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Title: EFFECTS OF TEMPERATURE, SALINITY, FEEDING, SUBSTRATE, AND STORAGE ON THE SETTING AND SURVIVAL OF COMMERCIALLY-REARED EYED LARVAE OF THE PACIFIC OYSTER, CRASSOSTREA GIGAS

Abstract approved: Herbert F. Frolander
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A series of factorial experiments were conducted using eyed oyster larvae (Crassostrea gigas) reared at a commercial hatchery in Netarts, Oregon. The objectives of the study were to obtain the highest percentage of setting larvae and the best survival of the spat.

Experiments on the combined effects of temperature and salinity indicated that temperature had a highly significant effect on the setting of Pacific oyster larvae. For temperatures ranging from $15^{o}$-$30^{o}$C, the percentage of larvae setting during a 48-hour period increased as temperature increased. There was no significant difference in setting for salinities ranging from 15-30 ppt. In one setting experiment with Kumomoto larvae (a different variety of C. gigas), the differences among the sixteen combinations of temperature and salinity were statistically insignificant.

Feeding larvae 120,000 cells per milliliter of the alga Pseudoisochrysis paradoxa did not significantly improve setting during a 48-hour period, and in one experiment temperature and feeding interacted

to the detriment of setting.

There was no significant difference in the percentages of attaching larvae when oyster, cockle, or butter clam shells were used as substrates. Dipping oyster or butter clam shells in an aqueous extract of oyster tissue improved setting, but the presence of the extract did not improve setting on razor or littleneck clam shells.

Storing eyed larvae at $5^{o}C$ for several days before setting them appeared to increase the percentage of attaching larvae. The best temperature for setting stored larvae was $25^{o}C$.

Oyster larvae that attached to shell cultch during the setting experiments were held in a tank of raw seawater in the laboratory. After eight to nine months, the percentages of surviving spat were calculated. Survival was poor for larvae set at $15^{o}C$, and there was some evidence of higher mortality among larvae stored for several days before being set. Because of the laboratory conditions, these studies may not have provided a realistic measure of spat survival under natural conditions, and field studies should be carried out to confirm these results.

# Effects of Temperature, Salinity, Feeding, Substrate, and Storage on the Setting and Survival of Commercially-reared Eyed Larvae of the Pacific Oyster, *Crassostrea gigas*

by

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# THE EFFECTS OF TEMPERATURE, SALINITY, FEEDING, SUBSTRATE, AND STORAGE ON THE SETTING AND SURVIVAL OF COMMERCIALLY-REARED EYED LARVAE OF THE PACIFIC OYSTER, CRASSOSTREA GIGAS

## INTRODUCTION

### Historical Background

Early inhabitants of the Pacific coast of the United States found local bays populated by a small oyster, Ostrea lurida. An industry developed around this species with Washington state accounting for 95% of west-coast oyster production (Galtsoff, 1929). The slow growth and labor-intensive culture required by O. lurida combined with sulfite pollution from pulp mills to drastically deplete native oyster populations, and west-coast oyster growers began to look for another oyster to cultivate (Steele, 1957; Matthiessen, 1970).

Shortly after completion of the first transcontinental railroad in 1870, the native east-coast oyster, Crassostrea virginica, was introduced to the Pacific coast. Mortalities were high, and survivors failed to grow or fatten satisfactorily. Dissatisfied growers soon abandoned attempts to commercially cultivate the eastern oyster.

In 1899, the Washington state fish commissioner requested information from the University of Tokyo on growing Japanese oysters in the Pacific Northwest (Galtsoff, 1929). An oyster from northern Japan was suggested as the most promising candidate for importation. It was the Miyagi variety of the species Crassostrea gigas, as named by Thunburg in 1795 (Quayle, 1969).

Experimental shipments of Japanese oysters (also called Pacific or Miyagi oysters) began in 1902 (Woelke, 1957). Initial commercial

cultivation started in 1919 when 400 cases of oysters were shipped from Japan to Samish Bay, Washington (Steele, 1964). Most of these oysters died en route, but they were spread on the tidelands anyway. After a few months, many young oysters were growing where the dead ones had been scattered. Juvenile oysters (called spat or seed), attached to the shells of the dead adults, had survived the transoceanic journey and were growing in Washington waters.

This experience taught oystermen that juvenile oysters attached to empty shells (called cultch) were more likely to survive the two-week trip to the United States than were adult oysters. The business of exporting seed oysters soon developed in Japan. Glud (1949) and Cahn (1950) have described in detail the Japanese methods of collecting and shipping oyster seed.

Significant commercial production of Pacific oysters in the United States began in 1928 when 8000 cases of seed were imported into Washington (Woelke, 1957).[1] By the 1930's, the industry had expanded into British Columbia, Oregon, and other regions in Washington.

## Sources of Oyster Seed

Obtaining seed oysters probably has been the biggest problem faced by the Pacific oyster industry throughout its history. Waters along the western coast of the United States are generally too cold to permit Crassostrea gigas to reproduce, so oyster growers must purchase seed

[1] A standard case of oyster seed has a capacity of four cubic feet and contains 10,000 to 20,000 spat attached to pieces of cultch (Galtsoff, 1929; Steele, 1964).

oysters to place on their growing grounds. According to Steele (1964), a pioneer Washington oysterman, "The success or failure of an oyster industry is the same in one respect as any upland crop. It is entirely dependent upon being able to secure good seed at a reasonable price."

Initially almost all seed was imported from Japan. From 1924 to 1976, Pacific oystermen received annual shipments of Japanese seed, except during the war years, 1941-1946 (Steele, 1964; Im et al., 1976). The quality of Japanese seed varied widely during a given season and from year to year. Shipping mortalities of five to twenty-five percent were common. In some years, Japanese growers were unable to produce enough seed to meet buyers' demands (Woelke, 1957).

In Hood Canal and Willapa Bay (Washington) and in Pendrell Sound (British Columbia), hydrographic and weather conditions sometimes create an environment conducive to spawning of Crassostrea gigas. As early as 1932, Pacific oysters in these regions began to produce natural sets of young oysters (Steele, 1964).[2]

In Washington, natural spatfalls do not occur annually. Water temperatures often fail to get high enough to trigger spawning, or larvae may be flushed from the bays before setting takes place (Korringa, 1976). In Hood Canal, commercial-size spatfalls usually occur in seven out of ten years; setting is more sporadic in Willapa Bay (Chew, 1979). Setting occurs nearly every year in Pendrell Sound (Quayle, 1969), but U.S.

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[2]The terms set, setting, and spatfall are used interchangeably to refer to the attachment of free-swimming larvae to a substrate. After attaching, larvae undergo a metamorphosis to become juvenile oysters.

oyster growers prefer seed from Washington or Japan. They believe Canadian spat grow more slowly thus taking longer to reach marketable size.

As early as 1924, Kincaid wished to reduce the Pacific oyster industry's dependence on natural spatfalls by producing seed under artificially controlled conditions (Steele, 1964). At that time, laboratory methods for rearing oyster larvae to the setting stage had not been developed, and Kincaid's attempts were unsuccessful.

## Development of West Coast Oyster Hatcheries

Present hatchery technology evolved from a series of scientific investigations on the artificial propagation of oysters. Loosanoff (1971) and Clark and Langmo (1979) have reviewed significant developments, including the contributions of Winslow (1884), Wells (1920), Prytherch (1934), Loosanoff and Davis (1963), and Walne (1964).

During the 1970's, several west-coast hatcheries opened to provide another source of seed for Pacific oyster growers. Most experienced limited success, because hatchery seed was more expensive than domestic or imported seed. In recent years, the cost of domestic and imported seed has increased, and with continued technological advancements, hatchery seed may soon be cost-competitive (Chew, 1979).

Between 1960 and 1974, the price of Japanese seed tripled due to rising labor costs in Japan and increased shipping rates (Im et al., 1976). Little if any Japanese seed was purchased by west-coast growers

in 1981 due to high costs.[3]

The price of domestic seed will increase as escalating fuel prices cause transportation costs to climb. Each year Washington oyster growers prepare strings of oyster shells and transport them to natural setting areas to collect wild spat, with no guarantee that shells will collect enough spat to be commercially useable. One grower annually transports 20,000 shell strings from Willapa Bay to Hood Canal, a distance of over 100 miles. The bulky cultch is conveyed by a truck which carries only 2000 strings at one time (Korringa, 1976). Oregon and California growers must depend on imported or hatchery seed, since these states lack natural setting areas.

Handling cultch is a significant cost in the production of hatchery seed also. Traditionally, the hatchery operator has acquired shell from the growers, set the larvae (i.e. provided a substrate and allowed larvae to attach to it), and transported the shell back to the growers (Clark and Langmo, 1979). Im and Langmo (1977) estimated that cultch preparation accounted for 15-26% of hatchery seed-production costs. One hatchery operator in Netarts, Oregon, has reduced production costs by selling oyster larvae rather than selling seed. Setting the larvae on cultch becomes an activity of the grower, not of the hatchery.

Since prices for seed have risen faster than have prices for marketable oysters (Im et al., 1976), more west-coast growers are interested in buying and setting larvae. In 1981, one million oyster larvae

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[3]Interview with Wilbur P. Breese, Professor of Fisheries, Oregon State University, Corvallis, Oregon, June, 1981.

cost $100, and the hatchery operator guaranteed that 20% of the larvae would settle.[4]

## Setting and Metamorphosis of Oyster Larvae

Attachment to a substrate occurs only when oyster larvae are completely developed. Fujita (1934) has described the anatomical development of Crassostrea gigas from gastrula to setting stage. Figure 1 shows the major stages in the development of Pacific oyster larvae. A fully developed larva possesses a swimming organ, the velum; a crawling organ, the foot; and two pigment spots, one on each side of the body.

These pigment spots are often called eyes, although there is no conclusive evidence that they are light-sensitive (Prytherch, 1934; Cole and Knight-Jones, 1939; Galtsoff, 1964). Larvae with well developed pigment spots are called eyed larvae. Pigment spots develop during the last few days before settlement and indicate that a larva is ready to end its free-swimming life and seek a solid surface on which to attach and transform into an adult.

The behavior of attaching oyster larvae has been described by many authors, including Nelson (1924), Prytherch (1934), Cole and Knight-Jones (1939), Yonge (1960), Galtsoff (1964), and Cranfield (1973a). A fully developed larva swims with the foot and velum projecting from between the shell valves. When a larva touches a solid surface, it adheres with the foot and begins to crawl. This may lead to final attachment, or the foot

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[4] Interview with Lee Hanson, owner, Netarts Bay Oyster Hatchery, Netarts, Oregon, June, 1981.

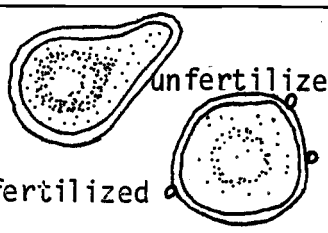
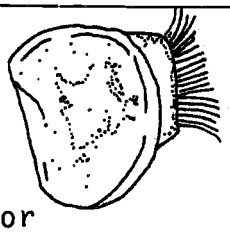
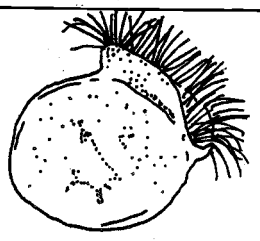
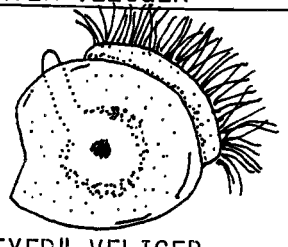
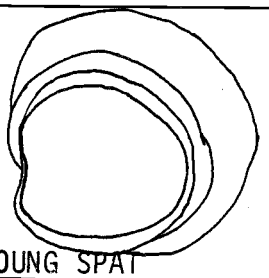
| STAGE | AGE | SIZE |
|---|---|---|
|   EGG | 0-24 hours | 55 microns |
|  "D" or STRAIGHT-HINGE VELIGER | 1-6 days | 75-120 microns |
|  LATER VELIGER | 7-14 days | 130-200 microns |
|  "EYED" VELIGER | 14-21 days | 200-300 microns |
|  YOUNG SPAT | 21 days | up to about 4 millimeters |

Figure 1. Major stages in the development of Pacific oyster larvae and their approximate sizes and ages (After Breese and Malouf, 1975).

may be withdrawn, the velum projected, and swimming resumed. Crawling, alternating with periods of swimming, can continue for several days if no suitable surface is encountered (Cole and Knight-Jones, 1939). Settlement is completed when the larva presses its left valve into a cement secreted by pedal glands. The cement hardens in a few minutes, and metamorphosis begins immediately.

Physiological changes that occur during the metamorphosis from larva to juvenile oyster have been described by Cole (1939), Yonge (1960), and Galtsoff (1964). Rapid growth of the adult shell begins during the second day after attachment (Yonge, 1960).

## Factors That Affect Settlement

Numerous physical and chemical factors influence the settlement of marine invertebrate larvae, including oysters (Crisp, 1974). Kinne (1957) cited temperature, salinity, and substrate as the most important environmental variables for marine and brackish-water organisms.

Effects of temperature on metabolism and activity have been reviewed by Kinne (1963) who stated, "With respect to life as a whole, temperature is presumable the most important single environmental entity." Temperature studies on setting-stage oyster larvae have shown mixed results.

For the American (or eastern) oyster, Crassostrea virginica, rapidly increasing the temperature from 24° to 29°C stimulated setting (Lutz et al., 1970). At low temperatures, the setting period was prolonged, and fewer larvae successfully completed metamorphosis (Loosanoff and Davis, 1963; Davis and Calabrese, 1964; Loosanoff, 1965).

Varying temperature within the range of 19° to 23°C had no effect on setting of the European oyster, Ostrea edulis (Bayne, 1969).

Another factor that may affect setting oyster larvae is salinity. General effects of salinity on the structure, metabolism, activity, and distribution of marine organisms have been reviewed by Kinne (1964).

Prytherch (1934) observed heavy sets of American oysters near river mouths and at the stage of tide when salinity was lowest. His laboratory experiments failed to show a specific salinity or a change in salinity that induced setting, but salinity appeared to affect the time required for a larva to attach. In other laboratory studies, Davis (1958) obtained significantly more Crassostrea virginica spat at a salinity of 17.5 parts per thousand (ppt) than at other test salinities ranging from 7.5 to 26 ppt.

Prytherch (1934) suggested that salinity influenced setting by affecting glands in the larval foot. At relatively high or low salinities, larvae seemed to have difficulty ejecting the attachment cement, consequently, fewer larvae successfully completed the metamorphosis into juvenile oysters.

For sessile animals, such as oysters, the selection of a settlement site is particularly important for survival (Thorson, 1964). When oyster larvae are fully developed, they will attach to almost any clean non-toxic substrate. In nature they settle on rocks, gravel, cement, wood, mollusk shells, marsh grass, tin cans, rubber boots, tires, glass, and plastic (Galtsoff, 1964). In tank and laboratory experiments, limed tiles (Cole, 1939), cement board (Butler, 1954), glass (Galtsoff, 1964), and plastics (Loosanoff, 1958; Galtsoff, 1964; Walne, 1979) have been

used to collect spat.

The setting rate and the percentage of attaching larvae can be increased by providing a preferred substrate or by chemically treating a substrate. If old clean oyster shells and artificial collectors are placed together, the oyster shells will collect more spat (Loosanoff, 1965). Extracts of oyster tissue applied to shells or slate panels induced settlement in European (Bayne, 1969; Walne, 1966), American (Crisp, 1967; Keck et al., 1971; Veitch and Hidu, 1971), and Pacific oysters (Lund, 1972).

The concentration of food cells in the water may have an effect on the settlement of oyster larvae. In large outdoor tanks, Cole (1939) found dense spatfalls of Ostrea edulis associated with high levels of algal flagellates, and in laboratory experiments, food-cell concentrations of 100,000 cells per larva stimulated settlement (Bayne, 1969). Chanley (1975) has recommended tripling food rations as a general rule for setting bivalves in the laboratory.

Light is another factor that might influence settlement. Thorson (1964) stated that the larvae of most intertidal species select shaded areas for attachment and metamorphosis. The results of studies on the effects of light on settling oyster larvae are inconsistent.

Many investigators have reported that fully developed oyster larvae preferred the lower shaded surfaces of spat collectors, especially during daylight (Cole and Knight-Jones, 1939; Medcoff, 1955; Yonge, 1960; Walne, 1974, 1979). Korringa (1952), Butler (1954), and Quayle (1969), however, obtained the opposite results; the majority of larvae settled on upper surfaces.

Walne (1966, 1979) hypothesized that some light prior to settlement was stimulatory, but the intensity should not exceed that used during the setting period. He found spatfalls occurred earlier and were more prolonged in light than in dark. Bayne (1969) observed that high light intensities induced settlement of European oysters.

Some authors have suggested that factors such as siltation or competition from other organisms might mask the effects of light. Prytherch (1924) reported heavy setting on both light and dark sides of shell collectors that were equally clean. According to Butler (1954), a layer of sediment 1/75 of an inch thick will prevent attachment on what would normally be a preferred surface and will cause larvae to settle on surfaces they would normally avoid.

## Statement of the Problem

The oyster hatchery in Netarts, Oregon, sells fully developed eyed larvae. Oyster growers put the larvae in large tanks filled with seawater and old oyster shells. The larvae settle, attach to the shells, and undergo the metamorphosis into juvenile oysters. Shells and spat are moved to a bay or an estuary to grow.

To realize the greatest economic benefits, growers setting eyed larvae want to achieve:

1) the highest percentage of larvae attaching to the shells.

2) the greatest growth and survival of the spat.

This study was designed to investigate factors, which an oyster grower could control, that might affect the percentage of setting larvae and the subsequent survival of the spat. Larvae were obtained from the

Netarts hatchery and were handled as closely as possible to the way they would be handled by an oyster grower.

Each experiment was a two-factor analysis designed to allow examination of possible interactions between variables. Due to its importance in biological processes (Kinne, 1963), temperature was a variable in all experiments except one. Other variables were salinity, feeding, type of substrate, and storage time before setting.

## GENERAL METHODS

Eyed larvae of the Pacific oyster, Crassostrea gigas, were obtained from a commercial hatchery in Netarts, Oregon. Spawning and rearing methods at the hatchery followed those of Loosanoff and Davis (1963).

Larvae were removed from 6400-gallon fiberglass rearing tanks by draining water from the tanks through a 230-micron-mesh nylon screen. Larvae were washed from the screen and wrapped in nylon cloth. The bundle of larvae was moistened with seawater, wrapped in damp paper towels, and placed inside a styrofoam chest for transport.

After returning to the laboratory, the larvae were refrigerated until the experiment began, usually the same day. In general, a new batch of larvae was used for each experiment.

Larvae were placed in a 250-milliliter glass beaker with approximately 200 milliliters of filtered, pathogen-free seawater. The water in the beaker was agitated with a perforated plexiglass plunger to obtain a uniform suspension of larvae. One milliliter aliquots were removed with an Eppendorf volumetric pipette.[5]

In each experimental container, aliquots were added to three liters of seawater. Every third aliquot was preserved in a small vial. The larvae in these vials were counted and the numbers were averaged to determine the quantity of larvae in each experimental container. This method for determining means was accurate to about $\pm$ 10 percent. Among experiments, the number of larvae per container varied from 1050 to 1650,

[5]Oyster larvae reportedly can withstand vigorous stirring before quantitative samples are taken (Calabrese and Davis, 1970).

however, within one experiment, all containers were assumed to contain an equal number of larvae.

Cylindrical one-gallon plastic jars, obtained from a fast-food restaurant, were used as experimental containers. Treatments were assigned to the containers at random; two jars received each treatment. Rectangular containers have not been recommended for bivalve culture, because larvae congregate in the corners. This may lead to mortality due to lack of food, lack of oxygen, or build-up of toxic metabolites (Walne, 1974; Culliney et al., 1975). Calabrese and Davis (1970) found no evidence of toxicity in white or natural-color polyethylene, polypropylene, or polycarbonate plastics, and a bioassay carried out on the containers used in these experiments showed them to be non-toxic.

The jars were covered to reduce evaporation and were placed in thermostatically-regulated water baths or in temperature-controlled rooms. Individual containers were not aerated. Loosanoff and Davis (1963) stated that aeration or mechanical agitation was unnecessary if the water in the culture containers was changed frequently, and Lund (1972) recorded better setting of Crassostrea gigas larvae when individual jars were not aerated. The respiratory activity of oyster larvae reportedly has little effect on the gas content of the water (Walne, 1964, 1966), although bivalve larvae can tolerate very low oxygen concentrations at least for short periods (Loosanoff and Davis, 1963).

The jars or water baths were covered with black plastic during the experiments. Crisp (1974) emphasized that larval reactions to light in laboratory experiments do not always correspond to field observations, and Galtsoff (1964) concluded that Crassostrea virginica larvae in the

laboratory do not discriminate between light and dark surfaces. Since the influence of light on settlement is unclear, the containers were covered to eliminate light as a variable.

Larvae settled on the plastic containers or on mollusk shells used as cultch. Plastics appear suitable for attachment of oyster larvae and have been used as spat collectors in some hatcheries (Loosanoff, 1958; Walne, 1979). The mollusk shells had approximately equal surface areas for setting, and they were assigned to the experimental containers at random. Hidu (1969) reported that individual oyster shells showed no differential attractiveness to setting Crassostrea virginica larvae. Using a marking pen with permanent black ink, each shell was labelled with the experiment number, the jar number, and a code number indicating the orientation of the shell in the jar. In each container, half the shells were oriented with the cupped side up and half with the cupped side down.

Under normal conditions, most larvae should attach within twenty-four hours (Matthiessen, 1970; Lund, 1972). In these experiments, the larvae were allowed 48 hours to settle.

After the 48-hour experimental period, all jars were treated identically. They were emptied, and any unattached larvae were rinsed out using a squirt bottle filled with seawater. Jars of clean water at room temperature and ambient salinity were ready, so larvae were not exposed to air for more than a few seconds. Culliney et al. (1975) recommended this procedure, although other authors have reported that oyster larvae can withstand sharp changes in their environment, including complete water changes (Walne, 1964; Loosanoff, 1965).

In experiments by Lund (1972), Crassostrea gigas larvae retained on nylon screens for up to one hour apparently were unharmed.

Spat were fed the unicellular flagellate, Pseudoisochrysis paradoxa,[6] at a concentration of approximately 120,000 algal cells per milliliter of water in the experimental containers. Algal cells were counted with a Coulter Counter.[7]

After two or three days, the larvae that had settled on the smooth inner surfaces of the shells were counted under a dissecting microscope. Davis and Calabrese (1964), Lund (1972), and others followed this procedure, because it is nearly impossible to accurately count settled larvae on the convoluted outer surface of oyster shells. Crisp (1967) reported that Crassostrea virginica larvae preferred the smooth internal surface of oyster shells to the rough outer surface, especially when shells were oriented with the cupped side down. Spat were considered attached if they were not dislodged by gentle stroking with a camel-hair brush (Lund, 1972).

After the spat were counted, shells were placed in stackable plastic baskets in a large tank of raw seawater in an indoor laboratory of the Oregon State University Marine Science Center. This was to

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[6]The original culture of Pseudoisochrysis paradoxa was obtained from the Virginia Institute of Marine Science where it was isolated by Dr. John Dupuy. Large cultures of P. paradoxa provide the principal food for bivalves used in shellfish research at Oregon State University.

[7]The Coulter Counter operates on the following principle: as particles suspended in an electrically conductive fluid (in this case algal cells in seawater) pass through a small aperture, they alter the resistance between two electrodes producing a series of voltage pulses that are electronically counted. Coulter Electronics Industrial Division, Instruction Manual, Coulter Counter/Industrial Model B. (Franklin Park, Illinois), Section 2.

simulate the oysterman's practice of moving spat directly from setting tanks to a bay or an estuary. Approximately 8.5 liters of raw seawater from Yaquina Bay flowed through the laboratory tank each minute. Shells in the baskets were oriented with their cupped sides down to prevent spat from becoming smothered with silt.

The larvae that settled on the plastic jars were held an additional two to five days to allow them to grow enough to be counted with the naked eye. Clean water and food were added to the containers every two to three days. After the attached juveniles were counted, the jars were scrubbed with hot water before being reused. No soap was used, because many detergents, including the widely used Alconox, are toxic to larvae (Culliney, et al., 1975).

The number of larvae that settled on the shells was added to the number settled on the corresponding jar to obtain a total number of larvae setting in each experimental container. These totals were transformed into the percentages of larvae that settled out of the number that initially were placed in each jar. The percentages were subjected to a statistical analysis of variance to determine the significance of the variables on setting.

The shells that had been placed in raw seawater in the laboratory were removed after eight to nine months. They were sprayed with a strong jet of water to remove any debris or unattached animals (Butler, 1954). The number of surviving spat were counted and compared to the number that originally settled on the shells. The percentages of surviving spat were examined to determine how the temperature, salinity, feeding, and type of substrate used during the setting period affected the

survival of the juvenile oysters.

# SETTING EXPERIMENTS

## Combined Effects of Temperature and Salinity on Setting

Temperature and salinity are master ecological factors for many aquatic organisms. Temperature can enlarge, narrow, or shift the salinity tolerance of a species; salinity can similarly alter temperature tolerance (Kinne, 1957, 1964). Interactive effects of temperature and salinity on the structure, metabolism, and activity of marine and brackish-water organisms have been reviewed by Kinne (1964).

In experiments with American oysters, Crassostrea virginica, and hard-shell clams, Mercenaria mercenaria, Davis and Calabrese (1964) found that at near-optimum salinities young larvae survived and grew over a significantly wider range of temperatures than at salinities near the lower limits of their tolerance.

Embryos and larvae of the coot clam, Mulinia lateralis, (Calabrese, 1969), the mussel, Mytilus edulis, (Brenko and Calabrese, 1969; Lough, 1973), and the boring bivalve, Adula californiensis (Lough and Gonor, 1973) showed similar responses. At unfavorable salinities, the temperature tolerance narrowed; at unfavorable temperatures, the salinity tolerance narrowed. When both temperature and salinity were at acceptable levels, there was no interrelationship between the factors.

Most experiments on setting-stage oyster larvae have considered the individual effects of temperature or salinity, rather than the combined effects. In studies with Crassostrea virginica, Davis and Calabrese (1964) found that larvae reared to setting size at 27$^{o}$C could

set at temperatures as low as 12.5°C, however, the percentage successfully completing metamorphosis decreased with each decrease in temperature. In laboratory studies, all Crassostrea virginica larvae reared at 27°C and transferred to 25°C settled within ten days. Those transferred to 20°C required up to 20 days to set (Loosanoff, 1965).

Prytherch (1934) observed that salinity influenced the length of time required for individual C. virginica larvae to complete their attachment. He concluded that the optimum salinity for setting American oysters was 15-25 ppt, although setting occurred at salinities from 9-29 ppt. Beyond these limits, only a small percentage of larvae attached.

In studies with the European oyster, Ostrea edulis, Davis and Ansell (1962) noted that larval growth and setting intensity were reduced at suboptimal salinities.

Amemiya (1928) first observed a relationship between temperature and salinity in Pacific oyster development. He found that Crassostrea gigas embryos developed over a wider range of salinities at a lower temperature. At 25°C, the optimum salinity range for embryonic development was 20-26 ppt. At 16°C, the optimum was 17-26 ppt, although development was retarded at this temperature.

Experiments 1, 2, 3A, and 3B were designed to investigate the combined effects of temperature and salinity on the settlement of Pacific oyster larvae. Temperatures and salinities were chosen from within known limits for C. gigas development.

The optimum temperature for embryonic and larval development of the Pacific oyster is around 25°C (Seno et al., 1926; Cahn, 1950;

Korringa, 1976; Helm and Millican, 1977). Seno et al. (1926) found that the lowest temperature at which eggs developed into shelled larvae was 15°C and the highest was 30°C.

In general, temperatures used for these experiments were 15°, 20°, 25°, and 30°C. Thermostatically-regulated water baths maintained the three higher temperatures. Experimental containers at 15°C were held in a refrigerated room.

The optimum salinity range for development of embryos and larvae of Crassostrea gigas is 23-28 ppt (Seno et al., 1926; Cahn, 1950; Korringa, 1976). At salinities greater than 30 ppt or less than 15 ppt, embryos are often deformed, and larvae develop abnormally. Formation of the larval shell may be retarded at unsuitable salinities (Amemiya, 1928).

Salinities used in these experiments were prepared from seawater pumped from Yaquina Bay to the Oregon State University Marine Science Center and diluted with tap water. The seawater was sand-filtered and sterilized with ultraviolet light. Toxic chlorine was removed from city water with charcoal filters. Salinities were measured with a hydrometer.

## Experiment 1

Temperatures of 15°, 20°, 25°, and 30°C and salinities of 15, 20, 25, and 30 ppt were used in a four by four factorial design for Experiment 1. Two jars were prepared at each of the 16 treatments. Each container received 1647 eyed larvae.[8] Procedures differed somewhat from

[8]This number was determined by averaging the number of larvae in preserved aliquots, as described under General Methods.

those described under General Methods. Rather than using a 48-hour test period, experimental temperatures and salinities were maintained throughout the ten-day experiment. Every two to three days, each jar was emptied through a 240-micron-mesh nylon screen and was refilled with clean water. Larvae retained on the screen were washed into vials and preserved with formalin. Vials were numbered to correspond to the experimental containers. These preserved larvae were examined when determining the total number of metamorphosed larvae, but this procedure is discussed later.

At each water change, food (Pseudoisochrysis paradoxa) was added at the rate of 20,000 cells per milliliter (60,000 cells/larva).

Larvae that settled on the bottoms and sides of the plastic containers were counted after ten days. Table 1 shows the numbers of larvae settling at each combination of temperature and salinity. Numbers in parentheses are the percentages of larvae that settled of the 1647 initially placed in each jar.

Table 1. Numbers and, in parentheses, percentages of setting Pacific oyster larvae as affected by temperature and salinity (Experiment 1).[a]

| Salinity (ppt) | Temperature 30°C | 25°C | 20°C | 15°C | Mean |
|---|---|---|---|---|---|
| 30 | 96 ( 6)<br>414 (25) | 413 (25)<br>305 (19) | 24 ( 1)<br>126 ( 8) | 0 ( 0)<br>15 ( 1) | (10.62) |
| 25 | 383 (23)<br>463 (28) | 306 (19)<br>404 (25) | 134 ( 8)<br>110 ( 7) | 9 ( 1)<br>8 ( 0) | (13.88) |
| 20 | 201 (12)<br>323 (20) | 343 (21)<br>27 ( 2) | 183 (11)<br>27 ( 2) | 8 ( 0)<br>3 ( 0) | ( 8.50) |
| 15 | 300 (18)<br>388 (24) | 149 ( 9)<br>283 (17) | 132 ( 8)<br>200 (12) | 5 ( 0)<br>5 ( 0) | (11.00) |
| Mean | (19.50) | (17.12) | ( 7.12) | ( 0.25) | |

[a] The combination of temperature and salinity at which the most larvae settled is enclosed by solid lines. Other satisfactory combinations are enclosed by dashed lines.

The highest percentage of larvae settled at 30°C and 25 ppt. Sets were also good a 25°C when salinity was 25 or 30 ppt.

A two-way analysis of variance (Table 2) indicates that temperature had a statistically significant effect on the percentage of setting larvae. At higher temperatures, more larvae attached. After ten days at 30°C, an average of 19.50% of the larvae were attached; 17.12% were attached at 25°C. Fewer larvae settled on jars held at lower temperatures. The average percentages attached at 20°C and at 15°C were 7.12% and 0.25%, respectively.

Varying the salinity from 15 to 30 ppt had no statistically significant effect on the percentage of setting larvae, and there was no evidence of interaction between temperature and salinity.

Table 2. Analysis of variance on the percentage-setting data given in Table 1. N.S. denotes that the difference was not significant.

| Source of Variation | d.f. | Mean Square | F Ratio | p |
|---|---|---|---|---|
| Temperature | 3 | 640.917 | 18.118 | .001 |
| Salinity | 3 | 39.083 | 1.105 | .375 N.S. |
| Interaction | 9 | 26.667 | .754 | .658 N.S. |
| Residual | 16 | 35.375 | | |

As the spat on the jars were counted, many were detached when stroked with the camel-hair brush (Lund, 1972). These detached larvae were placed in a Petri dish and were examined under a dissecting microscope. The number that showed adult shell growth (evidence that they had successfully completed metamorphosis) was recorded for each experimental container. The vials of larvae removed from the screen at water changes were similarly scrutinized for larvae with adult shell. These numbers were added to the jar counts to determine the total number of larvae in each jar that successfully completed metamorphosis. The numbers and percentages of larvae completing metamorphosis at each treatment are shown in Table 3.

Table 3. Numbers and, in parentheses, percentages of metamorphosing Pacific oyster larvae as affected by temperature and salinity (Experiment 1).[a/]

| Salinity (ppt) | Temperature 30°C | 25°C | 20°C | 15°C | Mean |
|---|---|---|---|---|---|
| 30 | 223 (14)<br>625 (38) | 769 (47)<br>493 (30) | 234 (14)<br>233 (14) | 42 ( 3)<br>46 ( 3) | (20.83) |
| 25 | 529 (32)<br>748 (45) | 532 (32)<br>659 (40) | --<br>342 (21) | 85 ( 5)<br>34 ( 2) | (25.29) |
| 20 | 274 (17)<br>489 (30) | 485 (29)<br>-- | 424 (26)<br>182 (11) | 156 ( 9)<br>174 (11) | (19.00) |
| 15 | 473 (29)<br>588 (36) | 370 (22)<br>385 (23) | 432 (26)<br>390 (24) | 101 ( 6)<br>-- | (23.71) |
| Mean | (30.13) | (31.86) | (19.43) | ( 5.57) | |

[a/] The combination of temperature and salinity at which the most larvae metamorphosed is enclosed by solid lines. Other satisfactory combinations are enclosed by dashed lines.

The most larvae metamorphosed at 30°C, 25 ppt and at 25°C, 30 ppt. The percentage metamorphosing at 25°C and 25 ppt was high also.

A temperature of 25°C was slightly better than 30°C. An average of 31.86% of the larvae in jars at 25°C successfully metamorphosed; 30.13% metamorphosed at 30°C. The percentages at 20°C and 15°C were much lower, 19.43% and 5.57%, respectively.

An analysis of variance (Table 4) shows the same results as the original analysis. More larvae metamorphosed at higher temperatures, salinity had no significant effect, and no interaction was observed.

Table 4. Analysis of variance on the percentage-metamorphosing data given in Table 3. N.S. denotes that the difference was not significant.

| Source of Variation | d.f. | Mean Square | F Ratio | p |
|---|---|---|---|---|
| Temperature | 3 | 1021.677 | 17.039 | .001 |
| Salinity | 3 | 33.820 | .564 | .648 N.S. |
| Interaction | 9 | 73.800 | 1.231 | .355 N.S. |
| Residual | 13 | 59.962 | | |

Cole and Knight-Jones (1939) noted that Ostrea edulis larvae, dislodged after setting, did not attempt to reattach. Cranfield (1973b) observed that the pedal cement glands discharge completely during settlement. The larval foot is reabsorbed completely within three days after attachment (Yonge, 1960; Galtsoff, 1964). Commercial oystermen are uninterested in larvae that become detached after setting. These larvae either will die or will be lost when cultch is transferred from setting tanks to a bay or an estuary. Because of this, and because the effects of temperature and salinity were the same whether the analysis was carried out on the percentages of setting larvae or on the percentages of metamorphosing larvae, in Experiments 2-7, only setting percentages were determined.

Experiment 2

Experiment 2 was also a four by four factorial design. Temperatures were $15^{o}$, $20^{o}$, $25^{o}$, and $28^{o}$C; salinities were 15, 20, 25, and 30 ppt. There were duplicate cultures at each of the 16 combinations of temperature and salinity.

Two scrubbed oyster shells were placed on the bottom of each jar

as substrate for the settling larvae. (Oystermen expressed concern that larvae might set differently on oyster shells than on plastic containers.) One shell was oriented with the cupped side up, the other with the cupped side down. Based on the average of the preserved aliquots, each container received 1588 larvae. Experimental temperatures and salinities were maintained for two days. (Oyster growers usually leave eyed larvae in setting tanks for 24-48 hours.)

After 48 hours, the jars were emptied and were refilled with water at room temperature (20$^{o}$C) and ambient salinity (34 ppt). Food was added, and the jars were left an additional two days to allow time for the spat to begin producing adult shell. Then the cultch was removed from the jars, and the spat were counted. The spat were placed in baskets in the tank of raw seawater in the laboratory.

Spat that settled on the jars in Experiment 2 were counted seven days after the start of the experiment. Table 5 shows the numbers and percentages of larvae that settled at each combination of temperature and salinity. The best set occurred at 28$^{o}$C and 20 ppt.

Table 5. Numbers and, in parentheses, percentages of setting Pacific oyster larvae as affected by temperature and salinity during a 48-hour setting period (Experiment 2).[a/]

| Salinity (ppt) | Temperature 28°C | 25°C | 20°C | 15°C | Mean |
|---|---|---|---|---|---|
| 30 | 21 ( 1)<br>71 ( 4) | 34 ( 2)<br>28 ( 2) | 33 ( 2)<br>13 ( 1) | 13 ( 1)<br>7 ( 0) | (1.62) |
| 25 | 25 ( 2)<br>74 ( 5) | 24 ( 2)<br>40 ( 3) | 17 ( 1)<br>7 ( 0) | 11 ( 1)<br>8 ( 1) | (1.88) |
| 20 | 122 ( 8)<br>117 ( 7) | 20 ( 1)<br>22 ( 1) | 12 ( 1)<br>12 ( 1) | 5 ( 0)<br>3 ( 0) | (2.38) |
| 15 | 71 ( 4)<br>8 ( 1) | 17 ( 1)<br>19 ( 1) | 24 ( 2)<br>8 ( 1) | 10 ( 1)<br>13 ( 1) | (1.50) |
| Mean | (4.00) | (1.62) | (1.13) | (0.62) | |

[a/] The combination of temperature and salinity at which the most larvae settled is enclosed by solid lines.

The statistical analysis of the data (Table 6) shows an interaction between temperature and salinity.

Table 6. Analysis of variance of the percentage data given in Table 5. N.S. denotes that the difference was not significant.

| Source of Variation | d.f. | Mean Square | F Ratio | p |
|---|---|---|---|---|
| Temperature | 3 | 17.865 | 17.323 | .001 |
| Salinity | 3 | 1.198 | 1.162 | .355 N.S. |
| Interaction | 9 | 4.059 | 3.936 | .008 |
| Residual | 16 | 1.031 | | |

Figure 2 illustrates that in general setting increased with temperature for all salinities. For Experiment 2, a synergistic effect on setting appeared when larvae were set at 28°C and 20 ppt.

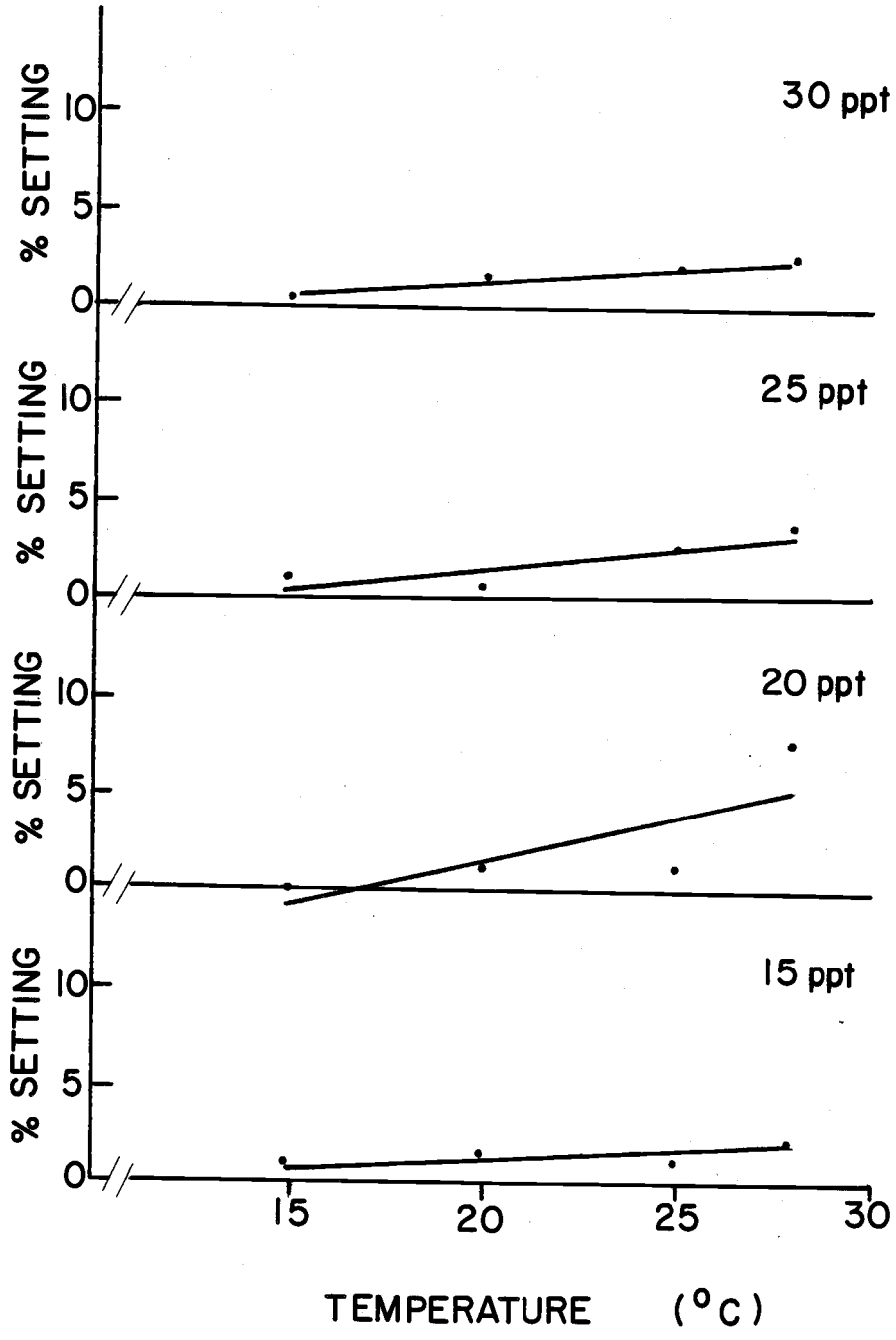


Figure 2. Effect of temperature on the percentage of setting Pacific oyster larvae at four experimental salinities (Experiment 2).

In Experiment 2 and in many ensuing experiments, the percentages of setting larvae were far below the 20% guaranteed by the hatchery operator. Part of this resulted from counting larvae on only one side of the oyster shells, but this alone fails to explain the low setting percentages.

Loosanoff and Davis (1963) reported considerable differences in performance among batches of Crassostrea virginica larvae grown under identical conditions. Cole (1937) concluded that a proportion of tank-reared Ostrea edulis larvae were inherently nonviable, although the non-viable individuals could not be detected microscopically.

Spawning and rearing conditions at the hatchery may have affected the percentages of settling larvae. Walne (1966) noted that populations of O. edulis larvae grown rapidly to a large size had a smaller proportion of eyed larvae at that size than did populations grown more slowly. Larvae grown too slowly, however, lacked the vigor to metamorphose. The first larvae removed from a rearing tank at the Netarts hatchery were those that grew fastest and reached setting size first. Fewer larvae may have been physiologically ready to settle in these early batches. The last batches removed from a rearing tank would have included slower-growing larvae that may have been unable to metamorphose.

Schaefer (1938) and Loosanoff (1954) suggested that late-season spawnings produced feeble larvae, although Davis and Chanley (1956) questioned this conclusion. Loosanoff and Davis (1963) reported no significant difference in the quality of spawn produced by adult oysters of different ages or sizes. Bruce et al. (1940) stated that the viability of O. edulis larvae depended upon factors influencing the adult

breeding stock prior to spawning, but Walne (1964) found little evidence that the physiological or genetic condition of parent oysters influenced growth and survival of larvae.

Detailed hatchery records were not available when the present experiments were conducted, but future studies at Oregon State University are planned to investigate the effects of spawning and rearing conditions on setting variability among batches of hatchery larvae.

Alternatively, the poor sets may have been caused by something in the laboratory system at the O.S.U. Marine Science Center. Experiment 1 and several preliminary experiments were carried out in the autumn of 1979 in an older building of the Marine Science Center. The results showed high percentages of setting larvae. Few larvae attached in Experiments 2-7 which were conducted in a new laboratory during the summer and autumn of 1980. Good sets occurred in Experiments 4A and 4B, although they were carried out in the new laboratory.

Calabrese and Davis (1970) reported differences in growth of oyster larvae reared in old and new laboratories at the Bureau of Commercial Fisheries Laboratory in Milford, Connecticut. They were unable to determine whether the seawater system of the new laboratory was initially toxic or whether the growth abnormalities resulted from seawater differences. Natural variations in seawater can inhibit growth or cause mortality of oyster larvae (Chanley, 1975; Culliney et al., 1975).

In Experiment 2 and in other experiments with poor sets, high percentages of the attaching larvae settled on the oyster shells placed in the jars as cultch (see Appendix). If more shells had been used, sets might have been better.

Both experimental conditions and differences in larvae could have affected the results of these experiments. Something in the water system of the new building possibly was harmful to the setting larvae. Conditioning or genetic differences in batches of larvae could account for the good results in Experiments 4A and 4B.

Experiment 3A

Crassostrea gigas exists throughout Japan in several varieties. These varieties differ in environmental requirements and in the size, shape, and coloration of their shells.

The Pacific oysters of the western United States originated in the Miyagi prefecture of northern Japan. They have large shallow shells and are adapted to cold water. Oysters from the more southerly Kumomoto prefecture are more deeply cupped, darker in color, and adapted to both cold and warm waters. Other varieties of C. gigas are found in the Hokkaido (most northerly) and Hiroshima (central) regions of Japan. The differences among these varieties are hereditary and persist through generations of inbreeding (Imai and Sakai, 1961).

A few growers in Washington and Oregon cultivate the Kumomoto variety of C. gigas. Kumomoto oysters are smaller and slower-growing than the Miyagi strain. Most Kumomotos are sold to restaurants as cocktail or half-shell oysters. Experiments 3A and 3B were conducted with Kumomoto oysters. (All other experiments used the Miyagi variety of C. gigas.)

Procedures for Experiment 3A were the same as those for Experiment 2. Experimental temperatures were $16^{o}$, $20^{o}$, $25^{o}$, and $30^{o}$C; salinities

were 15, 20, 25, and 30 ppt. Based on the average of the preserved aliquots, each container received 1136 eyed larvae. There were two jars at each combination of temperature and salinity. After the 48-hour experimental period, all jars were refilled with water at 20°C and 29 ppt.

Table 7 shows the number and percentage of larvae settling in each container. The highest percentage settled at 30°C and 20 ppt, but setting was good at 20°C and 30 ppt also. An analysis of variance (Table 8), however, shows that the differences between treatments were statistically insignificant.

Table 7. Numbers and, in parentheses, percentages of setting Kumomoto oyster larvae as affected by temperature and salinity during a 48-hour setting period (Experiment 3A).[a]

| Salinity (ppt) | Temperature 30°C | 25°C | 20°C | 16°C | Mean |
|---|---|---|---|---|---|
| 30 | 97 ( 9)<br>45 ( 4) | 71 ( 6)<br>56 ( 5) | 35 ( 3)<br>185 (16) | 89 ( 8)<br>83 ( 7) | (7.25) |
| 25 | 62 ( 5)<br>55 ( 5) | 35 ( 3)<br>22 ( 2) | 77 ( 7)<br>38 ( 3) | 38 ( 3)<br>66 ( 6) | (4.25) |
| 20 | 120 (11)<br>121 (11) | 89 ( 8)<br>60 ( 5) | 16 ( 1)<br>45 ( 4) | 35 ( 3)<br>36 ( 3) | (5.75) |
| 15 | 67 ( 6)<br>66 ( 6) | 26 ( 2)<br>61 ( 5) | 75 ( 7)<br>49 ( 4) | 26 ( 2)<br>31 ( 3) | (4.38) |
| Mean | (7.12) | (4.50) | (5.62) | (4.38) | |

[a] The combination of temperature and salinity at which the most larvae settled is enclosed by solid lines. Other satisfactory combinations are enclosed by dashed lines.

Table 8. Analysis of variance of the percentage data given in Table 7. N.S. denotes that the difference was not significant.

| Source of Variation | d.f. | Mean Square | F Ratio | p |
|---|---|---|---|---|
| Temperature | 3 | 13.031 | 1.610 | .226 N.S. |
| Salinity | 3 | 15.781 | 1.950 | .162 N.S. |
| Interaction | 9 | 10.642 | 1.315 | .303 N.S. |
| Residual | 16 | 8.094 | | |

Experiment 3B

Commercial oystermen often are unable to set eyed larvae immediately after receiving them. Usually larvae are refrigerated until they can be used. Experiment 3B was designed to determine how storing larvae before setting them influenced their responses to temperature and salinity during the setting period.

Larvae used for this experiment were Kumomoto larvae from the same batch used for Experiment 3A. After obtaining larvae from the hatchery, half were used immediately for Experiment 3A. The rest were wrapped in damp nylon cloth and wet paper towels and were held for eight days at $5^{o}$C before being used for Experiment 3B.

Temperatures for Experiment 3B were $15^{o}$, $20^{o}$, $25^{o}$, and $30^{o}$C; salinities were 15, 20, 25, and 30 ppt. Two jars were prepared at each combination of temperature and salinity, and 1083 larvae were placed in each jar. In all other respects, this experiment was identical to Experiment 3A.

Table 9 shows the numbers and percentages of larvae setting at each of the 16 combinations of temperature and salinity. The best set occurred at $25^{o}$C and 30 ppt.

Table 9. Numbers and, in parentheses, percentages of Kumomoto oyster larvae setting at various combinations of temperature and salinity after being held for eight days at 5°C (Experiment 3B).[a/]

| Salinity (ppt) | Temperature 30°C | 25°C | 20°C | 15°C | Mean |
|---|---|---|---|---|---|
| 30 | 68 ( 6)<br>70 ( 6) | 84 ( 8)<br>125 (12) | 42 ( 4)<br>62 ( 6) | 12 ( 1)<br>4 ( 0) | (5.38) |
| 25 | 52 ( 5)<br>75 ( 7) | 69 ( 6)<br>106 (10) | 49 ( 5)<br>39 ( 4) | 8 ( 1)<br>6 ( 1) | (4.88) |
| 20 | 78 ( 7)<br>78 ( 7) | 75 ( 7)<br>80 ( 7) | 38 ( 4)<br>52 ( 5) | 10 ( 1)<br>3 ( 0) | (4.75) |
| 15 | 38 ( 4)<br>51 ( 5) | 82 ( 8)<br>30 ( 3) | 21 ( 2)<br>34 ( 3) | 3 ( 0)<br>1 ( 0) | (3.12) |
| Mean | (5.88) | (7.62) | (4.12) | (0.50) | |

[a/] The combination of temperature and salinity at which the most larvae settled is enclosed by solid lines.

Analysis of this data (Table 10) indicates that the effect of temperature on setting was highly significant. The best temperature for setting was 25°C, although sets were low at all combinations of temperature and salinity. A temperature of 15°C was particularly unsuitable for setting stored larvae; the mean set at 15°C was only 0.50%.

In this experiment, the effect of salinity on setting was significant at the 5% level. More larvae attached at the highest salinity. The mean set at 30 ppt was 5.38%. An average of only 3.12% settled at the lowest salinity of 15 ppt. There was little difference in the average percentage of setting larvae at 20 ppt (4.75%) or 25 ppt (4.88%). There was no interaction between temperature and salinity.

Table 10. Analysis of variance of the percentage data given in Table 9. N.S. denotes that the difference was not significant.

| Source of Variation | d.f. | Mean Square | F Ratio | p |
|---|---|---|---|---|
| Temperature | 3 | 74.115 | 33.404 | .001 |
| Salinity | 3 | 7.615 | 3.432 | .042 |
| Interaction | 9 | 1.476 | .665 | .728 N.S. |
| Residual | 16 | 2.219 | | |

Comparing the results of Experiments 3A and 3B suggests that Kumomoto eyed larvae stored for eight days at 5°C can still settle and metamorphose successfully.

The average percentage of larvae attaching at 25°C was higher for larvae that had been held in the refrigerator before setting than for larvae set soon after receipt from the hatchery. This apparent enhancement of setting after storage is discussed in conjunction with results from Experiments 4A and 4B.

Stored larvae seemed more sensitive to temperature and salinity conditions during setting than did those set initially, but more experiments should be conducted before definite conclusions are drawn.

## Discussion

Results from Experiments 1-3 suggest that temperature during a 48-hour setting period has a highly significant effect on the percentage of eyed larvae that successfully attach and metamorphose. For temperatures ranging from 15°-30°C, setting increased as temperature increased. The present observations support Lund's (1972) conclusion that temperatures less than 24°C are unsuitable for massive setting of Pacific oysters.

Davis and Calabrese (1964) noted that at temperatures of 12.5° and 15°C, the only Crassostrea virginica larvae that settled were those that were mature and ready to set at the time of transfer to those temperatures. Smaller immature larvae survived for more than 20 days after transfer, but their development was arrested.

Setting-stage larvae of bryozoans, Bugula spp. (Lynch, 1947, 1949, 1952), soft-shell clams, Mya arenaria (Stickney, 1964), and American oysters, Crassostrea virginica (Loosanoff and Davis, 1963; Davis and Calabrese, 1964; Loosanoff, 1965), have shown responses similar to those observed here for Crassostrea gigas; metamorphosis decreased as temperature decreased.

Numerous authors have reported that C. gigas larvae will develop throughout a range of salinities corresponding to the natural adult habitat, 10-36 ppt (Amemiya, 1928; Chanley, 1975; Korringa, 1976). Helm and Millican (1977) found no significant difference in growth and survival of later-stage C. gigas larvae placed in salinities from 15-34 ppt. Lund (1972) investigated the response of setting Pacific oyster larvae to salinities ranging from 10 to 40 ppt and concluded that the optimum salinity for setting was 22-34 ppt. The experiments described in the present paper fail to show a significant relationship between settling of C. gigas larvae and salinities ranging from 15 to 30 ppt.

Variations in the results of salinity experiments can be caused by differences in the physiology or conditioning of parent oysters or of larvae. For American oysters, both the optimum salinity and the salinity range for embryonic development appear to be governed by the salinity at which the parent oysters develop gonads (Davis, 1958; Loosanoff and

Davis, 1963; Calabrese and Davis, 1970). Davis (1958) suggested that larvae from oysters that developed gonads at low salinities might survive better and grow faster at low salinities than did larvae from oysters conditioned at higher salinities. Soft-shell clam larvae (Mya arenaria), from parents having dissimilar environmental backgrounds, showed different responses to both salinity and temperature (Stickney, 1964). Muranaka (1981) observed that survival of Crassostrea gigas larvae was influenced by the salinity used to condition adults for spawning. Since records were not kept on the adult oysters used for spawning at the Netarts hatchery, it is impossible to determine whether conditioning effects were important in the experiments described in this paper.

Although these experiments fail to show a single combination of temperature and salinity that yields the greatest percentage of setting Pacific oyster larvae, the data suggest that good results can be obtained by setting commercially-reared eyed larvae at temperatures of 25-30$^{o}$C and salinities of 20-25 ppt. A salinity of 30 ppt may be suitable for setting Kumomoto larvae. (The normal salinity of the native habitat of the Kumomoto oyster, Yatsushio Bay, Japan, is 25-30 ppt.[9]) For Kumomoto larvae held eight days at 5$^{o}$C, the best temperature for setting was 25$^{o}$C, and the best salinity was 30 ppt, however, further experimentation is needed to confirm these results.

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[9]Charles E. Woelke, "Introduction of the Kumomoto oyster Ostrea (Crassostrea) gigas to the Pacific Coast." Washington State Department of Fisheries, Fisheries Research Papers 1 (1955): 41-50.

## Combined Effects of Temperature and Feeding on Setting

Over forty years ago, experimenters observed that in large outdoor tanks, rapid growth and settlement of European oyster larvae (Ostrea edulis) were associated with high levels of naked flagellates (Cole, 1937, 1939). In nature oyster larvae consume a variety of small plankton including flagellates, diatoms, and bacteria. Crassostrea virginica larvae apparently cannot utilize bacteria or organic detritus, and these materials pass through the digestive system without being assimilated (Cahn, 1950; Davis, 1953; Loosanoff, 1954). In laboratory cultures, motile unicellular algae of the order chrysomonadales seem to be the best foods for C. virginica larvae (Davis and Guillard, 1958). The food requirememts of Crassostrea gigas larvae are reportedly the same as those of other Crassostrea larvae (Loosanoff and Davis, 1963).

Temperature influences the results of feeding experiments. High temperatures can destroy food organisms causing reduced larval growth and vitality (Ukeles, 1961; Davis and Calabrese, 1964; Brenko and Calabrese, 1969; Helm and Millican, 1977). At lower temperatures, larvae may ingest foods but be unable to digest and assimilate them (Davis and Calabrese, 1964).

Some Pacific oyster growers believe that if food is added to setting tanks, a higher percentage of eyed larvae will attach. Experiments 4A, 4B, and 5 were designed to investigate the combined effects of temperature and feeding on the setting of Crassostrea gigas larvae.

Experiment 4A

Temperatures used in Experiment 4A were 15°, 20°, 25°, and 30°C; salinity was 29 ppt. Four jars were prepared at each temperature. Two received the chrysomonad Pseudoisochrysis paradoxa at a concentration of 120,000 algal cells per milliliter (250,000 cells/larva). The other two received no food during the setting period. Based on the average of the preserved aliquots, 1434 larvae were added to each container.

Four scrubbed oyster shells were placed in each jar. Holes were punched in the shells, and they were strung on a piece of nylon twine. Pieces of tygon tubing separated the shells. Figure 3 shows the orientation of shells in the jar. Two shells were placed with the cupped side up and two with the cupped side down.

Korringa (1952) stated that under natural conditions Ostrea edulis larvae are uniformly distributed in the water, and Galtsoff (1964) reported that the same is true for artificially-grown Crassostrea virginica larvae undisturbed by stirring or aeration. Variations in setting on shells in different positions in a jar should have had little effect on the results of Experiment 4A, since only the total number of spat in each container was used in the analysis.

After the 48-hour test period, the jars were emptied and refilled with water at 20°C and 29 ppt. Food was added to all jars at this time. After an additional two days, the shells were removed from the experimental containers, and the spat were counted. The shell strings were hung from a dowel rod placed across the laboratory tank of raw seawater.

Spat that settled on the jars were counted eight days after the

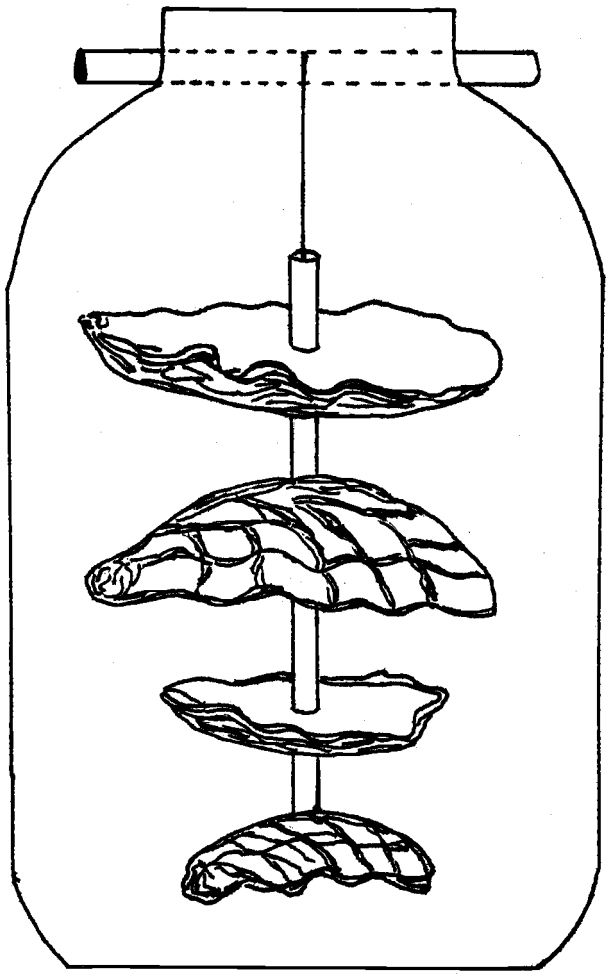

Figure 3. Orientation of oyster shells strung on nylon twine and suspended in experimental containers for use as cultch in Experiments 4A and 4B.

start of the experiment. Table 11 shows the numbers and percentages of larvae setting at each combination of temperature and feeding used during the setting period.

Table 11. Numbers and, in parentheses, percentages of setting Pacific oyster larvae as affected by temperature and feeding conditions during a 48-hour setting period (Experiment 4A).[a/]

| | Temperature | | | | |
|---|---|---|---|---|---|
| | 30°C | 25°C | 20°C | 15°C | Mean |
| Food | 515 (36)<br>200 (14) | 295 (21)<br>316 (22) | 154 (11)<br>173 (12) | 42 ( 3)<br>48 ( 3) | (15.25) |
| No Food | 284 (20)<br>313 (22) | 263 (18)<br>234 (16) | 150 (10)<br>210 (15) | 72 ( 5)<br>61 ( 4) | (13.75) |
| Mean | (23.00) | (19.25) | (12.00) | ( 3.75) | |

[a/] The combination of temperature and feeding at which the most larvae settled is enclosed by solid lines. Other satisfactory combinations are enclosed by dashed lines.

The most larvae settled at 30°C in jars that received food during the setting period. Sets were also good at 25°C in jars with food and at 30°C in jars without food.

An analysis of variance (Table 12) indicates that temperature had a significant effect on the percentage of setting larvae. At 30°C, the mean percentage of setting larvae was 23.00%. The average set at 25°C was 19.25%. At 20°C, an average of 12.00% settled, but only 3.75% attached at 15°C.

The presence or absence of food during the 48-hour test period had no statistically significant effect on setting, and there was no interaction between the factors.

Table 12. Analysis of variance of the percentage data given in Table 11. N.S. denotes that the difference was not significant.

| Source of Variation | d.f. | Mean Square | F Ratio | p |
|---|---|---|---|---|
| Temperature | 3 | 288.833 | 8.887 | .006 |
| Food or No Food | 1 | 9.000 | .277 | .613 N.S. |
| Interaction | 3 | 10.167 | .313 | .816 N.S. |
| Residual | 8 | 32.500 | | |

Experiment 4B

Experiment 4B was designed to investigate the effects of temperature and feeding on larvae that were refrigerated at 5°C for five days before being set. It was thought that feeding might improve the setting percentages if larvae had been held out of water for several days before setting. Larvae were from the same batch used for Experiment 4A. Those not used for Experiment 4A were left in the refrigerator wrapped in damp cloth and paper towels.

Each jar in Experiment 4B was assumed to contain 1228 eyed larvae, the mean number of larvae in the preserved aliquots. Half the jars at each temperature received 120,000 cells per milliliter of *Pseudoisochrysis paradoxa* (290,000 cells/larva). Temperatures were 15°, 20°, 25°, and 30°C. Procedures were identical to those described for Experiment 4A.

The numbers and percentages of setting larvae at each of the eight treatments are shown in Table 13.

Table 13. Numbers and, in parentheses, percentages of Pacific oyster larvae setting at various combinations of temperature and feeding after being held for five days at 5°C (Experiment 4B).[a/]

| | Temperature | | | | |
|---|---|---|---|---|---|
| | 30°C | 25°C | 20°C | 15°C | Mean |
| Food | 392 (32)<br>905 (74) | 712 (58)<br>567 (46) | 164 (13)<br>85 ( 7) | 1 ( 0)<br>10 ( 1) | (28.88) |
| No Food | 338 (28)<br>365 (30) | 759 (62)<br>589 (48) | 239 (19)<br>142 (12) | 3 ( 0)<br>9 ( 1) | (25.00) |
| Mean | (41.00) | (53.50) | (12.75) | ( 0.50) | |

[a/] The combination of temperature and feeding at which the most larvae settled is enclosed by solid lines. Other satisfactory combinations are enclosed by dashed lines.

The highest percentage of larvae attached at 25°C in jars receiving no food. Good sets also occurred at 25°C and at 30°C in cultures that were fed during the 48-hour experimental period.

An analysis of the data (Table 14) shows that temperature had a highly significant effect on the percentage of setting larvae. The best temperature for setting stored larvae was 25°C. At this temperature, the average set was 53.50%. Of the larvae at 30°C, 41.00% attached. Fewer larvae settled at lower temperatures. At 20°C and at 15°C, the mean sets were 12.75% and 0.50%, respectively.

Feeding larvae during the 48-hour setting period had no significant effect on attachment, and there was no interaction between temperature and feeding.

Table 14. Analysis of variance of the percentage data given in Table 13. N.S. denotes that the difference was not significant.

| Source of Variation | d.f. | Mean Square | F Ratio | p |
|---|---|---|---|---|
| Temperature | 3 | 2404.729 | 17.529 | .001 |
| Food or No Food | 1 | 60.063 | .438 | .526 N.S. |
| Interaction | 3 | 185.062 | 1.349 | .326 N.S. |
| Residual | 8 | 137.187 | | |

Comparing the results from Experiments 4A and 4B suggests that eyed larvae stored for five days at 5°C settled well at 25°C and 30°C whether or not cultures were fed during the setting period. In fact, at these temperatures, the percentages of setting larvae were considerably higher when larvae were held before being set. At 30°C in Experiment 4A, an average of 23.00% of the larvae attached; after storage, the mean percentage setting at 30°C was 41.00%. At 25°C, the mean set in Experiment 4A was 19.25%, while 53.50% settled in Experiment 4B. Similar results have been observed by Henderson.[10]

In any batch of larvae from the hatchery, not all individuals will be ready to settle. During five days in the refrigerator, larvae that were not initially ready to metamorphose may have developed to the setting stage using food reserves. Walne (1966) reported that larval shell growth in Ostrea edulis continued for at least two days in the absence of food. Enhanced setting in Experiment 4B may indicate that a higher percentage of larvae were physiologically ready to settle when

[10]Interview with Bruce Henderson, Graduate Student, Oregon State University Department of Fisheries and Wildlife, July, 1981.

the experiment began.

Alternatively, the higher setting percentages in Experiment 4B may have resulted because ready-to-settle larvae were denied access to a suitable substrate.

The ability to postpone metamorphosis is common among marine invertebrate larvae, including oysters, as a mechanism for increasing the probability that a pelagic larva will encounter a substrate suitable for survival as a benthic adult (Cole and Knight-Jones, 1939; Thorson, 1950; Wilson, 1952; Crisp, 1974). When metamorphosis is delayed, a larva maintains its level of organization but does not continue to grow (Bayne, 1969). Lynch (1952) viewed the inhibition of metamorphosis as a condition analagous to anaesthesia, in which larval activity was reduced to a minimum.

Normally when a fully developed larva encounters stimuli of its adult habitat, neural and possibly hormonal reactions trigger its metamorphosis. If a suitable habitat is withheld, the stimulus required to initiate metamorphosis diminishes, and the pre-settlement behavior pattern shortens or is eliminated. This may cause the larva to lose its ability to discriminate between habitats (Crisp, 1974).

More larvae may have settled in Experiment 4B because the stimulation required to trigger their metamorphosis declined, or because they were less selective after being held in the refrigerator.

The enhancement of setting was less pronounced in Experiments 3A and 3B than in Experiments 4A and 4B. In both cases, the most favorable temperature for setting shifted from 30°C before storage to 25°C after storage, but in Experiment 3B, setting was enhanced only at 25°C.

Poor sets may have obscured the effects of storage in Experiments 3A and 3B. The percentages of larvae setting in these experiments were condiderably lower than those in Experiments 4A and 4B.

Varietal differences could account for the varied responses in the two sets of experiments. Kumomoto larvae were used for Experiments 3A and 3B, while Experiments 4A and 4B were conducted with the Miyagi variety of Crassostrea gigas.

Holding time may be an important factor influencing the attachment of larvae that are not used immediately after being received from the hatchery. The results of preliminary experiments, in which C. gigas eyed larvae were held 1-14 days before being set, suggest that a settlement peak occurs when larvae are held four to six days before being set. For larvae held one to six days, the number of setting larvae increased as storage time increased. Setting declined as storage time was extended beyond six days.[11]

Experiment 5

Experiment 5 investigated the effects of temperature and feeding on larvae that had been stored eight days at 5°C. Experimental temperatures were 20°, 25°, and 30°C; salinity was 25 ppt. Four jars were prepared at each temperature, and Pseudoisochrysis paradoxa was added to half the jars at the rate of 120,000 cells per milliliter (280,000 cells/larva). Two scrubbed oyster shells were placed on the bottom of each jar. Based on the average of the preserved aliquots, each container received 1277

[11] Ibid.

eyed larvae. Procedures followed those described under General Methods.

Table 15 shows the numbers and percentages of larvae that settled at each treatment.

Table 15. Numbers and, in parentheses, percentages of Pacific oyster larvae setting at various combinations of temperature and feeding after being held for eight days at 5°C (Experiment 5).[a/]

| | Temperature | | | |
|---|---|---|---|---|
| | 30°C | 25°C | 20°C | Mean |
| Food | 15 ( 1)<br>27 ( 2) | 25 ( 2)<br>40 ( 3) | 4 ( 0)<br>0 ( 0) | (1.33) |
| No Food | 50 ( 4)<br>53 ( 4) | 44 ( 3)<br>35 ( 3) | 1 ( 0)<br>0 ( 0) | (2.33) |
| Mean | (2.75) | (2.75) | (0.00) | |

[a/] The combination of temperature and feeding at which the most larvae settled is enclosed by solid lines.

The most larvae settled at 30°C in cultures that were not fed, however, very few larvae settled at any combination of temperature and feeding.

Analysis of the data from Experiment 5 (Table 16) shows an interaction between temperature and feeding.

Table 16. Analysis of variance of the percentage data given in Table 15.

| Source of Variation | d.f. | Mean Square | F Ratio | p |
|---|---|---|---|---|
| Temperature | 2 | 10.083 | 60.500 | .001 |
| Food or No Food | 1 | 3.000 | 18.000 | .005 |
| Interaction | 2 | 1.750 | 10.500 | .011 |
| Residual | 6 | .167 | | |

The mean percentage of larvae settling at each combination of temperature and feeding is recorded in Table 17 and illustrated graphically in Figure 4.

Table 17. Mean percentage of Pacific oyster larvae setting at various combinations of temperature and feeding after being held for eight days at 5°C (Experiment 5).

| Temperature | | Mean Set (%) |
|---|---|---|
| 30°C | Food | 1.50 |
| | No Food | 4.00 |
| 25°C | Food | 2.50 |
| | No Food | 3.00 |
| 20°C | Food | 0.00 |
| | No Food | 0.00 |

A temperature of 20°C was unfavorable for setting eyed larvae that had been stored for eight days. Feeding the larvae during the 48-hour setting period did not improve setting at 20°C.

At 25°C, the mean number of setting larvae was approximately the same in cultures that were fed during the test period and those that were not fed. In jars receiving food, an average of 2.50% of the larvae settled. Slightly more, 3.00%, settled in jars at 25°C without food.

The difference between treatments was greatest at the highest temperature tested. At 30°C, the mean set was 1.50% in containers that received food during the setting period, while 4.00% settled in those without food.

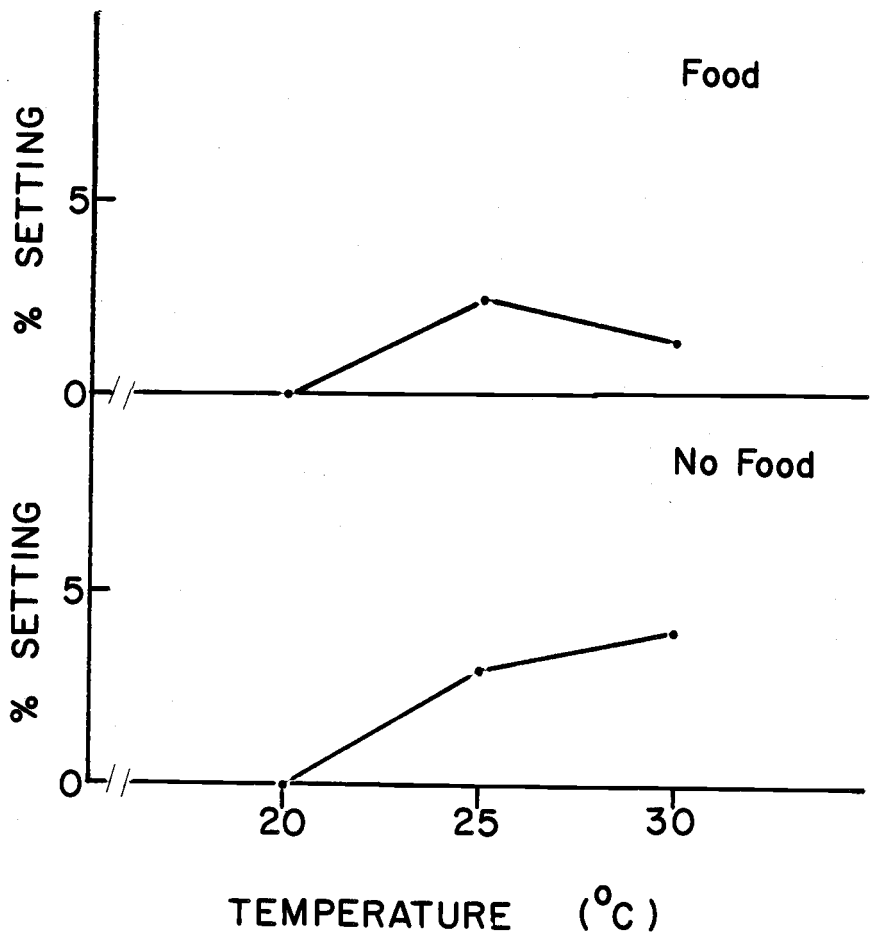


Figure 4. Effect of temperature and feeding on the percentage of setting Pacific oyster larvae (Experiment 5).

## Discussion

Results from the three experiments on the combined effects of temperature and feeding support the previous conclusion that temperature during a 48-hour test period significantly affects the percentage of setting Pacific oyster larvae, with temperatures of 25°-30°C being most favorable.

Feeding eyed larvae during the setting period did not improve setting. Since these experiments were carried out in the dark, the food value of Pseudoisochrysis paradoxa may have been diminished, and there may have been little difference in the jars with food and those without food. Larvae of many aquatic species cease to feed during metamorphosis, since the digestive system is reorganized, so these oyster larvae may have been anatomically unable to eat. Figure 5 illustrates the relocation of digestive organs that occurs when a fully developed oyster larva metamorphoses into a juvenile oyster (Galtsoff, 1964).

It is difficult to explain why temperature and feeding interacted to the detriment of setting in Experiment 5. Ukeles (1961) reported that some chrysomonads fragment, clump, and settle out of the water at temperatures above 27°C. Calabrese and Davis (1970) stated that cells of Isochrysis galbana and Monochrysis lutheri died and the cell walls disintegrated at temperatures above 27.5°C. If Pseudoisochrysis paradoxa was destroyed at 30°C, the poor set in Experiment 5 may have resulted from larval reactions to the increase in bacteria associated with decomposition. Bacterization may cause even good food to become toxic to larvae (Calabrese and Davis, 1970). Walne (1966) noted a

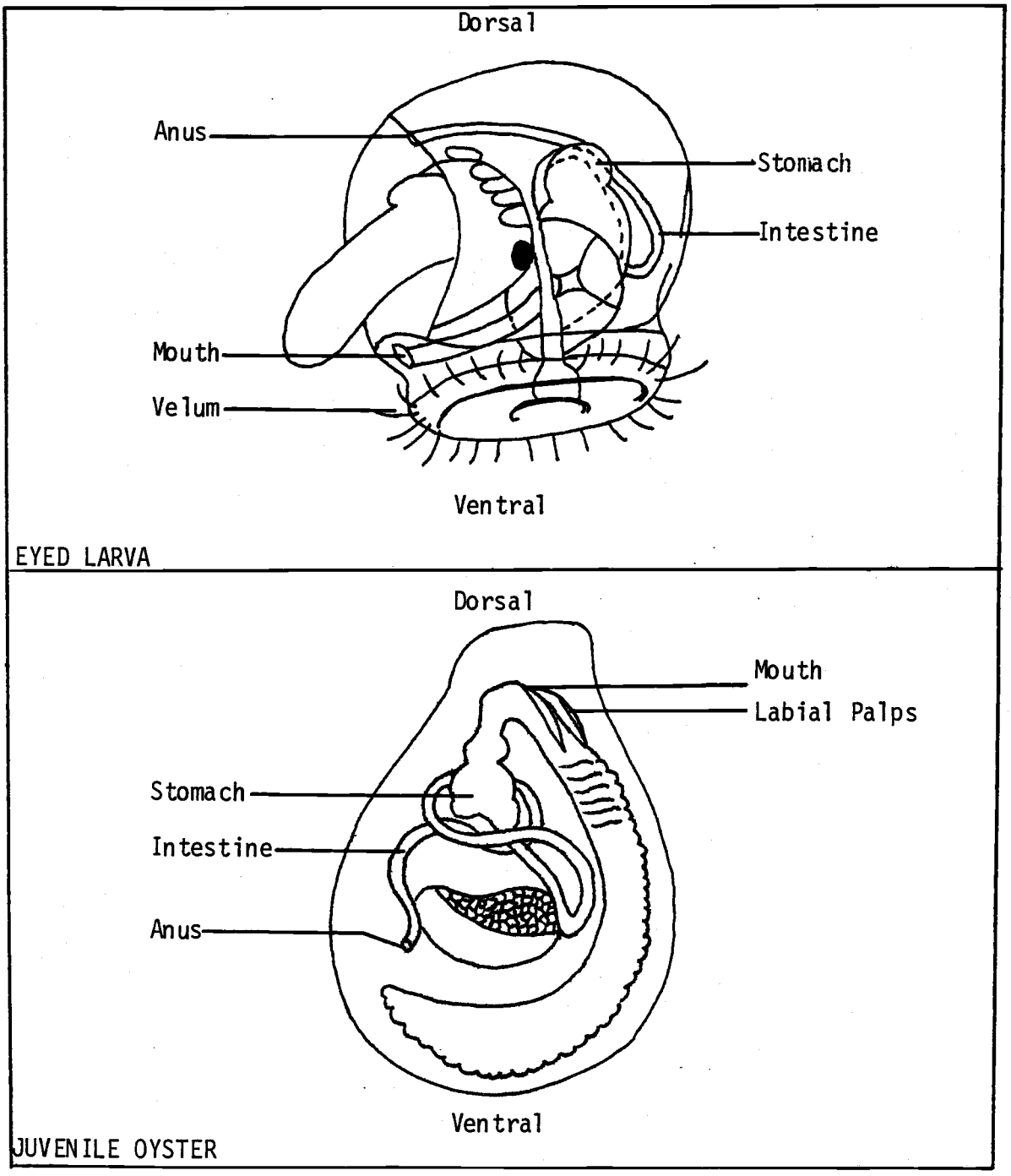


Figure 5. Location of various organs of the digestive system in a a fully developed eyed larva and in a young spat (after Galtsoff, 1964).

reduced growth rate in Ostrea edulis larvae when large numbers of bacteria were present. In experiments with Crassostrea gigas larvae, Helm and Millican (1977) reported that algal foods were unable to survive at 32°C, and cultures were fouled with algae and bacteria.

Settling food cells might have made the surfaces of jars and cultch unsuitable for larval attachment. Galtsoff (1964) stated that the success of setting of American oyster larvae (Crassostrea virginica) depends primarily on the availability of clean surfaces. Since the shells in Experiments 4A and 4B were suspended on strings, they might have been less affected by bacteria or settling food cells than were shells placed on the bottoms of containers in Experiment 5. More experiments should be conducted to determine if Pseudoisochrysis paradoxa is destroyed at higher temperatures and if food-cell destruction influences the results of this type of feeding experiment.

The present studies agree with Lund's (1972) conclusion that larvae will set rapidly, regardless of the feeding rate, if they are well fed during their free-swimming period in the hatchery. In laboratory experiments with Crassostrea gigas, he tested food concentrations of 0-100,000 cells per milliliter, of either Isochrysis galbana or Monochrysis lutheri, and found that setting increased with food availability. His experiments lasted six to ten days, however, and larvae that initially were not fully developed could have matured and settled during the course of an experiment.

In 24-hour experiments with Ostrea edulis, Bayne (1969) found that settlement increased with increasing food concentrations up to 100,000

cells of Isochrysis galbana per larva. Settlement was reduced when a concentration of 200,000 cells per larva was used. Experiments 4A, 4B, and 5 used food concentrations of 120,000 algal cells per milliliter, but the concentration per larva varied from 250,000 to 290,000 cells.

Many factors can influence the results of feeding experiments, so one must exercise caution when comparing the work of different authors. The effects of temperature on food organisms were discussed previously.

The food value of different algae may influence the results of feeding experiments. Davis (1953) pointed out that different species of flagellates induced different growth rates in larvae fed equal numbers of food cells, and Davis and Guillard (1958) tested the value of numerous microorganisms as food for clam and oyster larvae. Pseudoisochrysis paradoxa has not been used widely as a food for oyster larvae, and results of experiments with P. paradoxa may differ from those using other food organisms.

Methods of measuring food densities vary among investigators and may influence their conclusions. In cultures of Crassostrea gigas, Lund (1972) used absolute food concentrations of 0-100,000 algal cells per milliliter of water. Bayne (1969) measured food densities as concentrations of food cells per larva. He observed no consistent relationship between settlement of Ostrea edulis and absolute food-cell concentrations, as opposed to concentrations per larva. Malouf (1971) hinted that a complex relationship exists between food supply and larval density. His studies with Crassostrea gigas larvae yielded significantly different growth rates when larvae were fed different cell concentrations but the same amount of algae per larva.

The major conclusion that can be drawn from Experiments 4A, 4B, and 5 is that feeding does not significantly increase the percentage of Pacific oysters attaching during a 48-hour setting period, if eyed larvae are set in the dark at temperatures of $15^{o}$-$30^{o}$C.

## Effects of Substrate on Setting

### Experiment 6

Experiment 6 was designed to test the combined effects of temperature and substrate on the percentage of Pacific oyster larvae setting during a 48-hour experimental period.

Most bivalves will settle without a specific substrate, however, the percentage of setting larvae may be influenced by the specific substrate provided (Chanley, 1975).

Temperatures used for Experiment 6 were $20^{o}$, $26^{o}$, and $30^{o}$C; salinity was 29 ppt. Oyster, cockle, and butter clam shells were used as substrates. Two shells were placed in each experimental container. One shell was oriented cupped side up, the other cupped side down. Each jar contained only one type of substrate. Based on the average of the preserved aliquots, 1041 eyed larvae were placed in each container. Duplicate cultures were prepared at each combination of temperature and substrate.

After two days, all jars were emptied and refilled with water at $20^{o}$C and 29 ppt. After an additional two days, the spat on the shells were counted and placed in plastic baskets in the raw seawater tank. The larvae that settled on the sides and bottoms of the jars were

counted seven days after the start of the experiment.

The numbers and percentages of larvae attaching at each combination of temperature and substrate are recorded in Table 18.

Table 18. Numbers and, in parentheses, percentages of setting Pacific oyster larvae as affected by temperature and the type of substrate used during a 48-hour setting period (Experiment 6).[a]

| Substrate | Temperature 30°C | 26°C | 20°C | Mean |
|---|---|---|---|---|
| Butter clam shell | 135 (13)<br>159 (15) | 27 ( 3)<br>24 ( 2) | 9 ( 1)<br>7 ( 1) | (5.83) |
| Cockle shell | 155 (15)<br>160 (15) | 33 ( 3)<br>32 ( 3) | 4 ( 0)<br>2 ( 0) | (6.00) |
| Oyster shell | 64 ( 6)<br>165 (16) | 35 ( 3)<br>14 ( 1) | 18 ( 2)<br>45 ( 4) | (6.00) |
| Mean | (13.33) | ( 2.50) | ( 1.33) | |

[a] The optimum combination of temperature and substrate is enclosed by solid lines. Other satisfactory combinations are enclosed by dashed lines.

The greatest percentage of larvae settled at 30°C in jars with cockle shells. Good sets also occurred at 30°C with butter clam and oyster shells as substrates.

The analysis of variance in Table 19 indicates that temperature had a highly significant effect on the percentage of setting larvae. The highest temperature tested, 30°C, was by far the best for setting. At 30°C, the mean set was 13.33%. An average of only 2.50% attached at 26°C, and the set at 20°C averaged 1.33%. The type of substrate had no significant effect on the percentage of setting larvae, and there was no interaction between temperature and the type of substrate used.

Table 19. Analysis of variance of the percentage data given in Table 18. N.S. denotes that the difference was not significant.

| Source of Variation | d.f. | Mean Square | F Ratio | p |
|---|---|---|---|---|
| Temperature | 2 | 262.722 | 41.850 | .001 |
| Substrate | 2 | .722 | .115 | .893 N.S. |
| Interaction | 4 | 6.556 | 1.044 | .436 N.S. |
| Residual | 9 | 6.278 | | |

These results support Galtsoff's (1964) conclusion that oyster larvae are relatively nondiscriminating in their choice of substrates. Cole and Knight-Jones (1949) noted that the crucial factor in determining suitability of cultch for attachment appeared to be freedom from silt. They observed that oyster and cockle shells were equally efficient spat collectors for Ostrea edulis, and the same appears to be true for Crassostrea gigas. In this experiment, fully developed eyed larvae seemed to settle and attach equally well to a variety of mollusk shells and to the plastic surfaces of the experimental containers.

Experiment 7

Cole and Knight-Jones (1949) first noted a gregarious tendency in setting oysters when they observed that Ostrea edulis larvae attached more readily to shells bearing recently settled spat than to shells with the spat removed. They suggested that larvae were stimulated to settle by a substance secreted by the spat.

American oyster larvae exhibit this gregarious response also. Hidu (1969) found that shells with 24-hour or two-month-old spat stimulated more setting of Crassostrea virginica than did nonspatted shells.

Certain substances from adult oysters appear to induce larval settlement. In tank and laboratory experiments, two to three times more Ostrea edulis spat settled on slate or glass plates painted with an aqueous extract of oyster meat than on untreated plates (Walne, 1966; Bayne, 1969). Oyster shell liquor (Veitch and Hidu, 1971; Hidu, 1975), oyster feces and pseudofeces (Keck, et al., 1971), water pumped by adult oysters (Veitch and Hidu, 1971; Hidu, 1975), and water in which adults have soaked (Crisp, 1967) may stimulate settlement of American oysters.

Crisp (1974) has reviewed the chemical characteristics of extracts that promote gregarious settlement of oyster larvae, and he and Bayne (1969) have examined numerous hypotheses that explain how larvae detect these substances.

British workers generally have held the opinion that gregarious setting is triggered by direct contact of larvae with compounds adhering to the setting surface (Crisp, 1967, 1974; Bayne, 1969). American workers have reported that larvae respond to a waterborne substance released by juvenile oysters (Hidu, 1969; Keck et al., 1971; Veitch and Hidu, 1971). Recent experiments by Hidu et al. (1978) demonstrated that the setting response could be triggered in both European and American oyster larvae without larvae contacting the treated surface. The mode of action and the mechanism for larval detection of waterborne materials are still unknown.

Experiment 7 was designed to investigate the effects of an aqueous extract of oyster tissue on setting, to determine whether substrates not normally used by an oysterman could be made as attractive to setting

larvae as the oyster shells usually used as cultch.

The shell contents of one adult Pacific oyster (Crassostrea gigas) were homogenized in one liter of filtered 30 ppt seawater. This mixture was refrigerated for several hours to allow large particulates to settle and to prevent the decay of organic compounds that might trigger settlement.

Matched shells of razor clams, butter clams, littleneck clams, and oysters were used as substrates. One shell of each matched set was dipped for five seconds in the oyster extract; the other was dipped for five seconds in 30 ppt seawater. All shells were left to air-dry in the refrigerator.

Two shells were placed in each experimental container. Only one type of substrate was used in each jar, and both shells were either treated with the extract or untreated. Seawater of 25$^{o}$C and ambient salinity (33 ppt) was placed in each container along with 1478 eyed larvae. Precedures followed those described under General Methods.

Table 20 shows the number and percentage of larvae settling in each experimental container.

Table 20. Numbers and, in parentheses, percentages of setting Pacific oyster larvae as affected by the type of substrate and the presence or absence of oyster extract during a 48-hour setting period (Experiment 7).[a/]

| Substrate | Extract | No Extract | Mean |
|---|---|---|---|
| Razor clam shell | 10 ( 1)<br>11 ( 1) | 9 ( 1)<br>8 ( 1) | (1.00) |
| Butter clam shell | 25 ( 2)<br>8 ( 1) | 4 ( 0)<br>1 ( 0) | (0.75) |
| Oyster shell | 58 ( 4)<br>57 ( 4) | 14 ( 1)<br>15 ( 1) | (2.50) |
| Littleneck clam shell | 3 ( 0) | 1 ( 0) | (0.00) |
| Mean | (1.86) | (0.57) | |

[a/] The optimum combination of substrate and extract is enclosed by solid lines.

The best set occurred on oyster shells that had been dipped in oyster-tissue extract, but the setting percentages were extremely low for all treatments.

Analysis of the data (Table 21) indicates a significant interaction between the type of substrate and the presence or absence of osyter extract.

Table 21. Analysis of variance of percentage data given in Table 20.

| Source of Variation | d.f. | Mean Square | F Ratio | p |
|---|---|---|---|---|
| Substrate | 3 | 3.536 | 42.429 | .001 |
| Extract or No Extract | 1 | 5.786 | 69.429 | .001 |
| Interaction | 3 | 1.821 | 21.857 | .001 |
| Residual | 6 | .083 | | |

The mean percentage of setting larvae is shown in Table 22 for each type of substrate, with or without the oyster extract.

The percentages of larvae attaching to oyster or butter clam shells increased when shells were dipped in the oyster extract, but the presence of the extract did not improve setting on razor or littleneck clam shells.

Table 22. Mean percentage of larvae setting on various substrates in the presence or absence of an aqueous extract of oyster tissue.

| Substrate | | Mean Set (%) |
|---|---|---|
| Razor clam shell | Extract | 1.00 |
| | No Extract | 1.00 |
| Butter clam shell | Extract | 1.50 |
| | No Extract | 0.00 |
| Oyster shell | Extract | 4.00 |
| | No Extract | 1.00 |
| Littleneck clam shell | Extract | 0.00 |
| | No Extract | 0.00 |

The results of this experiment suggest that littleneck clam shells are an unsuitable substrate for settling Pacific oyster larvae. Razor clam shells compared favorably to oyster shells in this experiment, however, the fragility of razor clam shells would make them impractical for commercial operations in which cultch receives considerable handling.

The presence of oyster extract on butter clam shells made them slightly more attractive than oyster shells without extract, but oyster shells with extract were by far the best substrate tested.

Dipping shells in an aqueous extract of oyster tissue may increase their attractiveness to setting larvae, but this procedure is not recommended for commercial oystermen due to the difficulties of dipping

large quantities of shell and of contending with the rapid decomposition that accompanies homogenization of adult oyster tissues.

## Summary of Setting Studies

The first objective of this research project was to achieve the highest percentages of setting Pacific oyster larvae by manipulating temperature, salinity, feeding, and the type of substrate used during a 48-hour setting period. The recommendations in Table 23 are based on the results of four experiments on the combined effects of temperature and salinity, three experiments on temperature and feeding, and two experiments on the type of substrate used for setting.

Table 23. Recommendations for setting commercially-reared eyed larvae to obtain the highest percentage of attaching larvae.

| Factor | Recommendation |
|---|---|
| Temperature | $25^{o}$-$30^{o}$C ($77^{o}$-$86^{o}$F) |
| Salinity | No significant difference in the percentage of larvae setting for salinities in the range of 15-30 ppt. |
| Feeding | Feeding larvae 120.000 cells per milliliter of Pseudoisochrysis paradoxa did not significantly improve setting during a 48-hour period. |
| Substrate | No significant difference between oyster, cockle, and butter clam shells as substrates.<br>Poor set on littleneck clam shells. |
| Storage of Eyed Larvae | Setting may be enhanced by refrigerating eyed larvae at $5^{o}$C ($41^{o}$F) for several days before setting them.<br>The best temperature for setting stored larvae was $25^{o}$C. |

## SURVIVAL STUDIES

The second phase of this project was intended to evaluate the survival of spat that attached to shell cultch during the setting experiments. Shells were kept in baskets in a tank of raw seawater, and after eight to nine months, the percentages of surviving spat were calculated. The data were examined to determine how spat survival, under these laboratory conditions, was affected by the temperature, salinity, substrate, and feeding conditions used during the original 48-hour setting period. Survival data was examined for six of the setting experiments.

Because of laboratory conditions, these results do not provide a realistic measure of spat survival under natural conditions. The seawater entering the O.S.U. Marine Science Center comes from an area uninhabited by oysters. The rate of water flow through the laboratory tank was much lower than spat would experience during tidal changes in most bays and estuaries. These two factors probably combined to starve the spat and increase mortality.[12]

### Combined Effects of Temperature and Salinity on Survival

#### Experiment 2

The shells from Experiment 2 were kept in raw seawater for eight months before the spat were counted. Table 24 shows the percentage of spat that survived at each combination of temperature and salinity that

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[12] Interview with Wilbur P. Breese, Professor of Fisheries, Oregon State University, Corvallis, Oregon, August, 1981.

was used when setting the eyed larvae. Data from duplicate cultures were combined to determine survival percentages, except as noted. In some cases, shells were lost or the markings used to identify them were illegible. In other cases, larvae initially settled on shells in only one of the two jars receiving a particular treatment.

Table 24. Percentages of Pacific oyster spat surviving eight months as affected by the temperature and salinity used during the initial 48-hour setting period (Experiment 2).[a/]

| Salinity (ppt) During Setting | Temperature During Setting | | | | |
|---|---|---|---|---|---|
| | 28°C | 25°C | 20°C | 15°C | Mean |
| 30 | 5 | 0 | 0 | 0 [b/] | 1.25 |
| 25 | 5 | 4 | 0 | 0 | 2.25 |
| 20 | 3 | [10] | 5 | 0 | 4.50 |
| 15 | 4 [b/] | 4 | 0 | 0 | 2.00 |
| Mean | 4.25 | 4.50 | 1.25 | 0.00 | |

[a/] The combination of setting temperature and salinity at which the most spat survived is enclosed by solid lines.

[b/] Based on data from only one experimental container

The highest percentage of surviving spat had been set at 25°C and 20 ppt, however, survival was low for all treatments. Figure 6 illustrates the percentage of spat surviving at each treatment. Pacific oyster larvae attaching at 15°C did not survive. Five percent of the eyed larvae settling at 20°C and 20 ppt survived; when set at 20°C and 15, 25, or 30 ppt, none were alive after eight months. More spat survived among those settling at 25°C and 28°C.

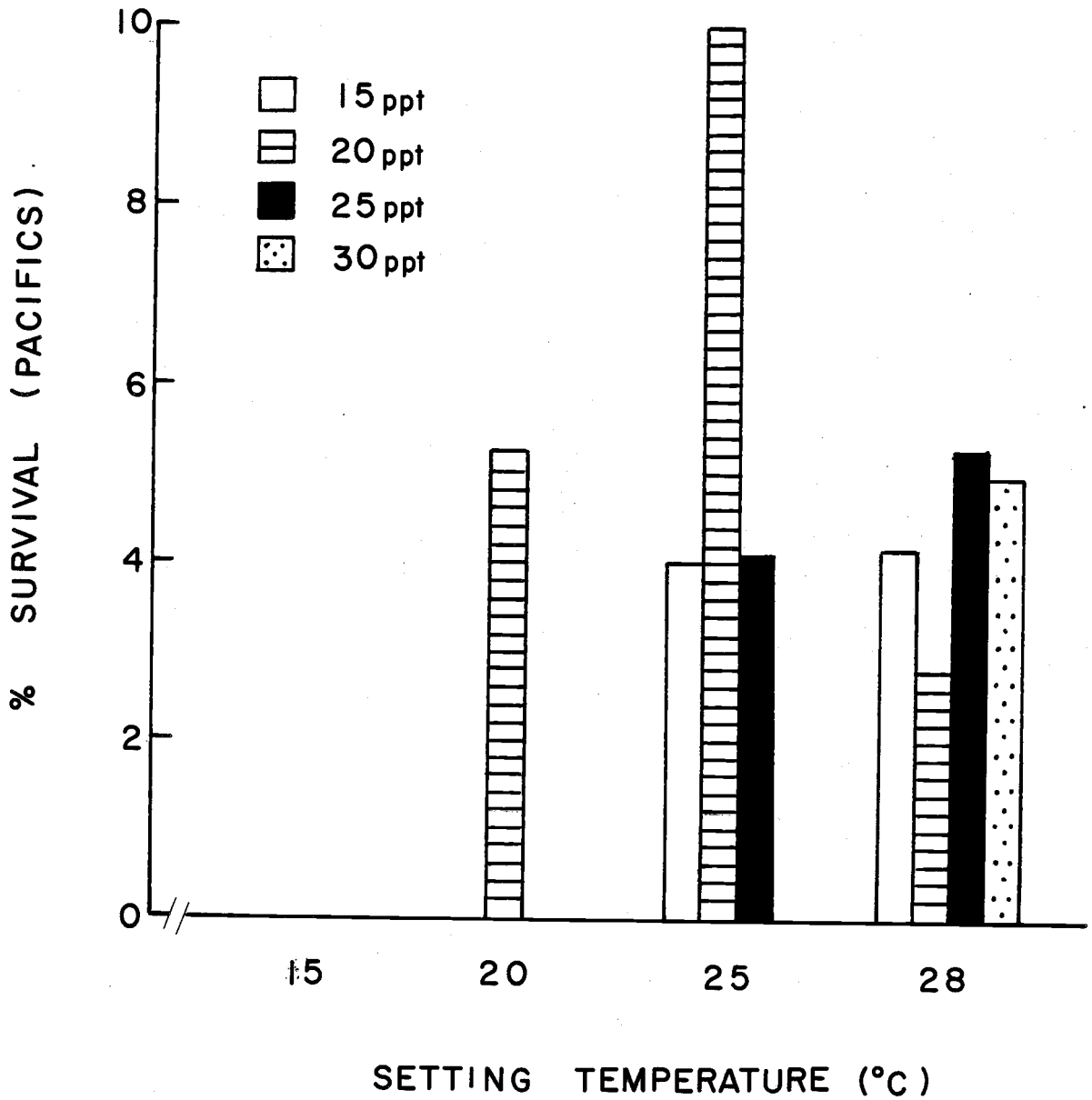


Figure 6. Effect of setting temperature and salinity on the percentage of surviving Pacific oyster spat (Experiment 2).

Experiment 3A

The Kumomoto larvae that settled on shells in Experiment 3A were held nine months before being counted. The percentages of surviving spat are given in Table 25.

Table 25. Percentages of Kumomoto spat surviving nine months as affected by the temperature and salinity used during the initial 48-hour setting period (Experiment 3A).[a/]

| Salinity (ppt) During Setting | Temperature During Setting | | | | |
|---|---|---|---|---|---|
| | 30°C | 25°C | 20°C | 16°C | Mean |
| 30 | 28 | 14 | 89[b/] | 31 | 40.50 |
| 25 | 12 | 39 | 15 | 23 | 22.25 |
| 20 | 4[b/] | 12 | 43 | 22 | 20.25 |
| 15 | 5 | 14 | 29 | 18 | 16.50 |
| Mean | 12.25 | 19.75 | 44.00 | 23.50 | |

[a/] The combination of setting temperature and salinity at which the most spat survived is enclosed by solid lines. Other satisfactory combinations are enclosed by dashed lines.

[b/] Based on data from only one experimental container

The greatest survival occurred for larvae set at 20°C and 30 ppt, although only one observation was available for this treatment. Survival was also good for larvae set at 20°C and 20 ppt and at 25°C and 25 ppt. The upper graph in Figure 7 shows the percentage of surviving larvae at each combination of temperature and salinity used for setting. At salinities of 15, 20, or 30 ppt, a setting temperature of 20°C was best for spat survival. Larvae set at 25 ppt survived best when the setting temperature was 25°C.

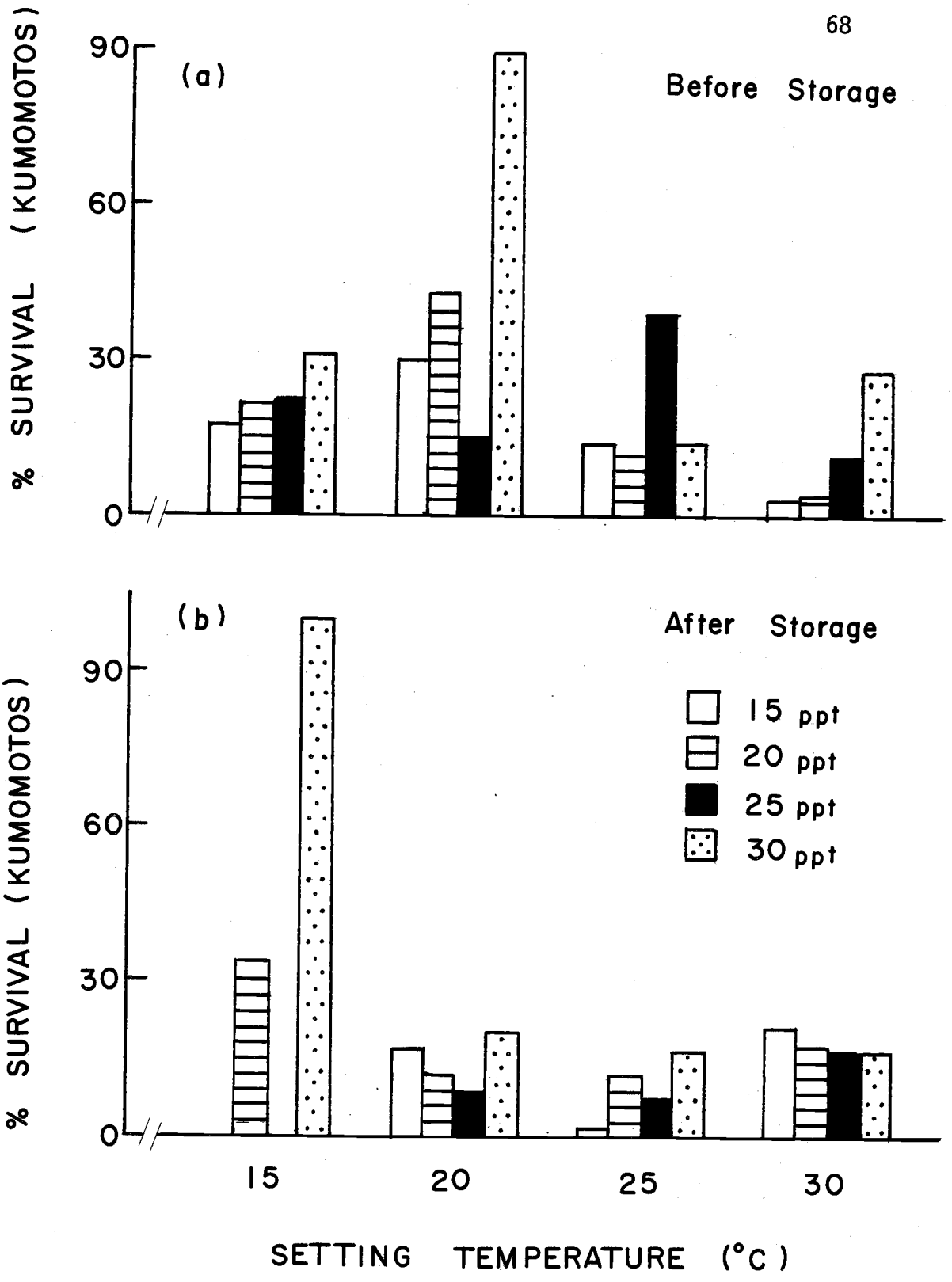


Figure 7. Effect of setting temperature and salinity on the percentage of surviving spat when Kumomoto eyed larvae were set (a) before storate (Experiment 3A) or (b) after storage for eight days at 5°C (Experiment 3B).

Experiment 3B

The Kumomoto larvae used for this experiment were those that had been stored for eight days at 5°C before being set. Spat were counted after nine months, and the survival percentages are recorded in Table 26. The 100% survival at 15°C and 30 ppt may be misleading, since it reflects the survival of only one oyster. Survival was good for larvae that attached at 15°C and 20 ppt.

Table 26. Percentages of Kumomoto spat surviving nine months as affected by the temperature and salinity used during the initial 48-hour setting period (Experiment 3B).[a]

| Salinity (ppt) During Setting | Temperature During Setting 30°C | 25°C | 20°C | 15°C | Mean |
|---|---|---|---|---|---|
| 30 | 17 | 17 | 20 | 100[b] | 38.50 |
| 25 | 17 | 8 | 9 | 0 | 8.50 |
| 20 | 18[b] | 12 | 12 | 33[b] | 18.75 |
| 15 | 22 | 2 | 17[b] | 0 | 10.25 |
| Mean | 18.50 | 9.75 | 14.50 | 33.25 | |

[a] The combination of setting temperature and salinity at which the most spat survived is enclosed by solid lines. Other satisfactory combinations are enclosed by dashed lines.

[b] Based on data from only one experimental container

At setting salinities of 20 and 30 ppt, the highest percentages of spat survived among those set at 15°C. Only one observation was available for each of these treatments, however, so these results should be viewed cautiously. A setting temperature of 30°C was apparently best for larvae set at 15 and 25 ppt. The lower graph in Figure 7 shows the

percentages of surviving spat at each combination of temperature and salinity used during setting in Experiment 3B.

## Discussion

These three experiments fail to reveal a combination of setting temperature and salinity that will result in the best spat survival. A comparison of the graphs in Figure 7 suggests that survival may be lessened if larvae are held several days before being set. For most combinations of setting temperature and salinity, survival was better for larvae set immediately upon arrival from the hatchery than for those stored eight days before being set. The enhanced attachment at 25°C in Experiment 3B (discussed on pages 46-47) was too small to offset the higher mortality for larvae that had been stored before being set. Survival among larvae attaching at 25°C in Experiment 3A averaged 19.75%, while an average of only 9.75% survived from those set at 25°C in Experiment 3B.

## Combined Effects of Temperature and Feeding on Survival

Experiments 4A and 4B were selected to study the survival of spat that had been exposed to different temperatures and feeding conditions during the setting period. The larvae for both experiments were from the same batch. Those used for Experiment 4B had been held five days at 5°C before being set.

Experiment 4A

Spat from Experiment 4A were counted eight months after they were placed in raw seawater. Table 27 shows the percentages of spat surviving at various temperatures when larvae were or were not fed during the setting period.

Table 27. Percentages of Pacific oyster spat surviving eight months as affected by the temperature and feeding conditions used during the initial 48-hour setting period (Experiment 4A).[a/]

| | Temperature During Setting | | | | |
|---|---|---|---|---|---|
| | 30°C | 25°C | 20°C | 15°C | Mean |
| Food During Setting | 45 | 45 | 9 | 0 | 24.75 |
| No Food During Setting | 16 | 30 | 32 | 0 | 19.50 |
| Mean | 30.50 | 37.50 | 20.50 | 0.00 | |

[a/] The combination of temperature and feeding, used for setting, at which the most spat survived is enclosed by solid lines. Other satisfactory combinations are enclosed by dashed lines.

Survival was best for larvae that settled at 30°C and 25°C in containers that received food during the setting period. It was also good for larvae attaching at 20°C and 25°C in containers without food.

The upper graph in Figure 8 illustrates that none of the larvae attaching at 15°C were alive after eight months in the laboratory. At setting temperatures of 25°C and 30°C, more spat survived among those that received food during the setting period. Survival was higher for larvae in jars without food when the setting temperature was 20°C.

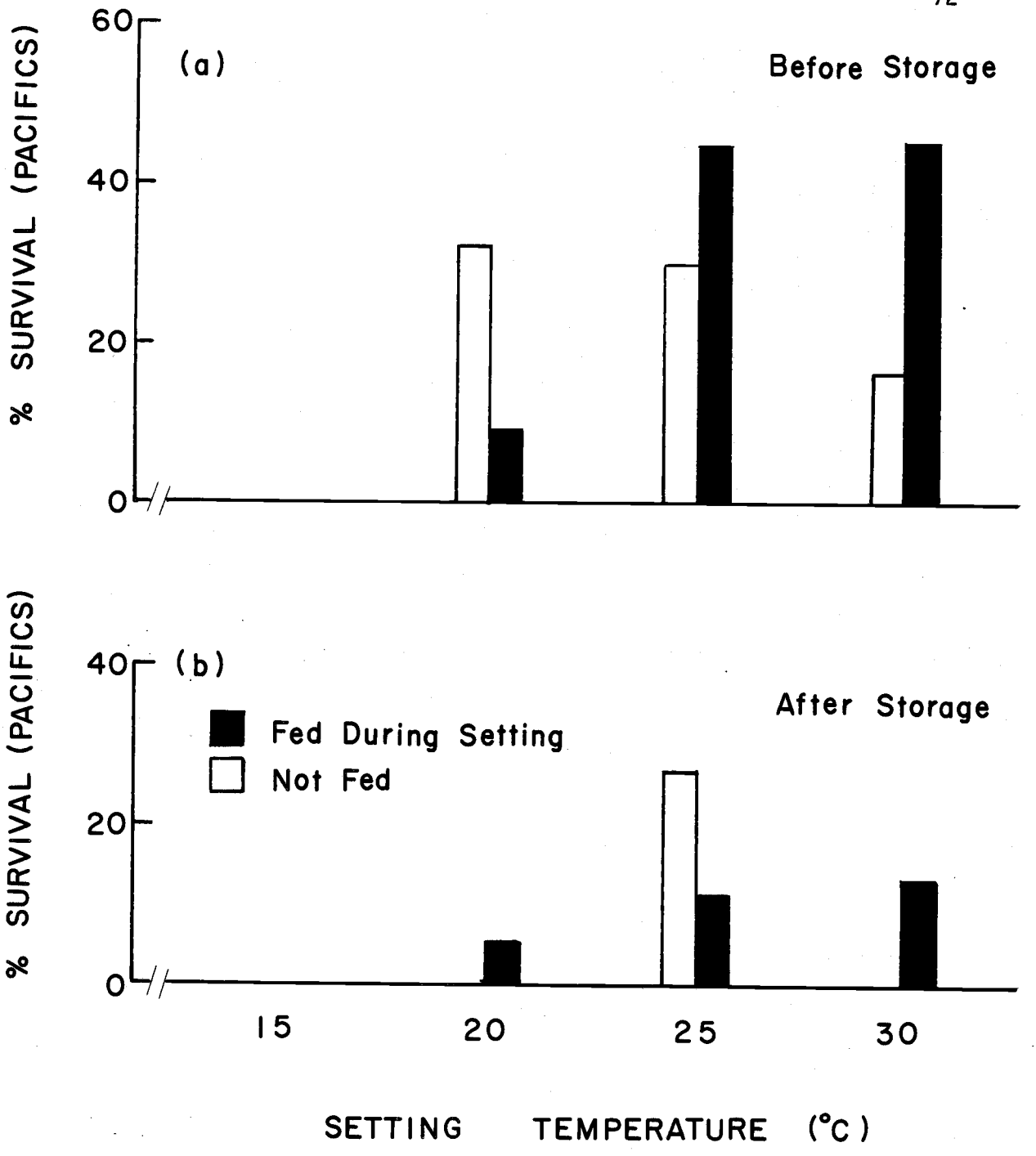


Figure 8. Effect of setting temperature and feeding conditions on the percentage of surviving spat when Pacific oyster eyed larvae were set (a) before storage (Experiment 4A) or (b) after storage for five days at 5°C (Experiment 4B).

Experiment 4B

Table 28 gives the percentages of surviving spat after the attached larvae from Experiment 4B had been held for eight months. The highest percentage of surviving spat had been set at 25°C in jars without food.

Table 28. Percentages of Pacific oyster spat surviving eight months as affected by the temperature and feeding conditions used during the initial 48-hour setting period (Experiment 4B).[a/]

| | Temperature During Setting | | | | |
|---|---|---|---|---|---|
| | 30°C | 25°C | 20°C | 15°C | Mean |
| Food During Setting | 14 | 12 | 6 | 0 | 8.00 |
| No Food During Setting | 0 | 27 | 0 | 0 | 6.75 |
| Mean | 7.00 | 19.50 | 3.00 | 0.00 | |

[a/] The combination of temperature and feeding, used during setting, at which the most spat survived is enclosed by solid lines.

The lower graph in Figure 8 illustrates that survival increased as setting temperature increased for spat that had received food during the setting period. No larvae survived at temperatures of 15°, 20°, or 30°C in clutures without food, but the best survival occurred for larvae set at 25°C in containers without food.

Discussion

Pacific oysters seem to survive well when set at temperatures of 25°-30°C. It is unclear whether adding food to cultures during a 48-hour setting period influences survival.

Comparing the graphs in Figure 8 again suggests that survival is

lessened for larvae that are held for several days before being set. In Experiment 4B, setting was enhanced at 20°, 25°, and 30°C by holding larvae for five days at 5°C before setting them (discussed on pages 46-47). Only at 25°C did enough additional larvae attach to offset the higher mortality among larvae that had been stored before being set. Assuming that 19.50% of the larvae in jars at 25°C initially attached in Experiment 4A (Table 11) and that 37.50% of those survived (Table 24), seven percent of the larvae originally placed in the jars were alive after eight months. In Experiment 4B, approximately ten percent survived, assuming that 53.50% set initially (Table 13) and 19.50% of those survived (Table 25).

## Effects of Substrate on Survival

The original purpose of Experiment 6 was to investigate the effects of temperature and type of substrate on the setting of eyed larvae. Those larvae that settled on the cockle, clam, and oyster shells in Experiment 6 were left in raw seawater for nine months. The percentages of surviving spat are recorded in Table 29 and illustrated graphically in Figure 9.

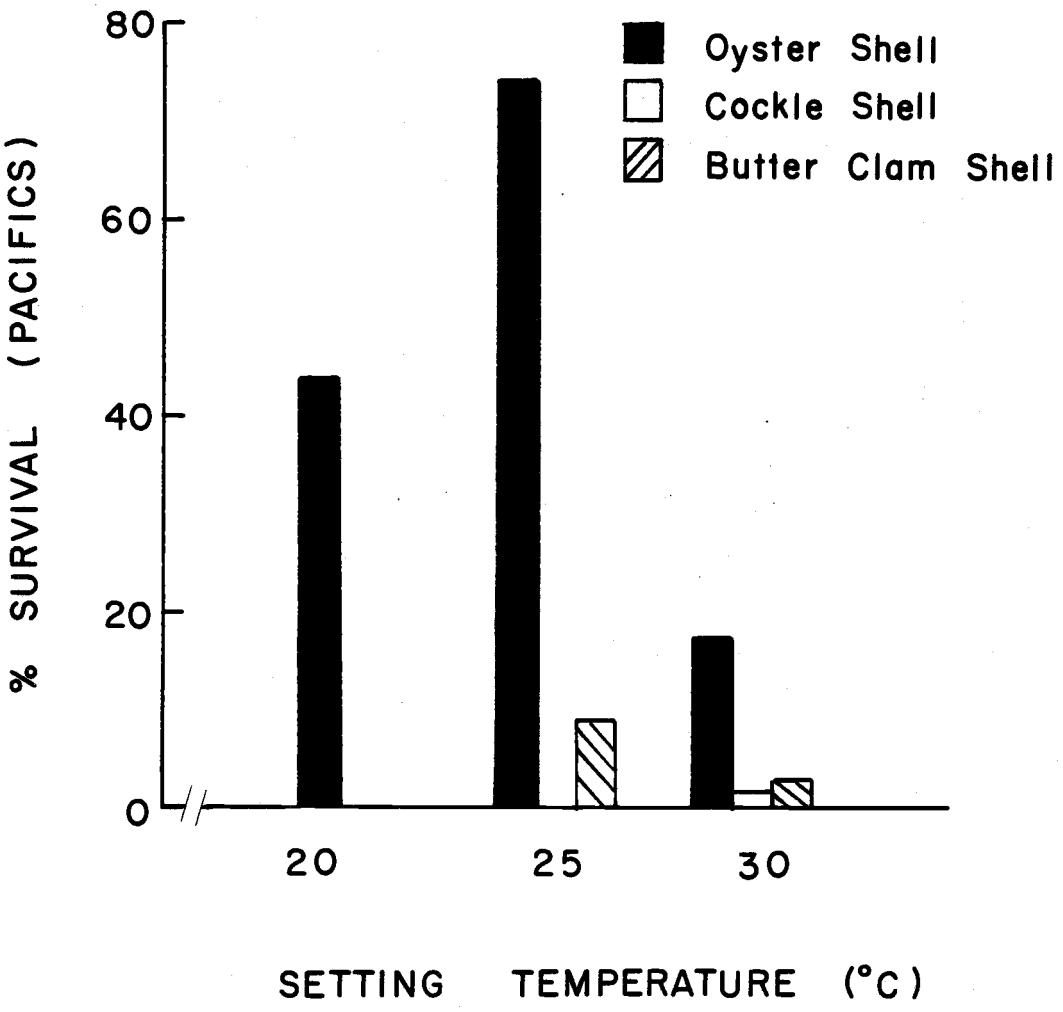


Figure 9. Effect of setting temperature and type of substrate on the percentage of surviving Pacific oyster spat (Experiment 6).

Table 29. Percentages of Pacific oyster spat surviving nine months as affected by the temperature and type of substrate used during the initial 48-hour setting period (Experiment 6).[a]

| Substrate | Temperature During Setting 30°C | 26°C | 20°C | Mean |
|---|---|---|---|---|
| Butter clam shell | 3 | 9 | 0 | 4.00 |
| Cockle shell | 1 | 0 | -- | 0.50 |
| Oyster shell | 18 | 74 | 44 | 45.33 |
| Mean | 7.33 | 27.67 | 14.67 | |

[a] The combination of setting temperature and thpe of substrate at which the most spat survived is enclosed by solid lines. Other satisfactory combinations are enclosed by dashed lines.

Survival was best for larvae set at 26°C on oyster shells. Larvae that had settled on oyster shells at 20°C and 30°C also survived well. When oyster or butter clam shells were used as substrates, survival was best for larvae set at 26°C. Almost no spat survived among those attached to cockle shells.

Experiment 7

Experiment 7 was designed to investigate the effectiveness of oyster shells and various clam shells as substrates for setting Pacific oyster larvae. Half the shells used in Experiment 7 had been dipped in an aqueous extract of oyster tissue to test for a gregarious response in setting larvae. The rest had been dipped in seawater. None of the spat that attached in Experiment 7 were alive after eight months.

## Discussion

The results from the two experiments examining the effects of substrate on spat survival suggest that oyster shells are the only satisfactory substrate among those tested. Although dipping shells in an aqueous extract of oyster tissue enhanced setting, this treatment did not increase spat survival.

## Summary of Survival Studies

In these laboratory experiments, the percentages of surviving spat ranged from 0-100%, with an average survival of 16%. In nature, survival should be much higher. Amemiya (1928) stated that once having fixed and grown some, Crassostrea gigas spat possess the power to resist an unsuitable environment. During the time the spat were held in the laboratory (September, 1980 to June, 1981), the salinity and temperature of seawater entering the tank were within the limits for C. gigas survival.

Salinity ranged from 4 to 36 ppt but usually was in the range of 20-30 ppt (Figure 10). Loosanoff (1952) and Chanley (1957) stated that juvenile oysters differ from larvae in their salinity requirements and respond similarly to adults. Crassostrea oysters generally are euryhaline (Korringa, 1957) and published reports of the salinity tolerance of Crassostrea gigas adults range from 10 to 39 ppt (Wakiya, 1929; Galtsoff, 1932; Hopkins, 1936; Korringa, 1976; Malouf and Breese, 1978).

Temperatures varied from $9^{o}$C to $17^{o}$C during the months the spat were kept in the laboratory (Figure 11). Wakiya (1929) reported the temperature limits of C. gigas as $15^{o}$-$30^{o}$C, but Elsey (1933, cited by

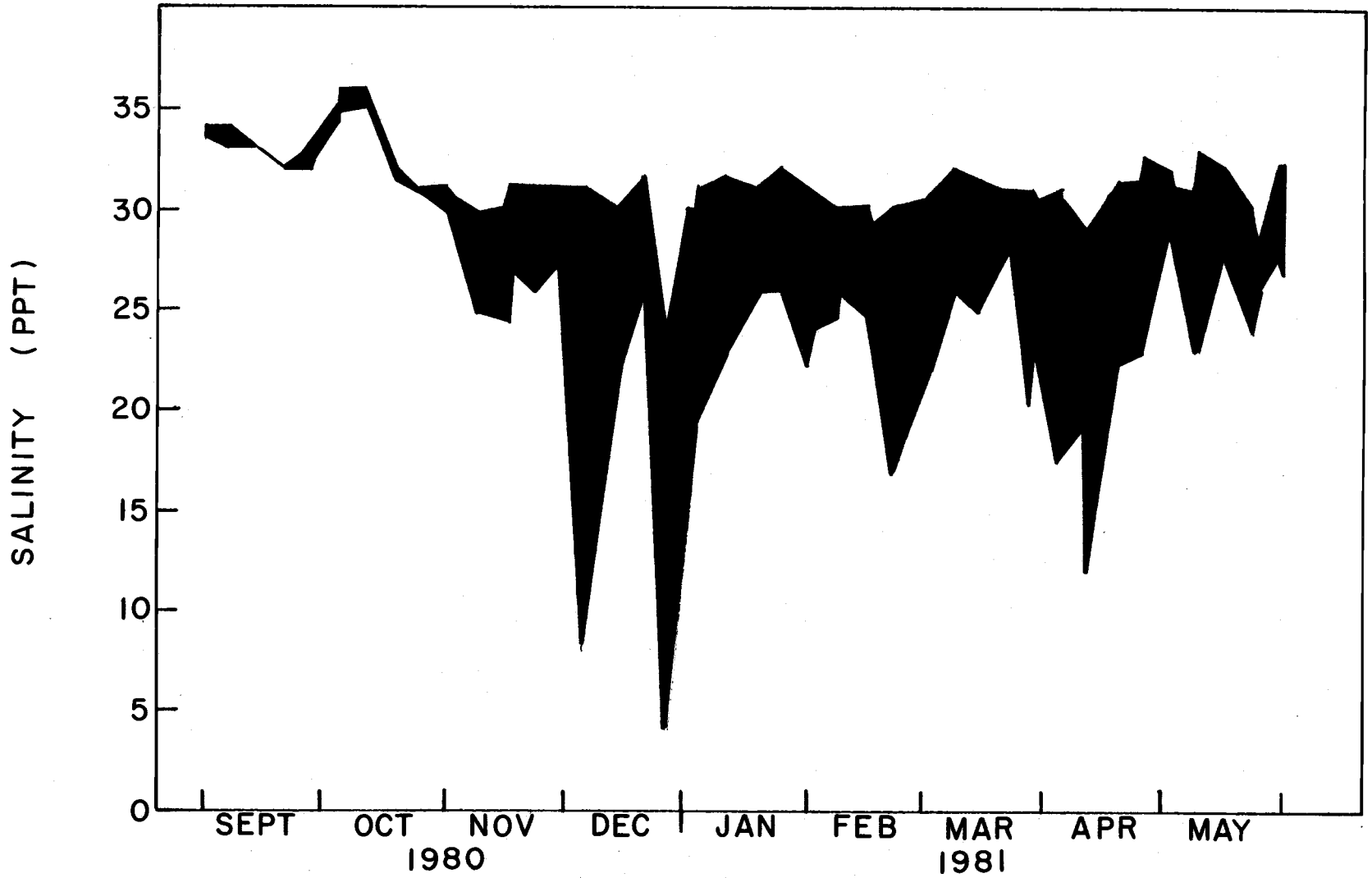


Figure 10. Approximate salinity of water from Yaquina Bay entering the Oregon State University Marine Science Center during the months that spat were held in the laboratory.

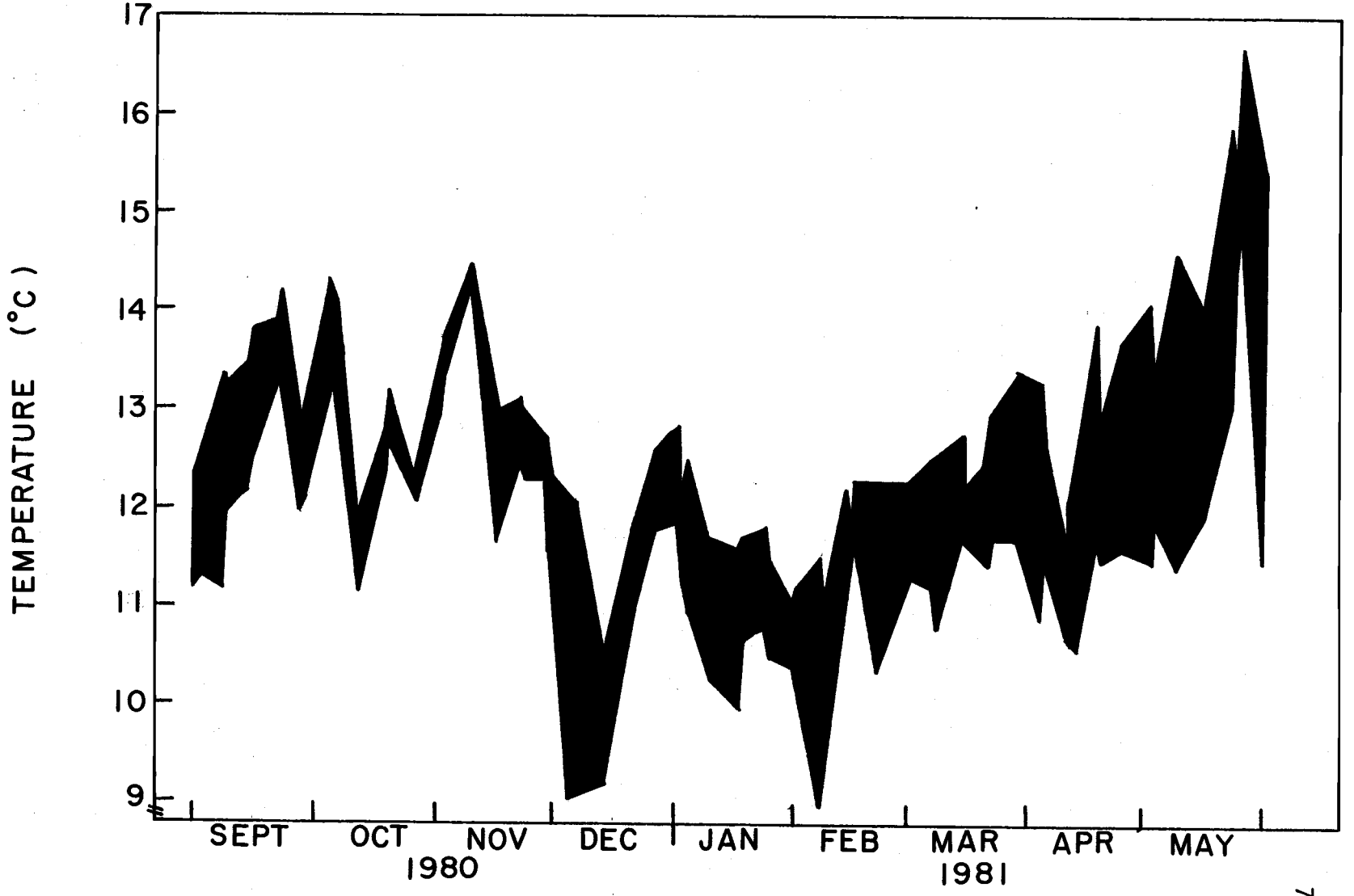


Figure 11. Approximate temperature of water from Yaquina Bay entering the Oregon State University Marine Science Center during the months that spat were held in the laboratory.

Barrett, 1963) reported that Pacific oysters can feed at temperatures as low as 3°C. Quayle (1951) noted little or no growth of Pacific oysters when temperatures were less than 9°C. Malouf and Breese (1978) reported optimum growth of juvenile Crassostrea gigas at 15°C They observed little growth advantage to water temperatures greater than 15°C, but mortality was always higher at elevated temperatures.

Walne (1979) found high mortalities among Ostrea edulis spat moved to outdoor tanks a few days after setting. Knight-Jones (1949) attributed high mortalities among recently-settled O. edulis spat to activities of oyster pests. In the present experiments, screens around the seawater intake in Yaquina Bay should have prevented most predators from reaching the spat. Invertebrate larvae did enter the system, however, and competition from these organisms may have reduced spat survival (Korringa, 1976). The lack of food has been discussed previously as another possible cause of mortality in these experiments.

No specific recommendations can be given to oyster growers based on these survival studies, since the laboratory set-up did not adequately reflect conditions found on oyster grounds. It might be advisable to conduct further experiments to determine the relationship between setting and survival for eyed larvae that are refrigerated for several days before being set.

# RECOMMENDATIONS TO OYSTER GROWERS

Based on the laboratory studies described in this paper, the following recommendations are made for setting Pacific oyster eyed larvae (Table 30).

Table 30. Recommendations for setting commercially-reared eyed larvae of the Pacific oyster, Crassostrea gigas.

| Factor | Recommendation | Comments |
|---|---|---|
| Temperature | 25°-30°C (77°-86°F) | Setting was best at 25°C if larvae were stored for 5-8 days at 5°C before being set. |
| Salinity | 20-25 ppt<br>20-30 ppt for Kumomotos | There was no significant difference in results for salinities in the range of 15-30 ppt. |
| Feeding | Unnecessary | There was no advantage to feeding larvae during setting when the setting period was 48-hours and the larvae were set in the dark. |
| Substrate | Oyster Shell | There was no difference between oyster, cockle, butter clam, or razor clam shells as setting substrates, but spat may not survive as well on clam shells. |
| | Littleneck Clam Shells Unsuitable | Dipping oyster or butter clam shells in an aqueous extract of oyster tissue improved setting, but this is not a practical procedure for commercial oystermen. |
| Storage of Eyed Larvae | Not Recommended without Further Study | Preliminary results indicate that setting may be enhanced by refrigerating eyed larvae at 5°C (41°F) for several days before setting them, but spat mortality may be higher among larvae that are held before being set. |

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# APPENDIX

Appendix 1. Percentage of total set attaching to shells placed in experimental containers as cultch (Experiment 2).

| Temperature (°C) | Salinity (ppt) | % of Total Set Attaching to Shells |
|---|---|---|
| 28 | 30 | 90 |
| | | 87 |
| | 25 | 60 |
| | | 82 |
| | 20 | 96 |
| | | 83 |
| | 15 | 34 |
| | | 38 |
| 25 | 30 | 88 |
| | | 75 |
| | 25 | 71 |
| | | 80 |
| | 20 | 45 |
| | | 50 |
| | 15 | 65 |
| | | 74 |
| 20 | 30 | 52 |
| | | 38 |
| | 25 | 24 |
| | | 57 |
| | 20 | 83 |
| | | 75 |
| | 15 | 29 |
| | | 38 |
| 15 | 30 | 31 |
| | | 86 |
| | 25 | 45 |
| | | 25 |
| | 20 | 20 |
| | | 33 |
| | 15 | 10 |
| | | 31 |

Appendix 1. Percentage of total set attaching to shells placed in experimental containers as cultch (Experiment 3A).

| Temperature (°C) | Salinity (ppt) | % of Total Set Attaching to Shells |
|---|---|---|
| 30 | 30 | 78 |
| | | 73 |
| | 25 | 85 |
| | | 89 |
| | 20 | 81 |
| | | 83 |
| | 15 | 79 |
| | | 47 |
| 25 | 30 | 86 |
| | | 75 |
| | 25 | 94 |
| | | 82 |
| | 20 | 87 |
| | | 83 |
| | 15 | 62 |
| | | 56 |
| 20 | 30 | 26 |
| | | 28 |
| | 25 | 53 |
| | | 68 |
| | 20 | 75 |
| | | 40 |
| | 15 | 7 |
| | | 24 |
| 16 | 30 | 31 |
| | | 31 |
| | 25 | 30 |
| | | 30 |
| | 20 | 77 |
| | | 28 |
| | 15 | 38 |
| | | 23 |

Appendix 1. Percentage of total set attaching to shells placed in experimental containers as cultch (Experiment 3B).

| Temperature (°C) | Salinity (ppt) | % of Total Set Attaching to Shells |
|---|---|---|
| 30 | 30 | 28 |
| | | 31 |
| | 25 | 40 |
| | | 19 |
| | 20 | 27 |
| | | 42 |
| | 15 | 18 |
| | | 22 |
| 25 | 30 | 14 |
| | | 38 |
| | 25 | 16 |
| | | 26 |
| | 20 | 31 |
| | | 13 |
| | 15 | 44 |
| | | 80 |
| 20 | 30 | 21 |
| | | 56 |
| | 25 | 22 |
| | | 28 |
| | 20 | 13 |
| | | 21 |
| | 15 | 29 |
| | | 0 |
| 15 | 30 | 8 |
| | | 0 |
| | 25 | 13 |
| | | 17 |
| | 20 | 30 |
| | | 0 |
| | 15 | 33 |
| | | 100 |

Appendix 1. Percentage of total set attaching to shells placed in experimental containers as cultch (Experiment 4A).

| Temperature (°C) | Food | % of Total Set Attaching to Shells |
|---|---|---|
| 30 | Yes | 9 |
| | | 42 |
| | No | 2 |
| | | 17 |
| 25 | Yes | 14 |
| | | 12 |
| | No | 10 |
| | | 7 |
| 20 | Yes | 9 |
| | | 7 |
| | No | 4 |
| | | 9 |
| 15 | Yes | 8 |
| | | 2 |
| | No | 2 |
| | | 6 |

Appendix 1. Percentage of total set attaching to shells placed in experimental containers as cultch (Experiment 4B).

| Temperature (°C) | Food | % of Total Set Attaching to Shells |
|---|---|---|
| 30 | Yes | 3 |
| | | 7 |
| | No | 3 |
| | | 3 |
| 25 | Yes | 1 |
| | | 4 |
| | No | 2 |
| | | 2 |
| 20 | Yes | 1 |
| | | 10 |
| | No | 7 |
| | | 7 |
| 15 | Yes | 0 |
| | | 11 |
| | No | 0 |
| | | 10 |

Appendix 1. Percentage of total set attaching to shells placed in experimental containers as cultch (Experiment 5).

| Temperature (°C) | Food | % of Total Set Attaching to Shells |
|---|---|---|
| 30 | Yes | 33 |
| | | 22 |
| | No | 34 |
| | | 23 |
| 25 | Yes | 24 |
| | | 48 |
| | No | 41 |
| | | 49 |
| 20 | Yes | 25 |
| | | 0 |
| | No | 100 |
| | | 0 |

Appendix 1. Percentage of total set attaching to shells placed in experimental containers as cultch (Experiment 6).

| Temperature (°C) | Substrate | % of Total Set Attaching to Shells |
|---|---|---|
| 30 | Butter Clam | 26 |
| | | 55 |
| | Cockle | 32 |
| | | 51 |
| | Oyster | 70 |
| | | 82 |
| 26 | Butter Clam | 33 |
| | | 54 |
| | Cockle | 85 |
| | | 34 |
| | Oyster | 37 |
| | | 43 |
| 20 | Butter Clam | 11 |
| | | 29 |
| | Cockle | 0 |
| | | 0 |
| | Oyster | 39 |
| | | 20 |

Appendix 1. Percentage of total set attaching to shells placed in experimental containers as cultch (Experiment 7).

| Substrate | Extract | % of Total Set Attaching to Shells |
|---|---|---|
| Razor Clam | Yes | 50 |
| | | 73 |
| | No | 11 |
| | | 38 |
| Butter Clam | Yes | 48 |
| | | 50 |
| | No | 100 |
| | | 0 |
| Oyster | Yes | 69 |
| | | 82 |
| | No | 100 |
| | | 80 |
| Littleneck Clam | Yes | 67 |
| | No | 100 |