DISCUSSION

The propeller guard prevented scouring of bottom which was a problem in early trials (Dickie and MacPhail 1957).

We assume that observed effects on clams 1 1/4 to 1 3/4 inches long are typical of effects on all sub-legal sizes. In digging in 30 inches of water they were seldom carried more than 100 feet (Table 1) and in 50-inch depths seldom more than 75 feet. All tables show that most clams are carried backward by water currents set up by the harvester and deposited "behind" the place where they entered the harvester. There are exceptions. After the harvester passes, tidal currents in digger tracks, which form shallow channels, apparently carry some clams "ahead" as much as 50 feet. Such distributions are indicated by negative values in the tables. The general result of all disturbances is that most clams come to rest near their former homes where they should find equally good conditions.

The harvester deposited about 90% of test clams on the loose track soil and live ones burrowed in quickly. Ebb-tide currents in tracks washed some clams out of their plots. Sometimes we visited the flats before they were completely exposed and when this washing-out process was still going on. The flats dried off before some of the washouts had a chance to dig in and counts of surfacing clams were accordingly high (Table 3). This leaves opportunity for attacks by strictly surface-feeding clam enemies. And looseness of track soil might leave buried clams more than ordinarily susceptible to attack by gulls (Medcof 1949), flounders (Medcof and MacPhail 1952a) and clam drills (Medcof and Thurber 1958). It is not believed that this risk is greater than that involved in manual harvesting even though track erosion sometimes affects almost as many more clams as lie in the

Table 3. Distribution counts of small, live, planted clams recovered at next low tide after harvester cut through plots. "T" indicates clams found in harvester track. "S" indicates clams found on shoulders beside track. In these tests clams were buried because they had burrowed into the soil.

Water depth when dug (inches)	34				34				32				40			
Soil type	Soft sand				Soft sand				Firm sand				Firm sand			
Disposition of clams	Surface		Buried		Surface		Buried		Surface		Buried		Surface		Buried	
	T	S	T	S	T	S	T	S	T	S	T	S	T	S	T	S
Distance behind plot (feet) -50-0	29	1	153	0	5	0	0	0	6	1	1	0	1	0	0	0
0-25	1	0	43	0	8	1	142	0	3	7	1	0	1	8	10	0
25-50	0	2	5	0	5	3	41	0	3	2	4	0	11	0	64	0
50-75	1	0	9	0	0	0	0	0	13	0	13	0	0	0	18	0
75-100	0	0	4	0	0	0	0	0	13	0	49	0	4	0	0	0
100-125	0	0	0	0	0	0	0	0	2	0	3	0	0	0	0	0
No. recovered	31	3	214	0	18	4	183	0	40	10	71	0	17	8	92	0
Distribution of recoveries	13%	1%	86%	0%	9%	2%	89%	0%	33%	8%	59%	0%	14%	7%	79%	0%
No. recovered / No. in path of digger	(248/158) 158%				(205/158) 129%				(121/158) 77%				(117/158) 74%			
Washouts (difference)	90 58%				47 29%				obscured				obscured			
No. broken / No. recovered	(8/248) 3%				(18/205) 9%				(17/121) 14%				(14/117) 12%			

harvester's direct path (Tables 2 and 3). Unpublished studies indicate that disturbance enhances clam growth. This effect would offset disadvantages of greater exposures to enemies.

It is obvious, even without making allowance for clam breakage caused by probing in search for disturbed animals, that the total breakage was slight. Table 1 suggests that after clams enter the scoop breakage increases by only 2 to 3%. An equal or slightly higher breakage is caused by the scoop cutting through the soil. This is not easy to estimate because its frequency is masked by the numbers of washouts which do not pass through the harvester. If no allowance is made for these, total breakage works out to about 7% (Table 2). If it were worked out from the number of clams directly in the harvester path (158) and we assumed complete recovery of these, then the breakage would work out to about 10%. Breakages exceeding 10%, like those reported in Table 3, are attributable to damage done experimentally in recovering live clams that burrowed in. However, even Table 3 values seem low compared with breakages caused by hand tools (Medcof and MacPhail 1952b).

Table 2 shows that the escalator harvester buries few clams (less than 5%) and buries these shallowly in soft soil where unbroken live clams can quickly establish themselves. This low burial frequency is attributed to differences in the settlement rates for soil and clams. Apparently almost all bottom soil disturbed by the harvester settles out first (Manning et al. 1959) and in the track. Clams are not much heavier than water and settle out last, landing on top of the soil. Thus, in accounting for damage done by the harvester, burial and smothering seem far less important than shell breakage. The reverse is true for damage caused by hand tools (Needler and Ingalls 1944) where burial and smothering are chiefly responsible for 50% mortalities at every digging. From this it is deduced that if hydraulic harvesting were adopted, damage incidental to digging would be reduced to about 20% of that now regularly inflicted by clam hoes.

The ordinary clam digger with his conventional hoe seldom harvests more than 60% of the marketable clams from the soil he turns (Medcof, unpublished MS report). The other 40% are hidden in soil clods and encourage him to return soon and redig the same ground. Every digging is attended by 50% mortality of the clams left behind. Experiments (MacPhail, unpublished MS report) have shown that the hydraulic digger harvests more than 90% of marketable-size clams in its path. This means that grounds exploited by hydraulic harvesters are likely to be redug at longer intervals than those dug with hoes. This might reduce digger damage to even less than 20% of that now being caused by hoes. Conservationwise our results agree in all essential respects with those reported from Maryland (Manning 1957) where harvesters have been used since 1950.

It is generally accepted (Glude 1951) that clam fishing is the chief of controllable factors regulating clam abundance in ex-

ploited areas. Other data (Needler and Ingalls 1944) show that indirect effects of fishing (damage incidental to digging with clam hoes) are more important in contributing to fishing mortality than direct effects (removal of marketable clams). Our experiments indicate that present indirect fishing mortality rates would be reduced by 80% or more if escalator harvesters were adopted. Such a reduction should substantially increase per-acre yields of clam grounds.

It must not be assumed that such benefits could be brought about in all clam areas. The whole Bay of Fundy region has such high tides that ordinary escalator harvesters could not work profitably. In many outer-coast Nova Scotia harbours tidal amplitudes are suitable but clam beds are too rocky, too small or too intricately shaped to make escalator harvesting practicable. In these areas conventional harvesting methods, or some other type of harvesting yet to be developed, will have to serve. There are many places, however, both on the outer coast of Nova Scotia, and in the Gulf of St. Lawrence, where tides are right, where soil texture is right and where extent and topography of well-stocked clam beds are right for successful escalator harvester operations. The majority of these beds are intertidal and more or less regularly fished, although wastefully, with clam hoes. But there are beds, such as those around Heron Island in Bay of Chaleur, that are permanently submerged and seldom fished. Use of escalator harvesters would be expected to bring about the most conspicuous per-acre yield benefits in these areas because they would then be efficiently fished for the first time. The harvester would be expected to effect smaller per-acre yield improvements in intertidal beds that are now dug. But, because of their vaster area, their improvement would likely mean more to the clam industry than development of the few currently unexploited areas like Heron Island.

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INHERITANCE OF SHELL MARKINGS AND GROWTH IN THE HARD CLAM, VENUS MERCENARIA

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ABSTRACT

Inheritance of Venus mercenaria (Mercenaria mercenaria) notata shell markings was followed through two generations. Offspring from three crosses of unmarked "white" clams were unmarked, while three crosses of white clams with clams having the "notata" marking, produced about half unmarked offspring and about half marked with the reddish-brown zigzag lines, typical of notata subspecies. When two clams with notata shell markings were crossed, about one-fourth of the offspring were unmarked and about half were marked with zigzag lines. The remaining one-fourth were almost solidly reddish-brown, with a light band from the umbo around the lunule, and two other light bands from the umbo to the margin of the shell, dividing it roughly into thirds. This marking is considered typical of clams homozygous for the color factor. The zigzag lines, which are commonly used to identify the notata subspecies, are considered the phenotypic markings of heterozygous clams.

Offspring from fast-growing sibling clams were 60 per cent larger, after 15 months, than offspring from clams randomly selected from wild stock. This suggests that only a few generations would be required to develop fast-growing races of clams.

INTRODUCTION

Advances in the laboratory culture of lamellibranch larvae have made possible the rearing of shellfish of known parentage. Because of these advances, studies of inheritance in shellfish are now possible. Knowledge of the principles of inheritance has permitted great improvements in agriculture in the past and knowledge of the principles of molluscan inheritance may be as beneficial to modern shellfish cultivation in the future.

Because of length of time required and difficulty in rearing larval stages, genetic studies of commercial bivalves are scarce. Interspecific crosses have been reported by Bouchon-Brandely (1883) and Davis (1950). Survival and growth of V. mercenaria, V. campechiensis and their hybrids have been studied in Virginia by Haven and Andrews (1957) and in North Carolina by Chestnut, Fahy and Porter (1957). Chanley (1955) reported that clam larvae from one set of parents grew more rapidly than those from other parents, but dealt only with larvae. Davis (1955) reared five generations of Olympia oysters (Ostrea lurida)

Table 1. Comparison of observed ratios of marked and unmarked offspring with those expected if the notata marking is a simple Mendelian character. The value of X^2 is given with the probability of a higher value of X^2 occurring by chance.

Cross	Observed Ratio		Expected Ratio		X^2	P
	Unmarked	Marked	Unmarked	Marked		
Parent Generation						
Marked* ♀ (A) x unmarked ♂ (A)	248	246	247	247	.008	>.90
Marked* ♀ (A) x marked* ♂ (B)	297	913	302.5	907.5	.133	>.70
F_1 Generation from ♀ (A) x ♂ (A)						
Unmarked ♀ (1) x unmarked ♂ (1)	968	32	1000	0	∞	
Unmarked ♀ (2) x unmarked ♂ (1)	999	1	1000	0	∞	
Unmarked ♀ (3) x unmarked ♂ (1)	999	1	1000	0	∞	
Unmarked ♀ (1) x marked* ♂ (2)	501	499	500	500	.004	.95
Marked* ♀ (4) x unmarked ♂ (1)	510	490	500	500	.400	>.50
Marked* ♀ (4) x marked* ♂ (2)	233	767	250	750	1.541	>.20

* Parent clams, listed as marked, have the typical reddish-brown zigzag lines commonly considered characteristic of the notata subspecies and illustrated as heterozygous in Fig. 1.

in an unsuccessful attempt to develop a strain that would survive New England winters, but there are few reports involving more than one generation.

The purposes of these experiments were (1) to study inheritance of the typical reddish-brown shell markings of V. mercenaria notata, and (2) to determine the feasibility of developing fast-growing races of hard clams by selective breeding.

RESULTS

Inheritance of Notata Markings

The subspecies V. mercenaria notata is easily identified by typical reddish-brown zigzag lines on both valves of the shell (Abbott 1954). It occurs along the east coast from Maine to Florida but is rather uncommon particularly over the northern part of its range (Miner 1950, Morris 1956). This form is rarely seen in Long Island Sound not only because it is uncommon but also because the shell markings are frequently obscured by shell discoloration caused by the substrate.

In studies of the inheritance of notata markings, two original crosses of Long Island Sound clams were involved. In each case female A, displaying notata markings, was used. In the first cross this female was crossed with unmarked male A. The ratio of notata-marked to unmarked offspring was close to the 1 to 1 ratio expected when a heterozygous individual is crossed with a homozygous recessive one (Table 1). When the same female, A, was crossed with male B, also bearing the notata markings, the ratio of marked to unmarked offspring was essentially 3 to 1. This is the expected phenotypic ratio in a cross of two individuals heterozygous for a simple Mendelian character.

Counts were not made until marked and unmarked clams had reached a minimum length of about 5 mm, since the notata markings were not always apparent in smaller clams. Even at this size markings were sometimes faint and difficult to detect. Subsequent counts, however, failed to show any appreciable change from the original ratios.

Offspring from the cross of marked female A with unmarked male A were raised to maturity and used in another series of crosses. Progeny from the female A by male B cross were not yet large enough to give adequate amounts of spawn at this time. Improved techniques in culture methods provided more clams than could be conveniently examined in these crosses and, therefore, random samples of 1,000 were counted.

The first three crosses of the F_1 generation involved unmarked clams (Table 1). Although a few marked clams were found among the offspring, we believe that these crosses actually produced no marked clams. It is possible that they were introduced accidentally from other groups during the handling that was necessary to rear the clams from eggs to more than a year old.

Both F_1 crosses involving a marked clam with an unmarked clam produced offspring roughly in a ratio of 1 marked to 1 unmarked. This is again the expected ratio for a cross of a heterozygous individual with a homozygous recessive.

When two notata-marked clams from the F_1 generation were crossed the offspring bore notata markings in a 3 to 1 ratio as expected in a cross involving heterozygous individuals. In this last cross two types of markings could be recognized (Fig. 1). The most common markings

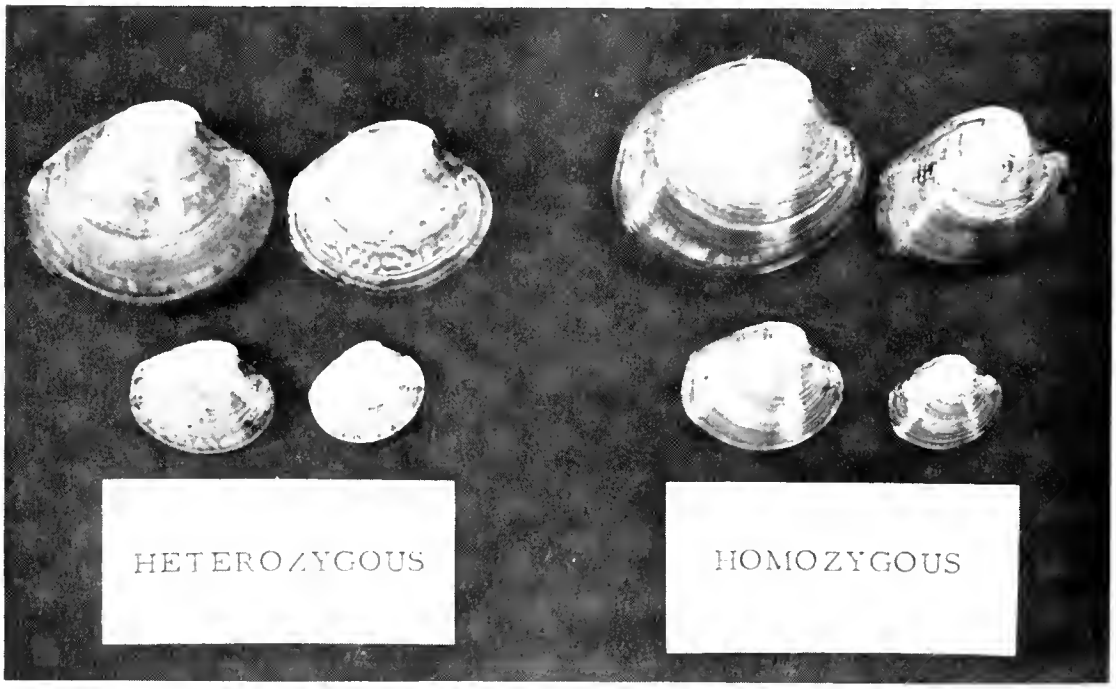

Fig. 1. Juvenile V. mercenaria notata showing the difference between homozygous and heterozygous markings.

were the typical reddish-brown zigzag lines. This marking was quite variable and ranged from faint fragments of lines to dark, heavy lines so concentrated that they produced almost a solid color. The other type of marking also varied, but typically the shells were almost solidly colored reddish-brown with a light band from the umbo around the lunule. Two other broad light bands extended from the umbo to the margin of the shell, dividing it roughly into thirds. The zigzag lines occurred only in lighter areas of the shell. Although this marking has not been described for the notata subspecies, it is probably the coloration characteristic of clams homozygous for the color factor, since it occurred only when two notata-marked clams were crossed. Apparently the more familar zigzag lines, accepted as characteristic of the notata subspecies, are actually a phenotypic blend exhibited by heterozygous clams.

A count based on these different markings gave 233 unmarked, 507 marked with zigzag lines, and 260 solidly colored with light bands. This is roughly the expected 1 to 2 to 1 genotypic ratio resulting when two heterozygous individuals are crossed. Chi square in this case is equal to 1.654, a value that would occur by chance in over 30 per cent of such crosses. Unfortunately, time and space limitations did not permit raising these clams so that homozygous marked clams could be crossed.

If we assume that the notata marking is a simple Mendelian character, either with complete dominance (marked vs. unmarked) or more probably with incomplete dominance in which the heterozygous individuals can be separated from the homozygous dominants by careful examination, then the chi square test shows no evidence that observed ratios are significantly different from the expected Mendelian ratios, except in three F_1 crosses of unmarked clams where a few marked individuals were found at the end of one year (Table 1).

Selective Breeding

The second phase of these studies was an attempt to demonstrate the feasibility of developing a fast-growing race of clams through selective breeding. The original plan involved rearing the fastest and the slowest growing offspring from a single pair of clams. Hereditary influence on growth would then be determined by comparing the rate of growth of offspring from each group. However, the slow-growing clams did not respond to spawning stimuli and parents from wild stock had to be substituted.

The experiments were begun by crossing one female hard clam with one male and rearing the surviving offspring. In several instances individual clams grew well during the first growing season and then poorly or not at all the second season. Undoubtedly, some variation in the rate of growth was caused by environmental factors, even though clams were reared under as nearly identical environmental conditions as possible.

At the age of 28 months these clams ranged from 14.5 to 42.0 mm in length. Of these, the 120 largest clams were selected as brood stock. When these clams were about 44 months old a large male was crossed with a large female. At the same time, one female and 2 male clams, chosen randomly from wild stock, were spawned and their larvae reared as controls.

Soon after setting it became apparent that the selectively-bred clams were growing more rapidly than the controls.

At the age of 10 weeks approximately 10,000 control clams had a total volume of 8.6 cc, while 10,000 selectively-bred clams were about 70 per cent larger with a total volume of 14 cc. When the clams were about 15 months old the total volume of 200 randomly-selected control clams was 53 cc, while the total volume of 200 randomly-selected selectively-bred clams was 86 cc or, roughly, 60 per cent greater (Fig. 2).

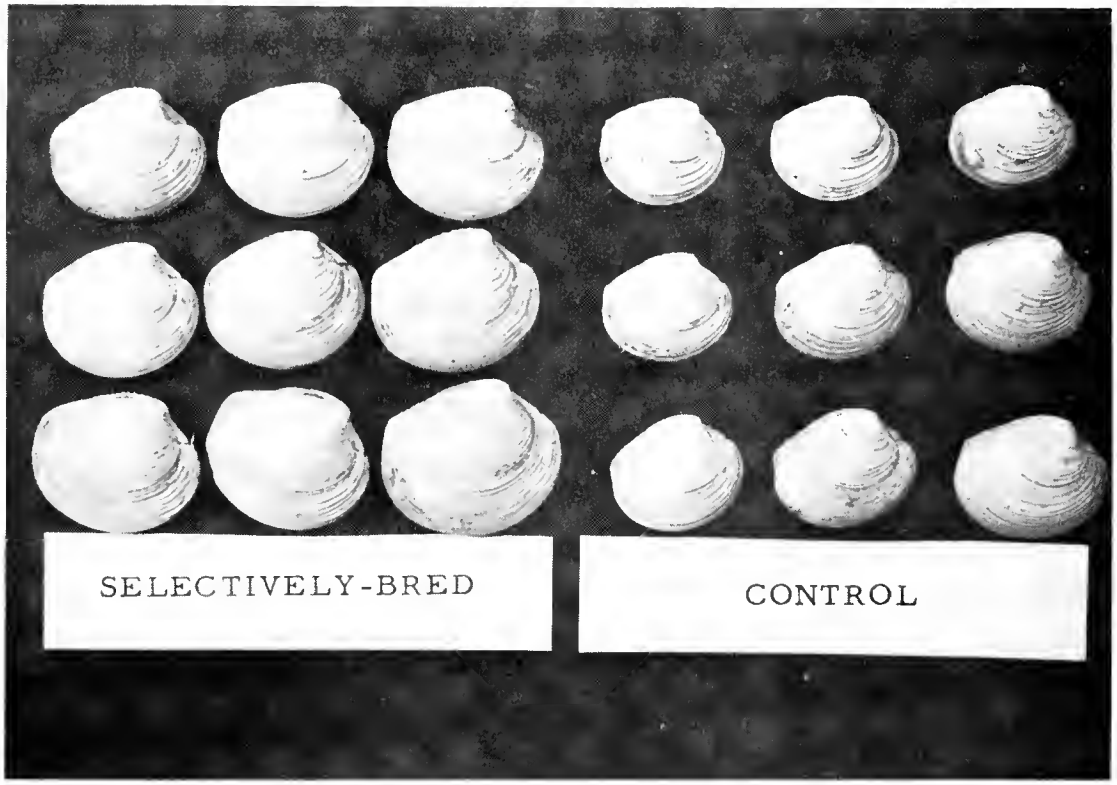

Fig. 2. Fifteen-month-old V. mercenaria, showing the largest clams from a group of selectively-bred clams compared with the largest clams from a group of clams that were not selectively bred.

At 15 months the selectively-bred clams ranged in length from 5.5 to 23.0 mm, while the control clams ranged from 6.6 to 19.0 mm. The average length of the selectively-bred clams was 13.2 mm, or 11.3 per cent greater than the average length of the controls, which was 11.8 mm.

Further selective breeding should result in even more rapid average growth of progeny, as the selection of brood stock becomes more stringent. These studies suggest that a rapid-growing strain of clams could be developed in only a few generations since in one generation of selective breeding, clams attained a 60 per cent larger size in 15 months than control clams.

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PROGRESS IN THE COOPERATIVE STATE-PUBLIC HEALTH SERVICE-INDUSTRY PROGRAM FOR THE CERTIFICATION OF INTERSTATE SHELLFISH SHIPPERS

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ABSTRACT

The Cooperative State-PHS-Industry Program for the Certification of Interstate Shellfish Shippers has been effective in preventing the interstate spread of disease due to shellfish, and is, therefore, of importance to health agencies. This program is also important to the shellfish industry since it insures public acceptance of their product. However, the program also has a pronounced impact on industry operations by regulating available sources of supply and by influencing costs of operations.

During 1958-1959 the program made considerable administrative and technical progress. Important administrative advances include: Adoption of new sanitation standards; a decision to develop equipment construction guides; the adoption of a new interim bacteriological standard for shucked oysters at the market level; the formation of regional shellfish sanitation seminars in the New York-New England, Gulf and West Coast areas; and the adoption of quarantine levels for paralytic shellfish poison in shellfish growing areas. In the technical field, progress was made in establishing bacteriological relationships for eastern shellfish; in development of a simple colorimetric test for the freshness of shucked oysters; and, in the perfection of better methods for assaying for paralytic shellfish poison. The National Institutes of Health have also supported a number of research projects which have application in the shellfish sanitation field. Training has been accomplished through a one week shellfish sanitation course held at a West Coast location.

The shellfish industry requires large estuarine areas which are essentially free of sewage pollution. Federal grants to communities for the construction of sewage treatment plants apparently have accelerated construction of such facilities. In 1958 contract awards reached $389 million versus $222 million in the period preceding the grants program. However, it is estimated that construction must be at a rate of $575 million yearly to meet the Nation's requirements.

The Cooperative State-Public Health Service-Industry Program for the Certification of the Interstate Shellfish Shippers is an unusual control program in which the regulated industry has an important administrative role. This cooperative program has been most successful in the prevention of disease transmission through fresh or frozen

shellfish. In almost 35 years of operations, there have been no major disease outbreaks attributed to commercially distributed oysters, clams and mussels. This is in sharp contrast to the pre-certification period when shellfish were frequently associated with disease. Since the economic health of the shellfish industry is almost entirely dependent upon the acceptability of its product by the public, it is obvious that this program is of great importance to them. In addition, the certification program has a significant impact on management through its influence on the availability of shellstock and on the unit cost of processing and marketing. Regulation of this degree--and the shellfish industry is probably one of the most strictly regulated industries in the food industry--could be extremely oppressive to management if the sanitary requirements adopted by the official agencies were unrealistic or were not well understood by industry. Industry therefore has both economic and moral reasons to take an active part in the administration of this program.

The informal organization of the certification program has posed many difficult administrative problems. It is essential, for example, that we avoid overlaps and conflicts between the Federal agencies which have adjoining responsibilities. The Public Health Service Act directs the Public Health Service to cooperate with the States in preventing the interstate spread of communicable disease. The Federal Food, Drug and Cosmetic Act gives the Food and Drug Administration, also of the Department of Health, Education, and Welfare, legal responsibility for safety and purity of foods shipped in interstate commerce. Also, by law, the Fish and Wildlife Service of the Department of Interior has primary responsibilities in the fishery field. In both cases, bilateral agreements have been developed which define each agency's responsibilities. These intragovernmental agreements are fulfilled through almost daily contacts at the working level and through more formal liaison meetings. By these means, we have been able to occasionally pool research resources, to avoid overlaps in inspectional operations, and to avoid conflicts of interest in which one Federal agency directs industry to follow one set of instructions while, at the same time, another agency is advising a quite different course. In the past we have also encountered many problems in maintaining adequate communication channels with State regulatory agencies and industry. Adequate communications are, of course, essential if these groups are to have an adequate voice in the direction of this program, and if they are to be kept acquainted with the technical and administrative problems that the Public Health Service encounters in its position as trustee for this program.

Real progress has been made in the administration of the program during the past year which will ultimately place it on a sounder technical basis. This will improve its public-health effectiveness without placing a substantially greater burden on the major part of the shellfish industry. This paper is essentially a progress report for the past year.

Very satisfying progress was made at the 3rd biennial Shellfish Sanitation Workshop held in Washington on August 26-27, 1958. This workshop--and it was a workshop in the truest sense of the word--was attended by about 93 persons representing some 43 State and Federal agencies. The shellfish industry had 9 representatives at this meeting. From the standpoint of the regulatory agencies, the most important accomplishment of the workshop was the completion of Part 1 of the Shellfish Sanitation Manual. This manual, which has been under development for the past two years, is an essential step toward the adoption of uniform appraisal methods which can be used by the Public Health Service in the evaluation of the adequacy of States' shellfish sanitation programs.

From the standpoint of industry, the most important change in the program is probably the adoption of the so-called 80% rule which is stated in the manual as follows:

> "Effective September 1, 1959, interstate shellfish shipper certificates are issued only to those establishments substantially meeting the construction requirements of Part II of this manual and which maintain a plant sanitation rating of at least 90 per cent during periods of operations. (The State shellfish control agency shall suspend or revoke certificates if a plant sanitation rating drops below 80 per cent or if significant individual sanitation item is violated repeatedly.) Ratings will be determined on the basis of compliance with the applicable provisions of Part II of this manual as measured by an inspection report comparable to that contained in appendix B of this manual."

Under this rule those shippers who have substandard plants or who fail to maintain reasonably sanitary conditions in their operations will not be included on the Public Health Service list of State certified shippers even though they may have valid operating permits from the State in which they are located. This new plant inspection system and the uniform scoring procedure has been in use for two years by our regional shellfish sanitation consultants in most parts of the country. Their experiences indicate that most shippers should have little difficulty in meeting these requirements if they give a reasonable amount of attention to plant operating procedures. The importance of good operating procedures should be emphasized since previous scoring procedures attached less importance to this aspect of shellfish sanitation. Plant inspections will, as in the past, be made by State officials with occasional check inspection by Public Health Service officers.

The problems which the shellfish industry has experienced in obtaining equipment which will meet the needs of the packer and yet be acceptable to the inspecting agency were also discussed at the workshop. This problem is not, of course, peculiar to the shellfish industry, and, in fact, has been faced by most of the food processing

industries. It was agreed that equipment construction and fabrication guides would help the shellfish industry secure equipment which would meet their needs, which could be manufactured economically, and which would also meet the sanitary requirements of the official agencies. Since the shellfish industry is quite small, and since there are only three or four equipment suppliers, it was decided that a formal organization such as the milk industries 3-A group was not necessary. The Public Health Service agreed to develop preliminary construction guides for review by the shellfish packers, the equipment fabricators, and official agencies. Such guides would not have any legal standing and would not constitute official agency approval of equipment.

Real progress has been made in the organization of regional shellfish sanitation seminars which will meet annually to discuss specific problems in the local administration of the cooperative program. Shellfish sanitation seminars have been organized in the New England-North Atlantic, Gulf and West Coast areas. These groups probably follow the same general pattern of operation as that of the Chesapeake Bay Seafood Seminar. These regional meetings, coupled with a biennial national shellfish sanitation meeting should insure that both States and the shellfish industry will have an ample voice in the organization and administration of the cooperative certification program.

The effort to develop workable bacteriological standards for shucked oysters at the market level was continued. The project is now nine years old. For many years a coliform MPN (Most Probable Number) or coliform score (also a statistical estimate of bacterial densities) and Standard Plate Count have been used as rough guides to the sanitary quality of oysters as marketed. More specifically, these tests have been used as an index of sanitary conditions in the packing plant and the adequacy of refrigeration since it has long been recognized that such laboratory tests cannot always distinguish between shellfish from polluted and non-polluted sources. To gain additional information on the validity of these tests for oysters as marketed, several of the Eastern and Southern States, the government of Canada, the New York and Chicago City Health Departments and the Public Health Service in 1955 undertook a cooperative study of the changes in the bacteriological quality of oysters during shucking and shipment to market.

The 1958 Workshop spent almost a full day in an evaluation of the results which had been obtained in this study and concluded that the interim standards adopted at the 1956 Shellfish Sanitation Workshop were of questionable value. Accordingly, the following interim standards were adopted for use during the 1958-59 and 1959-60 merketing season:

Satisfactory

A fecal coliform density (MPN) of not more than 78 per 100 ml

of sample as indicated by production of gas in E.C. liquid broth media and a Plate Count of not more than 100,000 per ml of sample, except that a fecal coliform density (MPN) up to and including 230 per 100 ml of sample and/or a Plate Count up to and including 500,000 per ml of sample will be acceptable in occasional samples. (For convenience these will be referred to as Class 1-A and 1-B samples, respectively).

The official agency in the receiving area should notify the shipper and shellfish control agency at the point of production of any Class 1-B results. If two consecutive Class 1-B shipments are found, the receiving area is justified in excluding the shipper from the market area until a satisfactory report on the shipper is received from the State control agency in the producing area.

Unsatisfactory

A fecal coliform density (MPN) of more than 230 per 100 ml of sample or a Plate Count of more than 500,000 per ml of sample. (For convenience these will be referred to as Class II samples).

The official agency in the receiving area should immediately notify both the shipper and shellfish control agency in the producing area of a Class II result. A single Class II result is justification for excluding the shipper from the market pending receipt of a satisfactory explanation from the official control agency in the producing area.

The same groups of agencies continued their studies of these bacteriological relationships in the 1958-59 marketing season. The results have not yet been subjected to statistical analysis or to evaluation by the bacteriologists. However, it appears that these new interim standards are realistic. The study will be continued for at least another year.

The real significance of this change in standards lies in the use of the Eijkman test. It is believed that these organisms are a more conclusive indicator of fecal pollution and should be much better adapted for use with shellfish sanitation work. Unfortunately, the use of the Eijkman positive coliform means discarding years of experience with the coliform group--an action which many regulatory agencies are reluctant to take.

The Workshop agreed that if these interim standards were to have real value, they should, if possible, be related to sanitary conditions in the growing areas. Accordingly, several States have agreed to undertake comprehensive studies of these organisms in their shellfish growing areas. The results of these studies over the next two years should do much to test the validity of the presently recommended

market standards. If proven successful, these new bacteriological procedures should make it possible for the regulatory agencies to make much more exacting sanitary surveys of shellfish growing areas.

Paralytic shellfish poison was also discussed at the 1958 Workshop, and, for the first time, definite quarantine levels were established for domestic production areas. This problem is not of immediate concern to the oyster industry; however, because of the trade inter-relationships, the oyster industry cannot ignore any such problem which affects other segments of the industry.

The Workshop agenda included a limited discussion of the relationships between shellfish production and radioactive waste disposal practices. It has been amply demonstrated that shellfish, like other marine animals and plants, can accumulate radioactive materials from their environment. It is, therefore, quite important that this characteristic be considered in any proposals for disposal of radioactive wastes in marine or estuarine areas. The use of nuclear reactors in merchant and naval vessels and the increasing tempo of construction of nuclear-powered electric generating stations may make this a problem which the control agencies and the shellfish industry will have to face in the quite near future.

The Public Health Service Shellfish Sanitation Laboratory, under the direction of Mr. C. B. Kelly, has been moved from Florida to the State of Washington to undertake long-range studies on the bacteriological relationships in Western shellfish. This is also a cooperative undertaking in which the Washington State Department of Health is providing the research facility and the Public Health Service the staff and equipment. The organized shellfish industry of the West Coast has taken an active part in getting this laboratory established and in planning the research activities which will be undertaken during the coming years.

An entirely new research development is found in the color test for oyster freshness. This simple chemical test, being developed by the Public Health Service Sanitary Engineering Center is based on a color change in a chromate solution. It will apparently indicate the age of shucked oysters and/or the temperature at which they have been stored. This simple colorimetric test is still in an early stage of development; however, the preliminary work with it has been quite promising. A traced-lot field trial will probably be undertaken.

During the past year a new brochure on the cooling rates of oysters was also published. This study, made by the American Can Company, gives information on the amount of time required to cool shucked oysters in various size cans.

The Public Health Service Sanitary Engineering Center has also continued experimental work on chemical and laboratory procedures for measuring the amount of paralytic shellfish poison present in shellfish.

Their collaborative efforts have resulted in the refinement of the bioassay procedure to a point at which it has been accepted as an Official Method by the Association of Official Agriculture Chemists. A second AOAC collaborative study of a chemical method for measurement of the poison has also been initiated.

The Public Health Service, through the National Institutes of Health, has also supported shellfish research by a number of Universites, and State and private research agencies. For example, the Maryland Department of Research and Education has undertaken a long-range study of the bacteriological relationships between the soft-shell clam and its aquatic environment, and of the effects of the hydraulic dredge on the bacteriological quality of the clam. At the Haskins Institute in New York City a research grant has made possible some very significant advances in the shellfish poisons field and which may greatly facilitate control of this problem. In another NIH supported study, which incidentally was carried on in Japan, a research group has apparently found that certain types of industrial wastes may be concentrated by shellfish and cause illnesses in consumers.

A specific sanitation program, if it is to be successful, must also include training activities. In this respect, we have been particularly successful in the shellfish program this past year. For the first time the training branch at the Sanitary Engineering Center arranged to present the one-week Shellfish Sanitation Course on the West Coast. Consequently, this course was very well attended by both representatives of the West Coast regulatory agencies and by plant management. In our opinion it was a great success. Detailed plans have been made with the States of Virginia and Maryland for two three-day courses in shellfish sanitation. These courses will emphasize plant sanitation practices.

The activities of the Public Health Service in the administration of the certification program are important to the economic health of the shellfish industry. However, the Water Pollution Control activities of the Service are also very important.

As presently operated in the United States, the shellfish industry requires large estuarine areas which are almost entirely free of sewage pollution. In years past it has not been difficult to find such areas because of our relatively low population density; however, this has changed rapidly in recent years! Our population has been expanding at a very rapid rate and gives every evidence of continuing do do so in the foreseeable future. The impact on the sanitary quality of our surface waters has been unmistakable.

The control of pollution depends on the construction and adequate operation of treatment facilities by both communities and industry. Many sewage treatment plants have been constructed in the United States over the years; however, the population has continued to increase at a rate faster than new treatment plants could be constructed. As a conse-

quence of this deficit in sewage treatment plant construction and the wearing out of existing facilities, the problem of water polution has become very acute in the United States. The Congress has recognized this problem and for the past several years has made Federal grants available to communities to assist in the construction of sewage treatment plants. During the five-year period from 1952 through 1956 immediately preceding the Federal grants program, contract awards for sewage treatment works construction averaged $222 million annually. In the first full year of the program, 1957, construction expanded 58 per cent over the previous annual average to reach $351 million. The second year of the program brought an even greater increase in construction, with contract awards reaching $389 million--75 per cent over the earlier five-year average. The lion's share of the increase in construction during 1957 and 1958 came from projects receiving Federal aid. This amounted to $118 million in 1957 and $143 million in 1958. These facts point strongly to the conclusion that had it not been for Federal grants, sewage treatment works construction would have remained at about the average level experienced during the five-year period preceding the grants program. They also indicated that a doubling of grant funds would further increase construction to about the required level of $575 million, the rate which has been necessary to eliminate our high backlog of construction needs and to compensate for plant obsolescence and population growth.

It should be quite apparent that without this surge in construction many additional coastal areas would have been lost to the shellfish industry. It should be equally apparent that this construction pace must be at least maintained if the industry is to avoid a further loss of growing areas. On the positive side of this, it should be pointed out that we now have a few instances in which growing areas have again become available for shellfish culture as a result of the construction of sewage treatment facilities.

From this discussion of our activities during the past year, it should be apparent that the Cooperative Program for the Certification of Interstate Shellfish Shippers is on reasonably solid ground, both from the administrative and research standpoints although there are some pressing administrative problems which have not been discussed in this paper. It is hoped that the program can continue to provide adequate public-health protection to shellfish consumers at minimum cost both to the taxpayer and to industry. However, this progress will require the wholehearted cooperation and support of industry, both as individuals and as an association, and of the interested State regulatory agencies.

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A BACTERIOLOGICAL STUDY OF THE NATURAL FLORA OF PACIFIC OYSTERS (CRASSOSTREA GIGAS) WHEN TRANSPLANTED TO VARIOUS AREAS IN WASHINGTON [1, 2]

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ABSTRACT

Total viable bacterial populations on oysters held experimentally on floating trays in areas of different water conditions fluctuated between 10^4 - 10^5 organisms per ml oyster liquid. Coliforms were never higher than 0.5% of the total viable bacterial flora. Study of 152 randomly isolated cultures (taken from gill, flesh, intestine and oyster liquid) indicated that Gram negative, asporogenous, rod-like bacteria of the Pseudomonas, Vibrio, Flavobacterium and Achromobacter groups predominated. The bacterial flora of fin fishes is overwhelmingly oxidative in nature, but 50% of the oyster bacteria tested were able to ferment glucose anaerobically.

INTRODUCTION

Most of the bacteriological work on oysters, from Cameron (1880) to the present day, has been concerned with the detection of human enteric bacteria. The studies of Fabre-Domergue (1912), Dodgson (1928) and Kelly and Arcisz (1954) revealed that molluscs contaminated with enteric bacteria will cleanse themselves in a few days when placed in clean, uncontaminated sea water. As a result of application of procedures based on these and similar findings, and of the very close control exercised on shellfish growing areas, the oyster is now the safe food product that it is.

Little information is available concerning the other types of bacteria which are present in oysters, the so-called saprophytic bacteria, harmless in the main to man, but known to be potent agents of spoilage in foodstuffs. Earlier workers have noted such groups in oysters as Spirochaeta (Dimitroff 1926), Proteus, Alcaligenes and Pseudomonas fluorescens (Geiger, Ward and Jacobson 1926) without actually analyzing the flora to determine the relative importance of each. Two reports concerning the bacterial types involved in the spoilage of oysters have been made. Eliot (1926) observed that during spoilage

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[2] Contribution No. 67, College of Fisheries, University of Washington, Seattle 5, Washington.

of shucked oysters at 20°C (68°F) "water" forms including green fluorescent, pigmented and non-pigmented bacteria, and "vibrio" types increased steadily in number while coliform types increased only during the first two days of storage. Tanikawa (1937) characterized bacteria from the groups which he considered of greatest importance in the spoilage of shucked oysters at 0°C (32°F), namely, Achromobacter, Pseudomonas, Flavobacterium and Micrococcus.

Similar types of bacteria are known to be important spoilage agents in the deterioration of fin fishes, post-mortem, and have been shown to be derived directly from the normal bacterial flora of the living fish (Shewan and Liston 1956).

The purpose of our study was to determine the natural flora within the oyster as a first step towards providing information which will enable shellfish technologists to take rational measures to deal with potential spoilage agents at an early stage in storage. The information was also sought to fill a considerable gap in our knowledge of the bacteriology of marine animals.

MATERIALS AND METHODS

Yearling Pacific oysters were maintained in floats in three areas, Willapa Bay, Oyster Bay and Hood Canal, and a control was kept in the seawater aquarium at the College of Fisheries (Sparks and Chew 1960). Most Probable Number (MPN) of coliforms and total viable counts were carried out on fluid extracted aseptically* from three oysters and also from seawater, taken from the aquarium weekly and from the floats at three-week intervals, according to procedures outlined in Standard Methods for the Examination of Water, Sewage and Industrial Wastes (1955). The medium chosen for the plate counts contained 0.8% nutrient broth, 0.5% yeast extract and 1.5% Bacto-agar in seawater (MacLeod, Onofrey and Norris 1954). From experimental data obtained in our laboratory, this medium appears to yield a maximum plate count, in that it affords good growth of non-marine and also marine types which are otherwise not picked up.

Cultures of microorganisms were obtained by random selection from the count plates inoculated from gill, intestine, body flesh and liquid, purified by routine methods, and maintained as pure cultures in seawater peptone broth (1% peptone in seawater) or in the basal agar medium described, depending on how fastidious the organism was.

The microbiological procedures applied in the tests for classification and identification are those described in the Manual of Microbiological Methods (1957).

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*Trial counts on gill, intestine, whole oyster (flesh and liquid), and oyster liquid indicated that oyster liquid provided the most consistent, high total viable counts.

Difco dehydrated media, including the Enterococci Presumptive Broth, Ethyl Azide Violet Broth (for enteroccocci), Brilliant Green Bile Broth and Eosin-Methylene Blue Agar (for coliforms and E. coli), S S Agar and Triple Sugar Iron Agar (for Salmonella, Shigella) were used to assist in the identification of possible members of the Enterobacteriaceae. Pure cultures of all the organisms isolated in this study were streaked on basal agar plates for colonial morphology and for tests of sensitivity to 0/129 vibriostat compound and to 2, 5, 10 unit Difco penicillin discs (Shewan, Hodgkiss and Liston 1954). Tests and media used were as follows: litmus milk, seawater nutrient gelatin, lead acetate agar slopes, methyl red, Voges-Proskauer, nitrate broth, indole, urea agar slopes, Koser's citrate broth, Hugh and Leifson oxidative and fermentative medium (Hugh and Leifson 1953), lactose, dextrose, maltose, mannitol, and sucrose fermentation tubes, and ammonia production. Temperature growth tests at 0°C, 25°C and 37°C were carried out in 1% peptone water containing 0.5% NaCl. Routine tests and identification media were inoculated and incubated at 25°C (RT), but selective media for enterobacteria were incubated at 37°C. One hundred fifty-two cultures were thus studied.

RESULTS AND DISCUSSION

The MPN of coliforms present in oysters and seawater during the course of the study (February-July 1959) is given in Table 1.

Table 1. Coliform content of oysters and seawater examined at three-week intervals (expressed as MPN coliforms per 100 ml sample)

Source	Time (Weeks)				
	0	3	6	9	12
Aquarium Oysters	0	0	0	0	0
Aquarium Seawater (Control)	0	0	0	0	0
Hood Canal Oysters	-	450	450	200	200
Hood Canal Seawater	-	0	0	2	2
Oyster Bay Oysters	-	0	200	0	0
Oyster Bay Seawater	-	0	0	0	0
Willapa Bay Oysters	-	450	1100	20	20
Willapa Bay Seawater	-	0	200	2	2

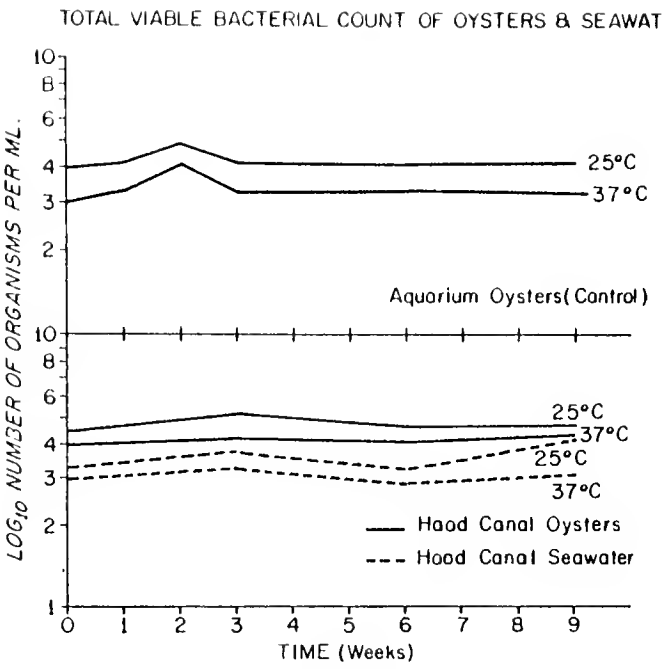

Fig. 1. Total viable count of bacteria per ml oyster fluid and per ml seawater for aquarium-held oysters (control) and Hood Canal.

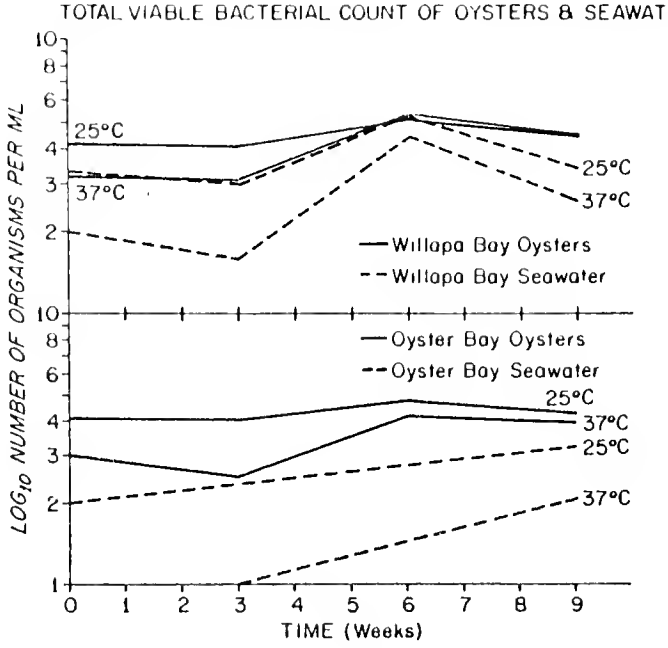

Fig. 2. Total viable count of bacteria per ml oyster fluid and per ml seawater for Willapa Bay and Oyster Bay samples.

There was a low degree of pollution, indicated by these tests, in the three areas except for a brief rise in the Willapa Bay area during the sixth week, and there was no apparent pollution in the aquarium. The ability of oysters to concentrate sewage bacteria from the surrounding water is demonstrated by the disparity in the counts for oysters and seawater at any given time. However, comparison of these results (recalculated on a weight basis) with the total 25°C count results given in Figures 1 and 2 reveals that at no time did the coliforms constitute more than 0.5% of the total flora. Numerically, therefore, the coliform bacteria represent an insignificant part of the oyster flora.

The total viable count in oysters at 25°C, which may reasonably be expected to include both mesophilic and psychrophilic bacteria, remained constant in all cases at about 10^4 organisms/ml of body fluid, while the count in the surrounding seawater was consistently lower and subject to rather large variation.

The generic distribution of the 152 strains obtained by random selection from the oyster plate counts is shown in Table 2. There is a remarkable similarity in the oyster flora in the four test areas. Gram negative rod-like bacteria, showing the characteristic marine properties

Table 2. Generic distribution of organisms isolated from oysters in controlled and natural environments.

	Salt-water Aquarium	Hood Canal	Willapa Bay	Oyster Bay	Total	% Distribution
Number of Isolates	43	50	30	29	152	100
Pseudomonas-Vibrio	20	27	18	14	79	52.0
Achromobacter	2	5	1	0	8	5.3
Flavobacterium	6	7	5	8	26	17.1
Coryneform	1	3	0	1	5	3.3
Alcaligenes	2	1	0	0	3	2.0
Micrococcus	6	5	2	3	16	10.5
Bacillus	4	1	2	0	7	4.6
Enterococci	1	0	1	0	2	1.3
Miscellaneous	1	1	1	3	6	3.9

of salt dependence and psychrophilic growth, predominated in each area. Outstanding among them was the Pseudomonas/Vibrio group which constituted about 50% of the flora in each case, while Flavobacterium present to ca. 17% were also prominent. Micrococcus alone among the Gram positive types was isolated in significant numbers, but was present only to ca. 10%. A corresponding identity of biochemical characteristics was observed among organisms isolated from the various areas. They were predominantly proteolytic and only weakly saccharolytic in nature. It thus appears that the natural flora of oysters is similar to the flora of free-swimming fish, the only other marine animal extensively studied bacteriologically.

The only major point of difference between the two floras is a biochemical one, since ca. 50% of the organisms isolated by us from oysters were observed to ferment glucose anaerobically by the Hugh and Leifson (1953) test. This is a property which has not been reported for many fish bacteria. The difference may be related to the composition of oysters and of fin fishes. From the practical point of view the property may be very significant in relation to spoilage. Eliot (1926) showed, and this has been confirmed repeatedly since, that there is a rapid fall in p^H during the early stages of spoilage of oysters. This may be due, at least in part, to the fermentative activity of these bacteria.

The types of bacteria identified by Eliot and Tanikawa as being of primary importance in spoilage are so similar to the microorganisms found in this study to constitute the natural flora of oysters, that it seems very likely that we are in fact dealing with the same groups of organisms.

It appears therefore that as in the case of fin fishes, the spoilage flora of oysters is derived from the natural population of the living animal and the generally proteolytic character of the natural flora lends verisimilitude to the hypothesis.

SUMMARY

The natural flora of oysters was characterized by study of pure cultures of bacteria isolated from plate counts of oysters held in floating trays in three areas of Washington: Southern Puget Sound, Hood Canal, and Willapa Bay, and the saltwater aquarium at the College of Fisheries, Seattle. Degrees of pollution were very low in each of the three natural environment areas, and there was none present in the saltwater aquarium, as detected by MPN of coliforms and standard plate counts at 25°C and 37°C.

From a total sample of 152 pure cultures extensively studied, it was concluded that the flora of the oyster consists primarily of the Pseudomonas/Vibrio, Flavobacterium and Achromobacter groups, i.e.

the Gram negative, asporogenous bacilli. Gram positive organisms, except for Micrococcus, were found to be a very minor fraction of the total population. These results are discussed in relation to the known data and theories concerning post-mortem spoilage of free-swimming fishes and of oyster meats.

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ANNUAL CONVENTION

The 1959 Convention of the National Shellfisheries Association and the Oyster Growers and Dealers Association of North America, Inc., was held July 27 to 30, 1959, at the Statler-Hilton Hotel, Washington, D.C. An interesting feature was a joint seminar at which reports on "Oyster culture in Europe" and "Oyster culture in Japan" were presented by V. L. Loosanoff and J. B. Glude. These reports contained observations from their recent foreign trips. The membership voted overshelmingly to increase Association dues from $2.00 to $4.00 to cover the cost of financing the Proceedings. The Oyster Institute of North America has continued to support publication of the Proceedings. Vice President Cronin distributed a list of titles of all papers presented at annual conventions since 1930. Most of these papers are out-of-print and no longer available.

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Original papers given at the Annual Association Convention and other papers on shellfish biology or related subjects submitted by members of the Association will be considered for publication. Manuscripts will be judged by the Editorial Committee or by other competent reviewers on the basis of originality, contents, clarity of presentation and interpretations. Each paper should be carefully prepared in the style followed in previous PROCEEDINGS before submission to the Editoral Committee. Papers published or to be published in other journals are not acceptable.

Manuscripts should be typewritten and double-spaced: original sheets are required but extra copies will facilitate reviews. Tables, numbered in arabic, should be on separate pages with the title at the top. Scientific names should be underlined. Do not underline section headings. Illustrations should be reduced to a size which fits on 8 x $10\frac{1}{2}$ inch pages with ample margins. Glossy photographs are preferred to originals. Illustrations smaller than a page should be carefully oriented and loosely attached to plain white paper with rubber cement. Legends should be typed on separate sheets and numbered in arabic.

Use the following style for literature citations: "Smith, Rebecca Joyce. 1958. Filtering efficiency of hard clams in mixed suspensions of radioactive phytoplankton. Proc. Natl. Shellfish. Assoc. 48: 115-124." Note in Volume 48 punctuation for literature citations in text. In abbreviations for names of serial publications, follow Biological Abstracts (see 29(5): v-xxxv, 1955). Abbreviations for units of weight and measure in the Handbook of Chemistry and Physics, 36th Edition, pages 3108-3134 will be followed. Punctuation will be strictly limited for abbreviations of common measurements, literature references in the text, and certain other usages. Note usage in recent PROCEEDINGS. Clarity of meaning and brevity of style are the keynotes of our policy.

Each paper should be accompanied by an abstract which is concise yet understandable without reference to the original article. It is our policy to publish the abstract at the head of the paper and to dispense with a summary. A copy of the abstract for submission to Biological Abstracts will be requested when proofs are sent to authors.

Reprints and covers are available at cost to authors. Master sheets will be retained for one year after publication. When proof sheets are returned to authors, information about ordering reprints will be given. The present agency from which authors may obtain reprints is the Duplicating Department, Bingham Y, University of North Carolina, Chapel Hill, N. C., Mr. J. Nelson Callahan, Head.

For inclusion in the PROCEEDINGS of the current year, all manuscripts should reach the Editor prior to October 1. Send manuscripts and address all correspondence to the Editor, Dr. Sewell H. Hopkins, Biology Department, Texas A&M College, College Station, Texas.

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